

EVOLUTION PROSPECTIVES OF BIOREACTORS

Abstract

Bioreactors are resourceful apparatus, simulating natural ambient conditions for the growth of cells in microbial or cell culture under optimal conditions and their volumetric capacity can be ranging from small, bench scale to large scale level. This chapter emphasizes the fundamentals of bioreactors with their major working parts and elucidates on the distinct types of conventional bioreactors in conjunction with the introduction of current modern bioreactors. Additionally, the current chapter updates the readers with the record of an assortment of modern bioreactors accessible in the market for their future uses.

Keywords: Bioreactors, Evolution, Conventional Bioreactors, Modern Bioreactors.

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I. INTRODUCTION

Bioreactors can be defined as a vessel basically used for carrying out biological reactions for culturing aerobic cells and to conduct enzymatic or cellular immobilization and usage of bioreactor can also be ascribed to be the scale-up process¹. Bioreactor offers a controllable ambiance favoring the biological, biochemical and mechanical requisites for the manufacture of engineered products. Owing to the fact that the intents to produce vital bio-based products, it is essential to monitor the process parameters viz. heat transfer, shear stress, internal and external mass transfer, fluid velocity etc². The fermentation process can be either aerobic (in the presence of oxygen) or anaerobic (in the absence of oxygen). The fundamental raw-materials for the desired product formation can be either simple or complex inorganic and organic sources. The major extracellular products generated from aerobic fermentation are enzymes, amino acids and vitamins; which are known as the primary metabolites³. The growth regulators, polysaccharides, antibiotics, lipid-lowering medication and cyclosporin A are established as the customary extracellular secondary metabolites⁴. The anaerobic process yields the fuel products such as ethanol, isobutanol and vital organic acids^{2,5}.

Bioreactor variegates from traditional chemical reactors as they control and support the biological entities and interestingly, bioreactor system offers a high level of control for contaminations and process upsets⁶. The bioreactor environment should be efficacious to aid the microbial systems to execute their function under defined conditions. This elucidates the requirement of panel of special features in the bio catalytic engineering processes⁷. Reduction in the undesired activities and maintenance of the desired bioactivity are dwelling as the major challenges owing to the fact that biological microbes could mutate and thereby alter the reaction biochemistry or physical traits of the biological organisms⁶. The vital factors to be maintained throughout the fermentation process are monoseptic conditions with optimal mixing cum uniform and meager shear rates⁸. In addition, the mass transfer becomes the most crucial factor in the aerobic fermentation. An insufficient supply of oxygen from the air to the media might result in production of inappropriate products and ultimately to perishing scenario of the microbes. Henceforth, the aerobic fermentation process always calls for a specific oxygen uptake rate (OUR) which is equal to the oxygen transfer rate (OTR) under the quasi steady-state conditions⁵.

As OUR differs throughout the aerobic fermentation, it is also necessitous to address that engineers should be able to design the optimal peak rates. The conventional processes consist of rates ranging from $100 \text{ mmol L}^{-1} \text{ h}^{-1}$ to $300 \text{ mmol L}^{-1} \text{ h}^{-1}$. However, the fermentation process of some modified strains of *E.coli*, consumes a higher oxygen level with a rate of $500 \text{ mmol L}^{-1} \text{ h}^{-1}$ wherefore requiring oxygen-enriched air as supply for feed gas⁵. Supplementarily, the maintenance of adequate heat transfer, appropriate flow condition and feeding of suitable substrate eliminating the under or overdosing is highly requisite for bioreactor fermentation. Supplementation of substrate, salts, vitamins should be ensured along with oxygen and water availability and moreover, scale-up, process control devices, sterilization, operational flexibility, antifoaming measures, compatibility with downstream and upstream processing are the vital requirements for a bioreactor².

Forasmuch as the conditions are microbiologically favorable inside the reactor system, the biological reactions can be treated as other chemical reaction, despite the fact that kinetic expressions would be quite complicated in comparison with the simple first or second order reactions⁵. The bioreactor size can differ from the microbial cell (few mm³) to shake flask (100-1000ml) to lab-scale fermenter (1-50L) to pilot (0.3-10m³) and plant scale (2-500m³) for large scale industrial applications².

1. Significant Systems in a Bioreactor: The salient features of a bioreactor are oxygen delivery system, agitator system, headspace volume, foam controller, pH and temperature controllers, sampling ports, sterilization and cleaning system and lines for emptying and charging the reactor⁹. The air supply system comprises of inlet and outlet air sterilization system, air sparger and compressor. An air compressor in the system, forces air into the bioreactor for generating the sufficient pressure to force the air through the sparger holes, filter system and into the liquid.

The compressor system used for large scale bioreactor is capable to produce air at 250 KPa. The air should be oil free and dry to avoid the blockage of inlet air filter or medium. The inlet and outlet air are sterilized by the process of filtration. These filters are commonly made of Teflon membranes housed in a polypropylene housing and these membranes are highly tough and reusable. The spargers are incorporated in the system for breaking the inlet air into small bubbles. The most common type of filter used in the bioreactors is sparger rings. It consists of a hollow tube with several small drilled holes. They are placed at the bottom of the agitator having an approximate diameter of an impeller. The agitator systems are comprised of impeller, baffles (for mixing) and external power drive.

The majority of agitators systems are commonly consist of Rushton turbine type impeller. Additionally, impellers offer enough shear condition needed for breakup of bubbles. The foam control is the indispensable part of the bioreactor as the formation of undesired bubbles results in the blockage of air exit filters in conjugation with the pressure builds up in the reactor.

The headspace volume is defined as the ratio of total volume (taken up by microbes, medium and gas bubbles) of the bioreactor and the working volume of the bioreactor. The general working volume of a reactor is 70-80% of the total bioreactor volume. The control system for pH is ascribed to control the pH of the media often through neutralizing agents and these agents should possess properties such as non-toxicity to cells with non-corrosive nature. The most common pH control agent is sodium carbonate for a small scale bioreactor. The temperature of the bioreactor system is controlled by the temperature probes. This is also aided by the heat transfer system (coil and jacket). The sampling ports are the injector ports through which the water, salts, nutrients and other requisites can be injected into the bioreactor system and it could also act as a sample collecting system.

The sterilization process is highly indispensable and essential process for fermentation, in order to eradicate the undesired contamination. For large-scale sterilization, the steam mediated thermal sterilization is accountable. On the other hand, the heat-sensitive equipments are often sterilized by chemical substances. The

sterilization can also be accomplished by UV rays (for surfaces), x-rays (for liquids) and also through membrane and depth filters. The emptying and charging lines are commonly used for the supply of reactants and withdrawal of outgoing products in the reactor system.

II. CONVENTIONAL BIOREACTORS

- 1. Stirred Tank Reactors (STR):** The STR are the most common and multifarious bioreactors. It works under the most ideal operating conditions. In these reactors, the solid retention time is equal to the hydraulic retention time. However, this system bears a major limitation viz. leakage of cells via outlet with the fermentation media; thereby distorting the proportion of cell and food, resulting in a underperformance of the reactor. The mode of operation for these systems can be either batch or continuous. During the continuous operating condition, the STR can be called as CSTR. In the batch mode of operation, feed is introduced at a time and the product is collected from the bioreactor only post to the biotransformation process. Moreover, the fed batch system shows a similar systematic operation which can be distinguished from the former with a division of total feed into distinct batches followed by its feeding at regular intervals¹⁰.
- 2. Continuous Stirred Tank Reactor (CSTR):** The CSTR are designed for submerged fermentation of microbial cultures. In these reactors, there is a continuous supply of fresh medium in conjunction with the removal of bioreactor fluid. Hence, the bioreactor could be operated for a long time without necessitating the shut down process. In these bioreactors, the distribution of the supplied heat is highly uniform and scum accumulation can be impeded. The operation and designing of CSTR is an easy process favoring the large scale productions. CSTR dwells as the prominent bioreactor for applications in fermentation arena¹⁰. These reactors are comparatively productive than batch reactors as they can be operated continuously and control of the microbial growth can be easily accomplished with CSTR¹¹.

The agitation process is achieved by an impeller with the formation of gas bubbles. The pH control of the fermentation medium can be achieved by addition of an acid or alkali. The composition of the liquid phase is uniform and in addition the temperature of the reactor can be maintained to be consistent with the passage of cold water into the cooling jacket, present as a surrounding attachment to the vessel. As an alternative option, glycol can also be used to replacing the cool water¹¹. These reactors can be run either through chemostat or turbidostat. For the chemostat based fermentation, the microbial growth is controlled by adjustment of the substrate concentration. While, in the case of turbidostat, microbial growth is maintained constant by the use of turbidity process in order to monitor the concentration of biomass.

The major assumptions made for CSTR analysis are (i) the mixing is done in such a way that the amount or concentration of all the components in the vessel is typically same in each and every part of the vessel (ii) for an aerobic fermentation, the dissolved oxygen concentration remains the same throughout the vessel (iii) a constant transfer factors are maintained throughout the system i.e., the removal of physiological factors such as heat is continuous (iv) the mixing is performed perfectly for achieving a balanced and same composition of the exit stream to that of the all parts of the vessel content¹⁰.

- 3. Plug Flow Reactors (PFR):** These reactors are also called as piston –flow or tubular reactors. It consists of a unidirectional and continuous flow in a steady state. The flow of the fluids is in the way that the reaction time remains the same for the any flowing material at any cross-sectional area of the tube i.e., during the movement of a fluid, via the channel or large pipe with higher Reynolds number, it executes a plug flow system, which means the negligible variation in the axial velocity along the cross-sectional area. Hence, it can be stated that there is an absence of temperature or concentration gradients in the radial coordinate¹¹.

In addition, the microbial and substrate concentrations diverge throughout the bioreactor system. These reactors offer high initial driving force, hence they are considered similar (functionally) to batch reactors¹². These reactors have a varying composition along their lengths and consist of a lateral mixing system¹⁰. Evincing contrasting properties with continuous stirred tank reactors (CSTR), a sterile feed supply to PFR signifies a negligible or zero level concentration of the biomass present in the effluents. In PFR, the bulk medium is added at once, which does not mix with previous culture present in the reactor. Hence, a productive option to eradicate this problem is to recycle, so that incoming media stream is inoculated prior to entering the reactor.

PFR also offers higher substrate conversion and product concentration. In case of microbial processes, the PFR amplifies the concentration of the effluent products. Despite this, the practical tediousness of PFR gas exchange and requisite of continuous inoculation often evinces to consequent in the recruitment of their analog reactors, if the concentration of the final product is highly essential. However, this type of reactor is not sufficient for strategies of exponential microbial growth¹¹.

- 4. Bubble Column Bioreactors (BCB):** The BCB reactors are air-driven, which could work in the absence of mechanical stirrer. The air bubbles passed from the sparger or perforated plate (at the bottom of the bioreactor), accounts for the proper agitation of the medium thus aiding its adequate mixing. The air reaches the system from the compressor via an air filter, which diffuses into the liquid gradually. The flow rate control is aided by flow meter or rotameter. The remaining air is released out from the reactor. BCB are commonly used for culturing the shear sensitive microbes (moulds) and plant cells^{10, 11}.

These systems are also well known for their meager maintenance and operating costs owing to the absence of the any moving ailments. However, there are some limitations for the scale-up by BCB such as back mixing, high drop of pressure (in case of long columns) and coalescence of bubbles. In this consistent view, the designing of the reactor is also quite tedious due to the complex transport processes viz. heat transfer, mass transfer, flow regime and unknown responses against distinct sets of design parameters like height to diameter ratio. These values of height to diameter ratio vary from 3:1 to 6:1. The overall mass transfer rate in BCB is controlled by the liquid film resistance. The bubble columns can be ascribed as the contactors through which a discontinuous gas phase moves in relation to the continuous phases in the bubble like forms. The most common continuous phase used in these reactors is water¹⁰.

BCB are widely used as prolific reactors for the production of commercial antibiotics such as penicillin. Extending their usage, these reactors are also extensively

used in the chemical processing industries. The fermentation products such as alcohol, baker's yeast and single cell protein are the most common products produced in the respective industrial sectors¹⁰.

- 5. Packed Bed Reactors (PBR):** The PBR reactors are immobilized reactors with solid matrix as an inert support material for providing a surface for microbial attachment throughout the reactor cell. These supports can be commercial glass beads, activated carbon beads and polyesters materials. Based on the feed entry, these reactors can be up or down flow bioreactors. A low hydraulic retention time (HRT) is effectuated owing to the flock retention and immobilization of the whole cell reactor. For the production of ideal flow within PBR, a turbulent flow is always preferable to laminar for enhanced mixing and less back mixing, as it is a major problem of PBR. Moreover, channeling and clogging are the auxiliary hindrances for scale-up processes using PBR¹⁰.

In PBR, the flow rate is attributed to be proportional to the pressure drop which develops across the reactor; where the pressure drop is directly proportional to the height of the packed bed, linear rate of flow, substrate dynamic viscosity. Contrastingly, the pressure drop evinces a inversely proportional relationship with the area of cross section of the immobilized enzymatic pellets. The immobilized enzyme catalysts are 1-3 mm in diameter as excessive flow rate could weaken the immobilized bed particles or distort them. These strategies of particle deformation reduce the surface area of the particles in contact with the substrate stream, flow restriction, reduction in external mass transfer characteristics and eventually resulting in a increased pressure drop across the reactor¹⁰.

The packed beds can be easily fouled by precipitating and colloidal material and the parameters such as pH could not be controlled through either acids or alkali. Conjugating with this, the temperature control is an added problem for PBR. There can also be deviations from ideal flow, owing to the back mixing problem within the reactor system. Moreover, the formation of channels can occur within the system due to the irregular packing, high pressure drop, and uneven supplementation of the substrate solution resulting in the flow rate differences across the packed bed. Hence, it is necessary to use the immobilized beads of uniform size in order to eradicate the non-ideal traits¹⁰.

The cells can be entrapped in carrageenan or agar. These gels offer larger surface area and effective cellular retention. The other entrapment materials for executing the cell immobilization are concrete blocks, plastic blocks, fibrous materials and wood shavings. Amidst the fibrous materials, the glass or plastics wools are most commonly used for catalytic entrapment objectives. The major applications of PBR are transformation of steroids, production of enzymes and amino acids and waste water treatments. In addition PBR acts as a biological filter in aquarium for recycling of water system¹⁰.

- 6. Fluidized Bed Reactor(FBR):** The cells in the FBR are entrapped in small particles. The relatively smaller size of these particles accounts to the mass transfer and surface area aiding the entrapment strategies. The phenomenon of fluidization of the particles in FBR accounts to the continuous turnover of the particle surface and also the FBR system has higher superficial fluid velocity (6-20 m/h) aiding the complete fluidization of the granules. These beds can be broadly classified as a non-aerated two phase system and

aerated three phase system. The size of the particle approximately varies from 0.2-1 mm with a recycle ratio of 5-500. The aptitude of the bed to expand ranges from 30 to 100%¹⁰.

The major application strategies of FBR are enzyme and cellular immobilization on solid matrix and commercial fermentative applications. The interesting advantages of the FBR are homologous distribution of solids, higher biomass concentration per unit volume of the reactor, higher surface area for bacterial growth and liquid contact enhancing the biotransformation process. In spite of this, there also arousal of some limitations viz. higher bed particle density, higher input of energy, separation of bed particles from bioreactor is also an added disadvantage which curtails the opportunity to recycle them for multiple practical uses¹⁰.

FBRd wells as a prolific propagation system for animal cells and hence, it is highly used as a reactor system for animal cell culture. The animal cells are immobilized or entrapped in gels or in micro-carriers. Therefore, FBR can be established as the prominent bioreactor choice for perfusion cell culture technology¹⁰.

- 7. Airlift Reactor (AR):** The Airlift type fermenter is the modified form of bubble column reactor¹⁰. The AR reactors are designed especially for aerobic culture cultivation. These reactors are devoid of an agitating device. The bioreactor should be filled with water, at the beginning of the operation and at the riser minimum height of the liquid should be maintained. In AR, a re-circulating flow of the liquid is executed and an increase in the air flow rate results higher velocity of the liquid. The suction force developed in the reactor is directly proportional to the air flow rate. The low airflow rate aids the homogenous bubble distribution; whereas, at the higher flow rate of air bigger bubbles are observed in the reactor^{13, 14}. Figure 1a-g, illustrates the schematic overview of the distinct conventional bioreactors.

The sparger placed at the bottom of the vessel, from which a filtered high pressure air is dispersed into the medium. The region into which the bubbles are sparged is called as the air –riser. These fermenters can be of two types such as internal or external loop reactor. Both the loop reactors consist of ascending and descending columns, which are responsible for regulating the flow of the fluid¹¹. These columns can be either parallel or concentrically oriented. This system is highly suitable for biological productions requiring higher rate of oxygen consumption¹⁰.

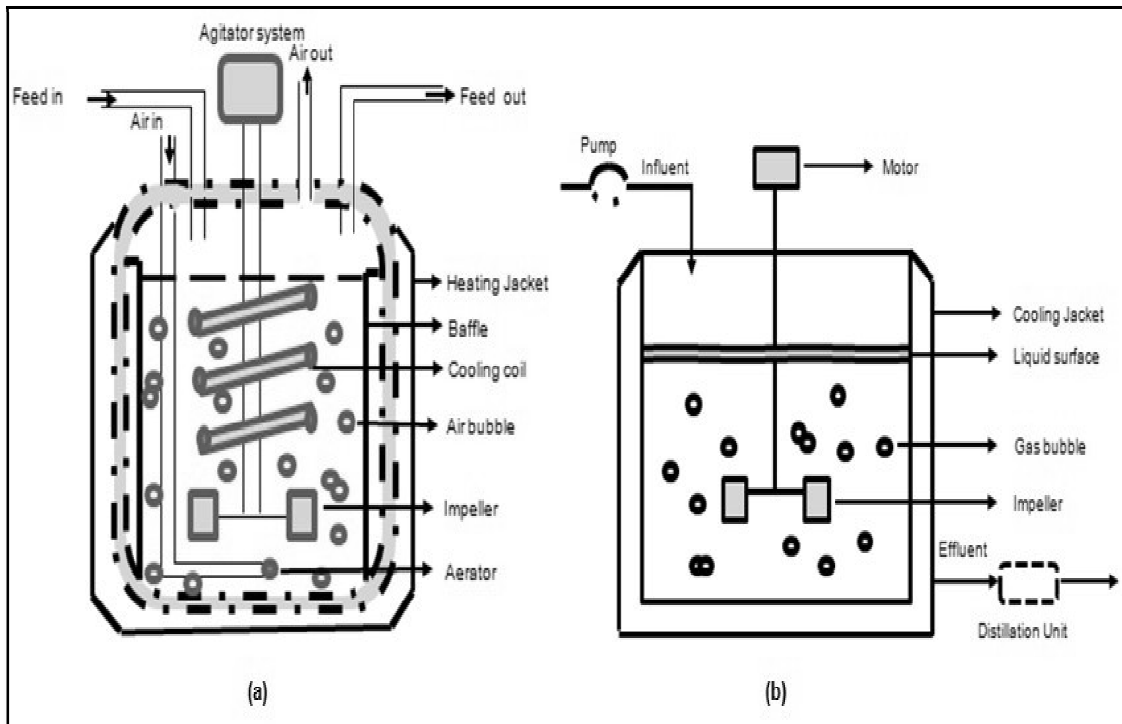


Figure 1(a), (b)

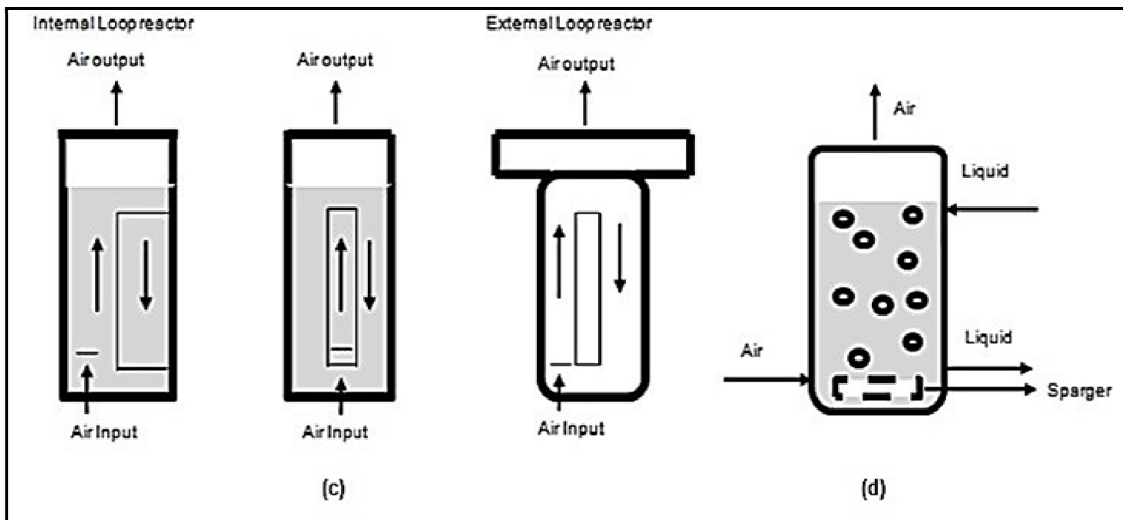


Figure 1(c), (d)

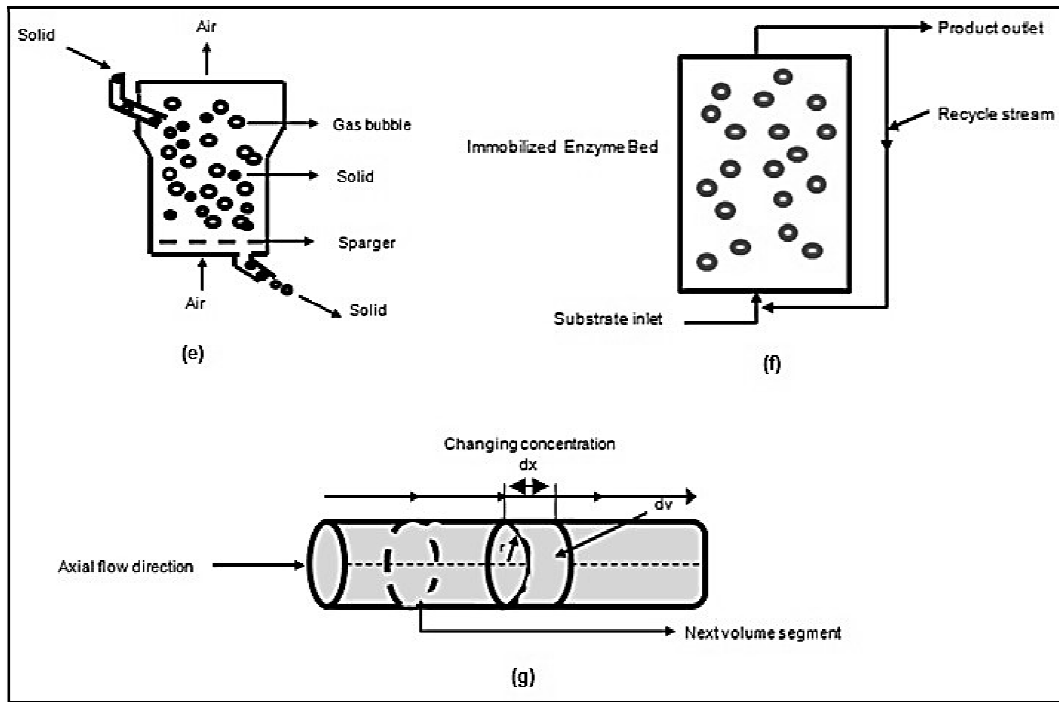


Figure 1(e), (f) and (g)

III. MODERN BIOREACTORS

1. Bench-Top Cell Culture and Bioreactor (BTCCB): The bench top bioreactors are indispensable and prominent modern reactor system for debottlenecking, troubleshooting the scale-down processes, process development and generating valuable proteins. The enthralling industrial practices liable to bench top-scale-up systems are: continuous or perfusion cell culture, batch and fed-batch and fermentation of microbes. These reactors are exclusively ranging from 2L to 20 L for industrial leeway space. The conventional stainless-steel reactors are used productively, although the advanced see-through or glass bioreactors are the current industrial hand-picked choices. These mini reactors are up for grabs with advanced sensor systems, certainly at large scale processes as space constraints can be unavoidable.

The reactor features head plate penetrations for dissolved oxygen probes (DO) and pH probes, along with three addition ports to harvest, exhaust, gas and overlay sparger. The temperature of the culture vessel is maintained by heat blanket (reusable) with a cutout window enabling the culture view. The DO can be monitored through a reusable noninvasive-polarographic probe. The surveillance of the pH parameter is achieved by disposable fluorescence sensor and optical pH probe. Interestingly, the bench top reactors (CelliGen BLU) introduced by New Brunswick are vastly advanced and the fascinating features: presterilized, single-use technology, prevalidated, true scalability, rapid turnaround, lower contamination and startup cost are yearning requisites for research targets.

CelliGen BLU bioreactors have out of the ordinary scope for the growth of ovarian cell line of Chinese hamster in the presence efficacious nutrients (glucose) in the

medium i.e., they display an ideal features viz. higher cell viability, when compared to similar miniature bioreactors such as CelliGen BLU bioreactor, CelliGen 310 bioreactor and Bag and rocker system. The compact control system in the fixed-speed pumps for both addition and harvesting purposes. Moreover, a built in control is also provided for DO, pH, mixing of three or four gases and temperature control. Further, effectuating the biotransformation, four heat mass flow controllers are found for sparging and in concomitant with this a gas overlay with rotameter or heat mass flow controllers is also available for independent gas control purposes entering the vessel via headspace. These reactors also customize the optional weight scale and gas analyzers for measurements¹⁵.

- 2. Disposable Bioreactors (DB):** In the last decades, the disposable bioreactors have been incorporated exclusively into preclinical, clinical and biotechnological sectors. The cultivation vessel of DB is made out of plastics (approved by Food and Drug Administration) such as polystyrene, polyethylene, polypropylene, ethylene vinyl acetate and polytetrafluorethylene unlike the usage of conventional glass or stainless steel as the latter material necessitates a validated cleaning and sterilization protocols incorporating the consumption of towering quality of water system¹⁶. To iterate simpler, the typical configurations of disposable systems is a non-instrumental cultivation container, thereby needs association with an external device viz. Carbon dioxide incubator and rotary shaker to maintain optimum ambiance aiding the cell development and bio production.

These reactors can be used with a volumetric culture capacity of 2000 L or more by their own unit for the control of process parameters and measurement^{17, 18}. For this, they are equipped with sensors: monitoring of dissolved oxygen (DO), temperature and pH.

The versatility is enlightened in these type of systems due to their support in cultivation of yeast, bacterial, plant cells¹⁹, mammalian cells²⁰ and insect cells²¹. The advantages of disposable reactors are easier handling, low costs and time saving with more flexible properties with a good showing titer-wise qualities like presterility²²⁻²⁵. These are also amenable for micro carrier or suspension types with a lower shear stress problems^{26-29, 20}. Moreover, they are comprised of good mixing facilities due to the involvement of distinct type of agitators such as stirred, paddle, orbital shaking and rotating type sparger for balancing the oxygen level in the medium²⁸.

However, these reactors are ascribed for their limited usage and inadequate strength of the plastic material^{18, 30}. Succeedingly, there can be chances for contamination in plastic bags, the system seeks an effective mixing device to enhance the mass transfer coefficient K_{La} value. Besides, the perfusion systems used in disposable reactors are explored limitedly. However, the applications liable to disposable reactors are production of monoclonal antibody (Mab), gene therapy vectors, veterinary and human vaccines and proteins^{26-29, 20}.

- 3. Tissue Cloning Bioreactor (TCB):** Recently the bioreactor technology has been a high prevalence in biotechnology spaces for cellular mass propagation of diverse plant and animal species³¹⁻³⁶. This is due to the expensive nature of the conventional micro propagation strategy as it is less apposite for larger and industrial scale production with an ability to resolve the manual handlings and workloads in distinct micro propagation

phases³⁴. Moreover, these reactors are capable to manage both physiological and chemical factors such as carbon dioxide concentration, gas exchange and photosynthetic photon flux; wherefore an efficient propagation is validated during the *in vitro* proliferation process³⁷⁻³⁹.

There are several recent findings on micro propagation using bioreactors for floral systems like blueberry, Coffee and 80 other crops⁴⁰⁻⁴². Extending their influence, on animal tissue culture viz. tissue propagation and engineering of calvarial osteoblast cells, hybridoma cells, blood cells, nerve cells, cardiac muscle cells and HepG2 cells⁴³⁻⁴⁵. The special demands required for tissue culture bioreactors are: gentle mixing and agitation, aeration (without damaging cells), a controlled and optimum environment for pH, dissolved oxygen, temperature, dissolved carbon dioxide concentration, production of higher biomass and products, lower possibilities for generation of toxic metabolites such as lactate and ammonia and adhering surface for cells.

The reactor system selection is accomplished based on the essential criteria such as production of desirable protein and single or multipurpose utilities⁴⁵. There are also recent development of bioreactors applying mechanical forces through compression/piston systems, hydrodynamic compression, substrate bending and shear stress⁴⁶⁻⁴⁹. For instance, a magnetic force bioreactor (MFB) is used for conditioning of stem cells, tissue engineering and dynamic screening *in vitro* levels. The MFB is based on the physical principles such as rotational and translational motion of ferrofluids, magnetic particles and materials in magnetic fields of high gradient. These systems use biocompatible nano or micro particles consisting of magnetic core coated with polymer; whereby the particles are attached with the cell membrane during the culture process either in multiwall plates or in polymeric scaffold in the bioreactor (prior to seeding).

The strong gradient of magnetic fields generated by an array of electromagnetic coils or rare earth magnets produce a translational force on nanoparticles along the vector (gradient) generating a tensile force on cells present in the scaffold⁵⁰. This force could be applied through a drive system in a time-varying approach for the alternating current application system to the coil system^{51, 52} and this force is amenable to the stimulation of loading of cell or plasma membrane mechanically even in the absence of direct cell access in the reactor system and without necessitating the stress transfer to the cells from the scaffold. By the variation of the magnetic properties or field strength of the nanoparticles. The cells with distinct magnetic properties can be seeded in distinct regions in the 3-D scaffold, generating a spatial variation of the force utilizing the geometry with same magnetic field.

- 4. The Envirostat (EVS):** Envirostats are chemically inert microfluidic chip based miniaturized analytic and cultivation system for microbes, channelizing a new trail for bio-based research. The cultivation can be performed for diverse microbes irrespective of the cell type or size under controlled environmental conditions^{53, 54}. It aids the visualization of single microbial cell with spatiotemporal resolution, therefore shedding light on into a single cell as the most primary unit beyond the vast microbial⁵⁵⁻⁵⁷. In addition, Envirostat systems make use of continuous perfusion to enable the user to decouple a single cell from fluctuations (extracellular). Control of the cellular micro-ambience in conjunction with the engineering favors the linking of cellular physiological

state and surrounding physicochemical state. Hence, it follows that for the understanding of multilevel physiological environment and matrix, the cellular description under controlled and standardized conditions with microfluidic ambience in the Envirostat systems is highly requisite^{58, 59}.

In the Envirostat system, 3-dimensional negative dielectrophoresis (nDEP) technique, can be used for octupole cage trapping and separation of single cell^{54, 60}. Moreover, during the cultivation process, it maintains a constant environmental condition⁵³ and this constant ambience favours the cultivation of microbial species like bacteria and yeast in the nDEP octupole cage with a significantly higher growth rate in comparison to the experiments based on population⁶⁰. A comparative study with distinct cultivation systems (single cell) can be a choice for excluding the negative influences of nDEP field forces on the phenotypes of the microbial cells in the Envirostat⁶¹. However, the Envirostat technique suffers from certain restrictions such as the usage of polymeric materials for the reactor manufacturing. This is ascribed to the reason that the usage of polymeric materials hinders the scrutiny of molecules viz. secretory microbial metabolites with meager concentration through adsorption (unspecific) of the target molecules. Therefore, it arises the need for the development of new strategical concepts giving rise to the chemically inert reactors to overcome these drawbacks and establish new enormous possibilities in the arena of single microbial cell analysis.

- 5. Miniature System Type Bioreactor (MSTB):** Miniature bioreactors are commonly used for development of growth medium and improvement of strains through metabolic engineering or by directed evolution; thereby bio prospecting the natural metabolites. Hence, eradicating the burdens of large bioreactor. Principally, MSTBs reduces intensity of labours and the expensive cost of materials for cellular cultivation for bioprocess development. Thereby, emerging as a current level of interest with the increase in the parallelism level⁶²⁻⁶⁴. MSTBs are less instrumented with meagre chances for off-line sampling owing to the usage of small volumes; in addition no devices have been able to yet satisfy the miniaturizing challenges i.e. favoring the large scale process conditions. Hence, it is productively requisite to develop multifarious and prolific bioreactors for process development.

MSTBs can be used variegatedly based on the agitation methods such as shaking, stirring and gas-sparging. The shake flask systems are have been used for the past fifty years by the scientists ranging from 10 ml to 500 ml⁶⁵. The shake flasks are either made of plastic or glass and for which the agitation can be achieved through orbital or linear shaking in the temperature controlled conditions. The significant factors affecting shake flask cultivation are fill volume, construction material, vessel size, battery geometries, plug types. It has been well asserted that 90% of microbial culture experiments for a diverse range of microbial system such as yeasts⁶⁶, fungi⁶⁷, bacteria⁶⁸ and mammalian cells⁶⁹. Moreover, there are also introduction of instrumented shake flask system for control of pH and dissolved oxygen levels^{66, 70}.

Bubble columns are based on gas sparging systems can be ascribed as stirred reactor system. The sparging system offers adequate oxygen mass transfer and promote proper mixing in the column for microbial cultivation. As an alternative option miniature bubble column reactors (MBCR) have been developed which are based on microtitre

plates with porous membranes (frits) acting as a complete base for each individual well⁷¹ and air permeates via fit and flows through each well and thereby supplying adequate oxygen for the wells. Where, each frit has identical degree of porosity with equal flow rate to the each column, which can be calculated; therefore preventing variation in the air-flow rate which could affect the results.

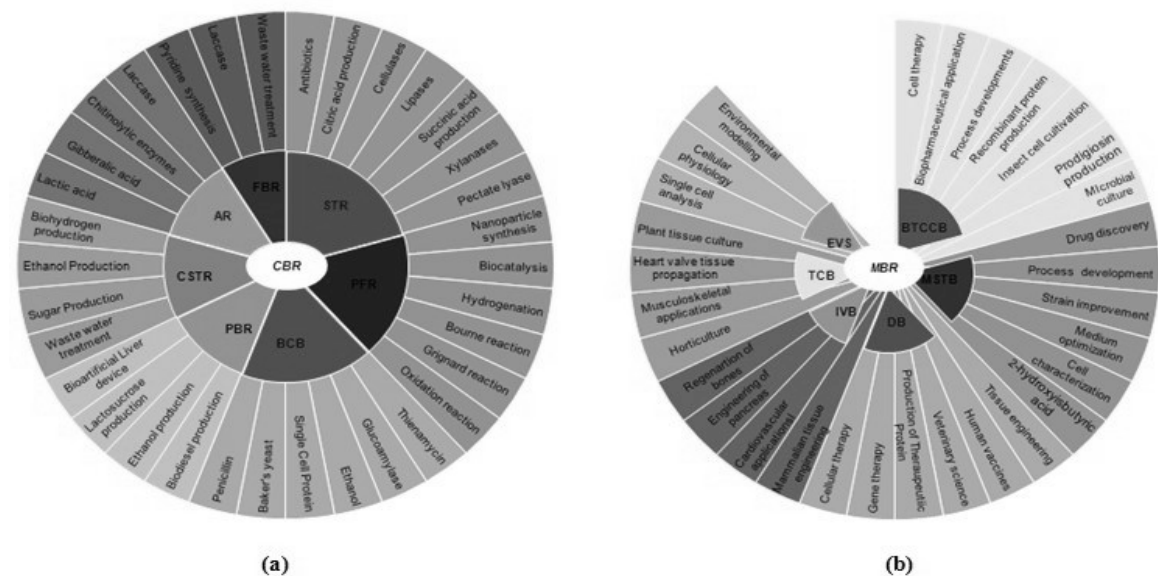
As an alternative to shaken systems, MSTBs have been developed on the basis of traditional stirred tank system for cellular characterization and process development objectives. Interestingly, these devices are designed like lab-scale bioreactors and hence acquiring greater aptitude for monitoring and controlling process in comparison with other miniature reactors. These are customary process volumetric intermediates between shake flasks and microtitre plates^{72, 73} and are constructed with broad spectrum of materials such as pyrex, poly-methylmethacrylate⁷³, perspex⁷² and stainless steel. These bioreactors are capable to mimic the conventional stirred tank system in terms of the oxygen demand, varying rheology and shear sensitivity; supplementarily they are potent systems for the growth of wide range of microbes as a result of their short mixing times and higher mass transfer coefficient (K_La) values. The effective mixing prospects allow the maintenance of homogenous conditions in case of viscous fermentation broth; while the higher (K_La) values channelizes the growth of quickly respiring microbes (*E.coli*).

- 6. In vivo Bioreactors (IVB):** The *in vivo* bioreactors display their profound potentiality in reconstruction of skeletal defects and other damaged tissues. These reactors can be of highly beneficial than tissue engineering approaches as the former could reduce the issues of donor site morbidity and other infections. To achieve these objectives large animal models are necessary to optimize and screen new strategies for the growth of required volumes of the tissues *in vivo*⁷⁴. A major strategy for bone tissue engineering: exploiting the natural osteogenic and angiogenic properties of the periosteum to act as an *in vivo* bioreactor to accomplish the formation of bones (vascularised) within implanted chambers of predetermined 3 dimensional shape^{75, 76}. The overall approach for the above process is (i) the image offers information of the patients on the 3D structure of the defect for reconstruction (ii) the computational models are used for determination of optimal structures (iii) designing of chambers for tissue reconstruction (iv) The chambers are supplied with scaffolds, growth factors and cells and implanted against the periosteum in the location away from the defect site (v) the reconstructed bone is transferred together with the periosteum to the recipient location in the form of a vascularised bone flap⁷⁴.

The bioreactors based on periosteum can be clinically applied for the vascular bone flap prefabrication for the transfer⁷⁶. For instance, to post excision of buccal cell carcinoma (squamous), the reconstruction was achieved firstly using the myocutaneous flap and a reconstruction plate. However, this approach failed and resulted in the incomplete reconstruction with a radial forearm flap, free thigh cutaneous flap (anterolateral) and a fibula flap. Henceforth, the meager remaining choices for the patients paved the way for the *in vivo* bioreactor based application using the morcellized graft of bone as the biomaterial. These morcellized grafts (harvested from the iliac crest) were filled in polymethylmethacrylate chambers, followed by which the chambers were subjected to implantation against the periosteum in the iliac crest. After a timeline of 8 weeks the bone was harvested and defective locus and histological samples signified the reconstructed bone. Table 1 records the details and specifications of various modern

bioreactors currently available in the market.

The competent model systems for this purpose are ovine and porcine, established for clinical bone tissue engineering *in vivo*. In addition, the non-human primates can be used as a modelling source, which are capable to offer broad battery of advantages⁷⁷. However, the high cost and strict regulations restricts their broader prospectives. The *in vivo* bioreactor should be consisting of the following traits in terms of the preclinical model: imitate clinical surgical approaches, supportive to the assessment of vascularised formation of bones in higher volume with complexity, acquiring high implantation site with probabilities of lower infection and higher regeneration, adaptable to distinct components of tissue engineering and allow results evaluation quantitatively^{74,75}. In spite of all these productive applications of *in vivo* bioreactors, it is an indispensable requisite to explore an optional and established sources to meet the existing demands in the clinical therapies^{78, 79}. Fig. 2a-b, enlists the various applications of conventional and modern bioreactor systems.



IV. FUTURE DIRECTIONS AND TRENDS

Emerging over eons bioreactors, have been used for diverse biological processes. Based upon the final required products, process variables and feed medium distinct types of bioreactors can be preferable suitable to the processes. In this consistent regard, the advancement and development of science and technology, has channelized new-fangled horizons for aiding the easier production of commercial products viz. recombinant proteins, novel antibiotics in addition to the cellular therapies and tissue engineering. These modern evolutions also feature the exceptional traits such as high cellular density, easier cell separation with reusability and easier compound separation. These inimitable specifications bring about the further fascinating interests in their exploration amenable to needs from industrial to clinical scale developments.

V. DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this book chapter.

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VII. FIGURE LEGENDS

Figure 1: Configurations of distinct conventional bioreactors a) Stirred tank reactor b) Continuous stirred tank reactor c) Airlift reactor d) bubble column bioreactor e) Fluidized bed reactor f) packed bed reactor & g) plug flow reactor.

Figure 2: Industrial and clinical applications of conventional and modern bioreactors.

Table 1: Summary of the Distinct Modern Bioreactors Currently Available in the Market with their Characteristic Specifications.

Reactor type	Bioreactor brand	Vendor	Volumetric capacity	Comments
Bench top bioreactors	LGR-1000	Emerald scientific	5L	Available with zero dead space drain and offers easy accessibility.
	Minifors 2	Infors HT	1.5 L, 3 L & 6 L	For microbial cultivation with good mixing and safety processing.
	Multifors 2		0.4 L, 0.75 L, 1.4 L (microbes) 0.4 L, 0.7 L & 1 L (cell culture).	For microbial and cell culture perspectives and consists of aseptic stirring system with time saving and highly precision pumps.
	Labfors 5		10L	For cell culture application with batch, fed batch and continuous mode of operation
	Labfors 5 Cleaning in place (CIP)		0.5 L to 10 L	Cultivation of microbes viz. E.coli, Pichia pastoris and phototrophic organisms with automatic cleaning.

	Xcellerex XDR-10	GE healthcare lifesciences	4.5 L to 10 L	Cultivation of broad range of cell lines with shear stress.
	DASGIP® benchtop bioreactors for cell culture	Eppendorff	2.5 L & 3.5 L	Autoclavable for cell culture application.
	DASGIP® bioreactors for Microbiology		2.5 L & 3.5 L.	Autoclavable for microbial fermentation.
	The BioFlo 120 (borosilicate glass)		1 L, 2 L, 5 L & 10 L.	Autoclavable, appropriate for all levels of research and development with digital
				Sensor technology for pH, CO ₂ , dissolved oxygen measurements.
	Reactor Ready pilot	radleys	5 L to 20 L	Specialized with self-aligning stirrer and universal pilot scale system.
	ambr® 15	Sartorius	0.012 L	Applicable for microbial fermentation strain selection, vector screening, feed and media development, early process optimization
	UniVessel® SU		2 L	Autoclavable, single-use and includes optical- holder for pH and dissolved oxygen control.
	BIOSTAT® B (BOROSILICATE GLASS)		1 L, 2 L, 5 L & 10 L	Cultivation of animal cells, insect cells, microbial cells, yeast cells, fungal and plant cells.
	Smart Glass bioreactors	Finesse	1 L, 3 L, 7 L & 15 L.	For yield optimization in research and development with current good manufacturing practices.
	Glass Autoclavable bioreactors	Applikon Biotechnology	1 L, 2 L, 3 L, 5L, 7 L, 15 L & 20 L.	Cultivation of cell and microbial cultures.

	CHEMRXNH UB SYSTEM	Chemglass Life sciences	0.3 L, 0.5 L, 1L, 2 L & 5 L.	Incorporated with Heidolph Motor and Mainfold/ Drain system and supports easier installation and removal of glassware and fits into bench top hoods.
	4523 BENCH TOP PRESSURE REACTOR SYSTEM	Parr instruments	0.97 L	Works good for highly viscous reaction mixtures of 25000 centipoise
	BioFlo 115 BenchtopFer mentor& Bioreactor	New Brunswick	1.L, 3 L, 7.5 L &14 L.	Cultivation of microbial cells, yeast cells, plant cells and mammalian cells by both aerobic or anaerobic fermentation.
	BioNet®	Broadley James	, 5 L, 7 L, 15 L & 20 L.	Appropriate for cell culture and microbial cultivation.
Disposable bioreactors	Mobius®	Merck	3L	Suitable for bench scale cell culture prospects and process developments
			50 L &200 L	Suitable for pilot scale cell culture prospects and process developments
			1000 L & 2000 L	Suitable for large scale cell culture applications with optimization of process parameters and their developments.
	Blonebenchto p single-use bioreactor system	Distek	2 L &5 L	Cultivation of mammalian cells and recombinant protein production.
	Hyperforma 5:1 Single- Use Bioreactor	Thermo Fischer scientific	50 L, 100 L, 250 L, 500 L, 1000 L & 2000L.	Cultivation of mammalian cells with application in process development, clinical trials and production of cGMP cell culture systems.
	Allegro™ Single- Use Stirred Tank Bioreactors	Pall Biotech	2000 L	Applicable for cell culture objectives and its scale-up.

	BIOSTAT® B	Sartorius	12.5 to 2000 L	Comprises of non-invasive sensors with online lactate and glucose measurement facilities.
	VMF50L/ 200L-SUB	Satake	50 L & 200 L.	Applicable for cell culture prospects.
	BactoVessel SUF	Cercell	0.5 L & 30 L.	Cultivation of microbial cells.
	Elara flat photo bioreactor	Solaris	40 L	Cultivation of bacteria, microalgae, plant cells and mass under optimal conditions.
	Jupiter		200 L, 400 L, 650 L, 800 L & 1000 L.	Cultivation of mammalian cells.
	Genesis		7.5 L, 10 L, 15 L & 20 L.	Cultivation of mammalian cells.
	M series		30 L to 200 L.	Cultivation of mammalian cells.
	Bioengineering		1.6 L, 2 L & 2.5 L.	Ideal for both scale-up and scale down processes
	Minifor fermenter-bioreactor	Lambda	0.3 L, 0.4 L, 1 L, 3 L & 7 L.	Cultivation of algal cells, animal cells, insect cells, bacterial cells, yeast cells, fungal and plant cells.
	CELL VESSEL	Cercell	All sizes ≤75 L	For microbial fermentation and scale-up prospectives.
The Envirostat	MICROFLU DIC LAB ON A CHIP	Provendis		For separation of polarizable of bio particles like bacteria cells from each other and enables their single cell analysis.
	MICROFLUI DIC CHIPS	Micalyne		For microfluidic mixing; genomic and proteomic analysis.
	PDMS MICROFLU DIC DEVICES	Flow JEM		Favours simultaneous appraisal of genome-wide RNA expression in thousands of individual microbial cells

	THERMOPLASTIC MICROFLUIDIC DEVICES			Made and offered in distinct thermoplastic devices based on thermal stability and chemical compatibility.
Tissue Cloning Bioreactor	SYNTHICON Rotary Cell Culture System™	MDxBioSciences	0.001L, 0.002L, 0.004L & 0.001 L.	Cultivation of human and animal cells with extreme fragility and their development into three dimensional model.
	WAVE Bioreactor System 200	GE healthcare life sciences	5L-100L.	For cell culture applications, where cells culture medium contact presterile chamber under rocking condition.
	NASA tissue cloning bioreactor	NASA	0.125 L	Growth of heart tissue, cancer tissue, skeletal tissue, ligaments, kidney and liver cells for therapeutic applications.
	LumaGen&CardioGen:	Bliss	0.06 L	Offers shear and pulsatile stress stimulation to the
	Pulsatile Pressure & Flow			vascular construct.
	LigaGen: Tension Bioreactor(L3 0-1C)		0.023 L	A single sample system, offering oscillatory stimulation of stress to ligaments and tendons.
	LigaGen: Tension Bioreactor(L3 0-4C)		0.08L	A multi sample system supporting the growth of ligaments and tendons.
	CartiGen: Compression Bioreactor (Single Sample: C10- 1)		NDA	A single sample bioreactor, for stimulation of stem cells and chondrocyte; meniscus regenerative medicine.
	CartiGen: Compression Bioreactor (Multi-sample & Imaging: C10-12 c)		NDA	A multi-sample reactor system for stimulation of stem cells.

	CartiGen: Compression Bioreactor (Multi-sample & Perfusion: C9-x)		NDA	A multi sample bioreactor system for tissue generation.
	OsteoGen: Perfusion Bioreactor (10 OsteoGen)		NDA	A single sample bioreactor system prospected in research applications for phenotypic analysis of bone stem cells and also in mineral deposition of bone marrow stromal cells.
	OsteoGen: Perfusion Bioreactor(12 0 OsteoGen)		NDA	A single sample multi chamber bioreactor system for bone stem cell analysis.
	DermiGen: Modified Tension Bioreactor (D70- 1DermiGen)		NDA	A single sample bioreactor system, favours co-culture of cells and development of skin like products.
	DermiGen: Modified Tension Bioreactor (D70- 4DermiGen)		NDA	A multi stimulator bioreactor system for tissue culture.
In vivo bioreactors	AcuSyst bioreactors	Cell culture company	NDA	For mammalian cell culture prospectives.
	Hollow Fiber Bioreactors	FiberCellSys tems	NDA	For mammalian cell culture; reduces the requirements of serum for cultivation of cells in <i>in vivo</i> -like manner.
Miniature Bioreactors	BioXplorer 100	HEL Group	0.02 L to 0.15 L	For accomplishment of cell culture microbial and C1 Gas fermentation
	Mini Bioreactors	Spinco Biotech Pvt Ltd	0.25 L, 0.5 L & 1L.	For cultivation of microbial cells and prospectives.
	Mini multiparous bioreactors- fermentor	KNIK bio	0.05 L, 0.1 L, 0.2 L, 0.3 L, 0.6 L &0.7 L.	In these reactors, pure oxygen by pass enhances DO function.

	MiniBio2	Applikon Biotechnology	0.25 L, 0.5 L & 1 L.	Facilitates the screening analysis along with media and process optimization
	Bioexplorer 100	HEL Ltd	0.02 L-0.15 L	Facilitates microbial fermentation and cell culture.
	Bioexplorer 400		0.05 L & 0.4 L	For bioprocess and process optimization.
	Bioexplorer 1000		0.4 L & 5 L	For microbial and mammalian cell culture.

*NDA-no data available.

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