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Bioreactor: Design, Functions and Fermentation innovations

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Abstract

Fermentation technology is the 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. Fermentation should not be seen merely as a process that is entirely focused on the happenings occurring in the fermenter alone. Fermentation is 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 (1).

Key words: Bioreactors, Frementation.

1. Introduction

Bioreactors are closed systems in which a biological process can be carried out under controlled, environmental conditions. It is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. A bioreactor system comprises a bioreactor, sensors, control system and software to monitor and control the conditions inside the bioreactor. Bioreactors are extensively used for the production of pharmaceuticals, food bio-based materials (such as poly-lactic acid), food processing, fermentation, bio-fuels and also in waste treatment, etc (2). The first truly large-scale aseptic anaerobic fermentation vessels were developed in the wake of the process developed (during the First World War, 1914-1918) by Weizmann and co-workers of U.K. to produce acetone by a deep liquid fermentation using *Clostridium acetobutylicum*. The large-scale aerobic fermentation vessels were first used in Central Europe during 1930s for the production of compressed yeast; these fermenter had large cylindrical tanks in which air was introduced at the base via a network of perforated pipes. In later modifications, mechanical impellers were used to improve mixing of broth and dispersal of air bubbles. Fermenter design

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was considerably improved during 1940s to accommodate the requirements of strict aseptic conditions, and good agitation and aeration for penicillin production from submerged cultures (3). Until the discovery of penicillin and its commercialization in 1943, bioprocessing was not performed on an industrial scale with equipment resembling that in a chemical processing plant. Since then, the number of products made by fermentation has grown, because bioprocessing generally uses less energy than other routes, usually uses inexpensive raw materials and sometimes makes products that cannot be made by any other way. Modern products include antibiotics, amino acids, enzymes, monomers, proteins, food cultures, biopolymers, ethanol, isopropanol, flavorings, perfume chemicals and many other organic chemicals.

2. A bioreactor should provide the following: (i) Agitation (for mixing of cells and medium), (ii) Aeration (aerobic fermenters; for O_2 supply), (iii) Baffles (to prevent vortex formation and to improve aeration efficiency), (iv) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc., (v) Sterilization and maintenance of sterility, and (vi) withdrawal of cells/medium (for continuous fermenters) (Fig. 1). Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

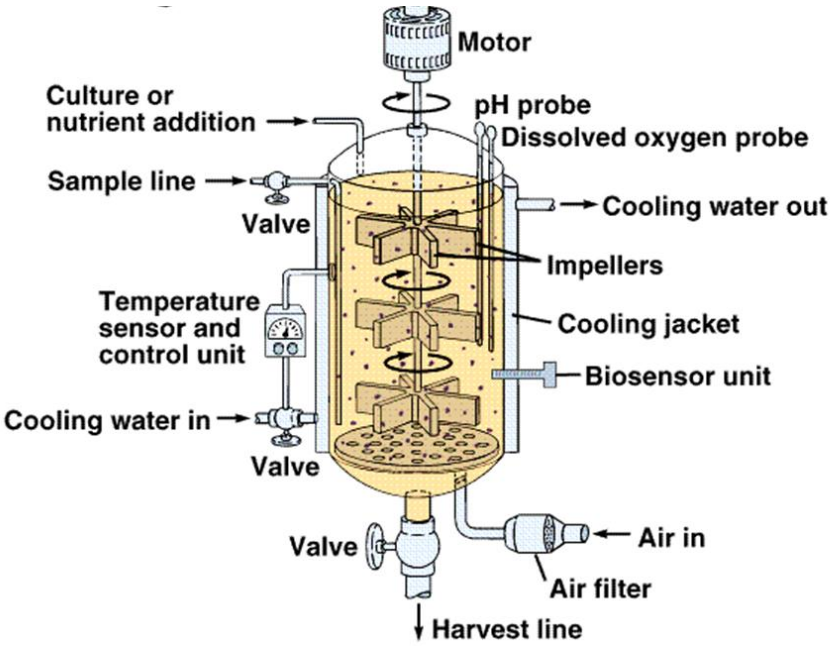

Fig.1 Design of Large scale Fermentation Unit (Prescott, 1999).

These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

Key issues in bioreactor design and operation

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The goal of an effective bioreactor is to control, contain and positively influence the biological reaction. To accomplish this, the chemical engineer must take into consideration two areas. One is the suitable reactor parameters for the desired biological, chemical and physical (macrokinetic)

System. The macrokinetic system includes microbial growth and metabolite production. Microbes can include bacteria, yeast, fungi, animal, plant, fish and insect cells, as well as other biological materials. The other area of major importance in bioreactor design involves the bioreaction parameters, including: controlled temperature, optimum pH, sufficient substrate (usually a carbon source) such as sugars, proteins and fats, water availability, salts for nutrition, vitamins, oxygen (for aerobic processes), gas evolution and product and byproduct removal (Fig. 2). In addition to controlling these, the bioreactor must be designed to both promote formation of the optimal morphology of the organism and to eliminate or reduce contamination by unwanted organisms or mutation of the organism (4).

Types of Fermentations Innovations

Innovations (new idea, device or process) are the application of better solutions that meet new requirements, in articulated needs or existing market needs. Fermentation types are classified into different classes:

1. Based on various aspects like based on feeding substrate to fermenter.
2. Based on need of supply of aeration
3. Based on need of light etc.

1. *Based on feeding substrate to fermenter:* There is Batch fermentation; Continuous fermentation and Fed-batch fermentation are the key modes. In batch fermentation, every material for process of fermentation including substrate, inoculum and all the process parameters are set and filled in a fermenter and the process is set on and until the total process comes to an end neither substrate is added into fermenter nor product is taken out of fermenter. It's a closed system. In continuous fermentation, the substrate is added continuously to the fermenter at a fixed rate which maintains the microbes at logarithmic growth phase and the products that are formed are taken out simultaneously and here we find growth associated products. In fed-batch mode we find both modes of operations of batch and continuous modes, where substrate is added at fixed time intervals during the fermentation process (5).

2. *Based on need of supply of aeration:* There are aerobic fermentation and anaerobic fermentation. Aerobic fermentations: many large-scale fermentation processes are carried out in presence of aerobic conditions where, the contents present in fermenter are agitated with the help of agitator and with the help of spargers by forcing sterilized air into the fermenter. There are several types of Microbial cell aerobic bioreactors: CSTR (suspended cells, mechanically agitated) and Airlift fermenter (Suspended cells, pneumatically agitated). Anaerobic fermentation: apart from intense need and presence of agitator and sparger to supply aeration, rest of the configuration of the fermenter is as same as aerobic fermentation. But the presence of agitator is made compulsion for the even distribution of temperature, pH, viscosity, nutrients etc. along the medium in the fermenter. Anaerobic bioreactions are used in

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applications such as ethanol production, winemaking, beer brewing and wastewater treatment.

3. *Based on need of light:* There are Photofermentation (only photosynthetic bacteria can undergo) and Dark fermentation. Photofermentation is a process of conversion of organic substances to other utilizable energy compounds following a series of biochemical reactions carried out by a specific group of bacteria named Photosynthetic bacteria, which only proceeds in the presence of light. Dark fermentation in every way it is similar to that of photofermentation, but in aspect of need of light, dark fermentation does not need any light to initiate the reactions and a diversified group of microbes are involved in dark fermentation.

Design of Bioreactor System used in Bioprocess Technology

Designing a bioreactor system involves mechanical, electrical and bioprocess engineering. The design process should be organized in such a way that systems can be used under the strictest of regulations. A number of new processes are being developed. One involves the use of isolated enzymes rather than whole cells to carry out a chemical change. The advantage is that this process does not require catering to the special requirements of living cells. However, enzymes, too, can undergo changes and, therefore, require determining the optimal conditions to express their catalytic activity. An additional problem is that using isolated enzymes is frequently an expensive undertaking for a single use application. Consequently, long reaction times may be necessary if cost factors necessitate that expensive enzymes must be used only in low concentrations. There are other disadvantages to their use, too, such as the need to remove the enzyme from the product once the desired bioreaction has taken place. Immobilized enzyme technology is now successfully solving some of these difficulties. With the enzyme immobilized in a bed or tube, the solution of substrate for conversion is then passed through for conversion to product. The product is continuously collected as effluent from the bioreactor. The design and operation of an immobilized system is similar to that of processes employing heterogeneous catalysis. Heterogeneous systems enable product recovery at lower separation costs than do corresponding homogeneous systems. Gas-liquid-solid contacting bioreactors have been investigated with a number of immobilized enzyme systems. Enzyme immobilization can take a variety of different forms and it has been studied on a range of supports. The method used in a particular application depends on the characteristics of the enzyme, its system, the substrate and the bioreactor fluid. Enzymes may be supported on a mesh-type or conventional mass-transfer structure, encapsulated in a film, supported by gel or silica-derived systems, on macroporous ion-exchange resins, or on other polymeric supports. One system that currently employs this technology is the trickle bed bioreactor. Not unlike certain types of biofilters traditionally used for emission control, this system features a screen onto which the enzyme is adhered and immobilized, and through which the substrate solution is passed for conversion. Membranes and hollow fibers have been tried for immobilized bioreaction systems. An example is hollow fibers with enzymes

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incorporated into their walls. The diffusion of the substrate through the tube wall allows contact with the gelled enzyme and conversion into product. Subsequent diffusion of the product provides the separation necessary for its recovery. Under the influence of the differential pressure across the tube wall, the product flows through to the inside of the tube, eventually to be collected at a multitube header.

The different types of bioreactors used in bioprocess technology are: (1) Submerged Culture Bioreactors. The major types of submerged culture bioreactor are: (i) Continuous Stirred Tank Bioreactors (ii) Bubble Column Bioreactors (iii) Airlift Bioreactors (iv) Fluidized Bed Bioreactors (v) Trickle Bed bioreactors. (2) Solid State Bioreactors.

1. Submerged Culture Bioreactors

(i) *Continuous Stirred Tank Bioreactor*: The most common type of aerobic bioreactor in use today is the stirred tank reactor, which may feature a specific internal configuration designed to provide a specific circulation pattern. Most production facilities and FDA approved production processes for biopharmaceuticals are based on the stirred tank bioreactor. Generally, the tank has an aspect ratio of between 1:1.5 (for mammalian cell culture) and 1:3 (for microbial fermentations). Baffles (enhance mixing) diameter is typically one tenth of the tank diameter. The impeller can either be marine impeller for axial mixing of the cell culture – having a diameter of between one third and one half of the tank diameter or multiple Rushton turbines for gad bubble breaking and axial mixing in microbial cultures. Gas is typically introduced below the mixing impeller and liquid additions are done from the top of the bioreactor. Stirred tank bioreactors are available from 0.05 litres up to 100 cubic metres in volume (6).

(ii) *Bubble Column Bioreactors:* These are the simplest type of tower fermenters; they consist of glass or metal tubes into which air is introduced into the bottom section through perforated pipes, plates or metal micro porous sparger for mixing and aeration purposes. Fermenter volumes from 3L to up to 950 L have been used, and the aspect ratio may be up to 16: 1. These tower fermenters have been used for citric acid and tetracycline production, and for a range of other fermentations based on mycelial fungi.

(iii) *Air lift Bioreactors:* Similar to bubble column reactors, these differ by the fact that they contain a draft tube. There are two types of draft tube: an inner tube (air-lift bioreactor with an internal loop); or an external tube (air-lift bioreactor with an external loop). The draft tube improves circulation and oxygen transfer and equalizes shear forces in the reactor. Air is typically fed through a sparger ring into the bottom of a central draught tube that controls the circulation of air and the medium. Air flows up the tube, forming bubbles, and exhaust gas disengages at the top of the column. The degassed liquid then flows downward and the product is drained from the tank. Airlift bioreactors are available from laboratory scale up to full production scale (7).

(iv) *Bioreactors with immobilized enzymes and cells*: In this category, there are Fluidized bed bioreactors and Packed bed bioreactors. Fluidized bed bioreactor is comparable to bubble column bioreactor except the top

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position is expanded to reduce the velocity of the fluid. The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out. These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocs. For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed. It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas to dense ones may settle at the bottom), and they are in a good suspended state. Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts. This enables good efficiency of bioprocessing. In case of Packed Bed Bioreactors, A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed bioreactor. The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. A nutrient broth flows continuously over the immobilized biocatalyst. The products obtained in the packed bed bioreactor are released into the fluid and removed. While the flow of the fluid can be upward or downward, down flow under gravity is preferred concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth. Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali. However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions. The packed bed bioreactors do not allow accumulation of the products to any significant extent.

(v) *Trickle Bed Fermenter*: These consist of a cylindrical vessel packed with support material (e.g. woodchips, rocks or plastic structures). The support has large open spaces, for the flow of liquid and gas and the growth of microorganisms on the solid support. A liquid nutrient broth is sprayed onto the top of the support material and trickles down the bed. Air may flow up the bed, countercurrent to the liquid flow. These fermenters are used in vinegar production, as well as in other processes. They are suitable for liquids with low viscosity and few suspended solids.

2) Solid State Bioreactors

These are used for processes where microorganisms are grown on moist, solid particles. The spaces between the particles contain a continuous gas phase and a minimal amount of water. SSF devices vary in technical sophistication, from very primitive banana leaf wrap pings, bamboo baskets and substrate heaps to the highly automated machines used mainly in Japan. The majority of Solid State Fermentation (SSF) processes involve filamentous fungi, although some also involve bacteria or yeasts. SSF is mainly used in food processes (8). The use of pressure vessels is not the norm for solid state fermentation. The commonly used devices are: Tray fermenters, Static bed fermenter, Tunnel fermenter, Rotary disc fermenter, Rotary drum fermenter, Agitated tank fermenter and Continuous screw fermenter (9).

Other Bioreactor designs

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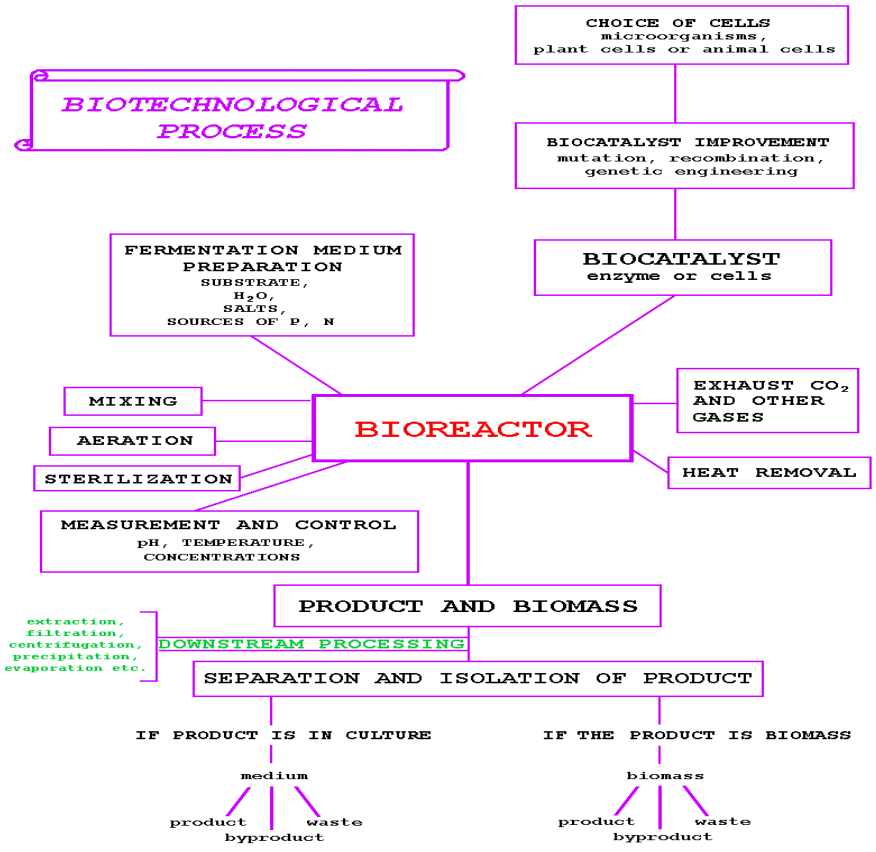
3) *Photo Bioreactors:* These are the bioreactors specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination. Since artificial illumination is expensive, only the outdoor photo-bioreactors are preferred. They are generally used for the cultivation of photosynthesizing organism (plants, algae and bacteria). Industrial scale photo bioreactors can also be open pond systems; and so are more sensitive to environmental influences. They are made up of glass or more commonly transparent plastic (Fig. 3). The array of tubes or flat panels constitutes light receiving systems (solar receivers). The culture can be circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps. It is essential that the cells are in continuous circulation without forming sediments. Further adequate penetration of sunlight should be maintained. The tubes should also be cooled to prevent rise in temperature. Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25-40°C. Microalgae and Cyanobacteria are normally used. The organisms grow during day light while the products are produced during night. Certain important compounds are produced by employing photo-bioreactors e.g., p-carotene, asthaxanthin.



ct (**Figure 4 to Figure 7**)

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Fig. 2 Bioprocesses: Biotechnological Processes (Source: www.slideshare.net

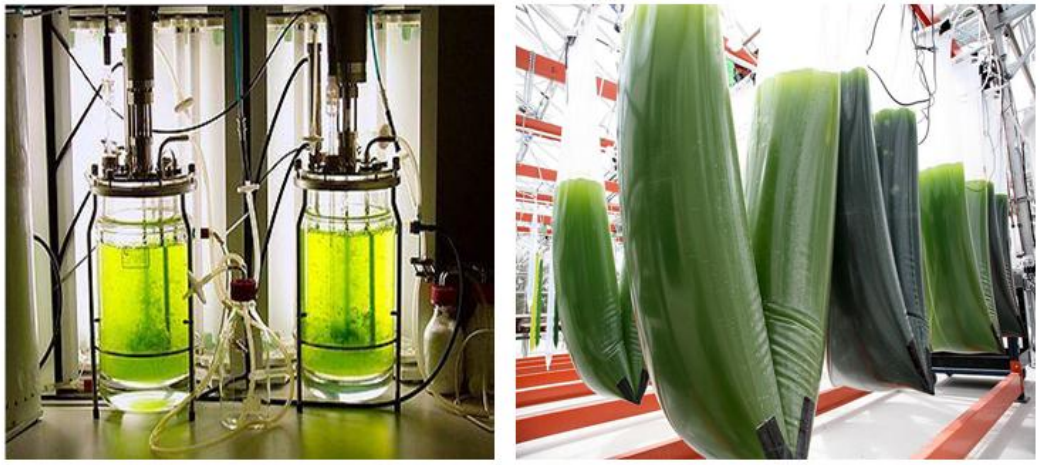

Fig. 3 a) Research Photobioreactor b) Photobioreactor- Plastic Bags

4) *Stem Cell Bioreactors:* A recent development is the stem cell bioreactor. Stem cells are very appealing for regenerative medicine, drug screening and biomedical applications. These cells have unlimited self renewal capacity and remarkable ability to produce mature cells with specialized functions, such as blood cells, nerve cells or cardiac muscle. However, the actual number of cells that can be obtained from available donors is very low. One possible solution for the generation of relevant numbers of cells is to scale up the culture of these cells in vitro (10). Several joint research programmes between industry and universities are focusing on the development of stem cell bioreactor systems. Applikon Biotechnology has participated in several of these projects and has developed a number of successful designs (11).

5) *Hollow Fiber Catridges:* Hollow fibers are small tube-like filters sealed into a cartridge shell so that cell culture medium pumped through the end of the cartridge will flow through the inside of the fiber, while the cells are grown on the outside of the fiber. Hollow fibers provide a tremendous amount of surface area in a small volume. Cells grow on and around the fibers at densities of greater than 1×10^8 per ml. Hollow fiber cell culture is the only means to culture cells at in vivo like cell densities. Cell culture at high densities can achieve a 10 to 100 time's higher concentration of secreted product compared with classic batch processes (12). The scalability of the hollow fiber system is limited, however, and so these types of bioreactor are mainly used at the laboratory scale. Smaller hollow fiber bioreactors are often used for selection and optimization of cell lines prior to stepping up to larger cell culturing systems (Gramer and Britton, 2000). Recently, hollow fiber bioreactors have been tested as novel platforms for the commercial production of high titer influenza A virus (13).

6) *Rocking Bag Bioreactors*: This system relies on the rocking motion of the bioreactor holder to mix a liquid volume contained in a

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plastic bag. This type of bioreactor is mainly used for cell cultivation, due to the low oxygen transfer rates and limited cooling capacity of such systems (14).

Overall, Bioreactors are the integral part for the development of many new high-value products and the replacement of existing chemical-based commodity processes. The proper selection and design of the bioreactor will determine the optimal commercial bioprocess and the corresponding capital investment. The bioreactor should not be regarded as an isolated unit, but as part of an integrated unit operation with both upstream (preparation) and downstream (separations) unit operations.

Funding

No

Ethical issue

No

Conflict of interest

no

References

4. Bailey JE and Ollis DF (1986) Biochemical engineering fundamentals 2nd ed., McGraw – Hill international edition. pp. 533-620.
5. Betts JI and Baganz F (2006) Miniature bioreactors: current practices and future opportunities. *Microbial Cell Factories*, 43(3): 113-119.
6. Chisti Y (1999) Fermenttaion (industrial): Basic considerations, In: Robinson R, Batt C and Patel P (eds) *Encyclopedis of Food Microbiology,*Academic press, London, pp. 663-674.
7. Chisti Y (1999a) Solid substrate fermentations, enzyme production, food enrichment. In: Flickinger MC and Drew SW (eds) *Encyclopedia of Bioprocess Technology*, New York: John Wiley, Vol. 5, pp. 2446-2459.
8. Decker EL and Reski R (2008) Individual cells exert individual conditions of a treatment. *Bioprocess Biosys Eng*, 31:3-9.
9. Gramer MJ and Britton TL (2000) Selection and isolation of cells for optimal growth in hollow fiber bioreactors. *Hybridoma,* 19(5): 407-412.
10. Jagani H, Hebbar K, Gang SS, Raj PV, Chandrashekhar R and Rao JV (2010) An overview of fermenter and the design considerations to enhance its productivity. *Pharmacology online,* 1: 261-301.
11. Kana G, Oloke EB, Lateef JK and Kana Z (2003) Constructional features of a 15 litre home made bioreactor for fed-batch fermentations. *African J Biotech*, 2(8): 233-236.
12. Liu N, zang R, Yang ST and Li Y (2014) Stem cell engineering in bioreactors for large scale bioprocessing. *Eng. Life Sci.*, 14(1): 4-15.
13. Rodrigues CAV, Fernanades TG, Diogo MM and Silva CL (2011) Stem cell cultivation in bioreactors. *Biotech Adv*, 29(6): 815-829.
14. Shuler ML and Kargi F (2004) Bioprocess Engineering, Basic concepts 2nd ed., Pearson education (Singapore) Pvt. Ltd. pp. 327-333.
15. Stanbury, P.F., Whitaker, A. and Hall, S.J. (2001) Principles of Fermentation technology 2nd ed., Pergamon Press, oxford.
16. Tapia F. *et al* (2014) Production of high titer human influenza A virus with adherent and suspension MDCK cells cultured in a single use hollow fiber bioreactor. *Vaccine,* 32: 1003-1011.

17. Thorat BN, Shevade AV, Bhilegaonkar KN, Aglawe RH and Veera UP (1998) Effect of sparger design and height to diameter ratio on fractional gas hold-up in Bubble Columns. *J Chem Eng*, 76: 492-497.