Chapter 7
Bioreactor Design and Analysis for Large-Scale Plant Cell and Hairy Root Cultivation

Chitra Srikantan and Smita Srivastava

Abstract Over the years, plant cells and hairy roots have been established as a successful and viable alternative for production of bioactive secondary metabolites and recombinant proteins, replacing the use of whole plants. Bioreactors are used for continuous and consistent in vitro production of these low-volume high-value bioactive/therapeutic molecules from plant cells and hairy roots at large scale. The design and operation of bioreactors for plant cell and hairy root cultivation differs from well-established microbial cultivation due to their size, aggregation, sensitivity to hydrodynamic stress, and viscous nature of the culture broth. The choice of bioreactor and nutrient feeding strategies to overcome substrate limitation and inhibition can be instrumental in enhancing the biomass and product productivity in plant cell and hairy root cultivations at large scale. Hence, this chapter deals briefly with the design and development of bioreactors to achieve maximum productivity in plant cell and hairy root cultivations. The overview of reactor operating parameters considered while designing bioreactors for plant cells and hairy roots are discussed. The chapter also includes application of mathematical modeling to optimize the design of bioreactors and in silico prediction of nutrient feeding strategies during fed-batch and continuous mode of bioreactor cultivation.

Keywords Bioreactors · Plant cell and hairy root cultures · Secondary metabolites · Bioreactor operating parameters · Mathematical modeling

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7.1 Introduction

The last 60 years have seen a steep increase in demand for production of biologically active molecules from cellular sources (Georgiev 2014). Living cells are capable of synthesizing the biomolecules in their most effective and stereo- and regiospecific form, giving an edge over the chemical synthesis (where the products are produced as racemic mixtures). The capacity to produce such biomolecules has been exploited in both prokaryotic (microorganisms) and eukaryotic (yeast, plant, and animal cells) systems. While microorganisms are most efficient in production of primary metabolites (e.g., ethanol, acetic acid, lactic acid, etc.) and less-structurally complex biomolecules, eukaryotic systems are required for production of specialized and chemically complex molecules as they have the cellular mechanism for posttranslational modifications (Huang and McDonald 2012).

Plants are known sources for secondary metabolites which are used for pharmaceuticals, flavors, fragrances, coloring agents, food additives, and agrochemicals (Wang et al. 2017). They are the major sources of medicinally active compounds, which have been used since ancient times and new ones being discovered for growing diseases and ailments. These plants have specific secondary metabolites (low volume, high value) which are produced mostly as defense-related compounds for survival of the plants against insects, pests and predators, etc. (Wink 2015). These are also non-growth associated and are not produced in large amounts, and their yield not only varies in different plants but also in different tissues of the same plant (Atanasov et al. 2015). These specific plants and trees are poached for extraction of very less amount of these biomolecules, making most of these plants endangered (Joe et al. 2015). As most of these biomolecules of interest in plants are defense-related compounds and with the development of plant cell and organ culture as successful alternative, it has led to the development of bioreactors for plant cell and organ cultures. Plant cells can also be engineered for recombinant protein production (they provide adequate posttranslational modification, being a eukaryotic system) and are advantageous over animal cell-based production systems due to lower production costs, easy scalability, and the absence of human pathogens (Kaldis et al. 2013).

Plant in vitro cultures are emerging as alternatives to replace whole plants, as a production platform for various biomolecules due to:

- Shorter production cycles (days or weeks) compared to months/years in natural and transgenic whole plants
- Consistency in product yield and quality and free of contamination
- Safer production platform in a closed bioreactor system, avoiding gene flow in the environment and contamination of the food chains
- Ease of compliance with cGMP (current good manufacturing practices) requirements, product registration process, etc.

As in vitro plant culture has been established as an efficient platform for biologically active molecule production, process optimization and engineering considerations for the factors affecting plant cells are needed for scaling it up to large bioreactor volumes (Fulzele 2000; De Muynck et al. 2010; Lienard et al. 2007; Franconi et al. 2010; Huang and McDonald 2012).

The bioreactors are suitably modified for cultivation of plant cell and hairy root cultures with low shear stress, adequate mixing, support system for organ cultures, and ease in scale-up (Honda et al. 2001). Plant cell suspensions grown in sterile bioreactors having guaranteed batch consistency in biomass and product productivity, and are more likely to proceed successfully and quickly through the regulatory approval system (Fischer et al. 2012). The enzyme taliglucerase alfa (for treatment of type I Gaucher's disease), produced as the drug Elelyso, became the first biological drug approved by the US Food and Drug Administration for human use that is manufactured in a genetically modified carrot cell suspension culture by the company Protalix (Fox 2012; Grabowski et al. 2014).

The goal of a plant cell/tissue-based bioprocess is to achieve high productivity (g product/l/day), high product yield (g product/g substrate), and high product concentration (g product/l) by selecting cell lines, optimum media, and bioreactor operating conditions (Srivastava and Srivastava 2007). This chapter describes the different factors influencing the bioreactor operating strategies and various types of bioreactors for plant cell and hairy root cultivation which can be chosen to commercialize the plant cell-based bioprocess.

7.2 Factors Influencing Plant Cells and Hairy Root Cultivation in Bioreactors

Bioreactors for plant cell and hairy roots have operating conditions similar to microbial bioreactors with modifications/features to aid in efficient growth of plant cell and hairy roots, owing to its characteristics (Table 7.1) (Chattopadhyay et al. 2002a, b, c). Plant cell cultures require aerobic bioreactors with low shear and good mixing. As plant cells are bigger than microbial cultures and form aggregates (cell suspension cultures) or organs (hairy root cultures), this makes the sampling of biomass from the bioreactor at constant intervals difficult. Measurement of the medium conductivity is an indirect way of estimating the biomass growth in the bioreactor (Hahlbrock et al. 1974; Madhusudhan et al. 1995, Maschke et al. 2015). Plant cell culture medium conductivity decreases continuously with the growth of plant cells inside the bioreactor as the growing cells take up the salts from the

medium (Eibl and Eibl 2002). Bioreactor operating parameters to be considered for designing of bioreactor for plant cell and hairy roots are as described briefly.

7.2.1 Aggregation and Adhesion

The plant cells and hairy roots are bigger in size compared to microbes (Table 7.1) and tend to grow in clumps (aggregates) as new cells need support to grow and are unable to separate after cell division. Aggregation is also due to the production of extracellular polysaccharides by the plant cells which help in the cell-cell adhesion (Sims and Bacic 1995). These polysaccharides also store the signaling molecules and other metabolites which are required for cell-to-cell communication. The cell-cell adhesion is also linked with secondary metabolite biosynthesis (Chattopadhyay et al. 2002a, b, c). Aggregated growth results in insufficient oxygen transfer, inefficient mixing, and sedimentation of cells in the bioreactor. Aggregates of large size (2–10 mm) make it difficult for oxygen to reach till the innermost cell and as a result cause death of cells in the core (Doran 1993). Aggregation maybe caused due to adhesion of cells on the bioreactor walls. At higher agitation speed, cells get deposited on the bioreactor walls when the biomass increases (Eibl and Eibl 2009). Bubbles coalescing on the walls are also responsible for cell adhesion to walls. Aggregation cannot be admonished completely as it leads to loss of viability (plant cells are unable to survive as single cell, like microbes) and product formation is related to aggregation of cells (Chattopadhyay et al. 2002a, b, c).

Table 7.1 Characteristics of plant, animal, and microbial cells for bioreactors

Characteristics	Microbial cells	Plant cells	Animal cells
Size	1–10 μm	40–200 μm	10–100 μm
Growth pattern	Individual cells/small aggregates	Small/large aggregates	Support required for growth
Doubling time	Hours (2–4 h)	Days (2–5 days)	Hours (12–20 h)
Shear sensitivity	Low	High	Very high
Product accumulation	Extracellular	Intracellular	Intracellular/extracellular
Posttranslational modifications	No	Yes	Yes
Contamination	Other microbes	Bacterial and fungal	Bacterial, fungal, and viral (human pathogens)
Culture medium components	Complex/synthetic, defined	Synthetic, defined	Complex (animal sources), not defined
Inoculum size	Low (1–2%)	High (5–10%)	High (5–10%)
Aeration rate	High	Low	Very low
Damage by aeration	Very less	Less	High
Cultivation time	Days	Weeks	Weeks
Oxygen demand	Very high	Low	Low

The high-value biologically important compounds produced by plant cells are generally defense related and in response to stress to the cells; which are produced, transported, and communicated among other cells in the aggregate (Gaurav and Roberts 2011). The product content and cell viability decreases when the culture is made to exist as single cells or smaller aggregates. Hence control of cell aggregation is an important parameter while designing large-scale plant cell/tissue culture systems (Chattopadhyay et al. 2005). To prevent the cells from sedimentation, the aggregate size should not exceed 1–2 mm having specific gravity of range 1.002–1.02. When the specific gravity increases above 1.03 and aggregate size is 0.5–1 cm, the plant cells sediment in the bioreactor (Takayama 2014). Aggregation can be reduced by addition of pectinase (enzyme) and polyvinylpyrrolidone with some loss in biomass (as the cells are more viable as aggregates due to adherence and cell-cell communication, separating them causes loss in cell viability), but overall increase in volumetric productivity of the product (Chattopadhyay et al. 2002a, b, c). Reduction in $CaCl_2.2H_2O$ concentration was found to decrease wall adhesion and retain the plant cells in bioreactor (Takayama 1991).

The aggregate sizes were correlated with paclitaxel production by Kolewe et al. (2011) and observed that smaller aggregates contained higher content of paclitaxel compared to bigger clumps. A population balance model was proposed, and the model was simulated to find an optimal breakage rate with minimal biomass loss to increase the paclitaxel concentration in cell suspension cultures of *Taxus* sp. in bioreactors.

Kolewe et al. (2012) developed a population balance equation to predict the aggregate formation in *Taxus* suspension cultures:

$$\frac{\partial n(v,t)}{\partial t} + \frac{\partial [g(v,S')n(v,t)]}{\partial v} + \Gamma(v)n(v,t) = 2(1-b)\int_v^\infty p(v,v')\Gamma(v')n(v',t)dv'$$

where $n(v,t)$ is the continuous number density function, $n(v,t)\mathrm{d}v$ is the number of aggregates in size range v to $v + \mathrm{d}v$ at time t, $g(v, S')$ is the growth rate for aggregates of size v and effective intracellular concentration of total sugar S', $\Gamma(v)$ is the breakage frequency for aggregates of size v, and $p(v,v')$ is the partitioning function describing the distribution of daughter aggregates of size v resulting from the breakage of mother aggregates of size v', assuming each breakage event results in two daughter aggregates b, representing the fraction of biomass which does not partition into daughter particles upon a breakage event.

The above equation was combined with the following equations which accounted for substrate depletion upon cell growth:

$$\frac{dS}{dt} = -\int_0^\infty \frac{g(v,S')}{Y} n(v,t)dv$$

$$\frac{dS'}{dt} = -\alpha(S - S')$$

where S is the total extracellular sugar concentration, S' is the intracellular sugar concentration, Y is a constant yield coefficient, and α is the rate constant for these lumped processes and describes how quickly cells respond to environmental changes. These equations were then used to predict the aggregate sizes to paclitaxel production by the authors.

7.2.2 Mixing and Viscosity

Mixing is required for effective transfer of nutrients and oxygen from liquid and gaseous phase to the cells without biochemical limitations. Mixing is achieved in a bioreactor, with either mechanically moving parts (shafts and impellers in an STR) or by sparging air at a high flow rate (airlift bioreactors). Agitation speed used for plant cell and hairy root cultivations (100–150 rpm) is lesser than microbial cultivation (>200 rpm) and is a major limiting factor for plant cell cultures (Doran 1999). Although plant cells have higher tensile strength in comparison to microbial cells, their large size, rigid cellulosic wall, extensive vacuole, and organ structure make them sensitive to shear stress, restricting the use of high agitation for efficient mixing (Bhojwani and Razdan 1996). Plant cells are, therefore, often grown in modified stirred-tank bioreactors at low agitation speeds due to their shear sensitivity to hydrodynamic stress (Meijer et al. 1993; Bronnenmeier and Märkl 1982).

Plant cell cultures tend to follow non-Newtonian rheological pattern, the change in viscosity, which affects the homogeneity in the culture (Raposo et al. 2010). Plant cells occupy 40–60% of the bioreactor volume under no limiting nutrient condition (Takayama 1991). At these high cell concentrations, rheological properties change as viscosity increases, and the plant cell culture starts to behave like non-Newtonian fluids (Jolicoeur et al. 1992). This behavior of culture affects effective heat and mass transfer in the bioreactor resulting in nonuniform maintenance of parameters (temperature, pH, and oxygen concentration in the bioreactor) and formation of dead pockets (no mixing/no air zone) (Bhojwani and Razdan 1996). Polysaccharide secretion (for aggregation) by the plant cells at the later stages of cultivation period also increases the viscosity rapidly. The apparent viscosity was observed to rise steeply after 10 g/l concentration of biomass (Tanaka 1982). By modifying the impeller design, adequate mixing can be achieved without the loss of viability in biomass.

Doran (1993) reviewed about the relationship between mixing time and circulation time in bioreactors for plant cells. Mixing in an STR can be expressed as a function of circulation time as follows (T_m is the mixing time, and T_c is the circulation time, i.e., time required for liquid to complete one full circulation in the bioreactor):

$$T_m = 4T_c$$

Time taken for mixing in airlift bioreactor is as follows:

$$\text{Internal loop } T_m = 3.5T_c\left(\frac{A_d}{A_r}\right)^{0.5}$$

$$\text{External loop } T_m = 5.2T_c\left(\frac{A_d}{A_r}\right)^{0.5}$$

where A_d is the downcomer cross-sectional area and A_r is the riser cross-sectional area in the airlift bioreactor.

For pneumatically driven bioreactors (bubble column bioreactor, airlift bioreactor, etc.), mixing is achieved by passing sterile air at a high flow rates (4–10 vvm) (Doran 2013). This air flow is responsible for providing oxygen to the cells and at the same time provides mixing due to the higher air flow rate.

7.2.3 Aeration Effects and Shear

Plant cells and hairy root cultivation require oxygen for growth, and if the culture is mixotrophic (uses energy from light and carbon source for growth)/phototrophic (uses only light as a source for energy), they also require CO_2 for photosynthesis (Bhojwani and Razdan 1996). Plant cell and hairy roots require oxygen (1–3 mmol O_2 g^{-1} h^{-1}) lesser than microorganisms (10–100 mmol O_2 g^{-1} h^{-1}) because of their slow metabolism (Bhojwani and Razdan 1996).

Plant cells are not damaged by aeration or air bubbles (unlike mammalian cells), so the bioreactor system for the plant cells is selected based on effective oxygen transfer characteristics and can be effectively grown in pneumatically driven bioreactors (Table 7.2) (Kieran et al. 2000; Takayama 2014). Effect of aeration and agitation is directly seen on the mass transfer coefficient, k_La (oxygen transfer

Table 7.2 Bioreactor configurations for plant cell cultures

Mechanically driven bioreactors	Hydraulically driven bioreactors	Pneumatically driven bioreactors	Immobilized bioreactors	Perfusion bioreactors
Stirred tank	Radial flow bioreactor	Bubble column bioreactor	Fluidized bed bioreactor	Filtration stirred tank bioreactor
Rotating drum	Jet-loop bioreactor	Airlift bioreactor	Trickle bed bioreactor	Spin filter bioreactor
Vibromixer bioreactor	Membrane bioreactor	Balloon-type bubble bioreactor	Mist bioreactor	Filtration bubble column

Adapted from Eibl and Eibl (2002), Eibl and Eibl (2009), Su (1995)

coefficient) values. It is a direct measure of effective oxygenation in the bioreactor to the plant cell cultures (Baldi et al. 2008a). To achieve a balance in good biomass and product yield, the k_La value has to be optimized for the cultures in the bioreactor. Initial k_La value was a key factor in cell suspension cultures of *Panax notoginseng* for production of ginseng saponin and polysaccharides in a 3 l STR with centrifugal impeller. At a k_La value of 30.2 h^{-1}, highest productivity of ginseng saponin, polysaccharide, and biomass dry weight (DW) was obtained. Increase in k_La increased the biomass yield, but caused a decrease in the ginseng saponin and polysaccharide yield (Zhang and Zhong 2004).

The oxygen transfer rate in a bioreactor can be estimated as:

$$OTR = k_La(C^* - C_L)$$

where OTR is oxygen transfer rate, kg m^{-3} s^{-1}; k_L is liquid-film mass transfer coefficient, m s^{-1}; a is interfacial area per unit volume of unaerated liquid, m^{-1}; C* is equilibrium concentration of oxygen in the liquid, kg m^{-3}; and C_L is actual oxygen concentration in the liquid, kg m^{-3}.

Higher air flow rates in bubble column/airlift bioreactor can lead to foaming in the bioreactor, which affects oxygen transfer, reduces homogeneity of culture, and reduces biomass (as cells carried by the foam bubbles settle on the walls). Foaming was reduced successfully by modifying a bubble column bioreactor to a balloon type (Paek et al. 2005). Addition of antifoam is effective, but frequent and higher use reduces the oxygen transfer efficiency (Kawase and Moo-Young 1990).

Aeration and agitation in the mechanically driven bioreactor also cause hydrodynamic stress to the plant cell cultures. The cells experience the stress and shear due to their bigger size, thick cell wall, and large vacuoles (Chattopadhyay et al. 2005). Impact of shear on cells can be observed by cell damage, loss of productivity, and change in cell morphology (Zhong et al. 1994; Kieran et al. 2000). Bioreactors operating without moving parts are favorable for shear-sensitive cultures as only the air bubbles cause the mixing. Shear for STR having flat blade turbine impeller is generalized as:

$\gamma_{av} = kN_i$ Metzner and Otto (1957)

where γ_{av} is the average shear, N_i is the number of impellers, and k is the proportionality constant. Many other empirical equations have been devised and used for calculating shear in a STR.

$\gamma = 4.2N\left(\frac{d_i}{d_T}\right)^{0.3}\frac{d_i}{W}$ Bowen (1986)

$\gamma = k_i\left(\frac{4n}{3n+1}\right)^{n/n-1N}$ Calderbank and Moo-young (1959)

$\gamma = \frac{0.367}{\mu}\left(\frac{P}{V}\left(\frac{V}{V_sN_p}\right)^{0.42}\right)^{0.55}$ Hoffmann et al. (1995)

$\gamma = \left(\frac{p}{V\mu_s}\right)^{0.5}$ Henzler and Kauling (1985)

$$\gamma_{max} = 9.7N\left(\frac{d_i}{d_T}\right)^{0.3}\frac{d_i}{W} \quad \text{Bowen (1986)}$$

$$\gamma_{max} = 3.3N^{1.5}d_i\left(\frac{\rho}{\mu}\right)^{0.5} \quad \text{Robertson and Ulbrecht (1987)}$$

$$\gamma_{max} = N(1+5.3n)^{1/n}\left(\frac{N^{2-n}d_i^2\rho}{K}\right)^{1/(1+n)} \quad \text{Robertson and Ulbrecht (1987)}$$

The empirical correlations used in literature to relate shear with bioreactor design parameters in a bubble column bioreactor are given below:

$$\gamma = \left(\frac{1}{K}g\rho U_g\right)^{1/(n+1)} \quad \text{Sánchez Pérez et al. (2006)}$$

$$\gamma = \left(\frac{\rho\varepsilon}{K}\right)^{\frac{1}{n+1}} \text{where } \varepsilon = \rho U_g \quad \text{Henzler and Kauling (1985)}$$

where γ_{av} is the average shear; N_i is the number of impellers; k is the proportionality constant; a, gas-liquid interfacial area per unit volume of liquid in bubble column (m^{-1}); d_i, diameter of the impeller (m); d_T, diameter of tank (m); H, height of fluid in tank (m); k_i, impeller constant; K, consistency index (Pa s^n); M, torque (N m); n, flow index; N, agitation speed (s^{-1}); N_p, power number; P, power input (W); Re, impeller Reynolds number; V, volume of fluid (m^3); V_s, volume swept by the impeller (m^3); W, width of impeller blade (m); ε, energy input per unit mass (W kg^{-1}); γ, average shear rate (s^{-1}); γ_{max}, maximum shear rate (s^{-1}); μ, viscosity (Pa s); μ_a, apparent viscosity (Pa s); and ρ, density of fluid (kg m^{-3}). Many other empirical equations have been devised and used for calculating shear in a STR.

Varying the aeration rate also enhanced production in STR with setric impeller with DO at 30%, and 176.3 mg/l of lignan were produced in a 5 l bioreactor for cell culture of *Linum album* (Baldi et al. 2008a).

7.2.4 Impellers

To achieve high density in plant cell cultivations, STRs are the most commonly used bioreactors due to their efficient nutrient mixing and aeration. Impellers are used in bioreactor cultivations to sustain mass homogeneity and oxygen dispersion (Doran 2013). The bioreactors used for microorganisms use high agitation speed and flat blade impellers for cultivation (Lawford and Rousseau 1991). Impellers used in microbial cultures have higher power input with great impeller tip speed to prevent formation of dead pockets in the bioreactor (Doran 2010). However, higher power input to impeller causes hydrodynamic shear on the cells. Microbial cultures due to their small size can withstand the high shear and grow, while the bigger sized, shear-sensitive plant cells experience stress under high hydrodynamic shear (Baldi et al. 2008b). The high-powered impellers used in microbial cultures are not suitable for the shear-sensitive plant cell cultures. Impellers for plant cell cultivations should have the following characteristics: (i) to transfer power over a large volume in the bioreactor, (ii) low impeller tip speed, and (iii) large surface area (Eibl and Eibl

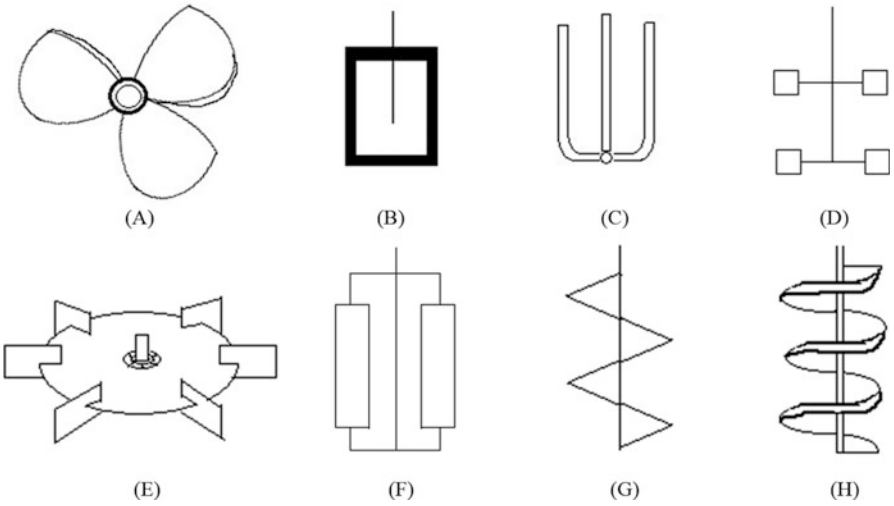

Fig. 7.1 Impellers used for plant cell cultivation: (**a**) marine propeller, (**b**) paddle, (**c**) anchor, (**d**) bladed, (**e**) rushton turbine, (**f**) spin, (**g**) helical, (**h**) helical screw

2002). Doran (1999) has deduced by analyzing various impellers for plant cell cultures that upward-pumping axial-flow turbine design of impellers is efficient in gas transfer and offers low shear to the plant cells.

Low-shear impellers have been developed by modifying an existing impeller used for microbial cell cultures or by designing a completely new one. Various impellers used for plant cell cultivations are shown in Fig. 7.1.

A low-shear helical impeller was designed and used for cell suspension cultures of *Catharanthus roseus* in a 100 l STR resulting in a very high accumulation of biomass (320 g/l of biomass in 16 days from an initial inoculum of 42.6 g/l cells) (Fulzele 2000). A novel low-shear setric impeller was used for cell suspension cultures of *Podophyllum hexandrum* and hairy root cultures of *Azadirachta indica* in STR successfully with no cell death (Chattopadhyay et al. 2002a, b, c; Srivastava and Srivastava 2012a). Cell suspension culture of *Harpagophytum procumbens* was cultivated in 3 l STR for production of anti-inflammatory phenylethanoid glycosides with a low-shear propeller impeller yielding highest biomass accumulation of 18.4 g/l (Georgiev et al. 2012).

7.2.5 Support System

Hairy root and organ cultures require a support system to be attached to while cultivated in a STR as the damage done by the impeller is high on hairy roots and organs than cell suspension cultures. Stainless steel attachment has been provided to retain the roots in a nutrient spray bioreactor and to reduce the liquid holdup by the hairy roots (Srivastava and Srivastava 2012b). The hairy root cultures can also be

separated by polyurethane foam in a STR to prevent shear from the impeller (Steingroewer et al. 2013). Autoclavable nylon mesh and baskets have also been used to separate the roots from the impellers and also to support the roots (Angelini et al. 2011; Gangopadhyay et al. 2011). A plastic nylon mesh was placed around the baffles in a zigzag fashion to provide more surface for the hairy roots of *Brugmansia candida* to grow in a 1.5 l STR for production of tropane alkaloids (Cardillo et al. 2010). Phytoremediation studies using *Brassica napus* hairy roots for removal of 2,4-dichlorophenol was studied in a 3 l STR by covering the hairy roots by an autoclavable nylon mesh covering the rushton turbine impeller (Angelini et al. 2011). *Plumbago indica* hairy roots were used for enhanced production of plumbagin in a 3 l STR, modified by addition of an autoclavable perforated basket 4 cm above the sparger (Gangopadhyay et al. 2011).

7.3 Mass Cultivation of Plant Cells and Hairy Roots in Bioreactors

Bioreactors were developed for cultivation of living cells under controlled conditions for production of biomass/biomolecules when supplied with required nutrients. Each system (microorganisms, plant, and animal) has varying characteristics which are to be considered while designing a bioreactor for production (Table 7.1). Characteristics of plant cells like larger cell size and shape, shear sensitivity, aggregation, slow growth rates, less oxygen requirement, increased mass transfer limitation, and product formation are to be considered while designing the bioreactor (Panda et al. 1989; Bisaria and Panda 1991). The following factors have to be considered while developing bioreactors for plant cells (Scragg 1995; Kieran et al. 1997):

- Homogeneous mixing for efficient nutrient transport, air-bubble dispersion, and optimum shear maintenance
- Aeration optimized for efficient oxygen uptake
- Maintenance of aseptic conditions for longer time (days/weeks)
- Light supply for phototrophic and mixotrophic cultures
- Control of physical parameters like temperature, pH, nutrients, and cell aggregate size
- Efficient mass transfers as the rheological characteristics tend to follow non-Newtonian pattern at high density.

7.3.1 Classification of Bioreactors

Since the demand for biotechnological products increased due to its low cost and high specificity, bioreactor technology has also emerged as a most sought after field for large-scale production of these products (Doran 2013). Various configurations of bioreactors have been developed to assist the biological system with efficient growth

and better product yield (Sharma and Shahzad 2013). For the cultivation of organized plant structures like hairy roots, somatic embryos, and micropropagation of plantlets, the bioreactors are modified (e.g., addition of mist spray, temporary immersion, mesh/basket) (Paek et al. 2005; Srivastava and Srivastava 2012a). Based on the energy input, plant cell bioreactors are operated as mechanically driven, hydraulically driven, and pneumatically driven bioreactors (Eibl and Eibl 2009). Few other configurations include the bed bioreactors and perfusion bioreactors (Table 7.2).

7.3.1.1 Mechanically Driven Bioreactors

Mechanically driven bioreactors (Fig. 7.2) use moving parts (impellers) inside the bioreactors which help in effective mixing and oxygen transfer. These bioreactors provide better control of temperature, pH, dissolved oxygen, and dissolved nutrients compared to other types of bioreactors (Choi et al. 2000). STR is the most used bioreactor (around 90%) in industries as its design, scale-up, and operation are well established. Though plant cells are sensitive to hydrodynamic stress due to powerful mixing, STR with modified impellers and low agitation speed have been successfully used for plant cell cultures to enhance biomass and product productivity (Fulzele 2000; Sharma and Shahzad 2013).

Rotating drum bioreactors vary in their oxygen supply mechanism and use a rotating vessel. Air is sent through the headspace of the bioreactor compared to sending it through the liquid medium in STR (Mitchell et al. 2006). The bioreactor is fitted with baffles in addition to impellers which can enhance the mixing process (Chattopadhyay et al. 2002a, b, c; Mitchell et al. 2006). Compared to other types of bioreactors, surface area to volume ratios are significantly higher in rotary drum bioreactors (Paek et al. 2005). The moving mechanical parts consist of rollers inside the bioreactor vessel, parallel to the rotating surface of the bioreactor vessel, which cause less shear stress to the plant cells. This bioreactor, owing to its low-shear, high-oxygen transfer characteristic, was suitable for high density and highly viscous cell suspension cultures of *C. roseus* (Tanaka et al. 1983). Kondo et al. (1989) were able

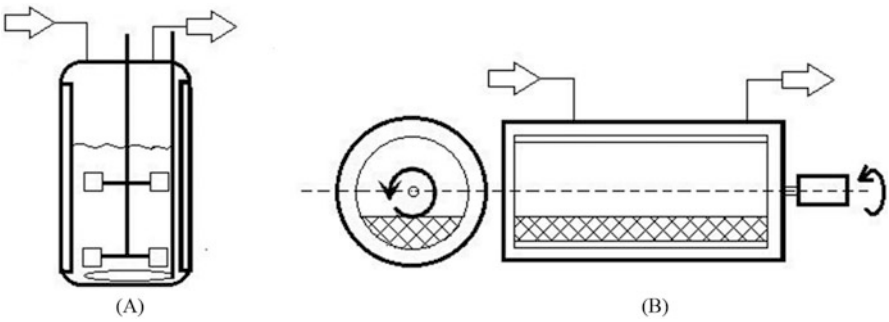

Fig. 7.2 Mechanically driven bioreactors: (**a**) stirred tank bioreactor, (**b**) rotating drum bioreactor

to achieve a maximum growth rate of 0.61 gl^{-1} d^{-1} after 30 days of cultivation of hairy roots of carrot in a glass vessel-based rotating drum bioreactor. The rotating drum bioreactors are not suitable for all types of plant cell/organ cultures and are difficult to scale up to higher volumes due to their vertical design and rotation. It also consumes much higher power than other bioreactors, due to which its use has reduced over the course of years (Sambamurthy and Kar 2006).

7.3.1.2 Pneumatically Driven Bioreactors

Pneumatically driven bioreactors (Fig. 7.3) use pressurized gas through a distributor (like nozzles, perforated plates, diffuser rings, injectors, etc.) to aid in mixing and aeration (Paek et al. 2005). Variation is observed for fluid mixing and dynamics in these bioreactors due to density differences between viscous liquid medium, bubble size and gas holdup (Eibl and Eibl 2009).

The design and operation are optimized for efficient gas holdup, which is the main criterion for designing and using pneumatically driven bioreactors. Gas holdup helps in understanding both mixing and mass transfer in these bioreactors (Takayama and Akita 1998). Variations in biomass, viscosity, and surface tension lead to foaming, floatation, and coalescence in the pneumatically driven bioreactors (Eibl and Eibl 2002).

Bubble column bioreactor has a simple design with a bioreactor vessel, gas sparger, and no moving parts (Kim et al. 2001). Gas sparging provides the necessary mixing and oxygen transfer to the plant cell/organs (Georgiev et al. 2012). The capital cost is lesser and can maintain better aseptic conditions than STR (Doran

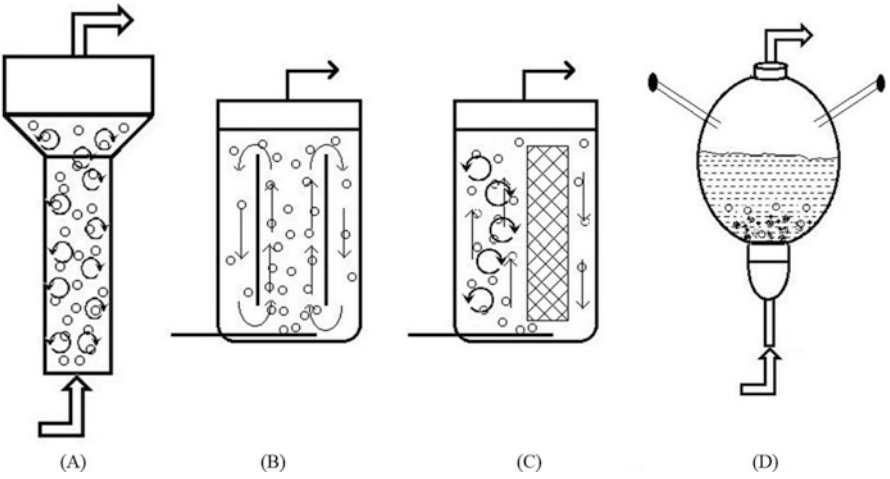

Fig. 7.3 Pneumatically driven bioreactors: (**a**) bubble column bioreactor, (**b**) airlift bioreactor (inner loop), (**c**) airlift bioreactor (outer loop), (**d**) balloon-type bubble bioreactor

2013). Airlift bioreactor is a modification of bubble column bioreactor with an addition of draught tube which aids in better mixing (Doran 2013). The flow gets divided in riser and downcomer in the draught tube, the density difference of which causes better mixing (Chattopadhyay et al. 2002a, b, c). Internal loop airlift bioreactors have the draught tube inside the system where the culture medium rises and falls inside. In an external loop airlift bioreactor, the downcomer is physically separated as an attachment to the main bioreactor vessel (Doran 2013). Mixing is achieved better in external loop bioreactors as the raiser and downcomer are separated physically, but the power consumed is more than the internal loop bioreactor. Production of betalain, a natural food dye and antioxidant from hairy roots of *Beta vulgaris*, was found to be 2.6 times higher in a bubble column bioreactor than produced in a STR. Additionally the doubling time of the hairy root cultures was also lower in bubble column bioreactor compared to STR (Georgiev et al. 2012). Balloon-type bubble bioreactors are a modification of bubble column bioreactor to reduce foaming and cell wall growth observed in bubble column bioreactors. Unlike bubble column bioreactor (where the diameter of vessel and top of the bioreactor are same), the sparger opens up to a balloon-type vessel (which reduces the foaming and cell wall growth) where the plant cell cultures are grown (Paek et al. 2005). These have been extensively used for large-scale plant micropropagation in a bioreactor (Paek et al. 2005; Cui et al. 2014).

7.3.1.3 Immobilized Bioreactors

Immobilized bed bioreactors (Fig. 7.4) are designed for the use of immobilized plant cells or organ cultures (like hairy roots). They are designed for passage of continuous or intermittent fluid flow which is responsible for transfer of nutrients and oxygen to the cells (Eibl and Eibl 2002). The bed is filled with immobilized particles and the fluid with nutrients and gas flows from the top of the bioreactor. These bioreactors face channeling problem. Channeling is a phenomenon when the fluid does not spend the designed residence time in the bioreactor but escapes through the channel formed between the particles (Shuler et al. 1986). This results in insufficient nutrient transfer and failure of the process. Channeling should be reduced to the minimum by efficient packing or sending the fluid at a very less flow rate to ensure it coats all the particles (Doran 2013).

Trickle bed bioreactors are the most used packed bed bioreactor for plant cells. Headspace of the column is integrated with various nozzles which spray nutrient solution on top of the packed cells, and air is introduced from base for aeration. When the nutrient is sprayed as a mist from the injector or ultrasonic nozzles in the headspace, trickle bed bioreactor gets modified to mist bioreactor. Mist bioreactors were developed to overcome the mass transfer limitations in growing organ cultures in submerged bioreactors (cells suspended in liquid medium and air passed through) (Eibl and Eibl 2008). Submergence increases the hyperhydricity of these cultures

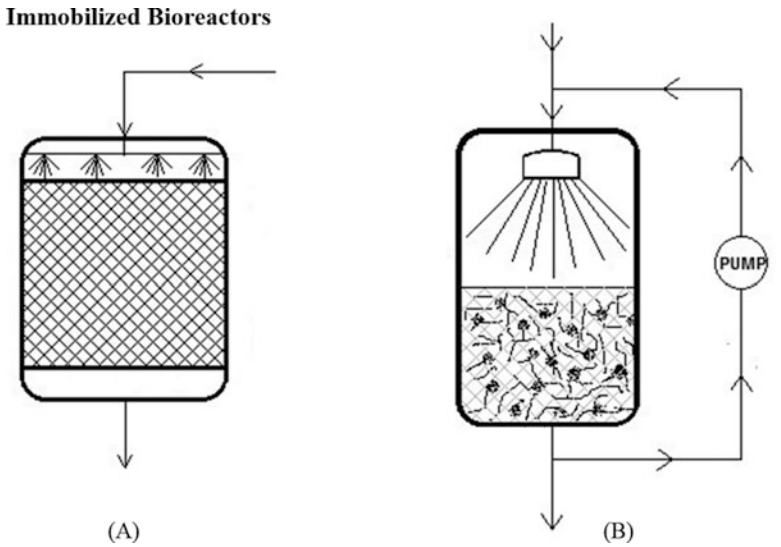

Fig. 7.4 Immobilized bioreactors: (**a**) trickle bed bioreactor, (**b**) mist bioreactor

(hydricity is the amount of moisture stored inside the cell). Rather than supplying oxygen (gas phase) via medium (liquid medium), mist bioreactors expose these organ cultures to continuous gas phase, and the nutrient medium is sprayed as a mist inside the bioreactor. Higher biomass (9.8 g/l) of *Azadirachta indica* hairy roots was obtained in a nutrient mist bioreactor for the production of biopesticide azadirachtin (volumetric productivity of 1.09 mg/l per day) compared to STR (no growth) and nutrient spray bioreactor (4.8 g/l biomass) (Srivastava and Srivastava 2012b).Similarly, a nutrient mist bioreactor was found to be better for production of mouse interleukin-12 (mIL-12) from transgenic tobacco hairy root line (5.3 μg/g fresh weight (FW) mIL-12), which was 49.5% more than the production in airlift bioreactor (Liu et al. 2009).

7.3.1.4 Perfusion Bioreactors

Perfusion bioreactors (Fig. 7.5) are used when there is a need to separate the cells from the medium continuously. It is used for continuous mode of production where the product is extracellular and leaches out in the medium. A porous membrane (pore size <50 μm) is used to segregate the cells from liquid medium. It can also be used for cultivation of immobilized cells which have to be retained inside the bioreactor (pore size is chosen based on the aggregate/organ size). Perfusion bioreactors are used when a certain nutrient or product (which is harmful for the cells) has to be removed continuously from the bioreactor. Membranes are incorporated in the

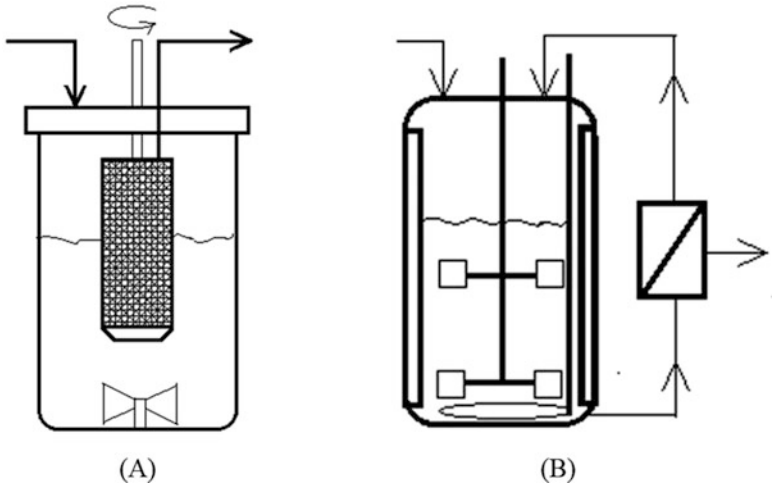

Fig. 7.5 Perfusion bioreactors: (**a**) spin filter bioreactor, (**b**) filtration stirred tank bioreactor

bioreactor where they can be inside the bioreactor, outside, or with medium recycle. The main advantage of using membrane is to prevent the deactivation of the immobilized particle by causing any shear stress and retaining the cells/particles.

Continuous cell/medium separation is a difficulty in perfusion culture. Continuous centrifugation or in situ filtration by membrane or steel mesh can lead to filter clogging when cell density is high (Kawahara et al. 1994). Gravitational sedimentation is considered the most effective way to separate cells from medium in the perfusion culture of plant cells. Su and Arias (2003) obtained complete cell retention and packed cell volume (PCV) of 60% by using a perfusion bioreactor based on cell sedimentation. Su et al. (1996) also reached maximum cell retention efficiency of 100 percent using an airlift bioreactor, which incorporated a cell sedimentation zone delimited by a rectangular baffle in the lower downcomer.

Spin filter bioreactors have a filter separating the medium and cells, which is coupled to a magnet and stirring plate. The filter also acts as an agitator and imparts low shear. The cells can be retained in such bioreactors for longer duration and is best suited for continuous culture of plant cells. However, such bioreactors with built-in cell-settling devices tend to have numerous cells accumulating at the bottom of the reaction tank, causing difficulties with liquid mixing and mass transfer. De Dobbeleer et al. (2006) developed a perfusion STR with four sedimentation columns fixed vertically on the lid of the reaction tank, but failed to find a suitable position for the gas sparger they used. Combining a high perfusion rate with high cell concentration for perfusion bioreactors with built-in cell-settling devices is thus highly challenging. Wang et al. (2010) grew suspension culture of *Glycyrrhiza inflata* in an STR with continuous filtration by gravimetric settling. This was done to remove the spent medium containing certain metabolites which are toxic for cell growth.

7.3.2 Cultivation Strategies

Plant cell systems can be grown based on product accumulation and cell growth. There are three relations for cell growth and product accumulation as follows:

- Growth associated
- Non-growth associated
- Mixed growth associated

When the biomass accumulation is directly proportional to the production of bioactive molecule, the product formation is growth associated. The product accumulates during the exponential growth phase of cells and stops when the cells enter the stationary growth phase (e.g., primary metabolites from microorganisms). In non-growth-associated product formation, the bioactive molecule gets accumulated in the stationary phase of the cell growth. The product of interest is generally a defense molecule produced by the cells, which are produced after cells have reached their maximum growth (e.g., secondary metabolites from microorganisms and plants). When the product is accumulated in the cells during both exponential and stationary phase, it is mixed growth associated (e.g., biomolecules from plant cell/organ cultures in flasks or bioreactor level) (Luedeking and Piret 1959). Based on the relation of cells and product, one of the following cultivation modes can be used.

7.3.2.1 Batch Cultivation

It is a mode of bioreactor operation in which there is no new addition to the system after initial inoculation of cells in the culture medium. It is a closed system where once the fixed volume of medium is inoculated with live cells; it is operated until a certain period of time determined by the shake flask kinetics (Doran 2013). The environment is dynamic with constant change of nutrient consumption and cell growth. The cells follow a sigmoidal pattern of growth. Its best suited for system where there is no substrate or product inhibition. It is suitable for any type of growth-product relation (Eibl and Eibl 2009). Scale-up of bioreactors is easy when the plant cell cultures are cultivated as batch. Batch culture data can be used for modeling the system to further configurations.

7.3.2.2 Fed-Batch Cultivation

It is variation of batch system, where the one or more nutrients are added slowly over a period of time, as high concentration will inhibit either growth or product formation. It is also suited for growth-associated product, where the cells have to be maintained at exponential phase. Production of mono-glucosylated stilbene from

cell suspension cultures of *Vitis vinifera* increased when the medium was replenished (fed) in the 1 l bioreactor after 14 days of growth and harvested after the next 14 days. Stilbene production increased from 0.63 μg/g FW in batch cultivation to 6 μg/g FW in fed-batch cultivation (Ferri et al. 2011). The production of recombinant human alpha-1-antitrypsin (rAAT) in semicontinuous batch mode from transgenic *Nicotiana tabacum* cells in a 2 l STR was 25-fold (603 μg/l) over batch culture (Huang et al. 2001).

7.3.2.3 Continuous Cultivation

It's an open system, where there is continuous exchange of medium. Fresh medium is added continuously, and the same volume is removed from it at the same time. Its main drawback is the maintenance of aseptic conditions in bioreactor for long term (Doran 2013). Contamination may cease every process initiated. Repeated batch (draw and fill) mode can be used for retaining the cells for a longer duration and increasing productivity.

Cultivation mode can be modified to enhance the production. In systems where product is non-growth associated, a two-stage cultivation strategy is used. The cells are grown in one bioreactor and transferred to another bioreactor for product accumulation (Chattopadhyay et al. 2002a, b, c). The medium composition will differ in both, as in the first bioreactor, it will mostly aid rapid growth and the medium in second bioreactor will favor product formation and not much cell growth. Multistage batch culture systems are used for production at large scale.

7.3.3 Bioreactors for Hairy Root Cultivation

Hairy root cultures are the most used organ cultures for development and production of plant-based products. Hairy roots are generated from dicotyledonous plant parts on interaction with gram-negative soil bacterium *Agrobacterium rhizogenes*. They are phenotypically and genotypically very stable. Hairy roots have been found to have stable production of biologically active compounds and have growth rates greater than normal roots. Hairy roots are more sensitive to physical damage (wounding) and shear stress than callus, due to which low-shear impellers and external support (stainless steel plate or styrofoam mesh) are used during cultivation in bioreactors. Excessive branching of hairy roots causes its self-immobilization in the bioreactor and reduces its own biochemical mass transfer of nutrients and oxygen.

Puerarin (an isoflavonoid) production from hairy roots of *Pueraria phaseoloides* was enhanced by 200-fold in a 2.5 l disposable airlift bioreactor with yield of 5570 μg/g DW compared to the yield from shake flask study (Kintzios et al. 2004). The yield of a recombinant protein, human tissue plasminogen activator (t-PA) produced from genetically modified oriental melon (*Cucumis melo*) in 18 l

Table 7.3 Bioreactors used for plant hairy root cultivations

Species	Product	Bioreactor type, volume	Yield	References
Astragalus membranaceus	Astragalosides	Airlift, 2 l	711 mg/l	Ionkava et al. (2010)
Artemisia annua	Artemisinin	Bubble column bioreactor	0.14 μg/g FW	Souret et al. (2003)
		Nutrient mist bioreactor	0.29 μg/g FW	
Panax ginseng	Flavonoids	Airlift balloon-type bioreactor, 5 l	4.8 mg/g FW	Ali et al. (2007)
Silybum marianum	Silymarin	Stirred tank bioreactor, 2.7 l	0.168 mg/g DW	Rahimi et al. (2012)
Stizolobium hassjoo	L-DOPA (3,4-dihydroxyphenylalanine)	Nutrient mist bioreactor, 3 l	0.644 g/l	Huang et al. (2004)
Echinacea purpurea	Cichoric acid	Balloon-type bubble bioreactor, 5 l	26.64 mg/g DW	Jeong et al. (2009)
Salvia sclarea	Diterpenoids	Nutrient sprinkle bioreactor, 10 l	67.5 mg/g DW	Kuźma et al. (2009)
Brugmansia candida	Anisodamine	Stirred tank bioreactor, 1.5 l	10 mg/g DW	Cardillo et al. (2010)
Hypericum perforatum	Hypericin	Balloon-type bubble bioreactor, 3 l	1.4 mg/g DW	Cui et al. (2010)
Harpagophytum procumbens	Iridoid glycosides	Bubble column bioreactor, 3 l		Ludwig-Müller et al. (2008)
Eleutherococcus koreanum	Eleutherosides	Bulb type bubble bioreactor, 3 l	246.41 μg/g DW	Lee and Paek (2012)

bioreactors, was 33 times higher than the production of t-PA in transgenic tobacco plants, suggesting that the mass cultivation of hairy roots in bioreactor is better than production from transgenic plants (Kim et al. 2012). Various bioreactors used for hairy root cultivation are presented in Table 7.3.

7.3.4 Bioreactors for Plant Cell Suspension Cultures

Large-scale bioreactors for production of plant cell-based products have been employed for plant cell suspension cultures (Taxol, shikonin, taliglucerase alfa, etc.). The cells can be homogeneous in suspension for cultivation in a bioreactor with a modified impeller for the hydrodynamic stress. Various configurations of

Table 7.4 Bioreactors used for plant cell suspension cultures

Species	Product	Bioreactor type, volume	Mode of cultivation	Yield	References
Azadirachta indica	Azadirachtin	Stirred tank bioreactor, 3 l	Batch	51 mg/l	Srivastava and Srivastava (2010)
Anchusa officinalis	Acid phosphatase	Perfusion stirred tank bioreactor, 3.3 l	Continuous	300 units/l/day	Su and Arias (2003)
Linum album	Lignan	Stirred tank bioreactor, 5 l	Batch	176.3 mg/l	Baldi et al. (2008a)
Taxus chinensis (cocultivated with *Fusarium mairei*)	Paclitaxel	Stirred tank co-bioreactor, 20 l (divided into two parts of 10 l by membrane)	Batch	25.63 mg/l	Li et al. (2009)
Curcuma zedoaria Roscoe	Essential oil and curcumin	Stirred tank bioreactor, 5 l	Batch	9.69% dry cell weight	Loc et al. (2008)
Commiphora wightii	Guggulsterone	Stirred tank bioreactor, 2 l	Batch	36 µg/l	Mathur and Ramawat (2007)
Nicotiana tabacum	Scopolamine	Stirred tank bioreactor, 5 l	Batch	35.5 mg/l	Moyano et al. (2007)
Pueraria lobata	Puerarin	Stirred tank bioreactor, 5 l	Batch	257 mg/l	Chen and Li (2007)

bioreactors, stirred tank bioreactor (STR), airlift, and bubble column with minor modifications have been successfully used for plant cell suspension cultures. Cell aggregation, foaming, and cell deposition are the common troubles faced with plant cell suspension cultures, which can be overcome with suitable low-shear impeller (less shear but effective in breaking the aggregates) and efficient aeration. Table 7.4 shows different bioreactors used for plant cell suspension cultures.

7.3.5 *Bioreactors for Micropropagation and Embryogenic Suspension Cultures*

Micropropagation is the cloning of a parent plant from any of its tissue to generate large number of progeny in nutrient medium under controlled physical and chemical conditions (Steingroewer et al. 2013). Micropropagation in a bioreactor can generate

Table 7.5 Bioreactors for micropropagation

Species	Bioreactor type, volume	Cultivation period	References
Vaccinium angustifolium	Temporary immersion bioreactor (RITA®)	4 weeks	Debnath (2011)
Vitis vinifera	Airlift, 2 l	6 weeks	Tapia et al. (2009)
Lessertia (Sutherlandia) frutescens	Balloon-type bubble bioreactor, 5 l	6 weeks	Shaik et al. (2010)
Daucus carota	Airlift bioreactor	30 days	Ziv (2010)

Table 7.6 Bioreactors used in embryogenic cultures

Species	Product	Bioreactor type, volume	Yield	References
Eleutherococcus sessiliflorus	Eleutherosides	Balloon-type bubble bioreactor, 3 l	0.1484 mg/g DW	Shohael et al. (2005)
Artemisia judaica	Flavonoids	Bubble column, 0.6 l	60 μg/l	Liu et al. (2004)
		Temporary immersion bioreactor, 0.6 l	100 μg/l	
Eleutherococcus senticosus	Eleutherosides	Balloon-type bubble bioreactor, 3 l	120 μg/g DW	Shohael et al. (2006)
Eleutherococcus senticosus	*E. coli* Entero-toxin B subunit	Airlift bioreactor, 130 l	0.36% TSP	Kang et al. (2006)

up to 10000 progenies with same characteristics in a single batch, which is consistent and efficient than conventional micropropagation, which has led to a less number of progenies in a single batch, and the clones may vary in each batch (Ducos et al. 2009). Some of the biologically active chemicals were found to be produced better in shoot/embryogenic cultivation than in cell suspension/hairy root cultivation. Shikimic acid was produced in a 2 l airlift bioreactor from sandalwood (*Santalum album*) from embryogenic suspension, yielding 0.08% (w/w) shikimic acid in 2–3 weeks (Misra and Dey 2013). Siberian ginseng somatic embryos were produced in a 500 l balloon-type bubble bioreactor (BTBB), where by inoculating 3.5 kg of Siberian ginseng IEDC – induced embryogenic determined cells – 60 kg of mature embryos were harvested after 30 days of culture (Paek et al. 2005). For naturally slow-growing *Stevia rebaudiana* leaf explants, direct shoot bud generation was done in a 1.75 l bubble column bioreactor, and high biomass of about 590 micro cuttings was achieved after 3-week cultivation. The regenerated shoots were then transferred to rooting medium and maintained under controlled conditions (Sreedhar et al. 2008). Sweet pepper (*Capsicum annuum*), a recalcitrant species, was successfully micropropagated in 1 l RITA® airlift bioreactors (Vitropic, France) in 60 days (Grozeva et al. 2009). Various bioreactors used for micropropagation and embryo cultures are in Tables 7.5 and 7.6.

7.4 Application of First Principle-Based Mathematical Modeling for Designing Nutrient Feeding Strategies in Bioreactors

A mathematical model is a real-time representation of the complex bioprocess occurring in the cellular state. The mathematical description of the bioprocess is developed to describe the complex intracellular reactions occurring during the metabolism of the cell which is converting the substrate to products in the fermentation reactions (iitd.vlab.co.in 2013) (Fig. 7.6).

First principle-based mathematical models can be used to simulate different process operating strategies to ensure the major nutrients are at non-limiting and non-inhibitory concentrations in the bioreactor throughout the fermentation process. These optimized cultivation strategies can be implemented in the bioreactor (experimentally) to achieve maximum productivity, thereby reducing the number of experiments required to enhance the efficiency of a particular fermentation process in minimum time without any trial and error fermentation process (Srivastava and Srivastava 2006). Bioprocess kinetic modeling could therefore serve as a biologically logical, yet simple, engineering approach in designing the fresh nutrient feeding strategies in order to obtain high productivity (Kaur et al. 2012).

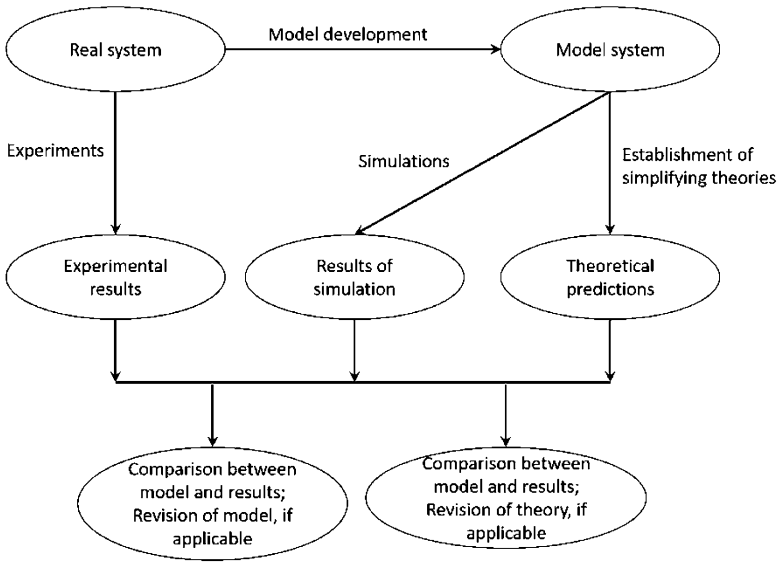

Fig. 7.6 Scheme of mathematical model development and validation (Adapted from Maschke et al. (2015))

Table 7.7 Types of cell kinetic models

Types of cell kinetic models	
Unstructured, distributed	Cells represented by a single component
	Homogeneous system
Unstructured, segregated	Cells represented by a single component
	Heterogeneous system
Structured, distributed	Multiple cell components interact with each other
	Homogeneous system
Structured, segregated	Cells composed of multiple components
	Heterogeneous mixture

Adapted from (Lee 2001)

7.4.1 Types of Models

During the course of growth, the heterogeneous mixture of young and old cells is continuously changing and adapting itself in the medium environment which is also continuously changing physically and chemically. As a result, accurate mathematical modeling of growth kinetics is impossible to achieve. Even with such a realistic model, this approach is usually useless because the model may contain many parameters which are impossible to determine. Therefore, assumptions are made to arrive at simple models which are useful for fermenter design and performance predictions. Various models can be developed based on the assumptions concerning cell components and population as shown in Table 7.7.

The simplest model is the unstructured, distributed model which is based on the following two assumptions:

1. Cells can be represented by a single component, such as biomass during balanced growth (as the biomass doubles, so does other cell components).
2. The population of cellular mass is distributed uniformly throughout the culture. The cell suspension is regarded as a homogeneous solution, and the medium is formulated so that only one component may be limiting the reaction rate. All other components are present at sufficiently high concentrations, so that minor changes do not significantly affect the reaction rate. Bioreactors are also controlled so that environmental parameters such as pH, temperature, and dissolved oxygen concentration are maintained at a constant level (Lee 2001).

For correlating growth rate of cells with substrate concentration in the bioreactor, Monod's model is widely used:

$$\mu = \frac{\mu_m S}{K_S + S}$$

where μ is the specific growth rate, μ_m is the maximum specific growth rate, S is the limiting substrate, and K_S is the Monod's saturation constant based on substrate affinity.

The limiting substrate may also inhibit the cell growth at very high concentration. The effect of inhibition on growth rate can be taken into account by fitting of experimental data into various models demonstrating inhibition kinetics. Few of the growth kinetic models which take into account substrate inhibition are as follows:

$$\mu = \mu_m \left[\frac{K_I}{K_I + S}\right] \quad \text{Prakash and Srivastava (2006)}$$

$$\mu = \mu_m \left[\frac{S}{K_S + S}\right] e^{\frac{-S}{K_I}} \quad \text{Gumel et al. (2014)}$$

$$\mu = \frac{\mu_m}{\left(1 + \frac{K_S}{S}\right)\left(1 + \frac{S}{K_I}\right)} \quad \text{Gumel et al. (2014)}$$

$$\left[\frac{\mu_i}{\mu_m}\right] = \left[1 - \left(\frac{s_i}{s_{mi}}\right)^{ni}\right] \quad \text{Srivastava and Srivastava (2006)}$$

where K_I is the inhibition constant.

Similarly, the product formation can be classified into three types, depending on the relation to the primary metabolism: direct, indirect, or not related (Maschke et al. 2015). In the Luedeking-Piret approach, the product formation rate can be divided into growth and a non-growth-associated component (Luedeking and Piret 1959):

$$\frac{dP}{dt} = \propto \frac{dX}{dt} + \beta X$$

where α and β represent the growth-associated and non-growth-associated product formation constants, respectively (Prakash and Srivastava 2006). Depending on the value of these parameters, product formation kinetics can be demonstrated as growth associated, non-growth associated, or mixed growth associated.

7.4.2 Modeling for Plant Cell and Hairy Root Cultivation

The use of modeling and simulation to study plant growth and developmental processes has increased tremendously over the past few years. By formulating a system of interacting mathematical equations, it becomes feasible for biologists to gain a mechanistic understanding of the complex behavior of biological systems (De Vos et al. 2012).

Mathematical models used for describing hairy root cultivations are highly complex, and yet a lot of potential exists for the identification of more reliable mathematical models (Patra and Srivastava 2015). As it is impossible to determine directly hairy root weight during a run, different techniques have been developed to estimate biomass growth. One of the most used is based on medium conductivity

Table 7.8 Use of mathematical model to enhance product productivity

Species	Product	Culture type	Yield enhancement	References
Azadirachta indica	Azadirachtin	Cell suspension	1.8 fold	Prakash and Srivastava (2006)
Artemisia annua	Artemisinin	Hairy roots	3.7 fold	Patra and Srivastava (2015)
Azadirachta indica	Azadirachtin	Cell suspension	3.8 fold	Prakash and Srivastava (2011)
Catharanthus roseus	Ajmalicine	Hairy roots	2.5 fold	Thakore et al. (2015)
Ajuga reptans	20-hydroxyecdysone	Hairy roots	Threefold	Uozumi et al. (1995)

measurement, which is dependent of the ionic concentrations. As a constant biomass yield from nutrients has been observed, online conductivity measurement has given accurate biomass estimation if there is no nutrient limitation (Mairet et al. 2010)

Few models have been proposed to describe hairy root growth. Different approaches can be discerned (Mairet et al. 2010):

1. Branching model: the increase of biomass is described by several rules concerning the branching kinetics (elongation of branch and formation of new branches). These rules are combined with a population balance approach: the model accounts for the difference between cells in different states.
2. Metabolic model: this approach is based on the metabolic network of the roots. The model uses intracellular nutrients as well as energy shuttles to describe metabolic regulation.
3. Oxygen limited growth kinetic model: this approach is based on fact that oxygen limitation plays a role in growth of hairy roots in shake flasks, considering its branching nature and oxygen limitation observed when scaled up to bioreactor (Palavalli et al. 2012).

These mathematical model-based strategies have been successfully used to enhance the product yield in plant cell and hairy root cultivation (Table 7.8).

7.5 Emerging New Designs of Bioreactors

Bioreactors for plant cell cultures are designed for increasing biomass and productivity. But varying characteristics of plant cells have generated a need to design even better bioreactors. These new bioreactors (Fig. 7.7) are designed to overcome the biochemical limitations, enhance mixing, and reduce the cost and ease of operation.

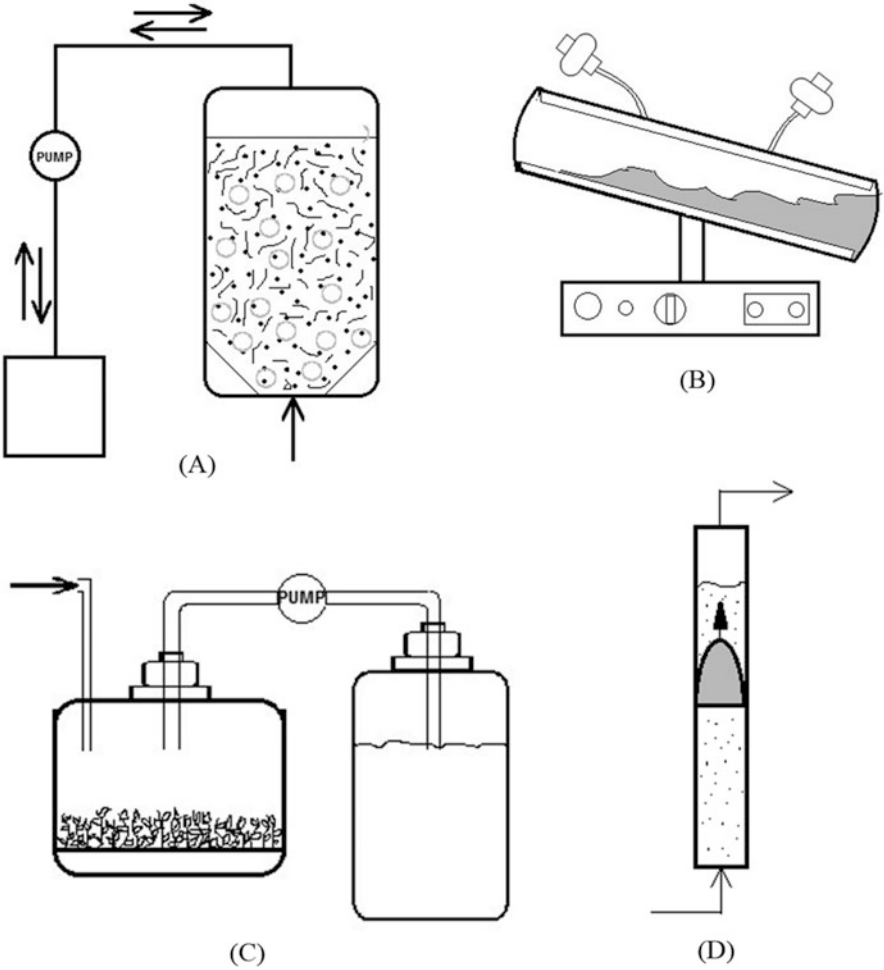

Fig. 7.7 Emerging new design of bioreactors for plant cell and organ cultures: (**a**) Ebb-and-flow regime bioreactor, (**b**) wave and undertow bioreactor, (**c**) temporary immersion bioreactor, (**d**) slug bubble bioreactor

7.5.1 Hydraulically Driven Bioreactors

Hydraulically driven bioreactors use the energy generated by pumping the fluids for mixing and aeration. The pumps ensure circulation of fluid through the loops. Their design is simple and work without moving mechanical parts inside the bioreactor. They cause low shear stress to the cells, and operation is easy. Wave-mixed bioreactor is a hydraulically driven bioreactor designed for shear-sensitive plant cell cultures, which can be grown in a sterile disposable bag made of non-gas permeable plastic. The mixing is provided by the rocking of the bag, to which all

the controllers are attached. They have the advantages of low cost and low shear stress. The mixing, mass, and heat transfer in the wave-mixed bioreactor are characterized by rocking rate, rocking angle, bag type and its geometry, and culture working volume (Huang and McDonald 2012). Oxygen is supplied from the air or gas mixture continuously through headspace aeration. While the wave-mixed bioreactor is rocking, the liquid surface of the medium in the bag is continuously renewed, and bubble-free surface aeration takes place resulting in oxygenation and bulk mixing with less shear stress to cultivated cells (Terrier et al. 2007).

7.5.2 Immersion Bioreactors

The temporary immersion bioreactor (TIB), consisting of two vessels (one for holding the plant tissue cultures and another for the liquid medium), was developed to allow cycling of the culture medium by using air pressure or a pump to push the medium from one vessel to the other to immerse the plant tissues and using gravity to withdraw the medium; thus the plant tissues or immobilized plant cells are exposed to the medium intermittently rather than continuously. A separate air or gas mixture is introduced through a sparger to aerate the plant cell or tissue cultures. TIB provides attractive advantages including adequate oxygen transfer and low shear stress to plant tissues (such as hairy root culture) due to the lack of mechanical agitation, although some limitations need to be addressed including vessel size at commercial scale, disposability, and insufficient mixing leading to the accumulation of inhibitory metabolites that can affect cell growth (Ducos et al. 2009). In addition, a modified TIB, consisting of a rigid box placed inside a transparent plastic bag, called a box-in-bag TIB, provides culture headspace between the immersion periods and allows horizontal distribution of biomass for better oxygenation and illumination than that in TIB or other types of immersion bioreactor (Ducos et al. 2009).

7.5.3 Microbioreactors

The microbioreactor is designed as a high-throughput platform for cell line selection and evaluation, bioprocess characterization (design space determination), media design and optimization (Betts and Baganz 2006; Diao et al. 2008), and as a scaled-down model to represent the production bioreactor for bioprocess scaling-up purposes (Micheletti et al. 2006). Microbioreactor platforms including microtiter plates (6, 12, 24, 96, with up to 384 wells with a few microliter to milliliter volumes), spin tubes (5–50 ml), shake flasks (25–1000 ml), and parallel miniature stirred and bubble column bioreactor systems (Betts and Baganz 2006) have been implemented for cultivation of many different host cell lines. Feeding, sampling, and harvesting can be automated by using a liquid handling system with an automation control system that can be programmed. Recently the optical sensing systems based on

noninvasive process analytical technology have been used for online measurements of pH, dissolved oxygen, and optical density in a microbioreactor (Zhang et al. 2007). Though microbioreactors are suitable for growing plant cells, there is no literature available on plant cells. Considering its effectiveness on animal cell culture studies, minibioreactor can prove to be successful with plant cells.

7.5.4 Ebb-and-Flow Regime Bioreactor

The ebb-and-flow bioreactor (EFBR) derives its name from the process behavior of its liquid medium which is characterized by its repetitive ebbing and flowing or periodic filling and draining. This bioreactor configuration is a mix between the two bioreactor configurations of the predominantly liquid-phase bioreactor (STR) and the predominantly gas-phase bioreactor (Mist bioreactor). The ebb-and-flow bioreactor has four characteristic operational phases which recur sequentially and intermittently as the liquid medium moves back and forth between the bioreactor vessel and its reservoir. These include the liquid dwell time (LDT), the drain time (DT), the gas dwell time (GDT), and the fill time (FT). The LDT is the phase where the whole reaction volume of the EFBR is completely submerged in liquid and where the bulk of the liquid medium is neither flowing upward nor downward. The GDT is that operational phase where the EFBR reaction volume is predominantly in the gas phase and where mass flow of the bulk liquid medium is not occurring. The operational phases where the bulk flow of the liquid medium takes place are the FT, when the bulk flow direction is upward, and the DT, when the bulk flow direction is downward (Cuello and Yue 2008).

Cuello et al. (2003) were able to successfully cultivate hairy roots of *Hyoscyamus niger* in a 2.5 l ebb-and-flow bioreactor which gave same productivity as in 250 ml Erlenmeyer flasks. It was cultivated in 2.5 l STR and 2.5 l EFBR for scaling up from Erlenmeyer flasks, and EFBR proved to be more efficient. EFBR has been observed to be successful for hairy root cultures, which tend to form clumps and are self-immobilizing.

7.5.5 Slug Bubble Bioreactor

The slug bubble (SB) bioreactor produces artificial slug bubbles and was developed to increase mixing of non-Newtonian fluid in the plant cell bioreactor. Bubble column bioreactors tend to form slug bubbles (based on column diameter and gas velocity) when gas is sparged at high velocity and the bioreactor is filled with fully grown plant cells and highly viscous media. Slug bubble generates significant changes in the hydrodynamic behavior of the system. There exists an onset of upward liquid circulation in the column center and downward liquid circulation

near the column wall. As a result more gas entry takes place in the center, leading to buildup of transverse holdup profile that enhances liquid circulation (Kantarci et al. 2005).

It consists of a vertical flexible plastic cylinder filled with medium up to 80% of its height. Agitation and aeration are achieved through the intermittent generation of large cylindrical single bubbles at the bottom of the system that rise to the top of the cylinder. The bubble size can be controlled by controlling the inlet pressure to form the bubbles. These bubbles are the slug bubbles, and the two-phase flow of gas-liquid formed is known as slug flow (Davies and Taylor 1950; Sousa et al. 2005). Slug bubbles can be described as long bullet-shaped bubbles, which nearly occupy the entire cross section of a pipe. Between the bubble and the pipe walls flows a thin film of liquid; the bubble moves upward at nearly constant speed, while the liquid flows downward as a falling film. The nose of the slug is a very stable region; on the contrary, the rear of the bubble is a region characterized by strong mixing, where all transfer processes are enhanced. Mixing and oxygen transfer are therefore achieved at the same time (Terrier et al. 2007). Terrier et al. (2007) were successful in using 24 l and 64 l of slug bubble bioreactor for production of isoflavones and monoclonal antibodies from suspension cultures of *Glycine max* and *Nicotiana tabacum* BY-2 (bright yellow-2), respectively. Nearly, twofold increase in the isoflavone content (in *G. max* cell line) was observed in slug bubble bioreactor compared to STR with pitched blade turbine impeller.

7.6 Conclusion

Plant cell and hairy root cultivation has proven to be efficient biofactories for production of medicinally/commercially important bioactive metabolites and recombinant protein. Large-scale production of these low-volume high-value compounds has led to modification of the existing bioreactors to suit the requirement of these cultures. Bioreactors have been succesfully developed for commercialization of few plant-based products (Taxol, shikonin, taliglucerase alfa, etc.). The successful use of disposable bag bioreactor vessels as a STR operated at batch mode for production of taliglucerase alfa with recombinant carrot cell suspension cultures is a good example of how far the bioreactor operating process and strategies have come along in the past few years. The overall cost and time required for cleaning of the bioreactor and harvesting of the culture reduces due to the disposable bags, and any chances whatsoever of contamination from the previous batch become negligible. Newer design approaches for better control of the bioreactors and real-time monitoring using artificial intelligence are being used for microbial cultivations. These can be applied to enhance productivity in plant cell bioreactors at a commercial scale. In application of computational tools of modeling and simulation, omic approaches for online monitoring of plant cell constituents also help in evolution of new bioreactors.

References

Ali MB, Hahn EJ, Paek KY (2007) Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. Molecules 12:607–621

Angelini VA, Orejas J, Medina MI, Agostini E (2011) Scale up of 2, 4-dichlorophenol removal from aqueous solutions using *Brassica napus* hairy roots. J Hazard Mater 185:269–274

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614

Baldi A, Srivastava AK, Bisaria VS (2008a) Effect of aeration on production of anticancer lignans by cell suspension cultures of *Linum album*. Appl Biochem Biotechnol 151:547–555

Baldi A, Srivastava AK, Bisaria VS (2008b) Improved podophyllotoxin production by transformed cultures of *Linum album*. Biotechnol J 3:1256–1263

Betts JI, Baganz F (2006) Miniature bioreactors: current practices and future opportunities. Microb Cell Factories 5:21

Bhojwani SS, Razdan MK (1996) Tissue culture media. In: Bhojwani SS, Razdan MK (eds) Plant tissue culture: theory and practice. Elsevier, Amsterdam, pp 39–62

Bisaria V, Panda A (1991) Large-scale plant cell culture: methods, applications and products. Curr Opin Biotechnol 2:370–374

Bowen RL Jr (1986) Unravelling the mysteries of shear-sensitive mixing systems. Chem Eng 93:55–63

Bronnenmeier R, Märkl H (1982) Hydrodynamic stress capacity of microorganisms. Biotechnol Bioeng 24:553–578

Calderbank PH, Moo-Young MB (1959) The prediction of power consumption in the agitation of non-newtonian fluids. Trans Inst Chem Eng 37:26–33

Cardillo AB, Otálvaro AÁM, Busto VD, Talou JR, Velásquez LME, Giulietti AM (2010) Scopolamine, anisodamine and hyoscyamine production by *Brugmansia* candida hairy root cultures in bioreactors. Process Biochem 45:1577–1581

Chattopadhyay S, Farkya S, Srivastava AK, Bisaria VS (2002a) Bioprocess considerations for production of secondary metabolites by plant cell suspension cultures. Biotechnol Bioprocess Eng 7:138–149

Chattopadhyay S, Srivastava AK, Bhojwani SS, Bisaria VS (2002b) Production of podophyllotoxin by plant cell cultures of *Podophyllum hexandrum* in bioreactor. J Biosci Bioeng 93:215–220

Chattopadhyay S, Srivastava AK, Bisaria VS (2002c) Optimization of culture parameters for production of podophyllotoxin in suspension culture of *Podophyllum hexandrum*. Appl Biochem Biotechnol 102–103:381–394

Chattopadhyay S, Srivastava AK, Bisaria VS (2005) Production of phytochemicals in plant cell bioreactors. In: Srivastava S, Narula P (eds) Plant biotechnology and molecular markers. Kluwer Academic Publishers, Dordrecht, pp 117–128

Chen G, Li L (2007) Nutrient consumption and production of isoflavones in bioreactor cultures of *Pueraria lobata* (Willd). J Environ Biol 28:321–326

Choi SM, Ho Son S, Rho Yun S, Woung Kwon O, Hoon Seon J, Yoeup Paek K (2000) Pilot-scale culture of adventitious roots of ginseng in a bioreactor system. Plant Cell Tissue Organ Cult 62:187–193

Cuello JL, Yue LC (2008) Ebb-and-flow bioreactor regime and electrical elicitation: novel strategies for hairy root biochemical production. Electron J Integr Biosci 3:45–56

Cuello JL, Walker PN, Curtis WR (2003) Design of ebb-and-flow bioreactor (EFBR) for immobilized "hairy root" cultures: part I. Preliminary design models and culture parameters. Trans Am Soc Agric Eng 46:1457–1468

Cui X-H, Chakrabarty D, Lee E-J, Paek K-Y (2010) Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. Bioresour Technol 101:4708–4716

Cui H-Y, Murthy HN, Moh SH, Cui Y, Lee E-J, Paek K-Y (2014) Protocorm culture of *Dendrobium candidum* in balloon type bubble bioreactors. Biochem Eng J 88:26–29

Davies RM, Taylor G (1950) The mechanics of large bubbles rising through extended liquids and through liquids in tubes. Proc R Soc A Math Phys Eng Sci 200:375–390

De Dobbeleer C, Cloutier M, Fouilland M, Legros R, Jolicoeur M (2006) A high-rate perfusion bioreactor for plant cells. Biotechnol Bioeng 95:1126–1137

De Muynck B, Navarre C, Boutry M (2010) Production of antibodies in plants: status after twenty years. Plant Biotechnol J 8:529–563

De Vos D, Dzhurakhalov A, Draelants D, Bogaerts I, Kalve S, Prinsen E, Vissenberg K, Vanroose W, Broeckhove J, Beemster GTS (2012) Towards mechanistic models of plant organ growth. J Exp Bot 63:3325–3337

Debnath SC (2011) Adventitious shoot regeneration in a bioreactor system and EST-PCR based clonal fidelity in lowbush blueberry (*Vaccinium angustifolium* Ait.). Sci Hortic (Amsterdam) 128:124–130

Diao J, Young L, Zhou P, Shuler ML (2008) An actively mixed mini-bioreactor for protein production from suspended animal cells. Biotechnol Bioeng 100:72–81

Doran PM (1993) Design of reactors for plant cells and organs. In: Bioprocess design and control. Springer, Berlin/Heidelberg, pp 115–168

Doran PM (1999) Design of mixing systems for plant cell suspensions in stirred reactors. Biotechnol Prog 15:319–335

Doran PM (2010) Bioreactors, stirred tank for culture of plant cells. In: Encyclopedia of industrial biotechnology. John Wiley & Sons, Inc, Hoboken, pp 1–35

Doran PM (2013) Bioprocess engineering principles. Elsevier, Oxford

Ducos JP, Terrier B, Courtois D (2009) Disposable bioreactors for plant micropropagation and mass plant cell culture. In: Eibl R, Eibl D (eds) Disposable bioreactors, Advances in biochemical engineering/biotechnology, vol 115. Springer, Berlin/Heidelberg, pp 89–115

Eibl R, Eibl D (2002) Bioreactors for plant cell and tissue cultures. In: Oksman-Caldentey KM, Barz WH (eds) Plant biotechnology and transgenic plants. Marcel Dekker, New York, pp 163–199

Eibl R, Eibl D (2008) Design of bioreactors suitable for plant cell and tissue cultures. Phytochem Rev 7:593–598

Eibl R, Eibl D (2009) Plant cell-based bioprocessing. In: Eibl R, Eibl D, Portner R, Catapano G, Czermak P (eds) Cell and tissue reaction engineering: principles and practice. Springer-Verlag, Berlin/Heidelberg, pp 315–355

Ferri M, Dipalo SCF, Bagni N, Tassoni A (2011) Chitosan elicits mono-glucosylated stilbene production and release in fed-batch bioreactor cultures of grape cells. Food Chem 124:1473–1479

Fischer R, Schillberg S, Hellwig S, Twyman RM, Drossard J (2012) GMP issues for recombinant plant-derived pharmaceutical proteins. Biotechnol Adv 30:434–439

Fox JL (2012) First plant-made biologic approved. Nat Biotechnol 30:472–472

Franconi R, Demurtas OC, Massa S (2010) Plant-derived vaccines and other therapeutics produced in contained systems. Expert Rev Vaccines 9:877–892

Fulzele DP (2000) Bioreactor technology for large scale cultivation of plant cell suspension cultures and production of bioactive compounds. BARC news. 41–46. http://www.barc.ernet.in/webpages/letter/2000/200010-04.pdf

Gangopadhyay M, Dewanjee S, Bhattacharya S (2011) Enhanced plumbagin production in elicited *Plumbago indica* hairy root cultures. J Biosci Bioeng 111:706–710

Gaurav V, Roberts SC (2011) Statistical optimization of single-cell production from *Taxus cuspidata* plant cell aggregates. Prep Biochem Biotechnol 41:219–235

Georgiev MI (2014) Design of bioreactors for plant cell and organ cultures. In: Paek KP, Murthy HN, Zhong JJ (eds) Production of biomass and bioactive compounds using bioreactor technology. Springer, Dordrecht, pp 3–15

Georgiev VG, Bley T, Pavlov AI (2012) Bioreactors for the cultivation of red beet hairy roots. In: Neelwarne B (ed) Red beet biotechnology. Springer US, Boston, pp 251–281

Grabowski GA, Golembo M, Shaaltiel Y (2014) Taliglucerase alfa: an enzyme replacement therapy using plant cell expression technology. Mol Genet Metab 112:1–8

Grozeva S, Rodeva V, Kintzios S (2009) Successful regeneration of sweet pepper in an airlift bioreactor: effect of medium phase and genotype. Open Hortic J 2:70–75

Gumel AM, Annuar MSM, Heidelberg T (2014) Growth kinetics, effect of carbon substrate in biosynthesis of mcl-PHA by *Pseudomonas putida* Bet001. Braz J Microbiol 45:427–438

Hahlbrock K, Ebel J, Oaks A, Auden J, Liersch M (1974) Determination of specific growth stages of plant cell suspension cultures by monitoring conductivity changes in the medium. Planta 118:75–84

Henzler HJ, Kauling J (1985) Scale-up of mass transfer in highly viscous liquids. In: Fifth European conference on mixing. BHRA fluid engineering, pp 303–312

Hoffmann J, Büscher K, Hempel DC (1995) Determination of maximum shear-stress in stirred vessels. Chemie Ing Tech 67:210–214

Honda H, Liu C, Kobayashi T (2001) Large-scale plant micropropagation. In: Zhong JJ (ed) Advances in biochemical engineering/biotechnology, vol 2. Springer, Berlin/Heidelberg, pp 157–182

Huang TK, McDonald KA (2012) Bioreactor systems for in vitro production of foreign proteins using plant cell cultures. Biotechnol Adv 30:398–409

Huang J, Sutliff TD, Wu L, Nandi S, Benge K, Terashima M, Ralston AH, Drohan W, Huang N, Rodriguez RL (2001) Expression and purification of functional human α-1-antitrypsin from cultured plant cells. Biotechnol Prog 17:126–133

Huang SY, Hung CH, Chou SN (2004) Innovative strategies for operation of mist trickling reactors for enhanced hairy root proliferation and secondary metabolite productivity. Enzym Microb Technol 35:22–32

iitd.vlab.co.in (2013) Development of mathematical model (steps in modelling): Bioreactor modeling & amp; Simulation lab: Biotechnology and Biomedical Engineering: IIT DELHI Virtual Lab. http://iitd.vlab.co.in/?sub=63&brch=177&sim=1542&cnt=3227. Accessed 19 Apr 2018

Jeong JA, Wu CH, Murthy HN, Hahn EJ, Paek KY (2009) Application of an airlift bioreactor system for the production of adventitious root biomass and caffeic acid derivatives of *Echinacea purpurea*. Biotechnol Bioprocess Eng 14:91–98

Joe MM, Benson A, Ayyanar M, Sa T (2015) Bioactive compounds and medical significance of some endangered medicinal plants from the Western Ghats region of India. In: Gupta VK, Tuohy MG (eds) Biotechnology of bioactive compounds. John Wiley & Sons, Ltd, Chichester, pp 163–193

Jolicoeur M, Chavarie C, Carreau PJ, Archambault J (1992) Development of a helical-ribbon impeller bioreactor for high-density plant cell suspension culture. Biotechnol Bioeng 39:511–521

Kaldis A, Ahmad A, Reid A, Mcgarvey B, Brandle J, Ma S, Jevnikar A, Kohalmi SE, Menassa R (2013) High-level production of human interleukin-10 fusions in tobacco cell suspension cultures. Plant Biotechnol J 11:535–545

Kang TJ, Lee WS, Choi EG, Kim JW, Kim BG, Yang MS (2006) Mass production of somatic embryos expressing *Escherichia coli* heat-labile enterotoxin B subunit in Siberian ginseng. J Biotechnol 121:124–133

Kantarci N, Borak F, Ulgen KO (2005) Bubble column reactors. Process Biochem 40:2263–2283

Kaur G, Srivastava AK, Chand S (2012) Mathematical modelling approach for concentration and productivity enhancement of 1,3-propanediol using *Clostridium diolis*. Biochem Eng J 68:34–41

Kawahara H, Mitsuda S, Kumazawa E, Takeshita Y (1994) High-density culture of FM-3A cells using a bioreactor with an external tangential-flow filtration device. Cytotechnology 14:61–66

Kawase Y, Moo-Young M (1990) Mathematical models for design of bioreactors: applications of Kolmogoroff's theory of isotropic turbulence. Chem Eng J 43:B19–B41

Kieran P, MacLoughlin P, Malone D (1997) Plant cell suspension cultures: some engineering considerations. J Biotechnol 59:39–52

Kieran PM, Malone DM, MacLoughlin PF (2000) Effects of hydrodynamic and interfacial forces on plant cell suspension systems. In: Schügerl K, Kretzmer G, Henzler H, Kieran P, Kretzmer G, MacLoughlin P, Malone D, Schumann W, Shamlou P, Yim S (eds) Influence of stress on cell growth and product formation, Advances in biochemical engineering/biotechnology, vol 67. Springer, Berlin/Heidelberg, pp 139–177

Kim Y, Wyslouzil B, Weathers P (2001) A comparative study of mist and bubble column reactors in the *in vitro* production of artemisinin. Plant Cell Rep 20:451–455

Kim SR, Sim JS, Ajjappala H, Kim YH, Hahn BS (2012) Expression and large-scale production of the biochemically active human tissue-plasminogen activator in hairy roots of Oriental melon (*Cucumis melo*). J Biosci Bioeng 113:106–111

Kintzios S, Makri O, Pistola E, Matakiadis T, Ping Shi H, Economou A (2004) Scale-up production of puerarin from hairy roots of *Pueraria phaseoloides* in an airlift bioreactor. Biotechnol Lett 26:1057–1059

Kolewe ME, Henson MA, Roberts SC (2011) Analysis of aggregate size as a process variable affecting paclitaxel accumulation in Taxus suspension cultures. Biotechnol Prog 27:1365–1372

Kolewe ME, Roberts SC, Henson MA (2012) A population balance equation model of aggregation dynamics in Taxus suspension cell cultures. Biotechnol Bioeng 109:472–482

Kondo O, Honda H, Taya M, Kobayashi T (1989) Comparison of growth properties of carrot hairy root in various bioreactors. Appl Microbiol Biotechnol 32:291–294

Kuźma Ł, Bruchajzer E, Wysokińska H (2009) Methyl jasmonate effect on diterpenoid accumulation in *Salvia sclarea* hairy root culture in shake flasks and sprinkle bioreactor. Enzym Microb Technol 44:406–410

Lawford HG, Rousseau JD (1991) Bioreactor design considerations in the production of high-quality microbial exopolysaccharide. Appl Biochem Biotechnol 28:667–684

Lee JM (2001) Biochemical engineering. Prentice Hall, Englewood Cliffs

Lee EJ, Paek KY (2012) Effect of nitrogen source on biomass and bioactive compound production in submerged cultures of *Eleutherococcus koreanum* nakai adventitious roots. Biotechnol Prog 28:508–514

Li YC, Tao WY, Cheng L (2009) Paclitaxel production using co-culture of Taxus suspension cells and paclitaxel-producing endophytic fungi in a co-bioreactor. Appl Microbiol Biotechnol 83:233–239

Lienard D, Dinh OT, van Oort E, Van Overtvelt L, Bonneau C, Wambre E, Bardor M, Cosette P, Didier-Laurent A, de Borne FD, Delon R, van Ree R, Moingeon P, Faye L, Gomord V (2007) Suspension-cultured BY-2 tobacco cells produce and mature immunologically active house dust mite allergens. Plant Biotechnol J 5:93–108

Liu CZ, Murch SJ, El-Demerdash M, Saxena PK (2004) *Artemisia judaica* L.: micropropagation and antioxidant activity. J Biotechnol 110:63–71

Liu C, Towler MJ, Medrano G, Cramer CL, Weathers PJ (2009) Production of mouse interleukin-12 is greater in tobacco hairy roots grown in a mist reactor than in an airlift reactor. Biotechnol Bioeng 102:1074–1086

Loc N-H, Diem D-T-H, Binh D-H-N, Huong D-T, Kim T-G, Yang M-S (2008) Isolation and characterization of antioxidation enzymes from cells of zedoary (*Curcuma zedoaria* Roscoe) cultured in a 5-l bioreactor. Mol Biotechnol 38:81–87

Ludwig-Müller J, Georgiev M, Bley T (2008) Metabolite and hormonal status of hairy root cultures of Devil's claw (*Harpagophytum procumbens*) in flasks and in a bubble column bioreactor. Process Biochem 43:15–23

Luedeking R, Piret EL (1959) A kinetic study of the lactic acid fermentation. Batch process at controlled pH. J Biochem Microbiol Technol Eng 1:393–412
Madhusudhan R, Ramachandra Rao S, Ravishankar GA (1995) Osmolarity as a measure of growth of plant cells in suspension cultures. Enzym Microb Technol 17:989–991
Mairet F, Villon P, Boitel-Conti M, Shakourzadeh K (2010) Modeling and optimization of hairy root growth in fed-batch process. Biotechnol Prog 26:847–856
Maschke RW, Geipel K, Bley T (2015) Modeling of plant *in vitro* cultures: overview and estimation of biotechnological processes. Biotechnol Bioeng 112:1–12
Mathur M, Ramawat KG (2007) Guggulsterone production in cell suspension cultures of the guggul tree, *Commiphora wightii*, grown in shake-flasks and bioreactors. Biotechnol Lett 29:979–982
Meijer JJ, ten Hoopen HJG, Luyben KCAM, Libbenga KR (1993) Effects of hydrodynamic stress on cultured plant cells: a literature survey. Enzym Microb Technol 15:234–238
Metzner AB, Otto RE (1957) Agitation of non-Newtonian fluids. AICHE J 3:3–10
Micheletti M, Barrett T, Doig SD, Baganz F, Levy MS, Woodley JM, Lye GJ (2006) Fluid mixing in shaken bioreactors: implications for scale-up predictions from microlitre-scale microbial and mammalian cell cultures. Chem Eng Sci 61:2939–2949
Misra BB, Dey S (2013) Shikimic acid (Tamiflu precursor) production in suspension cultures of east Indian sandalwood (*Santalum album*) in air-lift bioreactor. J Postdoc Res 1:1–9
Mitchell DA, Stuart DM, Hardin MT, Krieger N (2006) Group III: rotating-drum and stirred-drum bioreactors. In: Mitchell D, Berovic M, Krieger N (eds) Solid-state fermentation bioreactors. Springer-Verlag, Berlin/Heidelberg, pp 95–114
Moyano E, Palazón J, Bonfill M, Osuna L, Cusidó RM, Oksman-Caldentey K-M, Piñol MT (2007) Biotransformation of hyoscyamine into scopolamine in transgenic tobacco cell cultures. J Plant Physiol 164:521–524
Paek KY, Chakrabarty D, Hahn EJ (2005) Application of bioreactor systems for large scale production of horticultural and medicinal plants. Plant Cell Tissue Organ Cult 81:287–300
Palavalli RR, Srivastava S, Srivastava AK (2012) Development of a mathematical model for growth and oxygen transfer in *in vitro* plant hairy root cultivations. Appl Biochem Biotechnol 167:1831–1844
Panda AK, Mishra S, Bisaria VS, Bhojwani SS (1989) Plant cell reactors – a perspective. Enzym Microb Technol 11:386–397
Patra N, Srivastava AK (2015) Use of model-based nutrient feeding for improved production of artemisinin by hairy roots of *Artemisia annua* in a modified stirred tank bioreactor. Appl Biochem Biotechnol 177:373–388
Prakash G, Srivastava AK (2006) Modeling of azadirachtin production by *Azadirachta indica* and its use for feed forward optimization studies. Biochem Eng J 29:62–68
Prakash G, Srivastava AK (2011) Integrated yield and productivity enhancement strategy for biotechnological production of Azadirachtin by suspension culture of *Azadirachta indica*. Asia-Pac J Chem Eng 6:129–137
Rahimi S, Hasanloo T, Najafi F, Khavari-Nejad RA (2012) Methyl jasmonate influence on silymarin production and plant stress responses in *Silybum marianum* hairy root cultures in a bioreactor. Nat Prod Res 26:1662–1667
Raposo S, Azevedo F, Lima-Costa ME (2010) Development of an empirical correlation for Newtonian and non-Newtonian fluids in a stirred tank reactor, to predict oxygen mass transfer rates. J Biotechnol 150:395–395
Robertson B, Ulbrecht JJ (1987) Measurement of shear rate on an agitator in a fermentation broth. In: Ho CS, Oldshue JY (eds) Biotechnology processes: scale-up and mixing. American Institute of Chemical Engineers, New York, pp 72–81
Sambamurthy K, Kar A (2006) Pharmaceutical biotechnology. New Age International, New Delhi
Sánchez Pérez JA, Rodríguez Porcel EM, Casas López JL, Fernández Sevilla JM, Chisti Y (2006) Shear rate in stirred tank and bubble column bioreactors. Chem Eng J 124:1–5
Scragg AH (1995) The problems associated with high biomass levels in plant cell suspensions. Plant Cell Tissue Organ Cult 43:163–170

Shaik S, Dewir YH, Singh N, Nicholas A (2010) Micropropagation and bioreactor studies of the medicinally important plant *Lessertia (Sutherlandia) frutescens* L. S Afr J Bot 76:180–186

Sharma S, Shahzad A (2013) Bioreactors: a rapid approach for secondary metabolite production. In: Shahid M, Shahzad A, Malik A, Sahai A (eds) Recent trends in biotechnology and therapeutic applications of medicinal plants. Springer Netherlands, Dordrecht, pp 25–49

Shohael AM, Chakrabarty D, Yu KW, Hahn EJ, Paek KY (2005) Application of bioreactor system for large-scale production of *Eleutherococcus sessiliflorus* somatic embryos in an air-lift bioreactor and production of eleutherosides. J Biotechnol 120:228–236

Shohael AM, Ali MB, Yu KW, Hahn EJ, Islam R, Paek KY (2006) Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. Process Biochem 41:1179–1185

Shuler ML, Hallsby GA, Pyne JW, Cho T (1986) Bioreactors for immobilized plant cell cultures. Ann N Y Acad Sci 469:270–278

Sims IM, Bacic A (1995) Extracellular polysaccharides from suspension cultures of *Nicotiana plumbaginifolia*. Phytochemistry 38:1397–1405

Souret FF, Kim Y, Wyslouzil BE, Wobbe KK, Weathers PJ (2003) Scale-up of *Artemisia annua* L. hairy root cultures produces complex patterns of terpenoid gene expression. Biotechnol Bioeng 83:653–667

Sousa RG, Riethmuller ML, Pinto AMFR, Campos JBLM (2005) Flow around individual Taylor bubbles rising in stagnant CMC solutions: PIV measurements. Chem Eng Sci 60:1859–1873

Sreedhar RV, Venkatachalam L, Thimmaraju R, Bhagyalakshmi N, Narayan MS, Ravishankar GA (2008) Direct organogenesis from leaf explants of *Stevia rebaudiana* and cultivation in bioreactor. Biol Plant 52:355–360

Srivastava S, Srivastava AK (2006) Biological phosphate removal by model based continuous cultivation of *Acinetobacter calcoaceticus*. Process Biochem 41:624–630

Srivastava S, Srivastava AK (2007) Hairy root culture for mass-production of high-value secondary metabolites. Crit Rev Biotechnol 27:29–43

Srivastava S, Srivastava AK (2012a) *In vitro* azadirachtin production by hairy root cultivation of *Azadirachta indica* in nutrient mist bioreactor. Appl Biochem Biotechnol 166:365–378

Srivastava S, Srivastava AK (2012b) Azadirachtin production by hairy root cultivation of *Azadirachta indica* in a modified stirred tank reactor. Bioprocess Biosyst Eng 35:1549–1553

Steingroewer J, Bley T, Georgiev V, Ivanov I, Lenk F, Marchev A, Pavlov A (2013) Bioprocessing of differentiated plant *in vitro* systems. Eng Life Sci 13:26–38

Su WW (1995) Bioprocessing technology for plant cell suspension cultures. Appl Biochem Biotechnol 50:189–230

Su WW, Arias R (2003) Continuous plant cell perfusion culture: bioreactor characterization and secreted enzyme production. J Biosci Bioeng 95:13–20

Su WW, Jun He B, Liang H, Sun S (1996) A perfusion air-lift bioreactor for high density plant cell cultivation and secreted protein production. J Biotechnol 50:225–233

Takayama S (1991) Mass propagation of plants through shake- and bioreactor-culture techniques. In: Bajaj Y (ed) High-tech and micropropagation I, Biotechnology in agriculture and forestry, 17th edn. Springer, Berlin/Heidelberg, pp 495–515

Takayama S (2014) Bioreactors for plant cell tissue and organ cultures. In: Vogel H, Todaro C (eds) Fermentation and biochemical engineering handbook, 3rd edn. Elsevier, Amsterdam, pp 25–36

Takayama S, Akita M (1998) Bioreactor techniques for large-scale culture of plant propagules. Adv Hortic Sci 12:93–100

Tanaka H (1982) Oxygen transfer in broths of plant cells at high density. Biotechnol Bioeng 24:425–442

Tanaka H, Nishijima F, Suwa M, Iwamoto T (1983) Rotating drum fermentor for plant cell suspension cultures. Biotechnol Bioeng 25:2359–2370

Tapia E, Sequeida Á, Castro Á, Montes C, Zamora P, López R, Acevedo F, Prieto H (2009) Development of grapevine somatic embryogenesis using an air-lift bioreactor as an efficient tool in the generation of transgenic plants. J Biotechnol 139:95–101

Terrier B, Courtois D, Hénault N, Cuvier A, Bastin M, Aknin A, Dubreuil J, Pétiard V (2007) Two new disposable bioreactors for plant cell culture: the wave and undertow bioreactor and the slug bubble bioreactor. Biotechnol Bioeng 96:914–923

Thakore D, Srivastava AK, Sinha AK (2015) Model based fed batch cultivation and elicitation for the overproduction of ajmalicine from hairy roots of *Catharanthus roseus*. Biochem Eng J 97:73–80

Uozumi N, Makino S, Kobayashi T (1995) 20-Hydroxyecdysone production in Ajuga hairy root controlling intracellular phosphate content based on kinetic model. J Ferment Bioeng 80:362–368

Wang GR, Qi NM, Wang ZM (2010) Application of a stir-tank bioreactor for perfusion culture and continuous harvest of *Glycyrrhiza inflata* suspension cells. Afr J Biotechnol 9:347–351

Wang J, Li J, Li J, Li J, Liu S, Huang L, Gao W (2017) Production of active compounds in medicinal plants: from plant tissue culture to biosynthesis. Chin Herb Med 9:115–125

Wink M (2015) Modes of action of herbal medicines and plant secondary metabolites. Med (Basel, Switzerland) 2:251–286

Zhang ZY, Zhong JJ (2004) Scale-up of centrifugal impeller bioreactor for hyperproduction of ginseng saponin and polysaccharide by high-density cultivation of *Panax notoginseng* cells. Biotechnol Prog 20:1076–1081

Zhang Z, Perozziello G, Boccazzi P, Sinskey A, Geschke O, Jensen K (2007) Microbioreactors for bioprocess development. J Assoc Lab Autom 12:143–151

Zhong JJ, Fujiyama K, Seki T, Yoshida T (1994) A quantitative analysis of shear effects on cell suspension and cell culture of *Perilla frutescens* in bioreactors. Biotechnol Bioeng 44:649–654

Ziv M (2010) Bioreactor technology for plant micropropagation. Hortic Rev 24(24):1–30