Türk Bilimsel Derlemeler Dergisi
Turkish Journal of Scientific Reviews
E-ISSN: 2146-0132, 11 (2): 05-10, 2018

Mass Production of Medicinal Plants for Obtaining Secondary Metabolite Using Liquid Mediums Via Bioreactor Systems: SETIS™ And RITA®

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Received: June 11, 2018
Accepted: October 31, 2018

Abstract
Micropropagation techniques provide effective and important ways for plant mass propagation. While some cultivated species are still accepted as comparatively resistant to tissue culture, there are a lot of successful applications of somatic and zygotic embriyogenesis, organaogenesis, micropropagation have been reported from different type of plant species. *In vitro* liquid culture systems are thought to be more efficient than semi-solid culture systems because of having great advantages of them such as successful automation, big mass production, easy handling, simple calibration of medium components and culture conditions. Temporary immersion bioreactor systems (TIS) based on liquid medium usage have been provided many utilities such as reduction of production cost and higher proliferation rate. This review aimed to describe advantages of micropropagation protocols developed using two different temporary immersion bioreactor systems, SETIS™ and RITA®

Keywords: Micropropagation, plant breeding, plant clonal propagation, TIS

INTRODUCTION

Medicinal plant mass production from embryo, cell and/or tissue cultures via bioreactor systems is encouraging for large scale industrial propagation. Bioreactor systems are largely characterized as closed and controlled systems, aseptic requirements and controlled microenvironmental conditions such as pH, temperature, oxygen, aeration, designed for liquid medium and support for monitoring (Figure 1). In bioreactor systems, final product can be biomass production such as shoots or roots, embryonic or organogenic cells, enzymes or other different metabolites [1].

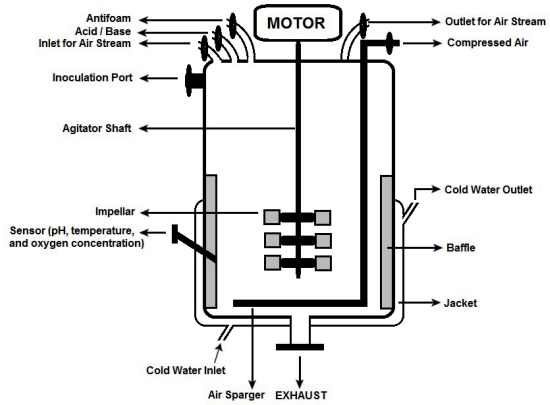

Figure 1. Typical structure of a stirred-tank bioreactor system [43].

The main purpose of cell cultures is production of economically important secondary metabolites such as pharmaceuticals, aromatic and sweetener chemicals. It is known that Approximately 20,000 metabolites have been obtained from plants, and each year about 1600 new secondary metabolites are added [2].

Most of improvement about bioreactors systems have been carried out by medicinal and cosmetic industry and there were many problems caused mass product limitation of plant cell cultures such as contaminations, low culture productivity, genetic instability, slow growth. Secondary metabolites production using bioreactor systems has big potential advantages, however, until now just only berberine, shikonin and ginsenosides have been produced in japan as big mass production [3].

Traditionally, secondary metabolites are obtained via extraction procedures using whole plants and tissues. Another conventional method for large scale secondary metabolites production is usage of high capacity *in vitro* plant cultures. Mass production using *in vitro* cultures has many advantages such as easy control of environmental conditions (independed vegetation periods of plant, all season production), standard and high quality yield production, prevention of biotic and abiotic factor effect and, opportunity of well developed system usage for big capacity production [1].

Automation of *in vitro* propagation using bioreactor systems has reduced cost of mass production of secondary metabolites [4, 5]. Liquid culture mediums are especially used in bioreactor systems for big-scale mass production of various plant cell and tissues. The first report of bioreactor system usage was on *in vitro* mass propagation of *Begonia* × *hiemalis*. In this study, erlenmeyer flasks containing Murashige and Skoog medium [6] supplemented with 1mg/ml kinetin were used and these flasks were shaken at 180 rpm for propagation of plantlets [7]. And, since that time different types of bioreactor system have been used for big scale micropropagation of different type plants species

and plant organs such as shoots, shoot tips, buds, somatic embriyos, bulbs, corms [8].

This review aimed to describe advantages of different type bioreactor systems designed for big scale mass production of medicinal plants containing economical important secondary metabolites and compared different protocols developed using two different temporary immersion bioreactor systems, SETIS™ and RITA®.

Workıng Prıncıples Of Basıc Bıoreactor Systems

Bioreactor systems provide big scale mass propagation of plant cells, tissues and organs containing zygotic and somatic embryos, nodal segments, corms, microtubers, shoots in liquid medium. For the first time usage of bioreactor system, stirred tank reactors with simple agitation turbines, extends to approximately fifty years ago. Today, many kind of bioreactor system used for different purposes are available for big scale mass production of wide range secondary metabolites from plant material. But there are still some requirements that must be overcome such as cell aggregation or fragile plant cell [9].

Agitation based bioreactor systems are grouped into three main tank systems according to their agitation construction, mechanically agitated, pneumatically agitated and non-agitated bioreactor systems. Stirred bioreactor systems (Figure 2A, B) has many limitations such as high electricity consumption and especially in tall bioreactors have sealing problems of rotating shafts. On the other hand, air lift bioreactor systems (Figure 2D-G) have many advantages such as simple design, low energy requirements and good mass transfer. There are many modify bioreactor system constructed by using advantages of traditional stirred bioreactors and air lift bioreactors [10].

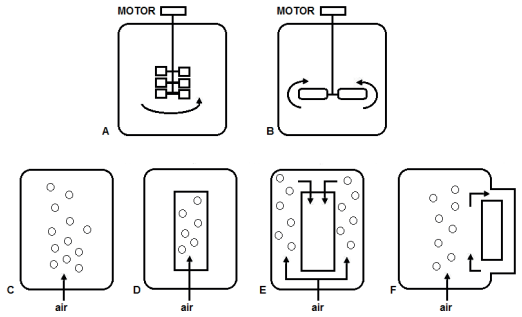

Figure 2. Structure of different types of bioreactor systems. Flat-blade turbine impeller (A) and marine propeller (B) agitation based bioreactor systems, bubble column (C, D), draft tube air lift (E) and external loop air-lift (F) bioreactor systems [43].

Air-lift bioreactor systems are very useful for mass propagation of various plant species cultures because of their simple design causing less degradation of cells, tissues, shoots and organs. However, these bioreactor systems have some disadvantages such as excessive foam formation and cell growing at the top of reactor tank. These problems can be solved by using antifoams and designing special type tanks such as larger top section diameter or balloon type bubble bioreactors [8]. Mechanically agitated, pneumatically agitated and non-agitated bioreactor systems have been utilised for the production of valuable secondary metabolites from various plant species (Table 1).

Table 1. In the last ten years, different secondary metabolite production from different plant species using different type bioreactor systems.

Bioreactor Type	Secondary Metabolite	Plant Species	Explant Type	Reference
shake flask system	hypericin	*Hypericum perforatum* L.	adventitious root	[25]
airlift bioreactor system	caftaric acid chlorogenic acid cichoric acid	*Echinacea purpurea* (L.) Moench.	adventitious roots	[26]
airlift bioreactor system	anthraquinones, phenolics, flavonoids	*Morinda citrifolia* L.	leaf cells	[27]
shake flask system	anthocyanin dyes	*Melastoma malabathricum* L.	callus cell cultures	[28]
balloon-type bubble bioreactors	ginsenosides	*Panax ginseng* Meyer	adventitious roots	[26]
shake flask system	Human serum albumin	*Oryza sativa* L.	transgenic rice seeds	[29]
airlift bioreactor system	mouse interleukin-12	*Nicotiana tabacum* L.	hairy roots	[30]
shake flask system	hepatitis B surface antigen	*Glycine max* (L.) Merr.	callus cell cultures	[31]
semicontinuous bioreactor	recombinant human alpha-1-antitrypsin (rAAT) glycoprotein	*Nicotiana benthamiana* Domin.	callus cell cultures	[32]
shake flask system	Human granulocyte-macrophage colony-stimulating factor (hGM-CSF)	*Oryza sativa* L.	callus cell cultures	[33]
two different types of bioreactor (stirred and airlift)	galphimine-B	*Galphimia glauca* Cav.	callus cell cultures	[34]
shake flask system	ginsenoside	*Panax japonicus* C.A. Mey. var. *repens*	callus cell cultures	[35]

Recently, two novel bioreactor systems based on a periodic immersion have been developed for mass propagation of different kind of plant species, SETIS™ and RITA®. The basic working principle of both bioreactor systems are to prevent complete immersion of plant material in liquid maintain medium using separated sides of culture vessels and evacuation system for periodic circulation of liquid medium. In these systems, a set of channel for liquid medium supports uniform growth of plant tissue. The liquid medium touches plant materials for different periods of time and then medium turns back to storage tank. this periodical process is controlled by an electronic system, depending on explant type.

Temporary Immersıon Systems (Tıs) For Secondary Metabolıte Productıon

In many times, liquid medium based bioreactor systems limit plant growth and complete immersion of whole plant tissues also causes malformation and loss of plant material because of hyperhydricity and asphyxia been unwanted physiological conditions [11, 12]. In these liquid cultures, complex morphological differentiation of plant material needs improvement of specific designed bioreactor systems, supporting sensitive and exclusive microenvironment for safe maintenance of cultures [13]. To overcome these limitations of liquid cultures, different kind of bioreactor systems such as temporary immersion systems TIS bioreactors have been developed. These systems are designed as simple automatic devices having many features to support ideal microenviroment, improved optimum aeration, ease nutrient intake, reduce mechanical stress and physiological disorders [14]. The optimum environmental conditions for *in vitro* propagation of plant tissues were provided by TIS bioreactor systems supplemented with periodically treatment of plant material with liquid medium [13].

Several type of TIS bioreactor systems have been developed and widely used for aromatic and medicinal plant micropropagation [15, 16]. In addition, due to their simple and functional design, these bioreactor systems have been used for aromatic and medicinal metabolite production, detoxification of toxic compound via phytoremediation, and also molecular farming [13]. Recently developed bioreactor systems, SETIS™ and RITA®, for production of secondary metabolites and foreign proteins, micropropagation, phytoremediation, molecular farming is discussed in this chapter.

SETIS™ BİOREACTOR SYSTEMS

SETIS™ (info@setis-systems.be, VERVIT, Belgium) is new style bioreactor system using TIS technology based on twin containers for mass production of plant tissues and organs (Figure 3) and these bioreactors have been used for mass production of many kind of plant species. These containers consist of two engaged vessels for liquid medium and plant material. Many limitations and problems of other TIS bioreactor systems have been solved by using SETIS™ bioreactors designed to provide the necessities of plant mass productions. These systems have been improved for optimum micropropagation of plant material. Basic, resistant, safe and ideal designed parts of these bioreactors have six components, culture and liquid medium containers, screw caps with silicone gaskets, filters for aeration, silicone tubes.

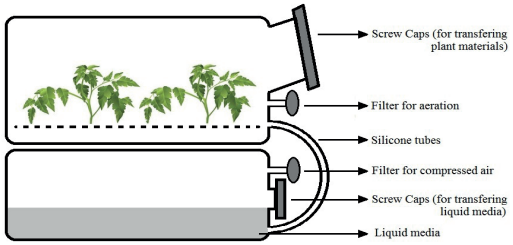

Figure 3. Structure of SETIS™ bioreactor system.

Working principles of SETIS™ bioreactor are based on three phase. First phase is constant phase, liquid media stay in the media vessel and plant material ventilates in this long phase. The second phase is immersion phase, liquid media is transferred by air compressing from medium container to growth container. During this phase, plant materials periodically intake nutrients and plant growth regulators from liquid media for mass production. The third phase is drain phase, liquid media returns to the medium container.

Rıta® Bïoreactor System

RITA® (https://www.cirad.fr, CIRAD, France) bioreactor system consisting of an autoclavable polypropylene container having two compartments has been developed for intensive mass propagation of plant tissues and organs (Figure 4). This compartments are separated by a table supported with grid and a central plastic pipe. The container is closed by a wide screw cap supported with centric connected with compressing air controlled using a timer and lateral ports secured with filters. The upper container is plant tissues and organs growth, whereas the lower container is for medium storage. RITA® is simple and safe bioreactor system and the system has compact design for supporting of enough relative humidity level for plant tissues. Connected internal elements of this bioreactor systems can be utilized as a single part for supporting mass production. Inadequacy of medium nutrient exchange and limited ventilation are the main disadvantages of these systems [17].

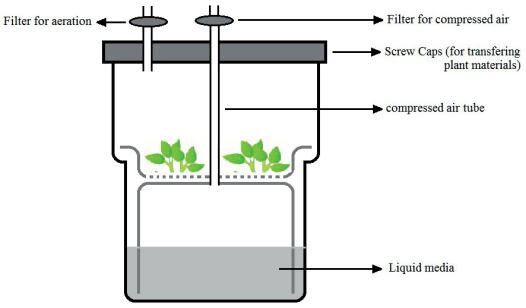

Figure 4. Structure of RITA® bioreactor system.

The RITA® bioreactor systems have been successfully used for mass production of medicinal and aromatic plants (Table 2).

Table 2. Studies on mass production of different plant species using RITA® [36].

Plant Species	Explant Type	Mass production (compared with semi-solid unless specified)	Reference
Eucalyptus grandis W.Hill ex Maiden	Hypocotyl segments	4 - 6 (in ½ time)	[37]
Hydrastis canadensis L.	Shoots	5,6	[38]
Hippeastrum x *chmielli* Chm.	Pieces of *in vitro* bulblets	2	[39]
Jacaranda decurrens Cham.	Nodal segments	1,4	[40]
Phoenix dactylifera L. (date palm)	Intact and fragmented juvenile leaves	2	[41]
Saccharum spp.	Leaf disks	9	[42]
Vaccinium angustifolium Ait. (lowbush blueberry)	3 node stem sections	3	[11]

Physıco-Chemıcal Factors Affectıng Mass Productıon Vıa Bıoreactor Systems

Biological needs for growth and development and mechanical requirements for supplying optimum conditions determined as physico-chemical factors such as dissolved oxygen and optimum ventilation, mixing of cultures, pH, medium composition, play an important role for large scale mass production of plant tissues and organs via bioreactor system.

Optimal ventilation and dissolved oxygen

The most important function of all bioreactor systems is to supply the optimal ventilation for regeneration and viability of plant tissues and organs. On the other hand, another important function of the bioreactor systems is to enrich the liquid medium with dissolved oxygen. For effective mass production, it is necessary to diffuse of oxygen into liquid medium, because of weak dissolved oxygen in water. Effective oxygen diffusion is achieved by arranging bioreactor parameters such as changing of agitation speed, aeration rate and redesigning of bioreactor configuration [18].

Mixing of liquid medium

The another important parameter is mixing of liquid medium for supporting of equal distribution of nutrients to cells, tissues and organs in the liquid phase [9]. Mixing of bioreactor contents is achieved by mechanical agitation, this agitation should be low for preventing of cell, tissue and organ damage caused from hydrodynamic forces, on the other hand, it should be support of enough uptake nutrients from liquid medium [1].

pH of liquid medium

The liquid medium pH is very effective on mass production of cells, tissues and organs in bioreactor systems. For example, liquid medium at pH 5.0 has inhibitory effect on embryogenesis of cultures [19]. The previous studies indicated that pH changes are caused by ammonium concentration of liquid medium [20,21]. Computer controlled bioreactor systems provide optimal conditions, like pH, for mass production of biological materials.

Nutrients of liquid medium

Another important factor affecting on big scale mass production is nutrients of liquid medium being a major chemical factor in bioreactor systems. To be obtain information of nutrient uptake from liquid medium, it can be provided by periodic individual nutrients measurement at different time for metabolite and mass production in bioreactors. One previous study about anlysis of different nutrient compound dynamics during *Lilium* bulblet culture in bioreactor system showed that phosphate, nitrate and ammonium run out from liquid medium, on the other hand, potassium, magnesium, calcium, sodium and chlorine were still present after sixteen weeks, but the most important limiting growth factor was carbon source in the medium [21]. There are many similar studies on different plant species, but effects of plant growth factor interactions and different compound dynamics for mass production are needed detailed future investigation.

Temporary immersion bioreactor systems for molecular farming

For production of very economical important recombinant proteins, plants provide an excellent source. Over the twenty years, molecular farming, obtaining of different proteins from plants or *in vitro* grown cells, tissues and organs of them, have been more attractive for industrial process. However, transgenic plants can produce very different product, if they grow in field and these products have limited shelf life, because they can impact from environmental conditions. These limitations can be overcome by using *in vitro* propagation techniques and there are several bioreactor systems adapted for foreign protein production from *in vitro* grown plant cell, tissue and organ cultures [22, 23]. For example, a vaccine antigen, green fluorescent protein and tetanus toxin (C fragment) were obtained by using temporary bioreactor systems from transplastomic tobacco shoots [24].

CONCLUDING REMARKS

Temporary immersion bioreactor systems are very effective for big scale mass production of economical important aromatic and medicinal plants and these bioreactors have also different usage for production of plant-derived secondary metabolites. At the same time, another application of TIS bioreactor systems are purification of phenol contaminated waste water using transgenic hairy roots but these phytoremediation applications are not still implemented for purification of industrial wastes. Because of full environmental control options and optimum nutrient uptake from liquid medium, TIS bioreactor systems are preferred techniques for mass production of transgenic plants and these systems can be also thought perfect eco-friendly bioreactors for big scale production of economical important recombinant proteins derived from transgenic plant cells, tissues and organs. These days many improvements on TIS bioreactor systems have aimed to developed low cost disposable variants such as SETIS™ and RITA®.

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