Bioreactors types and applications

This essay explores the evolution of bioreactor concepts and sheds light on its applications in various scientific aspects and its position in research. Definitions of bioreactors focus on designs and processes.

What is a bioreactor?

Bioreactors refer to certain devices or system which supports biologically active environment, where chemical processes are undergoing involving organisms or their derived biochemically active substances. Bioreactors can have any shape according to the application. Also processes inside vary as for the environment to be imitated differs, e. g. aerobic and/or anaerobic environment cycles. Bioreactors use also devices and/or system meant to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering (IUPA, 2009).

Bioreactor Design

Under optimum conditions there can be use of micro-organisms or cells, which are able to perform normal and/or with additional modified functions. Even small changes in the environment affect functions as reaching critical thresholds lead to major changes, e. g. gas (air, oxygen, nitrogen, carbon dioxide) flow rates, temperature, pH and dissolved oxygen levels, and agitation speed need to be closely monitored and controlled. Thus overall bioreactors design should consider details such as limitation on the production of hydrogen by proton gradients, carbon dioxide and requirements for carbon binding.

A bioreactor therefore consists generally of a vessel, sensors for cell growth conditions, and networks systems. Depending on how they are designed and

operated, they can be classified as batch, fed batch or continuous. One such continuous bioreactor is the chemostat (IUPA, 2009).

Continuous flow stirred tank reactors (CSTR or chemostat)

This is the type of bioreactor where the fresh medium containing growth factors is fed into the cells at a constant rate. To maintain a constant bioreactor volume, the waste media is made to leave the reactor at the same rate. To control the rate at which the cells grow, “ growth limiting factors” are fed together with the fresh medium. By controlling the rate at which these factors are fed, the rate of growth of the cells is indirectly controlled.

Problems associated with the Chemostat Bioreactor

Some of the problems associated with the chemostat bioreactor include:

Foaming- When feeding the fresh medium, some sort of foam can appear on top of the liquid. This can deprive the cells off oxygen and affect their growth.

Cells can be ruptured during the process of aeration.

” Some cells may adhere to other surfaces or grow on the walls” (Bonomi, 1976)

The medium is supposed to be mixed uniformly around the cells. This may not be the case.

In some cases, the medium may drip into the chamber rather than flow. This dripping causes pulses and fluctuations in the nutrients thus affecting the operation of the chemostat.

However, some of the problems above can be limited by:

The use of antifoaming agents to reduce the effects of foam

Aeration and conditioning of cells should be gentle.

If dripping of the medium is inevitable, drops should be made very small so as not to cause fluctuations. We can also use vessels with larger volumes to reduce the effects of drip

Isolation of cells

Cells can be isolated to change the environment or even to tailor them to another product depending on the target. Isolation of cells can take several forms including but not limited to blood samples where only white blood cells are capable of growth. This enables the cells to proliferate either random mutation or deliberate modification.

Cell culture

This refers to the general process of growth cells in a controlled environment by application of growth conditions. It involves separating cells from the original tissue source and was discovered in the 19th century (US EPA, 2002). Two types of cells can be cultured-

1. Anchorage dependent cells which are derived from normal tissues and diploid cell lines and
2. Mammalian cells which are form of cancerous or tumor cells.

Treatment of bioreactors

Bioreactors may have a fluid suspended medium or a solid through which the fluid is circulated to support cell growth. In the suspended growth systems, organic matter is degraded by bacteria to produce carbon dioxide and biomass which is recycled back or disposed off as sludge.

For bioreactors which have a solid support growth medium, the micro organisms are grown on the film of the supporting structure. These bacteria degrade the water/fluid as it passes through the solid matrix. This degraded water then helps in degrading the waste solid matter.

Oxygen is very necessary in aerobic process and keeping an optimum supply of oxygen can be a difficult task because of its poor solubility in water. Oxygen supply can be improved by constant and uniform mixing or stirring which also helps to mix nutrients in the process. This however has to be done at certain rates or speeds in order not to damage the organisms or cause unnecessary alterations in reactions.

Maintaining cells in culture

For cells to survive, they must be maintained at favorable temperature, gas composition and correct growth medium and factors such as PH, glucose concentration and other nutrients. Some cells need to be attached to a surface for them to grow while others (like cells in blood) can grow in suspensions. However, it is possible to tailor cells to grow in suspensions so as to increase their density which may not be possible in adhesive surfaces.

Cell line cross-contamination

When dealing with different types of cells, care must be taken not to cause cross contamination. This is because different cells require different growth conditions and environments. Therefore providing conditions which are meant for another cell may contaminate the other. This may be caused by misidentification of cells (Drexler, Dirks and Macleod 1999). “ Problems with cell line cross contamination have even been detected in lines from the NCI-60 panel, which are used routinely for drug-screening studies. Major cell line repositories including the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ) have received cell line submissions from researchers that were misidentified by the researcher (Drexler, Macleod and Dirks, 2001)”. Such contamination poses a problem for the quality of research produced using cell culture lines, and the major repositories are now authenticating all cell line submissions (Cabrera et al., 2006).

Manipulation of cultured cells

As cells generally continue to divide in culture, they generally grow to fill the available area or volume. This can generate several issues: Nutrient depletion in the growth media, Accumulation, Cell-to-cell contact can stimulate cell cycle arrest, causing cells to stop dividing known as contact inhibition or senescence of apoptotic/necrotic (dead) cells, Cell-to-cell contact can stimulate cellular differentiation. In the case of adherent cultures, the media can be removed directly by aspiration and replaced.

Passaging cells

Transferring a small number of cells into a new vessel requires the use of some kind of passage. This splitting is done to help the cells have enough area of growth and increase in density.

Established human cell lines

These are cells removed from a parent tissue of humans with or without their consent and used to establish or propagate their cell lines. These can be used as implants into other people are used for diagnosis (Liscovitch, 2007).

Generation of hybridomas

This is when an immortalized cell is combined with normal cells. This is mainly used in regenerative medicine to produce monoclonal antibodies.

Applications of cell culture

Cell cultures are very important in the field of biotechnology and viral vaccines. They can also be used to produce recombinant DNA (rDNA) contain synthetic hormones, anticancer agents, enzymes and immunobiologicals.

Tissue culture and engineering

Culturing cells involves a hectic and specialized process of maintaining very specific growth conditions in an environment external to the parent tissue. This is the basis of tissue engineering. This particular field makes it possible to culture and preserve cells for future use and studies are going on for their major applications in the stem cell industry.

Vaccines

Cells can be used in the process of making vaccines. Currently vaccines for measles, polio, rubila, chickenpox and mumps are being made. There are ongoing reserhes in the USA for the use of cells culture in making of influenza vaccines (Macleod et al, 2006) " or the use of adjuvants "(Masters, 1999).

Viral culture methods

Viruses can be cultured however, since viruses survive on mammals/animals, plants, fungi or bacteria, culturing viruses will require culturing of such cells as well. With the right combination of factors, it is possible to culture various combinations of viruses or viral products capable of fighting disease or viral replication.

Photobioreactor (PBR)

This is a bioreactor in which some kind of light source is used. This kind of bioreactor is mainly used to grow phototropic organisms. These organisms use light as a source of energy through photosynthesis. This type of bioreactor is said to be having lower risks of contamination as it is possible to have light of a specific frequency without much of an interference. (IUPA, 2009).

The use of bioreactors in sewage treatment

Bioreactors can be designed for sewage and waste water treatment. In this case the bioreactor is designed to hold wastewater in which an inert chemical media is supplied to enhance a bacterial reaction. Constant supply of oxygen and other factors have to be supplied to the reactor. The idea is to

develop bacteria that will nutralize the toxicity of wastes in the sewage and waste water so that it can be recycled. The product of the this neutralization process is water and biosolids. While water is recycled, the waste solids are collected and can be disposed off or used for other purposes such as manure.

The main goal in bioreactors is to grow tissue/s or cells for therapeutic purposes or experimental, when compared the design with industrial bioreactors is significantly different. The mammalian’s tissue/s and cells must have structural support or surface that to help to grow, as well as the agitated environments usually destructive to these kind of tissue/s and cells. Complex tissue/s need more complicated media and growth factors.

National Aeronautics and Space Administration (NASA) developed in USA a new bioreactor design which artificially grows tissue/s in cultures of cell. This kind of bioreactor has an ability to grow skeletal tissue, heart tissue , cancer tissue for study, ligaments, and other tissue/s type (Decker and Reski , 2008).

A bioreactor landfill

Other than designing a bioreactor for waste water treatment, a whole landfill can be designed in form of a bioreactor. Here the main bacterial growth conditions are water, air and the right temperatures. These are supplied in order to facilitate bacterial breakdown of solid waste. The landfill bioreactor differs from the traditional (dry tomb) municipal landfill approach in that the decomposition of waste is accelerated rather than the natural process relied

up on in the traditional landfill (Decker and Reski 2008). There are three types of this type of landfill bioreactor and they include:

“ Hybrid (Aerobic-Anaerobic) – This degrade material by use of “ a sequential aerobic-anaerobic treatment”. It operates in such a way that while the upper materials are degrading, the lower section of the bioreactor collects gas.

Aerobic -This bioreactor is composed of a bottom layer containing leachate and a storage tank containing liquid. The operation is in such a way that leachate from the bottom layer is piped to mix with the liquid before being recirculated back into the landfill. Air is injected to make the reaction faster.

Anaerobic – In this kind of bioreactor landfill, the moisture content of the waste mass is increased by re-circulating leachate. However, waste mass is deprived off oxygen and therefore the biodegradation is anaerobic, producing mainly methane, combustible green house gas.

Bioreactor function in Batch or continuous

The batch bioreactor is uncomplicated and less specific as compared to the rest because it takes in very many kinds of wastes and therefore its conditions widely vary. In the batch bioreactor, waste is added to a certain level and the reactor is then covered for a certain period of time. Bacteria is allowed to decompose the waste is an air tight environment with little or no oxygen. When it is determined that the reaction is complete, the bioreactor is opened and emptied. As the digestion of batch requires less equipment and lower levels of design work and simple, it is usually a cheaper digestion form. (Svoboda, (2003).

To maintain a constant and continuous bioreaction process, waste matter is constantly or periodically added to the bioreactor. This means that a way of constantly or periodically emptying the bioreactor without interrupting the bioreaction process has to be established. There are many examples forms of digestion of anaerobic that include, Upflow anaerobic sludge blanket (UASB), continuous stirred-tank reactors (CSTRs), Internal circulation reactors(IC) and Expanded granular sludge bed (EGSB). (Ciborowski, 2004).

For this kind of reaction to take place efficiently, temperature is a very important parameter. There are generally two temperature levels that are suitable for this process and these determine which kind of bacteria is responsible for the decomposition (Sleat, 2006). The types of bacteria responsible are usually mesophiles which operate at temperatures between 20-45 °C and thermophiles that work ell at araround 50-52 °C (Juniper, 2005).

A disadvantage of the thermophilic temperature operation is that it requires more heat to raise the temperatures thus increasing the energy consumption of the bioreactor. This, if not matched with an increase in the production of biogas may reduce the benefits of the bioreactor (Boone, 2006).

Tissue engineering

Tissue engineering is a multidisciplinary field that combines engineering and biological/life sciences. It applies engineering principles to help nature or grow biological organisms/cells that can be used for therapy in regenerative medicine, replacement of biological organs or their functions, or in the making of various kinds viral or gene targeted drugs. Its principles can also

be used in growing bacteria to enhance decomposition of solid waste and water purification or viruses to act as inhibitors to the proliferation of other viruses.

To understand all these, one has not to only be familiar with all the factors responsible for efficient growth of cells, bacteria/viruses, but also be able to control these factors. Therefore tissue engineering goes beyond just culturing cells but also designing of “ bioreactors” that provide conducive environments for such growths to take place.

Currently, tissue engineering though still relatively new, is quite an established field. It is enabling scientists and engineers to research on several was of increasing cell density and many other applications of such cultured cells are being developed.

However, the challenge still facing tissue engineering is growing enough tissue with all the mechanical characteristics for transplantation into the body. Mechanical loading of cells together with the right combination of growth factors still remains elusive.

The ultimate goal of tissue engineering is to be able to grow organs for eventual replacement of human parts, the success of which has to come from continued research.

Types of cells

Several types of cells exist depending on their source. Some of the common types include:

Autologus cells: In this type of cells, the human body is used as the bioreactor. Since the cells are from the body anyway, it is viewed as being able to provide the best growth factors for the cells. Therefore, problems associated with rejection and cross infections are limited.

However, relocation of cells from one part of the body to the other may still cause significant variations in their conditions and some precautions have to be taken to prevent this. The other problem could be the inability to source enough cells for the required function from the host person due to some kind of infections, age or for patients who are suffering from severe burns. The removal of these cells from one site and transplanting them into another involves some kind of surgical operation that may also turn out to be risky or even cause infections in both sites. The same procedures and risks may have to be incurred during the harvesting of these cells.

Problems may also arise with specificity of sites from where the cells are removed and where they are transplanted. They have to have relatively the same biological/physiological conditions. However, of recent it has been possible to use mesenchymal stem cells from fat and bone marrow. These cells have the capacity to differentiate into different kinds of cells given the right conditions. They can therefore be used to grow varied cells/tissue organs that include nerves and blood vessels, valves, bone and cartilage. It is also possible to obtain these cells in large quantities especially from fat.

Stem cells: This is another type of cells that are regularly used in cells culturing. They are “ undifferentiated cells with the ability to divide in

culture”. Stem cells can be characterized into two broad catergories- adult and embryonic stem cells.

Adult stem cells are said to be “ multipotent”. They can develop into several types of other cells but from the same or related lineage. On the other hand, embryonic stem cells are said to be pluripotent in that they can differentiate into even other lines of cells. Much as the use of embryonic stem cells shows some bright future, its application is still bogged down with lots of ethical issues.

Scaffolds

Just like in any other field, supporting structures are needed to enhance growth. Similarly for cells to grow, they need some kind of structure onto which they can be seeded and these are the scaffolds. Apart from providing the supporting structure for cells, scaffolds also make it possible to mechanical loading to be applied to cells. Some other functions performed by scaffolds include:

- Providing passages for cell migration and attachments
- Retention of cells and curring biochemical factors.
- Allowing diffusion of cells nutrients as well as oxygen to the cells.

Given the functions lined above, it is imperative that scaffolds must meet some specific requirements. The main requirement is being able to allow of nutrients by having pores of the right sizes. It should also meet requirements regarding mechanical properties such as tensile strength.

Materials for scaffolds

Scaffolds have to be designed from very specific materials in order to meet the above requirements. These materials can be synthetic or natural, permanent or biodegradable. Synthetic biodegradable materials are preferred because of the advantages they provide. They can be manufactured to specific detail and since they are degradable, they provide an opportunity for cells to support themselves and increase in density. Some of these synthetic biodegradable materials such as polyesters and collagen were even being used in the medical field long before tissue engineering came into place.

Research has gone ahead to engineer materials with “ customized funtionalization” and ideal properties such as “ injectability, non-immunogenicity, biocompatibility, nano-scale fibers, transparency, resorption rates, and low concentration”. This work is being pioneered by MIT labs of Zhang, Rich, Grodzinsky and Langer.

Because of their ability to perform various other functions as well as providing structural support, extracellular matrices are being investigated for their possible use as scaffolds. In particular, research and practical applications have shown that polysaccharidic materials such as chitosan orglycosaminoglycans (GAGs) and proteic materials such as fibrin or collagen have potentials of better performance as scaffolds.

However, some issues associated with cell- mediated immune response, and cell compatibility still remain a great concern. Therefore, the best choices of

materials appears to be a combination of GAGs hyaluronic acid and cross linking agents such as water soluble glutaraldehyde.

Tissue culture

In many cases, creation of biological structures and functional tissues in vitro requires extensive culturing to promote growth, inducement of functionality and survival. Generally, the essential cell requirements must be maintained in culture, that contain o2, humidity, PH, temp, osmotic pressure maintenance and nutrients

Bioreactor in up to date research

Orwin, Shah, Voorhee, and Ravi (2009) did some experiments to evaluate the suitability of using cornea tissue materials in bioreactor systems. They used human corneal stromal fibroblasts and performed inhibition and cytotoxicity analysis. In particular, they performed a seven days growth inhibition test and a one day cytotoxicity test based on the ASTM standard F813-01. They found that cell viability was affected by autoclaving of materials, different methods of preparation of materials surface and cell culture configurations.

In this experiment, it was found that titanium-6Al-4V and poly(etheretherketone) are the most appropriate materials for use in this kind of system.

In this situation, in around the world injuries and diseases to the cornea are common and via corneal transplants, corneal blindness may sometimes be repaired (Abuksis et al, 2004). Furthermore, these cells primarily take on the repair fibroblast or myofibroblast phenotype when seeded onto a 3D collagen

scaffold. (Orwin et al, 2003). It was concluded the material of construction must be able to withstand multiple autoclave cycles which means the bioreactor system must be reusable.

Sierad developed a conditioning system for pneumatic driven consist of a 3-chambered heart valve bioreactor, a reservoir tank, a pressurized compliance tank, pressure-retaining valves, one-way valves and pressure transducers. At 60 beats per minute a tissue derived heart valve substitute used to test the bioreactor’s functional capabilities. Pulsatile flows reaching 1400 mL per minute, and aortic pressures reaching 100 mmHg that means resulted in excellent opening and closing of the valve. Tissue engineered heart valves tested by bioreactor which made from lightlybone marrow-derived stem cells (valve 3) and decellularized and conditioned in the bioreactor for eight days. The bioreactor allowed for multiple mounting methods and created proper closing and opening of the heart valves.