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Chapter 2

APPLICATION OF DIFFERENT TYPES OF BIOREACTORS IN BIOPROCESSES

Michele Rigon Spier*, Luciana Porto de Souza Vandenberghe, Adriane Bianchi Pedroni Medeiros and Carlos Ricardo Soccol
Federal University of Parana, Bioprocess Engineering and Biotechnology Department, Paraná, Brazil

Abstract

This chapter presents bioreactors models applied to bioprocesses including submerged (stirred tank reactor, bubble column, air-lift, membrane, packed bed and fluidized bed bioreactors) and solid-state fermentation processes (horizontal drum, tray-type, packed bed bioreactor and bench scale bioreactors) for the production of important biomolecules for industrial applications. Some recent applications of bioreactors are mammalian cell culture, vegetable cell culture and photobioreactors for algae culture, which are also presented. An overview of bioreactors types, their design and properties will be considered for biomolecules production such as biomass, enzymes, organic acids, aroma compounds, spores, mushroom production, pigments, antibiotics and others. In this chapter some attention was given to bioreactors applied to animal cell culture and algae production that require some special specifications due to some factors that affect these biomolecules production. Some important details of mass transfer and scale-up of bioprocesses will also be described.

Introduction

The bioreactors are the main unit operations for industrial biochemical transformation in which the treated materials promote the biotransformation by the action of the living cells or by the cellular components such as enzymes (Pandey et al, 2008). Bioreactors are tanks or vessels in which cells or cell-free enzymes transform raw materials into biochemical products and or less undesirable byproducts. These reactors are commonly cylindrical, ranging in size from a liter to some cube meters, but differs depending on the design and the operation mode

* E-mail address: spier@ufpr.br. (Corresponding Author)

in industrial bioprocesses. Although the bioreactor may be simple or highly instrumental, its ability to produce the desired product or results is important to consider. The bioreactor is designed and operated to provide the environment for product formation selected by scientists, bakers, or winemakers. It is the heart of many biotechnological systems that are used for agricultural, environmental, industrial and medical applications (Schaechter & Lederberg, 2004). All the bioreactors present great importance due the generation of products in these equipments, which are the heart of the bioprocesses (Cinar et al, 2003). In some cases, the bioreactor may be applied for biomass production (e.g. single cell protein, Baker´s yeast, animal cells, microalgae); for metabolite formation (e.g. organic acids, ethanol, antibiotic, aromatic compounds, pigments); to transform substrates (e.g. steroids) or even for production of an active cell molecule (e.g. enzymes). The systems based on the mammalian or plant cells culture are usually referred as tissue cultures, while those based on the dispersed non-tissue-culture-forming culture of the microorganisms (bacteria, yeast, fungi) are loosely referred to as "microbial" reactors (bioreactor, fermenter). In the enzyme reactors, no live-cells are used for the transformation of the substrate. Frequently, these reactors employ immobilized enzymes where the solid supports are used to entrap (internally) or attach (externally) the enzyme (biocatalyst) so that it can be repeatedly used to economize the enzyme consumption (Bhattacharyya et al., 2008).

A bioreactor consists of a complex system of pipes, fittings, wires, and sensors; it is exposed to operational problems. With the aid of on-line monitoring and diagnosis tools, it is now possible to detect many things that can go wrong during the process (Cinar et al., 2003).

The bioreactor has origin in early history and some important marks are shown in Table 1. Before 500 B.C. the Babylonians still produce beer in tanks which had the function of a bioreactor. Wine was produced in wineskins, which were carefully selected for their ability to produce a beverage that met the approval of the king and other members of his sensory analysis. Early recorded history shows that some understood the importance of the components and the environmental or operating conditions of the reactor. This allowed leavened bread and cheese to be produced n Egypt more than 3000 years ago (Schaechter & Lederberg, 2004).

Bioreactor operation mode is classified in: batch processes, bed-batch and continuous processes. Normally these operations mode are used in submerged or liquid fermentations or during cell culture such as tissue culture or algae growth. Batch processes has increased significantly nowadays and are extensively used to produce specialty biomolecules for uses in chemical, biotechnological, pharmaceutical industries. The production of these high value-added bioproducts contributes to a significant and growing portion of the revenue and earnings of bioprocess industries (Cinar et al, 2003). Batch processes refer to a partially closed system in which most of the materials required are loaded onto the bioreactor aseptically and are removed at the end of the operation. In a batch bioprocess, the only material added and removed during the course of operation is air/gas exchange, antifoam and pH controlling agents. Most modern bioprocesses incorporate adjustments to the medium to control conditions and to supply nutrients and compounds that promote biosynthesis of the desired product (Cinar et al, 2003).

Bioreactors for fed-batch processes represent an important class of bioprocesses, mainly in the food industry and in the pharmaceutical industry but also e.g. for biopolymer applications (PHB). One of the key issues in the operation of fed-batch reactors is to optimize the production of a synthesis product such as enzymes and penicillin or even biomass

(Dochain, 2008). In fed-batch or also called semi-continuous bioreactor characterize by the feeding of sterile substrate, the absence of outflow from the fermenter and the increase in volume (accumulation of total mass) in the bioreactor. It can be used to demonstrate the important characteristics of quasi-ready state, linear growth, and use of alternative feed strategies (Dunn, 2003). Besides substrate, required nutrients also are added continuously or intermittently to the initial medium after the start of cultivation or from the point halfway through the batch process. Fed-batch processes have been utilized to avoid utilizing substrates that inhibit growth rate if present at high concentration, to overcome catabolic repression, to demand less initial biomass, to overcome the problem of contamination, and to avoid mutation and plasmid instability found in continuous culture (Nag, 2008).

In continuous culture, fresh medium is added into the batch system at the exponential phase of the microbial growth with a corresponding withdrawal of the medium containing the product. The continuous cultivation gives a near-balanced growth, with little fluctuation of the nutrients, metabolites, cell numbers or biomass (Binod et al, 2008).

Differ from submerged fermentation, solid-state fermentation (SSF) has been defined as the fermentation process which involves solid matrix and is carried out in absence or near absence of freewater; however, the substrate must possess enough moisture to support growth and metabolism of the microorganism. The operation mode of solid state fermentation most used industrially is the batch system. Commonly used SSF bioreactors can be divided into four types based on type of aeration or the mixed system employed. These are tray, packed-bed, horizontal drums and fluidized bead having their own advantages and disadvantages, which promoted the necessity to develop novel bioreactors with better design (Singhania et al, 2009). This process recycles agro-industrial residues without economic fate for many different applications in bioprocesses such as enrichment, biological detoxification, production of biomolecules such as enzyme, organic acids, food aroma compounds, biopesticides, mushrooms, pigments, xanthan gum, vegetable hormones (Soccol & Vandenberghe, 2003) and may be used different types of bioreactors for lead the solid state fermentation.

Batch operation systems can be applied for all types of bioreactors. However, fed-batch and continuous operation systems must be analysed according to the different models of bioreactors and the process itself. These operations present some advantages, compared to the batch systems, although they need some investments and rigorous instrumentation and control.

Table 1. Main events using bioreactors in the history

Year	Event
4000-3000 B.C.	Baking, brewer
2000 B.C.	Ethanol production and distillation (China)
1923	Commercial production of acid citric (Pfizer, USA)
1940s	Production of penicillin by fermentation (USA)
1950s	Design and scale-up pf large aerated fermenters
1970s	More than 100 new drugs and vaccines produced by bioprocesses
1980s	Control of fed-batch bioreactors

Source: Based on Heinzle et al (2006); Dochain (2008)

Submerged Bioreactors

Stirred Tank Reactors - STR

Bioreactors designed for the most efficient expression of the biological properties of the living cells must achieve optimal interactions between the cells and the culture media. In a closely controlled environment they have to provide efficient means of mixing, mass and heat transfer between the different phases. (Engasser, 1988). Current reactor technologies, new types of bioreactors are constantly being developed in order to optimize and improve productivities.

Because of its versatility and flexibility the mechanically stirred tank reactors (STR) remains the mainstay for industry. According to Najafpour (2008), there are three main types of fermenters that are used in industrial scale:

1- Non-mixed and non-aerated systems: approximately 70%
2- Non-mixed and aerated systems: approximately 10%
3- Mixed and aerated systems: approximately 20%.

Non-aerated and non-mixed tanks are used in the production of traditional products such as wine, beer and cheese. The major part of the new products are obtained from the cultivation of microorganisms, which is carried out in mixed and aerated tanks (Najafpour, 2008).

The main hole of the bioreactor is to provide an adequate and controlled environment for cell growth and product synthesis. In this way, there are several factors that must be considered in the construction of bioreactor. Among them sterility, aeration and mixing systems (when necessary), temperature and pH control, geometry, low energy consumption and adequate size and material (Stanbury, 1995).

The most important bioreactor for industrial applications is the conventional STR due to its low operation costs. The size of the tanks may vary between some dm^3 till hundreds of m^3. Laboratory-scale tanks with a volume of maximum 20 liters are made of glass. For higher volumes, they are generally made of staining steel. Different materials and their combinations can be used for their manufacture.

The relation between high and diameter may vary between 2:1 till 6:1, depending on the heat to be removed (Najafpour, 2008). The ration 1:1 is probably the most economic because it presents a lower superficial area and, consequently, it needs less material. However, when the aeration is required, the aeration rate must be higher to promote a higher contact between the air bubbles and the liquid, but also a higher hydrostatic pressure on the top of fermenter (Doran, 1995).

In the bioreactor, the homogeneity and bubble dispersion is achieved by mechanical agitation, which requires relatively high energy consumption per unit of volume (Doran, 1995). There is a great variety of impellers that are described, which produce different flow patterns in the tank.

Generally, 70-80% of the total volume of the STR is filled with liquid. In this case, there is a *headspace* for gas exhaustion and foam formation, which can be controlled by a foam breaker.

STRs are used for free and immobilized cells and enzymes. The sensibility of the biocatalyzers must be studied and analyzed in each case (Doran, 1995).

Continuous Stirred Tank Reactors - CSTR

The CSTR are defined as STR that works in a continuous operation including feeding and remove of mass and energy. An ideal CSTR can be modeled considering a perfect mixing, without temperature, concentration, fluid properties and reaction rate variations. It means that inlet and outlet streams have the same properties of the bioreactor medium. All properties of the CSTR are determined exactly as for the batch bioreactor. In this case, the instrumentation and control systems are different and more sophisticated (Laska & Cooney, 1999).

There are two types of CSTR operation strategies. One of them is the Chemostat that is used for cell culture in which all nutrients are added in excess and the liquid volume is kept constant by setting the inlet and outlet flow rates equal. The second one is the Turbidostat where the cell concentration is maintained constant by the monitoring of the culture optical density and the liquid volume is kept constant by setting the outlet flow rate equal to the inlet flow rate (Laska & Cooney, 1999). CSTRs can be used in series with more than one bioreactor with different conditions in each one.

Some Dispositive of STRs

Impellers

The mixing in bioreactors is done mechanically using impellers. They are normally in the center and suspended in the superior part of the tank. Actually, there is a great variety of impellers, specific for each kind of process. They are divided into two main groups: turbine impeller and paddle impeller.

The correct choice of impellers is based on the bioreactor dimensions, media and total volume, density and viscosity of the fluid to be mixed. The use of different types of impellers is defined according to the different scales of viscosity. The propeller type is recommended for the range of 1 to 10^4 centipoises.

When there is a satisfactory mixing, there is a high degree of interaction between the substrate and biocatalyzers (microorganisms and enzymes), which is positive to mass and heat transfer contributing for an efficient reaction. However, the intensity of mixing depends on the type of process. Some cells can suffer strongly with the shear stress generated by the mixing that causes their inactivation or a negative influence on them. So, it is critical to choose the impeller type that is best suited for the each process. Mammalian and plant cells are, for example, very sensitive and need a special attention and of course, if possible, the development of new models of impellers. Even so, with a wide range of impeller designs, it is difficult to choose the right one for a certain application (Mirro & Voll, 2009). Depending on the impeller used, there are three flux profiles: radial, axial and tangential.

Impeller designs are almost as varied as the types of cell lines they are designed to help grow. Mirro & Voll (2009) listed several impellers commonly used in fermentation showing the best suited for each type of cell culture processes.

Magnetic Mixing

Besides the mechanical impellers, there are also magnetic impellers for industrial applications. The magnetic agitator is directly connected to the bottom of the tank. It assures a very efficient mixing independently of the volume of the fluid. This fact eliminates the need of sealing that can provoke contamination or fluid losses.

Moreover, the use of magnetic agitators requires smaller engines, when compared to the ones with mechanical impellers, and facilitate the cleaning of the bioreactor.

Baffles

Baffles are vertical strips of metal mounted against the wall of the tank. They are installed to reduce vortexing and swirling of the liquid. Baffles are attached to the tank by means of welded brackets; four equally-spaced baffles are usually sufficient to prevent vortex formation. The optimum baffle width depends on the impeller design and fluid viscosity but is of the order 1/10-1/12 the tank diameter. For low-viscosity liquids, baffles are usually attached perpendicular to the wall. Alternatively, baffles can be mounted away from the wall with a clearance of about 1/50 the tank diameter, or set at an angle. These arrangements prevent sedimentation and development of stagnant zones at the inner edge of the baffle during mixing of viscous cell suspensions (Doran, 1995).

Mass and Heat Transfer in STRs

Fermentation broths containing mycelial cells frequently exhibit a pseudoplastic non-Newtonian rheological behavior, which can be described by the power-law model. This behavior exerts a profound effect on the bioreactor performance, affecting mixing pattern, power requirement, heat and mass transfer processes (Gavrilescu et al, 1993). The increase in the broth apparent viscosity (μ_{ap}) during aerobic fermentations can be partially compensated by increments in the operating conditions (N and Q), in order to maintain adequate k_La values. Nevertheless, high impeller speeds (N) lead to the formation of high shear zones close to the impellers, with consequent physical damage to the cells and a reduction in the process productivity (Smith at al, 1990).

STRs are used in a variety of process industries, but the prediction of k_La is extremely difficult because of the complexity of the gas–liquid hydrodynamics. Various investigators have correlated k_La values to power density (P_g/V) and superficial gas velocity (v_s) over one or two similar vessel sizes. Two types of correlations have been proposed for the volumetric oxygen transfer coefficient (k_La). The first does not make use of any dimensional criterion. In these correlations, k_La is related to the gassed power consumption per unit volume of broth (P_g/V) and the superficial gas velocity (v_s), as originally proposed by Cooper et al. (Cooper et al, 1944):

$$k_L a \;\alpha\; \left(P_g/V\right)^{a_1}.(v_s)^{b_1}$$

where the values of the constants a_1 and b_1 may vary considerably, depending on the system geometry, operational characteristics of the vessels, media composition, type, concentration and microorganisms morphology, the range of variables covered and the experimental methodology used (Bandino et al, 2001). Galaction et al, (2005) studied the oxygen mass transfer rate through the mass transfer coefficient, for a STR and different fermentation broths, using a large domain of operating variables. For quantifying the effects of the considered factors (concentration and morphology of biomass, specific power input, superficial air velocity) on k_La for submerged and surface aeration, the experiments were carried out for non-respiring biomass suspensions of *Propionibacterium shermanii*, *Saccharomyces cerevisiae* and *Penicillium chrysogenum*, mycelial aggregates (pellets) and free mycelia morphological structures.

Garcia-Ochoa & Gomez (2009) reported a very important review of the oxygen transfer rate (OTR) in bioprocesses to provide a better knowledge about the selection, design, scale-up and development of bioreactors. First, the most used measuring methods are revised; then the main empirical equations, including those using dimensionless numbers, are considered. The possible increasing on OTR due to the oxygen consumption by the cells is taken into account through the use of the biological enhancement factor. Theoretical predictions of both the volumetric mass transfer coefficient and the enhancement factor that have been recently proposed are described; finally, different criteria for bioreactor scale-up are considered in the light of the influence of OTR and OUR affecting the dissolved oxygen concentration in real bioprocess.

Scale-Up of STRs

Scale-up means reproducing in plant-scale equipment the results from a successful fermentation made in laboratory- or pilot-scale equipment (Hubbard, 1997). The scale-up process thus directly influences the production capacity and efficiency of a bioprocess. In this way, some parameters of the process must be maintained constant during the scale-up: reactor geometry, volumetric oxygen transfer coefficient KLa, maximum shear; power input per unit volume of liquid (P_g/V), volumetric gas flow rate per unit volume of liquid (Q/V or *VVM)*, superficial gas velocity (v_s), mixing time, impeller Reynolds number (Re) and momentum factor (Ju & Chase, 1992).

Normally, a current scale-up strategy is to maintain one or two of the in the order of criticality to the performance of the bioproces . Some examples of this strategy were presented by Ju & Chase (1992). The number of realizable factors, however is limited by the degrees of freedom available in process. Accordingly, the maximum number of criteria that can be maintained constant in a conventional scale-up strategy is three. For example, the following combinations of scale-up criteria have been suggested: 1) Geometric similarity (or constant *DI/DT)* , constant *k L a,* and constant *Q/V* (or *VVM) with* N determined by the k L a correlation; 2) Geometric similarity, constant *ICE a,* and constant maximum shear (or constant impeller tip speed *NDi) w*ith Q calculated from the k_La correlation; 3) Constant *kL a,* constant impeller tip speed *NDi,* and constant *Q/V with D t/D T* adjusted within the limits suggested by Oldshue (1966).

Bubble Column Bioreactors

Bubble column reactors (BCRs) are pneumatic mixed reactors, which were developed for sensitive cells culture such as filamentous fungus cells, mammalian and plant cells.

A bubble column reactor (BCR) is basically a cylindrical vessel with a gas distributor at the bottom. The gas is sparged in the form of bubbles into either a liquid phase or a liquid–solid suspension. These reactors are generally referred to as slurry bubble column reactors when a solid phase exists. Bubble columns are intensively utilized as multiphase contactors and reactors in chemical, petrochemical, biochemical and metallurgical industries (Degaleesan, 2001; Kantarcia et al, 2005).

BCRs usually consist of a cylinder with a ratio high:diameter of 2:1 or even 3:1, differently to STRs, in order to allow a better time of contact of the air and the liquid. For some applications it is possible to find the ratio high:diameter of 6:1. On the top of the BCRs the diameter of the cylinder is larger to facilitate the liberation of bubbles and foam break. The aeration is promoted with compressed air through some spargers that are installed in the bottom of the tank. There are not other internal components.

Gas sparger type is an important parameter that can alter bubble characteristics which in turn affects gas holdup values and thus many other parameters characterizing bubble columns. The sparger used definitely determines the bubble sizes observed in the column. Small orifice diameter plates enable the formation of smaller sized bubbles. Some common gas sparger types that are used in literature studies are perforated plate, porous plate, membrane, ring type distributors and arm spargers.

Gas holdup is a dimensionless key parameter for design purposes that characterizes transport phenomena of bubble column systems (Luo et al, 1999). It is basically defined as the volume fraction of gas phase occupied by the gas bubbles. All studies examine gas holdup because it plays an important role in design and analysis of bubble columns (Kantarci et al, 2005).

Some important applications of bubble columns are the production of industrially valuable products such as enzymes, proteins, antibiotics, etc. Several recent biochemical studies utilizing bubble columns as bioreactors will be presented in Table 2.

Although the construction of bubble columns is simple, accurate and successful design and scale-up require an improved understanding of multiphase fluid dynamics and its influences (Kantarci et al, 2005). The most important parameters in this type of bioreactor are the bubble ascending speed, the residence time, the “hold up”, the interfacial area and the mass transfer. Hold up is the proportion of liquid that is occupied by the gas or the bubble volume in relation to the liquid.

Air Lift Bioreactors

Airlift bioreactors (ARL) are a variation of the BCRs. The main difference is a central tube or other components (channels) that are responsible for an efficient mixing and re-circulation of the fluid. This fact reduces the coalescence of bubbles, which circulate through the reactor, and equalize the shear stress that is provoked by the mixing. The term Airlift is linked to the characteristics of pneumatic contact of the gas-liquid or gas-liquid-solid defined by the circulation of the fluids in a cyclic pattern (Flickinger & Drew, 1999).

There are two main basic configurations of the ALRs: External loop reactors and internal loop reactors. In the first one, the circulation of the fluids follow distintc channels; in the second one there is only a barrier strategically positioned in a unic vessel, which crieates some channels for the circulation or concetric tubes that creates a central and a periferic channel (Flickinger & Drew, 1999).

There are some different structures of external loop reactors and internal loop reactors. These configurations can be re-worked with the development of new possibilities for tha amelioration of the fluid dynamic and a better liberation of the gas in the liquid according to the different processes. For example, in the internal loop with concentric tubes, depending on the number and position of spargers, the ascendent gas flux can be produced at both the central or periferic part of the bioreactor.

Scale-up studies of ARLs pass through the same analysis made for BCRs, where the superficial gas velocity, holdup and dynamic of the fluids must be analysed.

Packed Bed Bioreactors

The Packed Bed Bioreactors (PBRs) typically consist of a packed-bed that supports the cells on or within carriers and a reservoir that is used to re-circulate the oxygenated nutrient medium through the bed. Two major configurations are possible, with the packed-bed compartment located either external to, or within, the reservoir of the medium (Wang et al, 1992a,b). A frequent approach in developing PBRs is to first use a small-scale model bed to identify the optimal packing matrix for the cell line of interest. An optimal matrix is one that provides the requisite combination of cell attachment, proliferation and productivity. This matrix is then used to optimize the operational parameters (e.g. packed-bed height and volume, medium perfusion rate, etc.) of the PBR through perfusion experiments that are generally performed at laboratory-scale (Meuwly et al, 2007).

There has been an increasing trend in identifying support materials that were compatible with different types of cells (microorganisms cells and mammalian cells). Higher internal porosities ranging from 0.80 to 0.95 were reached with the next generation of packing materials such as disks made of non-woven polyester and polypropylene screen, ceramic spheres and other shapes, glass fibers (Perry & Wang, 1989; Chiou et al, 1991), polyurethane and polyvinyl foams or resins (Meuwly et al, 2007).

PBRs can provide extremely high productivity within a compact size is the. PBRs have been used widely for perfusion culture of immobilized mammalian cells. Many authors presented the potential of the use of PBRs as “artificial organs” (Allen et al, 2001) in biomedical applications. A relatively well-known example of such application is the bioartificial liver device (BAL) (Allen & Bhatia, 2002).

Fluidized Bed Reactors

Fermenters for fluidized bed (FB) operation are tall columns, where the ratio of height to diameter (aspect ratio) is typically greater than 10:1. Dempsey [1994] reported that fermenters with an aspect ratio of either 20:1 or 40:1 have been designed and operated. The column diameter should ideally be at least 50-times the particle diameter; but in the

laboratory it is often necessary to compromise between this ideal ratio and the need to minimize the volume of the fermenter. Typically, lab-scale fermenters have a ratio between 25:1 and 40:1. The fluidizing medium can be gas, liquid, or a mixture of the two; with the flow being upwards for fluidization of particles more dense than the fluid, or downwards for particles of lower density, in the case of the fluidized bed fermentation (FBF)s, the fluidizing medium is usually the broth; and for biological fluidized bed (BFBs), the wastewater; with the liquid flowing up through the bed.

As well as applications in wastewater treatment, FB technology can also be applied to pure culture fermentations for the production of microbial metabolites or biomass.

Membrane Bioreactors

Since 1990, membrane bioreactors (MBR) are been used. They are maily composed by installations that are constructed in external configuration, in which case the membrane modules are outside the bioreactor and biomass is re-circulated through a filtration loop. After the mid 1990s, with the development of submerged MBRsystem, MBR applications in many areas extended widely (Meng et al, 2009).

Membrane bioreactor (MBR) technology is advancing rapidly around the world both in research and commercial applications. Several generations of MBR systems have evolved. Up to this date, MBR systems have mostly been used to treat industrial wastewater, domestic wastewater and specific municipal wastewater, where a small footprint, water reuse, or stringent discharge standards were required. It is expected, however, that MBRsystems will increase in capacity and broaden in application area due to future, more stringent regulations and water reuse initiatives (Cicek 2003; Visvanathan et al, 2000; Yang et al, 2006; Meng et al, 2009).

In the water and effluent treatment context, an MBR comprises a conventional activated sludge process coupled with membrane separation to retain the biomass. Since the effective pore size is generally below 0.1 μm, the MBR effectively produces a clarified and substantially disinfected effluent. In addition, it concentrates up the biomass and, in doing so, reduces the necessary tank size and also increases the efficiency of the biotreatment process (Santos et al, 2011). MBRs allow high concentrations of mixed liquor suspended solids (MLSS) and low production of excess sludge, enable high removal efficiency of biological oxygen demand (BOD) and chemical oxygen demand (COD), and water reclamation. However, membrane fouling is a major obstacle to the wide application of MBRs. Additionally, large-scale use of MBRs in wastewater treatment will require a significant decrease in price of the membranes (Meng et al, 2009).

Applications of Submerged Bioreactors

For many reasons STRs are the most widely used bioreactors in the biotechnological industrial applications. They have one or more impellers that are used to generate flow and mixing within the reactor. STRs offer unmatched flexibility and control over transport processes occurring within the reactor. However, as it was presented bellow, there are some variations and more recent developed models of bioreactors such as the bubble columns and

airlift bioreactors. Other models of bioreactors are also being exploited such as the packed bed and membrane bioreactors for immobilized cells and/or enzymes.

Some of the important applications of submerged bioreactors are presented in Table 2.

Table 2. Some applications of submerged bioreactors

Type of Bioreactor	Process	Reference
STR	Antibiotics	Ohta et al., 1995, Luti & Mavituna, 2011
	Citric acid	Papagianni et al., 1998
	Exopolysaccharides	Xu et al., 2006
	Cellulase	Szabo et al., 1996, Hreggvidsson et al., 1996, Kim et al., 1996, Reczey et al., 1996, Belghith et al., 2001, Shen and Xia, 2004, Szijarto et al., 2005, Jang & Chang, 2005, Ahamed & Vermette 2008a,b, 2010
	Chitinolytic enzymes	Chen et al., 2010
	Laccase	Galhaup & Haltrich (2001), Blánquez et al. (2002), Galhaup et al. (2002), Hess et al. (2002), Van der Merwe (2002), Fenice et al. (2003), Sedarati et al. (2003), Mohorčič et al. (2004), Sigoillot et al. (2004), Park et al. (2006), Tavares et al. (2006), Thiruchelvam and Ramsay (2007), Couto & Herrera, 2007
	Xylanase	Reddy et al., 2002
	Lipase	Brozzoli et al., 2009
	Pectic and pectate lyase	Gummadi & Kumar, 2008
	Polygalacturonases	Fontana et al., 2009
	Succinic acid	Isar et al., 2006
	Tissue mass culture	Martin & Vermette, 2005
Bubble Column	Algal culture	Mirón et al., 2000, Wu & Merchuk, 2002
	Chitinolytic enzymes	Chen et al., 2010
Air Lift	Antibiotic	Ohta et al., 1995
	Chitinolytic enzymes	Chen et al., 2010
	Exopolysaccharides	Xu et al., 2006
	Gibberelic acid	Chavez-Parga et al., 2008
	Laccase	Kim et al., 1997, Rancaño et al. 2003, Domínguez et al., 2005, Olivieri et al, 2006, Ryan et al., 2005, Rodríguez Couto et al. 2006
	Cellulase	Ahamed & Vermette 2010
	Lactic acid	Yin et al., 1997a,b, Zhang et al. 2007
	Polygalacturonases	Fontana et al., 2009
	Tissue mass culture	Martin & Vermette, 2005
Fluidized Bed	Laccase	Blánquez et al. (2004), Font et al. (2003),
Packed bed	Laccase	Schliephake et al. (2000), Kasinath et al. (2003), Prasad et al. (2005)
	Hydrogen	Leite et al., 2008
	Organic acids	Leite et al., 2008
	Mammalian cells	Meuwly et al., 2007
Membrane bioreactor	Alginate	Cheze-Lange et al., 2002
	Antibiotic	Schroën et al., 2009
	Cellulose hydrolisis	Gan et al., 2002
	Hydrogen production	Lee et al., 2009
	Water treatment	Williams & Pirbazari, 2007
	VOCs treatment	Mudliar et al., 2010

Bioreactors for SSF

Solid state fermentation (SSF) has several biotechnological advantages over submerged fermentation, although nowadays it is mainly used on a laboratory scale. May be listed higher fermentation productivity, the higher the concentration of end products, higher product stability, lower catabolic repression, cultivation of microorganisms specialized for water-insoluble substrates or mixed cultivation of various fungi. Besides these, the sterilization of the medium is not critical and often unnecessary due to low water activity used in SSF (Singhania et al, 2009).

Commonly used SSF bioreactors can be divided into four types based on type of aeration or the mixed system employed. These can be classified as follows: static bioreactors (fixed bed, perforated trays), stirred bioreactor (the horizontal drum or drum stirred), and bioreactors with or without forced aeration.

The construction of bioreactors must take into account the peculiarities of the SSF the variety of materials that can be used as growth media, and their characteristics such as composition, size, strength, porosity and water holding capacity, coupled with the fact low humidity of the substrate, which gives problems of heat transfer system. All items listed must be taken into consideration in the design and control strategies of a reactor that will operate on a solid state cultivation. The solid state fermentation is a process that occurs in the absence of free water, so filamentous fungi are microorganisms naturally adapted and appropriate for this type of condition. The morphology of the fungus, with respect to the presence of septate hyphae or not (which gives more or less mechanical resistance to possible unrest in the middle), and the necessity or otherwise of sterility in the process are other factors that influence the design of bioreactors for SSF. Thus, the bioreactor may or may not forced aeration, may be without stirring, stirring occasionally, or only with continuous rotation.

The number and types of bioreactors used in pilot and industrial scale is small compared to those used in the laboratory scale. They are mostly applied in laboratory scale due some important reasons and necessities which are:

- The hyphae can be damaged by mechanical agitation, especially if they are non septate, allowing very few drawings that meet the needs of aeration and heat removal;
- The solid medium can become compacted during the process, causing many problems;
- There are difficulties with the inoculation, control and sterilization of large volumes of medium;
- The maintenance and procedures for filling, emptying and cleaning of large reactors;
- The maintenance of uniformity is difficult for large volumes of biomass.

Applications of Bioreactors in SSF

Table 3 below shows some types of bioreactors used in the process of solid state fermentation. Most articles emphasizing the production of bioproducts by comparing different types of bioreactors,and its mode of operation and control.

Table 3. Examples of Bioreactors and products development in solid-state fermentation

Type of Bioreactor	Aeration system	Microorganism	Substrate	Product	Reference
Column fermenter	Forced aeration	*Aspergillus. niger*	Cassava bagasse	Citric acid	Vandenberghe et al (2000)
Erlenmeyer flasks	Natural	*Kluyveromyces marxianus*	Palm bran Cassava bagasse	Aroma compounds	Medeiros et al, (2000)
Horizontal drum and glass columns	Forced aeration	*Ceratocystis fimbriata*	Coffee husk	Aroma compounds	Medeiros et al, (2006)
Trays	Natural convection	*A. oryzae*	Red gram plant waste + wheat bran	α-Galactosidase	Shankar & Mulinami (2007)
Horizontal drum	Forced aeration	*A. niger*	Cassava bagasse	Citric acid	Soccol et al (2007)
Erlenmeyer flasks	Natural	*Monascus purpureus*	Jackfruit seeds	Pigments	Babitha et al, (2008)
Erlenmeyer flasks	Natural convection	*A. niger*	Sugarcane bagasse + soybean meal	Xylanase	Maciel et al, (2008)
Erlenmeyer flasks	Natural	*A. niger*	Citric pulp	Citric acid	Rodrigues et al, (2009)
Column bioreactors	Forced aeration	*Bacillus atrophaeus*	Sugarcane bagasse + soybean molasses	Spores	Sella et al, (2009)
Polyethylene bags; Erlenmeyer flasks	Natural convection	*Bacillus atrophaeus*	Sugarcane bagasse + soybean molasses	Spores	Sella et al, (2009)
Rotating drum	Forced aeration	*A. niger*	Mussel processing waste	Glucose oxidase	Mirón et al (2010)
Raimbault columns	Forced aeration	*A. niger*	Citric pulp bran	Phytase	Spier et al, (2011)

Bioreactor Type Perforated Trays

The tray reactors are characterized by being simple systems in which the substrate is laid out on trays, wooden or stainless steel, usually perforated to facilitate air convection. The substrate is placed in the tray in thin layers (typically 5 to 15 cm) and the trays are arranged on each other with a spacing of several inches in a chamber or room with controlled

temperature and humidity. Scaling up is easy (it increases the number of trays) but requires large area of operations, labour intensive and difficult to apply for sterile processes.

Problems can occur in the transfer of oxygen. The diffusion rate of O_2 transport properties depend on the tray. At the beginning, when the substrate is inoculated, the oxygen concentration is uniform in all layers of the substrate. Over time, the shrinkage of the substrate layer due to the formation of the mycelium changes the porosity and thus the effective diffusivity. The release of CO_2 and heat also prevents the transport of O_2 to the bottom. Under these circumstances, the oxygen concentration, which was once uniform, gives space to a gradient. In some deeper places oxygen concentration becomes small enough to reach zero. Obviously the reaction can not proceed in areas where there is no oxygen. Therefore, to have an efficient process, it must provide the profile of O_2 concentration in different layers.

Fixed Bed Bioreactors

The fixed bed reactors differ from the tray because they are closed systems where aeration is forced. These bioreactors have been considered interesting due to process control, especially by removing heat (which does not happen efficiently in large-scale processes).

Despite having several advantages for process control, forced aeration can result in water evaporation and drying the substrate. This can provide unfavorable low water activity (a_w) and humidity, resulting in poor microbial activity.

Column reactors, amongst those with implementing processes on solid medium, are the most studied in the laboratory scale. The use of column reactors minimizes problems with temperature gradients, due to the convection caused by air entering the reactor directly. Furthermore, the CO_2 released during metabolic reactions, can be eliminated, allowing its replacement by air. Temperature control is done by placing the reactor in a bath or using tactical terms columns jacketed with circulating coolant. However, the reduction in porosity of the bed, with the progress of fermentation, is a problem to be overcome in this type of reactor. The column reactors are associated with low bacterial contamination because they are closed systems, unlike the reactor tray. Moreover, the same reactor can be used for both fermentation and extraction of the final product.

Adopting mixing coupled with forced aeration is possible to increase the homogeneity of the cell population and the substrate concentration in SSF. The geometry of the stirred bed reactor is similar to that used in fixed bed, but includes a mixing system for biomass systems as bolt or other type of agitation device. The ability of this type of bioreactor is scalable to several tons (Pandey et al, 2008).

Drum Bioreactor

Agitated drum reactors are those that use agitation in the process. They are traditionally built in drum-shaped and may or may not contain baffles inside. There are also static drum reactors, in which other systems of agitation to ensure mixing of solid medium, such as a horizontal cylindrical vessel with a worm inside. The agitation in this type of reactor can be continuous or sporadic, and may lead to problems of shear and damage the structure of the

mycelium. Medeiros et al (2006) studied the production of aroma compounds in a 3kg drum bioreactor. The schematic system is showed in Figure (1).

Rotating drums have been used as bioreactors for solid-state fermentation since the 1930s and are already applied to make many products. Rotating drum bioreactors (RDB) provide relatively gentle and uniform mixing by improving baffle design, since there is no agitator within the substrate bed. The engineering principles of RDB have recently received interest for biofuels production using cellulosic materials (Wang et al, 2010).

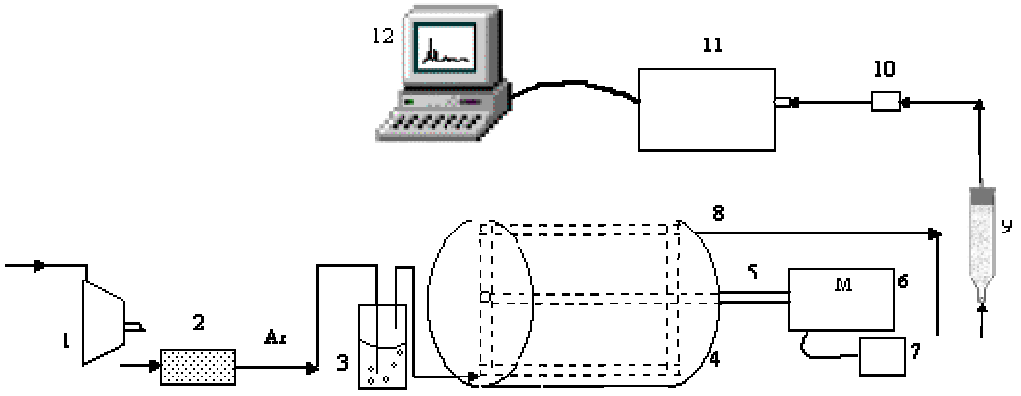

Figure 1. Horizontal stirred drum bioreactor system for solid state fermentation: (1) compressor, (2) Air filter, (3) humidifier, (4) horizontal drum, (5) stirrer, (6) motor, (7) speed controller, (8) air discharge, (9) silica gel columns, (10) (11) gas chromatograph (12) computer. Source: Medeiros et al (2006).

Examples of Laboratory-Scale Bioreactors

Laboratory-scale bioreactors are of simple configuration, based on monitoring (most often manual) of the important variables of the process. Several types of equipment are used for SSF. Erlenmeyer flasks, small perforated trays, Raimbault columns, Petri dishes, jars, Roux bottles and roller bottles offer the advantage of simplicity. Without forced aeration and agitation, only the temperature of the room, where they are incubated, is regulated. Easy to use in large numbers, they are particularly well adapted for the screening of substrates or micro-organisms in the first step of a research and development program (Durand, 2003). The different types of bioreactors are presented below.

Erlenmeyer Flasks

The Erlenmeyer flasks are used in laboratory scale reactors, often to initiate studies and optimization of procedures developed in laboratory scale. They are made of glass and have limited size. These bottles are closed using cotton plugs. The process occurs without agitation and aeration by diffusion. Its advantages are: ease of handling during research, low cost, allows multiple simultaneous tests, passive aeration. Its disadvantages are: inability to control parameters; inability to regulate the process.

Lab-Scale Columns

The column-type bioreactors (Raimbault & Germon, 1976) are in glass columns with 4 cm in diameter and 20 cm which are filled with the solid substrate, this being sterilized and inoculated separately. Subsequently, the columns are connected to air bubblers, and introduced into a water bath with controlled temperature. Aeration (saturated air is pumped through the columns) is adjusted to the desired amount of air flow and controlled with the aid of a flowmeter attached to the air outlet of the column. This model of bioreactors allows study of the influence of forced aeration on the process, and the evaluation of respirometry (measurements of O_2 consumed and CO_2 produced) of the microorganism. This study is of extreme importance for the understanding of the metabolism of microorganisms used in SSF process.

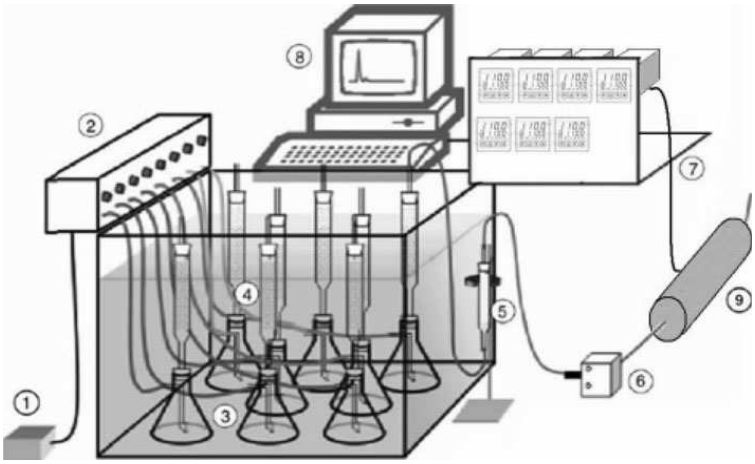

Figure 2. Typical lab-scale apparatus for solid state fermentation . Components are: (1) air pump; (2) air distribution system; (3) humidifiers; (4) fermentation columns immersed in a water bath with controlled temperature; reactor; (5) filter; (6) flow sensor; (7) controllers display; (8) computer with data acquisition and control software; (9) cylindrical sensor base, where the following sensors are installed: CO_2 andO_2, humidity and outlet temperature. Source: Spier et al (2011).

Due to the use of a few grams of solid medium and the geometry of the columns, the bed temperature fermentation can be maintained easily because the heat removal by the column wall seems to be efficient. The disadvantage of this type of reactor is the impossibility of withdrawing the sample from the column. Over cultivation is necessary to destructive sampling, removing a column in every certain interval. Figure (2) shows a model of columns reactors made of glass coupled with a system that analyses the oxygen and carbon dioxide composition in the exhaust airflow from the column.

Examples of Bioreactors to Pilot Scale / Industrial

As it was mentioned before, the number of bioreactor types used at pilot and industrial scale is less then in the laboratory scale. Basically, the configurations of bioreactors at pilot scale have more industrial instrumentation and control devices.

Trays are an example of SSF bioreactor without forced aeration. The system consists of the following elements: a controlled environment which has ejectors and air filters, inlet and outlet air, humidifier, heater, air recirculation, perforated trays and stands.

The tray bioreactors are applied on commercial scale especially for oriental products such as the famous Koji process. Wood, metallic or plastic trays may be perforated or not, and posses various sizes according to their design. The solid medium is disposed at a maximum depth of 15 cm, and the trays are incubated in thermostated chambers or rooms. Although it has been extensively used in industry, this technology requires large areas, intensive labour and the sterilization process is difficult. An alternative to maintain sterility is the use of sterilizable and microporous bags (Durand, 2003).

In a horizontal drum bioreactor with forced aeration, the agitation may occur continuously or intermittently. Discontinuously rotating drum operates like a tray reactor. The agitation is necessary to remove the heat accumulation in the substrate bed. The system consists of a horizontal drum and some auxiliary equipment such as compressor, air filter, humidifier, spindle, motor, speed controller, discharge air, silica column, autosampler, gas chromatography (respirometry), computer.

Rotating drum bioreactors with air circulation and continuously mixed are commonly used in pilot or lab scale process. According to Durand (2003) the largest reactor cited in the literature was a 200 L stainless steel rotating drum which used 10 kg of steamed wheat bran as substrate for kinetic studies of *Rhizopus*. The continuous mixing is necessary to maximize the exposure of the substrate particle to the air circulating in the headspace.

In packed-bed bioreactors, the air is introduced through a sieve which supports the substrate. Pre-pilot bioreactor was developed at for defining the control strategy and optimizing the air-inlet temperature, the airflow rate, the addition of water and agitation during a SSF process (Durand et al, 1988). This type of bioreactor consists of: a basket containing the solid medium; valves for adjusting the air flow, air temperature probe, humidity probe; valves for outlets; box, heating and thermal resistance.

Scale-Up Aspects

Bioreactors for solid state fermentation are used in large-scale traditionally processes such as Koji and Sake, whereas in other bioprocesses there is an absence of commercial designs of bioreactors. The major problems to overcome are related to the engineering aspects, such as lack of standardized processes and limited reproducibility of the experimental results. A large numbers of patents and publications are available on how to design, operate and scale-up SSF bioreactors. The scale-up of the bioreactor are difficulty mainly by intense heat generation and heterogeneity in the system. The gradients of temperature, humidity, substrate concentration, which may arise during the process, affecting not only those processes that involve static solid beds, but also processes involving agitation of the substrate, such as those performed in rotating drums.

Factors such as oxygen content, moisture level and temperature are important parameters in SSF which are difficult to control. The microbial growth under aerobic conditions in the bioreactor results in a considerable production of heat that causes fast increase in temperature. This effect is often undesirable especially in some, biotechnological processes in which heat sensible products or enzymes produced during the fermentation can be heat-denatured at the

end of the process. In the absence of a free aqueous phase, the produced heat is difficult to remove, for example, via the bioreactor double walls. Instead, the cooling of the process takes place through evaporation. However, this requires very high aeration rates because the rising metabolic activity and the associated increased heat production have to be overcompensated by high aeration intensity. The water lost by evaporation has to be in many cases replenished, and this can lead to undesirable local increase in water activity during static processing. In semi-sterile processes, this increased water activity can in turn have adverse effects by facilitating the growth of bacterial contaminants, whereas sterile fermentations, it may bring about local insufficient oxygen supply to the microorganisms. Substrate mixing may help but it should be noted that many microorganisms respond very sensitively to the shear stress caused by it. Another factor that is difficult to account for is the production of metabolic water by aerobic microorganisms, which can cause problems especially in the formation of conidiospores.

Bioreactors for Plant Cells Cultures

The interest in plant cell cultures for the production of valuable natural products such as pharmaceuticals, flavours and fragrances and fine chemicals has been growing. However, several problems have limited the commercial exploitation and application of plant cell cultures. The low and unstable cell productivity, slow growth, genetic instability, and an inability to maintain photoautotrophic growth are the main problems (Paek et al, 2005). Besides, distinct properties of plant cell cultures have to be considerate in bioreactor design including: large cell size and complex morphology, tendency for aggregation, time-dependent rheological behavior, foaming and wall growth, shear sensitivity, and relatively low growth and oxygen uptake rate (Xu et al, 2011). The implications of these factors on bioreactor design and operation have been comprehensively reviewed (Huang & McDonald, 2009).

Culturing plant cells in reactors has many similarities with the growth of mammalian cells, but with different nutritional and control requirements. The design and operation of bioreactors is critical for successfully developing large-scale production process using plant cell cultures. For large-scale culturing plant cells a variety of bioreactors are usually described according three classes of culture systems:

- Bioreactors for biomass production (cells or organonic or embryogenic propagules, shoots or roots as the final product),
- Bioreactors for metabolites and enzymes production
- Bioreactors used for biotransformation of exogenously added metabolites.

The conventional stirred tank reactor (STR) for microbial cultures have been modified to allow better conditions to plant cell cultures. The modifications include a variety of impellers design distinct from the blade turbines for agitation. Those bioreactors are fundamentally classified by agitation methods and vessel construction into:

- mechanically agitated (stirred tank bioreactor, rotating drum bioreactor, spin filter bioreactor)

- pneumatically agitated and non-agitated bioreactors (simple aeration bioreactor, bubble column bioreactor, air-lift bioreactor, balloon type bubble bioreactor-BTBB).

The goal in bioreactor design and operation is to provide a prolonged and sterile culture environment with efficient mixing and oxygen exchange in an effort to support optimized cell growth and achieve high productivity.

Stirred-Tank Bioreactor (STR)

Prakash and Srivastava (2007) successfully scaled-up *Azadirachta indica* suspension culture for azadirachtin production in a stirred tank bioreactor with two different impellers. The studies were carried out in a stirred tank bioreactor equipped with centrifugal impeller and compared with similar bioreactor with a setric impeller to investigate the role of O2 transfer efficiency of centrifugal impeller bioreactor on overall culture metabolism. The maximum cell mass for centrifugal impeller bioreactor and stirred tank bioreactor (with setric impeller) were 18.7 and 15.5 g/L (by dry cell weight) and corresponding azadirachtin concentrations were 0.071 and 0.05 g/L, respectively.

Bubble Column Bioreactor

Bubble columns are suitable for plant cell cultures due to low shear stresses, mechanical simplicity, easy operation, and good mixing. In bubble columns, the hydrodynamics of the liquid phase has a strong influence on column performance and cell response, particularly for long term cultures.

This type of bioreactor was used by Wu et al (2009). The authors investigated the effect of elicitation with linoleic and α-linolenic fatty acids in *Panax ginseng* C.A.Meyer adventitious roots cultured in balloon-type bioreactor.

Soccol et al (2008) developed a Bioreactor of Immersion by Bubbles. It was used to produce many plant species. The equipment is made with cylindrical Blindex® glass, with two compartments, divided transversally by a porous plate with 170 a 220 µm. The inferior part has 3.5 cm height, with the air entrance. The superior part has 24.5 cm height and at this compartment are placed the tripods with 2 to 5 cm height each, angle of 120º and the sieves (mash 18). All the internal components are made of stainless steel. The bioreactor totalizes 28 cm height with 90 mm of diameter, but can be changed according to the needs of the process. The equipment works with an interlinked system with hoses of flexible rubber, through which the plant tissues receive air and nutritive solution by bubbling (Figure 3).

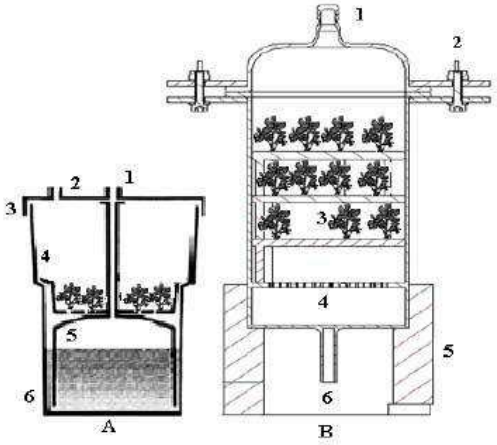

Figure 3. Front view (A) and side view (B) of a Bioreactor of Immersion by Bubbles; (1): Air exausting; (2) screws; (3): metallic perforated trays; (4): porous plate distribution; (5) support brackets; (6) Air inlet. Source: Soccol et al (2008).

Novel Bioreactors Types

Wave Bioreactors

The potential application of wave bioreactors for cultivating tobacco, grape and apple suspension cells have been demonstrated. Eibl and Eibl (2006) achieved high plant cell (*V. vinifera*) biomass productivities of 40 g/(L.day) with a doubling time of 2 days and observed that there was no significant change in cell morphology when compared to cultivations in stirred-tank bioreactors. Other advantages of wave bioreactors include time savings (the cleaning and sterilization processes are not needed), reduced foaming, easy operation and low risk of contamination.

Membrane Bioreactors

Membrane bioreactors utilize specialized membranes with specific molecular weight cutoff for either in situ aeration, nutrient supply, or product separation. They are designed to retain cell biomass and also possibly recombinant protein product in a cell compartment. Although, membrane bioreactors result in less shear stress to cultivated cells, studies indicated that mass transfer is an important consideration even for relatively slow growing plant cells (Huang & MacDonald, 2009). McDonald et al (2005) applied a membrane bioreactor for the production of recombinant human alpha-1-antitrypsin (AAT) using transgenic rice cell culture with a rice alpha amylase promoter, Ramy3D, resulting in extracellular product titre close to 250 mg/L and 4–10% of the extracellular total soluble protein. However, the membrane bioreactor is primarily used for small scale processes and is difficult to scale up for large-scale applications.

Disposable Bioreactors

The use of disposable bioreactors for large-scale plant cell culture with a goal of reducing production costs and minimizing validation efforts under GMP regulations. Disposable bioreactors are usually made of FDA-approved biocompatible plastics such as polyethylene, polystyrene, polytetrafluorethylene, polypropylene and ethylene vinyl acetate (Eibl et al, 2010). Since the bioreactor vessel is pre-sterilized and discarded after harvest, clean-up and re-sterilization steps are eliminated and associated risks with culture contamination are reduced (Xu et al, 2011).

Bioreactors for Mamalian Cell Culture

Animal and human cells are extremely sensitive to fluctuations in culture environment and the traditional depletion and toxic by-product accumulation associated with batch culture can adversely affect them. Tissue culture traditionally follows the methods of batch or semi-batch culture, with the carbon source present at the start of the culture. The majority of 2D tissue culture systems are T-flasks and well-plates in which cells are grown statically without any mixing. These static systems have limitations that arise from their non-homogeneous nature. Without mixing, concentration gradients of pH, nutrients, toxins, gases and growth factors will occur in the culture medium (Collins et al., 1998). This will give rise to different cell behaviour in different spatial locations in the culture and will also reduce culture reproducibility. It has been documented that the behaviour of cells in 2D can be very different in 3D culture (Abbott, 2003) and this has important implications for the in vitro generation of functional human tissues. Tissue growth is dynamic, non-steady state process where biological parameters, physical rate process and the local mechanical environment are continually changing with time – in essence tissue engineering is a 4D challenge. Some bioreactors used in mammalian models and in vitro are presented in Table 3.

Bioreactors are currently used for primary liver cell culture and clinical liver support as showed in Tabela 3, such as hollow fiber which exhibit two functional compartments. Another study pointed out, that four bioreactor compartments are necessary to enable integral oxygenation and distributed mass exchange with low gradients. A specific bioreactor was developed for clinical liver support that accommodated 400–800 g of primary cells (Gerlach et al., 2001) and can be used for growing liver progenitors (Table 3). A spontaneous reassembly of primary cells inoculated into a bioreactor established of a scaffold or biomatrix. A homogeneous mix of adult liver cells from organ collagenase digestion containing parenchymal hepatocytes, non-parenchymal cells (such as sinusoidal endothelial cells, stellate cells, and liver progenitor cells) will restructure after injection into specific bioreactors to form well-defined liver structures, such as neo-sinusoidal structures and neo-spaces of Dissé, reminiscent of the native liver (Gerlach et al, 2008).

Bioreactors provide a manner for efficient exchange of nutrients and mechanical stimulus necessary for tissue engineered bone grafts. In studies reported by Zhang et al (2009), a biaxial rotating bioreactor with fluidics through in-silico modelling was applied to generate osteogenic bone graft using polycaprolactone-tricalcium phosphate scaffolds seeded with human fetal mesenchymal stem cell. This bioreactor reached cellular confluence earlier (day 7) compared to static culture (day 28), and allowed the maintenance of cellular viability

beyond the limits of conventional diffusion, with increased proliferation and osteogenic differentiation both in vitro and in vivo.

Table 3. Bioreactors used in animal models and in vitro

Technology	Cell type	Reference
Stirred tank for the production of alpha-interferon to the clinical use in cancer and viral infection	Namalwa	Cartwright (1994)
Stirred tank for producing tissue plasminogen activator used for thrombolysis	CHO	Cartwright (1994)
Roller bottles	CHO	Cartwright (1994)
Hollow fiber-based bioartificial liver with integral oxygenation	Porcine liver cells	Gerlach et al (2001) Janke et al (1999)
Spirally wound flat sheet and hollow fiber-based bioartificial liver with integral oxygenation	Porcine hepatocytes	Flendrig et al (1999)
Flat plate bioartificial liver with integral oxygenation	Porcine hepatocytes	Shito et al (2003)
Hollow fiber-based renal tubule assist design	Human renal tubule cells	Humes et al (2002)
Early perfusion chambers	Chick heart fibroblasts, human malignant epithelial cells, Chinese hamster cells, hybridomas	Christiansen et al (1953) Rose (1954) Freed (1963) Katinger (1985)
Commercially available perfusion chambers	Bone marrow-derived osteoblasts	Minucells & Minutissue Vertriebs GmbH
Commercially available systems for non-adherent cells: VectraCell gas-permeable bags; Rotary Cell Culture System; Wave bioreactor; CELLine; miniPERM Bioreactor; CellMax; Tecnomouse	Hybridomas	Diagnostic Chemicals Limited; Synthecon, Inc; Wave Biotech LLC; Integra Biosciences AG; Sartorius AG; Spectrum Laboratories, Inc.; Integra Biosciences AG
Commercially available system for bone marrow expansion: AastromReplicell	Hematopoetic stem cells	Aastrom Biosciences, Inc.
Hollow fiber-based bioreactor with integral oxygenation	Human leukemic cell lines	Gloeckner & Lemke (2001)
Hollow fiber-based bioreactors	Mouse fibroblasts; Human choriocarcinoma cells; Reuber hepatoma cells; Human hepatocytes	Knazek et al (1972) Wolf & Munkelt (1975) Hager et al (1978, 1983)
Coaxial hollow fiber-based bioreactor with integral oxygenation	Rat hepatocytes	Macdonald et al (2001)
Hollow fiber-based bioartificial liver with integral oxygenation	Porcine and human liver cells	Gerlach et al (2003) Sauer et al (2002)
Flat sheet and hollow fiber-based bioartificial liver with integral oxygenation	Porcine hepatocytes	Flendrig et al (1997)
Titanium mesh bioreactor	Rat bone marrow stromal osteoblasts	Bancroft et al (2003)
Flat membrane bioreactor with integral oxygenation	Porcine hepatocytes	De Bartolo et al (2000)

Source: Gerlach et al (2008); Cartwright (1994)

Photobioreactors

Although some models of photo bioreactors have been proposed, only a few of them can be practically used biomass from algae. The main applications of photo bioreactors are in photosynthetic processes, involving vegetable biomass growth or microalgae growth under controlled conditions. According to Ugwu et al (2008), one of the major factors that limit their practical application in algal mass cultures is mass transfer, which is necessary for efficient operation of mass algal cultures. The biomass from algae is commonly used in water treatment, in the field of aquaculture, for the production of fine chemicals and as useful supplements in humans and animals, for biosorption of heavy metals, CO_2 fixation. The photo bioreactors range from laboratory to industrial scale models and besides they are classified in closed photo bioreactor, open ponds, flat-plate, horizontal/serpentine tubular airlift and inclined tubular photobioreactors (Ugwu et al, 2008). The introduction of more sophisticated cultivating methods of microalgae with higher productivities and capable of avoiding contamination, which are offered by different types of closed photobioreactors applied outdoors. The same author describe that the advantage of closed as compared to open ponds or tanks, is that the light path length is noticeably reduced leading to higher cell densities, which diminishes the chance of contamination and facilitates harvesting. And besides, reduces evaporative losses and temperature can be more easily controlled (Tredici, 2004).

In general, laboratory-scale photobioreactors are artificially illuminated using fluorescent or other light lamp distributors. Some of these reactors include open ponds, flat-plate, tubular, bubble column, airlift column, helical tubular, conical, torus, stirred-tank, seaweed type photobioreactors.

Open Ponds

Open ponds are easy to construct and operate, but presents some limitations such as poor light utilization by the cells, evaporative losses, diffusion of CO_2 to the atmosphere, requirement of large areas of land, and contamination by predators and other fast growing heterotrophy. Due to inefficient stirring mechanisms in open cultivation systems, their mass transfer rates are very poor resulting to low biomass productivity (Ugwu et al, 2008). Putt et al (2011) studied a system for carbonation and got a high biomass production during algae culture, reducing one of the limitations of the open pond photobioreactor, difficulty in CO_2 diffusion. The proposed device can be used with any exhaust gas stream with higher concentrations of CO_2 in conjunction with raceways for optimizing algae production. Park et al (2011) produced algal biomass for conversion to biofuels with minimum environmental impact using wastewater by *Chlorella* sp. and *Spirulina* sp. in open ponds photobioreactor.

Flat-Plate Bioreactors

In this type of photo bioreactor the photosynthetic microorganisms are cultivated in a large illumination surface area. Studies of vertical alveolar panels designs and flat plate reactors for mass cultivation of different algae were reported (Tredici & Materassi, 1992; Hu & Richmond, 1996; Zhang et al (2001); Hoekema et al, 2002). Generally, flatplate

photobioreactors are made of transparent of transparent materials for maximum utilization of solar light energy. Accumulation of dissolved oxygen concentrations in flat-plate photobioreactors is relatively low compared to horizontal tubular photobioreactors. It has been reported that with flat plate photobioreactors, high photosynthetic efficiencies can be achieved (Richmond & Cheng-Wu, 2001). Flat plate photobioreactors are very suitable for mass cultures of algae.

The flat-plate reactor geometry was chosen by Tamburic et al (2011) for H_2 production due to its superior surface-to volume ratio, which resulted in the highest observed photochemical efficiencies. This photobioreactor was divided in two compartments: the first compartments holds the algal culture and provide control measurements, while the second turbulent culture mixing was achieved by a circulating gas-lift system that is operated with a customised liquid diaphragm pump. An illustrative H_2 production experiment achieved a H_2 yield of 105 ml/L at a maximum H_2 production rate of 1.1 mL/L/h and a photochemical conversion efficiency of 0.24% (Tamburic et al, 2011).

A flat plate photobioreactor was applied for *Nannochloropsis* sp. cultive, a marine unicellular photoautotrophic alga producer of EPA (eicosapentaenoic acid, 20:5w3). This bioreactor was made of number of units (8-10 mm thick glass plates each unit), measuring 200 cm long, 110 cm high protected and sustained by dividers. The inside width composed by 10 cm light-path and form a 500 to 1000 L unit developed for outdoor production. It requires a relatively small ground area, utilizing strong light very effectively by having a large illuminated surface to volume ratio (i.e. 50 L. m^2, considering the area of all the illuminated reactor surfaces. The investment cost per liter culture was US$ 4 L^{-1} considered one of the lowest available for large-scale operation (Cheng-Wu, 2001).

Tubular Photobioreactors

Tubular photobioreactor is a type of reactor for outdoor biomass cultures with high surface area for illumination, constructed with glass or plastic tube. They can be in form of inclined, conical, horizontally serpentine, near horizontal photobioreactors. Efficient light distribution to the cells can be achieved by improving the mixing system in the tubes (Ugwu et al, 2005). The cultures are re-circulated either with pump or preferably with airlift system. An outdoor tubular photobioreactor were used for biomass culture of *Isochrysis* sp., a microalgae rich in docosahexaenoic fatty acid (polyunsaturated). The reactor tested consisted by horizontal serpentine-like, 60m (10m×6m) of acrylic tubes connected with 180 fits (photostage amounting to 200 L) and a 400-L fibreglass gas exchange tank (culture volume 200 L). The tubes had an inner diameter of 6.4cm (outer diameter 7.0 cm) and were submerged in a water bath in order to mitigate temperature variations. This work showed that outdoor tubular photobioreactor seemed to be particularly sensitive to the variable conditions outdoors and we suggest that new designs of closed photobioreactor try to achieve more stable and homogeneous conditions throughout the system and over the daily cycle (Bergeijk et al, 2010).

Vertical-Column Photobioreactors

Vertical-column photobioreactors are compact, low-cost, and easy to operate monoseptically (Sanchez Miron et al., 2002). Furthermore, they are very promising for large-scale cultivation of biomass algae. It was reported that bubble-column and airlift photobioreactors (up to 0.19 m in diameter) can attain a final biomass concentration and specific growth rate that are comparable to values typically reported for narrow tubular photobioreactors (Sanchez Miron et al, 2002). Some bubble column photobioreactors are equipped with either draft tubes or constructed as split cylinders. In the case of draft tube photobioreactors, intermixing occurs between the riser and the downcomer zones of the photobioreactor through the walls of the draft tube. *Chlorella* sp. was cultivated in 2 L bubble-column photobioreactor and investigated for biodiesel production. The photobioreactor consisted of different parts. Glassmade cylinder, four plastic tubes with a diameter of 6 mm which were used for gas inlet, gas outlet, feeding and sampling, porous bulk stone which was used for bubbling the air, air pump, top head plastic cover with four ports for plastic tubes. The biomass concentration of *Chlorella* sp. at the end of exponential phase was approximately 1.9 g/L. It has doubling time of approximately 15 h during the exponential phase (Rasoul-Amini et al, 2011). This type of photobioreactor has good light–dark cycling and low surface/volume (Mata et al, 2010).

The airlift photobioreactor was study by Ranjbar et al (2008) for cultivating *Haematococcus pluvialis* cells and astaxanthin production. This reactor consisted in a draft-tube internal-loop type made of Pyrex glass, with a working volume of 1.0 liter, exposed to certain light/dark cycles and by light intensity distribution and mixing inside the photobioreactor. The authors achieved the highest ever reported cell numbers ($7x10^6$ cells mL^{-1}) after 300 h cultivation, and the regular light/dark cycles and laminar flow had a positive effect on the astaxanthin accumulation. The increase of light intensity during the accumulation of astaxanthin resulted in a better result (600 mg L^{-1},) of this pigment, which was several times higher than previously reported values (Ranjbar et al, 2008).

Sánchez Mirón et al (2002) studied relatively large outdoor bubble column and airlift bioreactors that were compared for monoseptic fed-batch culture of the microalga *Phaeodactylum tricornutum* and concluded that the three photobioreactors produced similar biomass versus time profiles and final biomass concentration. Pigment-rich biomass is generated in light-limited culture. The carotenoids contents in *P. tricornutum* are much more sensitive to available light in light-limited culture than are the chlorophyll contents. Bubble column and airlift photobioreactors of up to 0.19m in diameter can attain a final biomass concentration and specific growth rate that is comparable to values typically reported for narrow tubes (e.g. 0.03m in diameter) of conventional horizontal tube reactors. The good performance of the large-diameter vertical reactors was explained by an absence of photoinhibition and photo-oxidation of the biomass and also the biomass in vertical reactors does not experience oxygen inhibition of photosynthesis (Sánchez Mirón, 2002).

Some photobioreactors can be internally illuminated with artificial light, mainly fluorescent lamps. This equipment is equipped with impellers for agitation of the algal cultures. Air and CO_2 are also supplied to the cultures. This type of photobioreactor can also utilize solar light.

Scale Up of Photobioreactors

For photobioreactor scale-up, several photobioreactors have been proposed for small scale, but most of them present limitations or difficulties to scale-up. Scale-up success depends on the good light supply to the cultivation of photosynthetic organisms. However, an appropriate method for harvesting the solar energy and distributing the light inside the photobioreactor is required. According to Ogbonna et al (1996), internal optical fibers for light distribution have been reported for scale up photobioreactors, although these fibers are expensive and may present technical problems.

Future Perspectives

Bioreactor design and the association with process optimisation are essential for transfering the parameters obtained from lab or pilot scale experiments to efficient production processes in industrial large scale bioreactors. It is necessary more efforts to improve bioreactor technologies and performance during operations. Besides, technical costs of construction and low energy losses should be considered, even if the product has high-value aggregated. The establishment of a more sustainable future industrial production is the key to develop new and innovative industrial bioprocesses.

Conclusion

Several operation models of bioreactor permit to cultivate since vegetable, algae, microbial and mammalian cells or its culture for obtain interest products. Even fermentation processes such as submerged and solid state fermentation are commonly used using microbial cells (bacteria, yeast and fungi) aim biomolecules or metabolic for commercial purposes. Most of them are projected for maximize processes conditions, but each of them presents limitations, depending to the application and the nature of the cell culture.

References

Abbott, A. Biology´s new dimension, *Nature*, 2003, 424, 870-872.

Bancroft, GN; Sikavitsas, VI; Mikos, AG. Design of a flow perfusion bioreactor system for bone tissue-engineering applications. *Tissue Engineering*, 2003, 9, 549-554.

Ahamed, A ; Vermette, P. Enhanced enzyme production from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* LMA grown as fed batch in a stirred tank bioreactor. *Biochemical Engineering Journal*, 2008, 42, 41–46.

Ahamed, A ; Vermette, P. Culture-based strategies to enhance cellulase enzyme production from *Trichoderma reesei* RUT-C30 in bioreactor culture conditions. *Biochemical Engineering Journal*, 2008b, 40, 399–407.

Ahamed, A. ; Vermette, P. Effect of mechanical agitation on the production of cellulases by Trichoderma reesei RUT-C30 in a draft-tube airlift bioreactor.
Biochemical Engineering Journal, 2010, 49, 379–387.

Allen, JW; Hassanein, T; Bhatia, SN. Advances in bioartificial liver devices. *Hepatology* 2001, 34, 447–55.

Allen, JW; Bhatia, SN. Improving the next generation of bioartificial liver devices. *Cell & Dev Biol*, 2002, 13, 447–54.

Babitha, S; Carvalho, JC; Soccol, CR; Pandey, A. Effect of light on growth, pigment production and culture morphology of Monascus purpureus in solid-state fermentation. *World Journal of Microbiology and Biotechnology*, 2008, 24, 2671–2675.

Badino Jr., AC; Facciotti, MCR ; Schmidell, W. Volumetric oxygen transfer coefficients (*k*L*a*) in batch cultivations involving non-Newtonian broths. *Biochemical Engineering Journal*, 2001, 8, 111–119.

Bergeijk, SA; Salas-Leiton, E; Cañavate, JP. Low and variable productivity and low efficiency of mass cultures of the haptophyte *Isochrysis* aff. *galbana* (T-iso) in outdoor tubular photo bioreactors, *Aquacultural Engineering*, 2010, 43(1), 14-23.

Belghith, H ; Ellouz-Chaabouni, S ; Gargouri, A. Biostoning of denims by *Penicillium occitanis* (Pol6) cellulases. *Journal of Biotechnology*, 2001, 89, 257–62.

Bhattacharyya, BC; Banerjee, S; Ghosh, TK. Bioreactors: Functions in fermentation processes. In: Pandey, A; Larroche, C; Soccol, CR; Dussap, CG (Editors). Advances in Fermentation Technology. New Delhi: *Asiatech Publishers*, Inc., 2008, 172-201.

Blánquez, P ; Caminal, G ; Sarrà, M ; Vicent, MT, Gabarrell, X. Olive oil mill waste waters decoloration and detoxification in a bioreactor by the white rot fungus Phanerochaete flavido-alba. *Biotechnology Progress*, 2002,18, 660–622

Blánquez, P ; Casas, N ; Font, X ; Gabarrell, M ; Sarrá, M ; Caminal, G ; et al. Mechanism of textile metal dye biotransformation by Trametes versicolor. *Water Research*, 2004, 38, 2166–2172.

BOMAX DO BRASIL®. AGIMAX®. Agitadores e misturadores verticais. 2010. Available in: http://www.bomaxdobrasil.com.br/pdfs/Agimax.pdf.

Brozzoli, V ; Crognale, S ; Sampedro, I ; Federici, F ; D'Annibale, A ; Petruccioli, M ; Assessment of olive-mill wastewater as a growth medium for lipase production by Candida cylindracea in bench-top reactor. *Bioresource Technology*, 2009, 100, 3395–3402.

Binod, P; Singhania, RR; Soccol, CR; Pandey, A. Industrial Enzymes. In: Pandey, A; Larroche, C; Soccol, CR; Dussap, CG (Editors), Advances in Fermentation Technology. New Delhi: Asiatech Publishers, Inc. *Bioreactors: Functions in fermentation processes*, 2008, 291-320.

Cartwright, T. Animal cells as bioreactors, *Cambridge University Press*, 1994, 184 p.

Collins, PC, Miller, WM, Papoutsakis, ET. Stirred culture of peripheral and cord blood hematopoietic cells offers advantages over traditional static systems for clinically relevant applications. *Biotechnology and Bioengineering*, 1998, 59, 534-543.

Chaudhuri, J; Al-Rubeai, M. Bioreactors for tissue engineering: Principles, design and operation. *Netherlands: Springer*, 2005, 375 p.

Chavez-Parga, MC ; Gonzalez-Ortega, M ; Negrete-Rodríguez, MLX ; Vallarino, IG ; Alatorre, GG ; Escamilla-Silva, EM. Kinetic of the gibberellic acid and bikaverin production in an airlift bioreactor. *Process Biochemistry*, 2008, 43, 855–860.

Cheze-Lange, H ; Beunard, D ; Dhulster, P ; Guillochon, D ; Caze, A-M ; Morcellet, M ; Saude, N ; Junter, G-A. Production of microbial alginate in a membrane bioreactor.*Enzyme and Microbial Technology*, 2002, 30, 656–661.

Chen, H-B ; Kao, P-M ; Huang, H-C ; Shieh, C-J ; Chen, C-J ; Liu, C-Y. Effects of using various bioreactors on chitinolytic enzymes production by *Paenibacillus taichungensis*. *Biochemical Engineering Journal*, 2010, 49, 337–342.

Cheng-Wu, Z; Zmora, O; Kopel, R; Richmond, A. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. Eustigmatophyceae, *Aquaculture*, 2001, 195, 35–49.

Cooper, CM; Fernstrom, GA; Miller, SA. *Ind. Eng. Chem.* 1944, 36, 504-509.

Couto, SR ; Toca-Herrera, JL. Laccase production at reactor scale by filamentous fungi. *Biotechnology Advances*, 2007, 25, 558–569.

Chiou, T-W; Murakami, S; Wang, DIC. A fiber-bed bioreactor for anchorage-dependent animal cell cultures: Part I. Bioreactor design and operations. *Biotechnology and Bioengineering*, 1991, 37, 755–761.

Christiansen, GS; Danes, B; Allen, L; Leinfelder, PJ. A culture chamber for the continuous biochemical and morphological study of living cells in tissue culture. *Exp. Cell. Res.*, 1953, 5, 10-15.

Xu, CP; Kim, SW; Hwang, HJ; Yun, JW. Production of exopolysaccharides by submerged culture of an enthomopathogenic fungus, *Paecilomyces tenuipes* C240 in stirred-tank and airlift reactors. *Bioresource Technology*, 2006, 97, 770–777.

Cinar, A; Birol, G; Parulekar, SJ, Undey, C. Batch Fermentation: Modeling Monitoring and Control, *CRC Press*, 2003, 648 p.

De Bartolo, L; Schweder, JV; Haverich, A; Bader, A. A novel full-scale flat membrane bioreactor utilizing porcine hepatocytes: Cell viability and tissue-specific functions. *Biotechnology Progress*, 2000, 16, 102-108.

Degaleesan, S; Dudukovic, M; Pan, Y. Experimental study of gasinduced liquid-flow structures in bubble columns. *AIChE Journal*, 2001, 47, 1913–1931.

Dempsey, MJ. Biofilms and Fluidized Bed Fermentation. *International Biodeterioration & Biodegradation*, 1994.

Dochain, D. *Automatic Control of Bioprocess*. London: John Wiley and Sons, 2008, 242 p.

Domínguez, A ; Rodríguez Couto, S ; Sanromán, MA. Dye decolourization by Trametes hirsuta immobilised into alginate beads. *World J Microbiol Biotechnol* 2005, 21,405–409.

Doran, PM. Bioprocess Engineering Principles. *Elsevier Science & Technology Books*, 1995.

Dunn, IJ. Biological reaction engineering: Dynamic modelling fundamentals with simulation examples. *Weinheim: Wiley-VCH Verlag*, 2003, 508 p.

Durand, A; De la Broise, D; Blachère, H. Laboratory scale bioreactor for solid state processes, *Journal of Biotechnology*, 1988, 8, 59–66.

Durand, A. Bioreactor designs for solid state fermentation *Biochemical Engineering Journal*, 2003, 13, 113–125.

Eibl, R; Eibl, D. Design and use of the wave bioreactor for plant cell culture, *Plant Tissue Culture Engineering*, 2006, 6, 203–227.

Eibl, R; Kaiser, S; Lombriser R; Eibl, D. Disposable bioreactors: the current state-of-the-art and recommended applications in biotechnology. *Applied Microbiology and Biotechnology*, 2010, 86, 41–9.

Engasser, J-M. Bioreactor Engineering: The Design and Optimization of Reactors with Living Cells. *Chemical Engineering Science*, 1988, 43, 1739-1748.

Fenice, M ; Sermanni, GG ; Federici, F ; D'Annibale, A. Submerged and solid-state production of laccase and Mn-peroxidase by *Panus tigrinus* on olive mill wastewater-based media. *Journal of Biotechnology*, 2003, 100, 77–85.

Flendrig, LM, Soe, JW; Jorning, GG; Steenbeek, A.; Karlsen, OT, Bowee, WM, Ladiges, NC, Te Velde, AA; Chamuleau, RA. In vitro evaluation of a novel bioreactor based on an integral oxygenator and a spirally wound nonwoven polyester matrix for hepatocyte culture as small aggregates. *Journal of Hepatology*, 1997, 26, 1379-1392.

Flickinger, MC ; Drew, SW. *Encyclopedia of Bioprocess technology: Fermentation, Biocatalysis, and Bioseparation*. 1rst ed. USA: John Wiley & Sons, Inc.; 1999.

Font, X ; Caminal, G ; Gabarrell, X ; Romero, S ; Vicent, MT. Black liquor detoxification by laccase of Trametes versicolor pellets. *J Chem Technol Biotechnol* 2003, 78, 548–554.

Freed, JJ, Cell culture perfusion chamber: *Adaptation for microscopy of clonal growth. Science*, 1963, 140, 1334-1335.

Gerlach, JC; Botsch, M; Kardassis, D; Lemmens, P; Schon, M; Janke, J; Puhl, G; Unger, J; Kraemer, M; Busse, B; Bohmer, C; Belal, R; Ingenlath, M; Kosan, M; Kosan, B; Sultmann, J; Patzold, D; Tietze, S; Rossaint, R; Muller, C, Monch, E; Sauer, IM; Neuhaus, P. Experimental evaluation of a cell module for hybrid liver support. *International Journal of Artificial Organs*, 2001, 24, 793-798.

Fontana, RC ; Polidoro, TA ; Silveira, MM. Comparison of stirred tank and airlift bioreactors in the production of polygalacturonases by *Aspergillus oryzae Bioresource Technology*, 2009, 100, 4493–4498.

Galaction, A-I ; Cascaval, D, Oniscub, C ; Turnea, M. Prediction of oxygen mass transfer coefficients in stirred bioreactors for bacteria, yeasts and fungus broths. *Biochemical Engineering Journal*, 2004, 20, 85–94.

Galhaup, C ; Haltrich, D. Enhanced formation of laccase activity by the white-rot fungus Trametes pubescens in the presence of copper. *Applied Microbiology and Biotechnology*, 2001, 56, 225–232.

Galhaup, C ; Wagner, H ; Hinterstoisser, B ; Haltrich, D. Increased production of laccase by the wood-degrading basidiomycete Trametes pubescens. *Enzyme Microb Technol*, 2002, 30, 529–536.

Gan, Q ; Allen, SJ ; Taylor, G, Design and operation of an integrated membrane reactor for enzymatic cellulose hydrolysis, *Biochemical Engineering Journal*, 2002, 12, 223–229.

Garcia-Ochoa, F; Gomez, E. Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnology Advances*, 2009, 27, 153–176.

Gavrilescu, M; Roman, RV; Efimov, V. *Acta Biotechnology*, 1993, 13, 59.

Gerlach, JC; Hout, M; Gage, K; Zeilinger, K. Liver Cell-Based Therapy – Bioreactors as Enabling Technology, *Principles of Regenerative Medicine*, 2008, 1086-1105.

Gerlach, JC; Mutig, K; Sauer, IM; Schrade, P; Efimova, E; Mieder, T; Naumann, G; Grunwald, A; Pless, G; Mas, A; Bachman, S; Neuhaus, P; Zeilinger, K. Use of primary human liver cells originating from discarded grafts in a bioreactor for liver support therapy and the prospects of culturing adult liver stem cells in bioreactors: a morphological study. *Transplantation*, 2003, 76, 781-786.

Gloeckner, H; Lemke, HD. New miniaturized hollow-fiber bioreactor for in vivo like cell culture, cell expansion, and production of cell-derived products. *Biotechnology Progress*, 2001, 17, 828-831.

Gummadi, SN ; Kumar, DS. Batch and fed batch production of pectin lyase and pectate lyase by novel strain *Debaryomyces nepalensis* in bioreactor. *Bioresource Technology*, 2008, 99, 874–881.

Hager JC, Carman, R; Porter, LE et al. Neonatal hepatocyte culture on artificial capillaries: a model for drug metabolism and the artificial liver. *ASAIO Journal*, 1983, 6, 26-35.

Heinzle, E; Biwer, AP; Cooney, CL. *Development of sustainable bioprocesses: modeling and assessment*, John Wiley and Sons, 2006, 294 p.

Hess, J ; Leitner, C ; Galhaup, C ; Kulbe, KD ; Hinterstoisser, B ; Steinwender, M ; et al. Enhanced formation of extracellular laccase activity by the white-rot fungus Trametes multicolor. Appl Biochem Biotech. 23rd Symposium on *Biotechnology for Fuels and Chemicals*, 2002, 98, 229–241.

Hoekema, S; Bijmans, M; Janssen, M; Tramper, J; Wijffels, RH A pneumatically agitated flat-panel photobioreactor with gas re-circulation: anaerobic photoheterotrophic cultivation of a purple non-sulfur bacterium, *International Journal of Hydrogen Energy*, 2002, 27(11), 1331-1338.

Hreggvidsson, GO ; Kaiste, E ; Holst, O ; Eggertsson, G ; Palsdottir, A ; Kristjansson AJ. An extremely thermostable cellulase from the thermophilic eubacterium *Rhodothermus marinus*. *Applied Environmental Microbiology*, 1996, 62, 3047–3049

Hu, Q; Richmond A, Productivity and photosynthetic efficiency of *Spirulina platensis as* affected by light intensity, algal density and rate of mixing in a flat-plate photobioreactor. *Journal Applied Phycology*, 1996, 8, 139-145.

Huang, TK; McDonald, K A. Bioreactor engineering for recombinant protein production in plant cell suspension cultures *Biochemical Engineering Journal*, 2009, 45, 168–184.

Humes, HD; Fissel, WH; Weitzel, WF; Buffington, DA; Westover, AJ; Mackay, SM; Gutierrez, JM. Metabolic replacement of kidney function in uremic animals with a bioartificial kidney containing human cells. *Am. J. Kidney Dis.*, 2002, 39, 1078-1087.

Hubbard, D W. Biotechnology processes. Scale-up and mixing. Eds. New York: AIChE, pp. 168-184. New York: AIChE; 1987

Isar, J ; Agarwal, L ; Saran, S; Saxena, RK. Succinic acid production from *Bacteroides fragilis*: Process optimization and scale up in a bioreactor. *Anaerobe*, 2006, 12, 231–237.

Jang, H; Chang, K. Thermostable cellulases from *Streptomyces* sp. scale-up production in a 50-l fermenter. *Biotechnology Letters*, 2005, 27, 239–242.

Janke, J; Gerlach, J; Kardassis, D; Bohmer, C; Rossaint, R. Effect of a hybrid liver support system on cardiopulmonary function in healthy pigs. *International Journal of Artificial Organs*, 1999, 20, 570-576.

Ju, L-K; Chase, GG; Akron, USA. Improved scale-up strategies of bioreactors. *Bioprocess Engineering*, 1992, 8 49-53.

Kantarci, N; Borak, F; Ulge, KO. Bubble column reactors. *Process Biochemistry*, 2004, 40, 2263–2283.

Kasinath, A ; Novotný, C ; Svobodová, K ; Patel, KC ; Sasek, V. Decolorization of synthetic dyes by Irpex lacteus in liquid cultures and packedbed bioreactor. *Enzyme Microbial and Technology*, 2003, 32,167–173

Katinger, H. Principles of animal cell fermentation. *Dev. Biol. Stand*, 1985, 66, 195-209.

Kim, SW ; Kang, SW ; Lee, JS. Celulase and Xylanase Production by *Aspergillus niger* KKS in Various Bioreactors. *Bioresource Technology*, 1997, 59, 63-67.

Knakek, RA; Gullino, PM; Kohler, PO; Dedrick, RL. Cell culture on artificial capillaries: an approach to tissue growth in vitro. *Science*, 1972, 178, 65-66.

Laska, ME; Cooney, CL. *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Separation*. 1ª ed. USA: Wiley, USA, 1999

Lee, D-Y ; Li,Y-Y ; Oh, Y-K ; Kim, M-S ; Noike, T. Effect of iron concentration on continuous H2 production using membrane bioreactor. *International Journal of Hydrogen Energy*, 2009, 34, 1244-1252.

Leite, JAC ; Fernandes, BS ; Pozzia, E ; Barbozab, M ; Zaiata, M. Application of an anaerobic packed-bed bioreactor for the production of hydrogen and organic acids. *International Journal of Hydrogen Energy*, 2008, 33, 579-589.

Luo, X; Lee, DJ; Lau, R; Yang, G; Fan, L. Maximum stable bubble size and gas holdup in high-pressure slurry bubble columns. *AIChE Journal*, 1999, 45, 665–685.

Khalid Jaber Kadhum Luti, KJK ; Mavituna, F. Elicitation of Streptomyces coelicolor with E. coli in a bioreactor enhances undecylprodigiosin production. *Biochemical Engineering Journal*, 2011, 53, 281–285.

Macdonald, JM; Wolfe, SP; Roy-Chowdhury, YI; Kubota, H; Reid, LM. Effect of flow configuration and membrane characteristics on membrane fouling in a novel multicoaxial hollow-fiber bioartificial liver. Ann. NY Acad. *Science*, 2001, 944, 334-343.

Maciel, GM; Vandenberghe, LPS; Bianca, ED; C; Pandey, A; Soccol, C.R. Xylanase Production by Aspergillus niger LPB 326 In Solid –State Fermentation Using Statistical Experimental Designs. *Food Technology and Biotechnology*, 2008, 46, 183-189.

Mata, TM; Martins, AA; Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renew Sustain Energy Rev*, 2010, 14, 217–32.

Martin, Y; Vermette, P. Bioreactors for tissue mass culture: Design, characterization, and recent advances. *Biomaterials*, 2005, 26, 7481–7503.

McDonald, KA; Hong, LM; Trombly, DM; Xie, Q; Jackman, AP. Production of human alpha-1-antitrypsin from transgenic rice cell culture in a membrane bioreactor. *Biotechnology Progress*, 2005, 21, 728–734.

Medeiros, ABP; Pandey, A; Freitas, RJS; Christen, P; Soccol, CR. Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology. *Biochemical Engineering Journal*, 2000, 6, 33–39.

Medeiros, ABP; Pandey, A; Vandenberghe, LPS;. Pastore, GM; Soccol, CR. Production and Recovery of Aroma Compounds Produced by Solid-State Fermentation Using Different Adsorbents, *Food Technology and Biotechnology*, 2006, 44(1), 47–51.

Meng, F ; Chae, S-R, Drews, A ; Kraume, M, Hang-Sik Shin, H-S ; Yang, F. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research*, 2009, 43, 1489 – 1512

Meuwly, F ; Ruffieux, P-A ; Kadouri, A ; von Stockar, U. Packed-bed bioreactors for mammalian cell culture: Bioprocess and biomedical applications. *Biotechnology Advances*, 2007, 25, 45–56.

Minucelis and Minutissue, Vertriebs GmbH, Bad Abbach, Germany.

Mirón, J ; Vázquez, JA; González, P; Murado, MA. Enhancement glucose oxidase production by solid-state fermentation of Aspergillus niger on polyurethane foams using mussel processing wastewaters. *Enzyme and Microbial Technology*, 2010, 46, 21–27.

Mirro, R ; Voll, K. Which Impeller Is Right for Your Cell Line? A Guide to Impeller Selection for Stirred-Tank Bioreactors. *BioProcess International*, 2009, 7, 52-57

Mohorčič, M, Friedrich, J, Pavko, A. Decoloration of the diazo dye Reactive Black 5 by immobilised Bjerkandera adusta in a stirred tank bioreactor. *Acta Chim Slov*, 2004, 51, 619–628

Mudliar, S ; Giri, B ; Padoley, K ; Satpute, D ; Dixit, R ; Bhatt, P ; Pandey, R ; Juwarkar, A ; Vaidya, A. Bioreactors for treatment of VOCs and odours – A review. *Journal of Environmental Management*, 2010, 91, 1039–1054

Nag, A. Biofuels refining and performance, *McGraw-Hill Professional*, 2008, 312 p.

Najafpour, GD ; Ghasem D. *Biochemical Engineering and Biotechnology*, 2008.

Ogbonna JC, Yada H, Masui H, Tanaka H., *Journal of Fermentation and Bioengineering*, 1996, 82(1), 61-67.

Ohta, N ; Park, SP ; Yahiro, K ; Okabe, M. Comparison of Neomycin Production from *Streptomyces fradiae* Cultivation Using Soybean Oil as the Sole Carbon Source in an Air-Lift Bioreactor and a Stirred-Tank Reactor. *Journal of Fermentation and Bioengineering*, 1995, 79, 443-448.

Oldshue, JY. Fermentation mixing scale-up technique. *Biotechnology Bioengineering*, 1966, 8, 3-24.

Olivieri G, Marzocchella A, Salatino P, Giardina P, Cennamo G, Sannia G. Olive mill wastewater remediation by means of Pleurotus ostreatus. *Biochemical Engineering Journal*, 2006, 31, 180–7.

Paek , KY; Chakrabarty, D; Hahn, EJ. Application of bioreactor systems for large scale production of horticultural and medicinal plants. Plant Cell, *Tissue and Organ Culture*, 2005, 81, 287–300.

Pandey, A; Larroche, C; Soccol, CR; Dussap, CG (Editors). Advances in Fermentation Technology. New Delhi: Asiatech Publishers, Inc. Chapter 7. *Bioreactors: Functions in fermentation processes.* 2008, p. 172-201.

Papagianni, M ;Mattey, M ; Kristiansen, B. Citric acid production and morphology of Aspergillus niger as functions of the mixing intensity in a stirred tank and a tubular loop bioreactor. *Biochemical Engineering Journal*, 1998, 2, 197-205.

Park, C ; Lee, B ; Han, EJ ; Lee, J ; Kim, S. Decolorization of acid black 52 by fungal immobilization. *Enzyme Microbial and Technology*, 2006, 39, 371–374.

Park, JBK; Craggs, RJ; Shilton A.N. Wastewater treatment high rate algal ponds for biofuel production, *Bioresource Technology*, 2011, 102, 35–42.

Prakash, G; Ashok, K. Srivastava . Azadirachtin production in stirred tank reactors by *Azadirachta indica* suspension culture. *Process Biochemistry*, 2007, 42, 93–97.

Prasad, KK ; Mohan, SV ; Bhaskar, YV ; Ramanaiah, SV ; Pati, BR. Laccase production using *Pleurotus ostreatus* 1804 immobilized on PUF cubes in batch and packed bed reactors: influence of culture conditions. *Journal of Microbiology*, 2005, 43, 301–307.

Yin, P; Nishina, N; Yahiro, Kyz; Park, Y; Kabe, M. Enhanced Production of L(+)-Lactic Acid from Corn Starch in a Culture of *Rhizopus oryzae* Using an Air-Lift Bioreactor. *Journal of Fermentation Bioengineering*, 1997, 84(3), 249-253.

Yin, P; YAahiro, K; Ishigaki, T; Park, Y; Okabez, ML. (+)-Lactic Acid Production by Repeated Batch Culture of *Rhizopus oryzae* in Air-Lift Bioreactor. *Journal of Fermentation Bioengineering*, 1998, 85(1), 96-100.

Perry, ST; Wang, DIC. Fiber bed reactor design for animal cell culture. *Biotechnology Bioengineering*, 1989, 34, 1–9.

Putt, R; Manjinder, S; Chinnasamy, S; Das, KC. An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer, *Bioresource Technology*, 2011, 102, 3240–3245.

PREMIERTEC®. Ultra Clean Magnetic Mixer®. 2010. Available from: http://www.premiertec.net/Ultra_Clean_Magnetic_Mixer/Ultra_Clean_Magnetic_Mixer.htm

Raimbault, M; Germon, JC. Procédé d'enrichissement en proteins de produits comestibles solides, *French Patent* no. 76-06-677 (1976)

Rancaño, G ; Lorenzo, M ; Molares, N ; Rodríguez Couto, S ; Sanromán, Á ; Production of laccase by *Trametes versicolor* in an airlift fermentor. *Process Biochem*, 2003, 39, 467–473

Ranjbar, R; Inoue, R; Katsuda, T; Yamaji, H; Katoh, S. High efficiency production of astaxanthin in an airlift photobioreactor, *Journal of Bioscience and Bioengineering*, 2008, 106(2), 204-207.

Rasoul-Amini, S; Montazeri-Najafabady, N; Mobasher, MA; Hoseini-Alhashemi, S; Ghasemi, Y. *Chlorella* sp.: A new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor, *Applied Energy*, In Press, Available online 8 Jan 2011.

Reczey, K ; Szengyel, ZS ; Eklund, R ; Zacchi,G. Cellulase production by *T. reesei*. *Bioresour Technol*, 1996, 57, 25–30

Reddy, V ; Reddy, P ; Pillay, B ; Singh, S . Effect of aeration on the production of hemicellulases by *T. lanuginosus* SSBP in a 30 l bioreactor. *Process Biochemistry*, 2002, 37, 1221–1228.

Richmond, A; Cheng-Wu, Z. Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoors, *Journal of Biotechnology*, 2001, 85(3), 259-269.

Rodrigues, C; Vandenberghe, LPS; Teodoro, J; Pandey, A; Soccol, CR. Improvement on Citric Acid Production in Solid-state Fermentation by Aspergillus niger LPB BC Mutant Using Citric Pulp. *Applied Biochemestry and Biotechnology*, 2009, 158, 72–87.

Rodríguez Couto, S ; Rodríguez, A ; Paterson, RRM ; Lima, N ; Teixeira, JA. High laccase activity in a 6 l airlift bioreactor by free cells of *Trametes hirsuta*. *Letters Applied Microbiology*, 2006, 42:, 612–616

Rose, G. A separable and multipurpose tissue culture chamber. *Tex. Rep. Biol. Med.*, 1954, 12, 1074-1083.

Ryan, DR ; Leukes, WD ; Burton, SG. Fungal bioremediation of phenolic wastewaters in an airlift reactor. *Biotechnology Progress*, 2005, 21, 1068–1074.

Sánchez-Mirón, A; García, MC; Camacho, FG; Grima, EM; Chisti, Growth and biochemical characterization of microalgal biomass produced in bubble columnand airlift photobioreactors: studies in fed-batch culture. *Enzyme and Microbial Technology*, 2002, 31(7), 1015-1023.

Sauer, I; Gerlach, J. Modeular extracorporeal liver support. *Art. Org.*, 2002, 26, 703-706.

Schaechter, M; Lederberg, *J. The Desk Encyclopedia of Microbiology*. New York: Elsevier Academic Press, 2004, 1149 p.

Sella, SRBR; Guizelini, BP; Vandenberghe, LPS; Medeiros, ABP; Soccol, CR. Lab-Scale production of Bacillus atrophaeus' spores by solid state fermentation in different types of bioreactors. *Brazilian Archives of Biology and Technology*, 2009, 52, 159-170.

Shankar, SK; Mulimani,VH. α-Galactosidase production by *Aspergillus oryzae* in solid-state fermentation. *Bioresource Technology*, 2007, 98, 958–961.

Shito, M; Tillers, AW; Tompkins, RG; Yarmuch, ML; Toner, M. Efficacy of an extracorporeal flat-plate bioartificial liver in treating fulminant hepatic failure. *Journal of Surg. Research*, 2003, 111, 53-62.

Singhania, RR; Patel, AK; Soccol, CR; Pandey, A. Recent advances in solid-state fermentation, *Biochemical Engineering Journal*, 2009, 44, 13–18.

Soccol, CR; Vandenberghe, LPS. Overview of applied solid-state fermentation in Brazil. *Biochemical Engineering Journal*, 2003, 13, 205–218.

Soccol, CR; Scheidt GN ; Mohan, R. Desenvolvimento de um biorreator do tipo imersão por bolhas (BIB) para as técnicas de micropropagação e cultura de células vegetais. *Brazilian Patent* (PI0801454-0), 2008.

Santos, A ; Ma, W ; Judd, SJ. Membrane bioreactors: Two decades of research and implementation. *Desalination*, 2011, 273, 148–154

Sedarati, MR ; Keshavarz, T ; Leontievsky, AA ; Evans, CS. Transformation of high concentrations of chlorophenols by the white-rot basidiomycete *Trametes versicolor* immobilized on nylon mesh. *Electron Journal of Biotechnology*, 2003, 6, 104–114

Schliephake, K; Mainwaring, DE; Lonergan, GT; Jones, IK; Baker, WL. Transformation and degradation of the disazo dye Chicago Sky Blue by a purified laccase from *Pycnoporus cinnabarinus*. *Enzyme Microbial and Technology*, 2000, 27, 100–7

Shen, X; Xia, L. Production and immobilization of cellobiase from *Aspergillus niger* ZU-07. *Process Biochemistry*, 2004, 39, 1363–1367

Schroën, CGPH; Van Roon, JL; Beefink, HH; Tramper, J; Boom, RM. Membrane applications for antibiotics production. *Desalination*, 2009, 236, 78–84

Sigoillot, C; Record, E; Belle, V; Robert, JL; Levasseur, A; Punt, PJ; van den Hondel, CAMJJ; Fournel, A; Sigoillot, JC; Asther, M. Natural and recombinant fungal laccases for paper pulp bleaching. *Applied Microbiology Biotechnology*, 2004, 64, 346–352

Smith, JJ; Lilly, MD; Fox, RI. *Biotechnology and Bioengineering*, 1990, 35, 1011.

Spier, M R;. Fendrich, RC; Almeida, P C; Noseda, M; Greiner, R; Konietzny, U; Woiciechowski , AL; Soccol, VT; SoccoL, CR. Phytase produced on citric byproducts: purification and characterization. World *Journal of Microbiology and Biotechnology* (2011) 27:267–274

Stanbury, PF. *Principles of Fermentation Technology*, Oxford: Pergamon Press; 1995.

Szabo, IJ; Johansson G; Pettersson, G. Optimized cellulase production by Phanerochaete chrysosporium: control of catabolite repression by fed-batch cultivation. *Journal of Biotechnology*, 1996, 48, 221–230.

Szijarto, N; Faigl Z; Réczey, K; Mézesc, M; Bersény, IA. Cellulase fermentation on a novel substrate (waste cardboard) and subsequent utilization of homeproduced cellulase and commercial amylase in a rabbit feeding trial. *Ind Crops Prod*, 2004, 20, 49–57.

Tamburic, B; Zemichael, FW; Crudge, P; Maitland, GC; Hellgardt, K. Design of a novel flat-plate photobioreactor system for green algal hydrogen production, *International Journal of Hydrogen Energy*, 2011, 36, 6578-6591.

Tavares, APM; Coelho, MAS; Agapito, MSM; Coutinho, JAP; Xavier, AMRB. Optimization and modeling of laccase production by Trametes versicolor in a bioreactor using statistical experimental design. *Applied Biochemistry Biotechnology*, 2006, 134, 233–248.

Tredici, MR, Materassi, R. From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *Journal of Applied Phycology*, 1992, 4, 221–231.

Tredici, MR. Mass production of microalgae: photobioreactors. In: Richmond, A. (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. *Blackwell Publishing*, Iowa, USA, 2004, 178–214.

Thiruchelvam, AT; Ramsay, JA. Growth and laccase production kinetics of Trametes versicolor in a stirred tank reactor. *Applied Microbiology Biotechnology*, 2007, 74, 547–554

Ugwu, CU; Ogbonna, JC; Tanaka, H. Characterization of light utilization and biomass yields of *Chlorella sorokiniana* in inclined outdoor tubular photobioreactors equipped with static mixers, *Process Biochemistry*, 2005, 40(11), 3406-3411.

Ugwu, CU; Aoyagi, H, Uchiyama, H. Photobioreactors for mass cultivation of algae, *Bioresource Technology*, 2008, 99, 4021–4028.

Uhl, VWl; Gray, JB. *Mixing: Theory and Practice*, New York: Academic Press, 1966.

Vandenberghe, LPS; Pandey, A; Soccol, CR; Lebeault, J. Solid State fermentation for the synthesis of citric acid. *Bioresource Technology*, 2000, 175-178.

Van der Merwe, JJ. Production of laccase by the white rot fungus Pycnoporus sanguineus. *Master Thesis*. Department of Microbiology and Biochemistry, University of the Free State, Bloemfontein, South Africa, 2002

Visvanathan, C; Ben Aim, R; Parameswaran, K. Membrane separation bioreactors for wastewater treatment. *Critical Review Environmental Science Technology*, 2000, 30, 1–48.

Wang, G; Zhang, W; Jacklin, C; Freedman, D; Eppstein, L; Kadouri, A. Modified CelliGen-packed bed bioreactors for hybridoma cell cultures. *Cytotechnology*, 1992a, 9, 41–49.

Wang, G; Zhang, W; Freedman, D; Eppstein, L; Kadouri, A. Animal cell technology: developments: process and products. In: *Spier RE*, Griffiths G, MacDonald D, editors. Continuous production of monoclonal antibodies in CelliGen packed bed reactor using Fibracel carrier. Oxford: Butterworth-Heinemann; 1992b, 460–464.

Wang, EQ; Li, SZ; Tao, L; Geng, X; Li, TC. Modeling of rotating drum bioreactor for anaerobic solid-state fermentation. *Applied Energy*, 2010, 87, 2839–2845.

Williams, MD; Pirbazari, M. Membrane bioreactor process for removing biodegradable organic matter from water. *Water Reseach*, 2007, 41, 3880–3893.

Wolf, CF; Munkelt, BE. Bilirubin conjugation by an artificial liver composed of cultured cells and synthetic capillaries. Trans. Am. Soc. Artif. Intern. *Organs*, 1975, 21, 16-27.

Wu, X; Merchuk, JC. Simulation of algae growth in a bench-scale bubble column reactor. *Biotechnology and Bioengineering*, 2002, 80, 156-168.

Wu, CH; Popova, EV; Hahn, EJ; Paek, KY. Linoleic and α-linolenic fatty acids affect biomass and secondary metabolite production and nutritive properties of Panax ginseng adventitious roots cultured in bioreactors. *Biochemical Engineering Journal*, 2009, 47, 109–115.

Xu, J ; Ge, X; Dolan, MC. Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures. *Biotechnology Advances*, 2011, 29, 278–299.

Yang, W; Cicek, N; Ilg, J. State-of-the-art of membrane bioreactors: Worldwide research and commercial applications in North America. *Journal of Membrane Science*, 2006, 270, 201–211.

Zhang, ZY; Jin, B; Kelly, JM. Production of lactic acid from renewable materials by *Rhizopus* fungi. *Biochemical Engineering Journal*, 2007, 35, 251–263.

Zhang, AY, Teoh, SH, Chong, WS, Food, TT, Chng, YC. Mahesh Choolani, Jerry Chan, A biaxial rotating bioreactor for the culture of fetal mesenchymal stem cells for bone tissue engineering, *Biomaterials*, 2009, 30, 2694–2704.

Zhang, CW; Zmora, O.; Kopel, R.; Richmond, A. An industrial-size flat plate glass reactor for mass production of Nannochloropsis sp. (Eustigmatophyceae), *Aquaculture*, 2001, 195, 35-49.