TEMPORARY IMMERSION BIOREACTOR

Engineering considerations and applications in plant micropropagation

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1. Introduction

Commercial laboratories need to produce a large number of high quality plants at the lowest possible costs of production which mainly includes labour cost, general overhead cost and the cost per unit space in the growth room. Large-scale plant propagation by using tissue culture technique is often criticized because of the intensive labour requirement for the multiplication process; thus, scaling-up of the production systems and automation of unit operations are necessary to cut down the production costs [1,2]. In order to achieve efficient and automated production in plant tissue culture, plant production systems have evolved from a small research scale to a large volume and high-yield culture system, and liquid media are preferably used to facilitate handling [3]. The use of bioreactors with liquid media for micropropagation is becoming more popular due to the ease of scaling-up [4] and the low production costs [5]. Bioreactor is a self-contained, sterile environment which capitalizes on liquid nutrient or liquid/air inflow and outflow systems, and is mainly designed for intensive culture. The basic function of a bioreactor is to provide optimum growth conditions by regulating various chemical and/or physical factors. More specifically, it affords the maximal opportunity to monitor and control over micro-environmental conditions such as agitation, aeration, temperature and pH of the liquid medium. Several types of bioreactors are currently available such as air lift-bioreactor, stirred tank bioreactor, rotating drum bioreactor, column bioreactor etc. In these bioreactors, the plantlets or explants are cultured under complete submerged condition in the liquid medium which may limit the gas exchange of the plant materials and consequently result in vitrification or hyperhydricity of plant tissues [6]. Vitrification is a severe physiological disorder involving apoplastic water accumulation, due to the extended contact between the explants [7,8]. Symptoms of vitrification include chlorophyll deficiency, cell hyperhydricity, hypolignification, reduced deposition of epicuticular waxes and changes in enzymatic activity and protein synthesis [7,8]. To avoid the problems associated with liquid culture in bioreactor, different systems have been developed, such as membrane raft system, nutrient mist bioreactor, temporary immersion bioreactor etc. [9]. Among those, temporary immersion
bioreactor has gained popularity mainly due to its simplicity and high production rate with minimum physiological disorders. In the current chapter the definition, brief historical description, designing, benefits and related problems of the system will be provided with special reference to the development of a new scaled-up system.

2. Requirement of aeration in bioreactor: mass oxygen transfer

Generally for normal plant cell metabolism, oxygen is required and only the dissolved oxygen can be utilized by plants growing in an aqueous culture medium. Therefore, in a bioreactor where oxygen transport limitations can usually be observed, aeration is required to promote the mass transfer of oxygen from the gaseous phase to the liquid phase. To meet the demand of the actively respiring plant tissues, forced-diffusion of oxygen in the liquid nutrient medium is required and this can be achieved by aeration of the liquid medium, agitation of the system, continuous shaking of the container etc. Gas-liquid oxygen transfer can be explained by using the equation of Leathers et al. [3]:

\[ OTR = K_L a (C_x - C_L) \]  

Where, \( OTR \) is the volumetric oxygen transfer rate (mmol l\(^{-1}\) h\(^{-1}\)), \( K_L \) is the mass transfer coefficient (m h\(^{-1}\)), \( a \) is the specific gas-liquid interfacial area. The terms \( K_L \) and \( a \) are generally considered together and thus \( K_L a \) in the current equation can be termed as oxygen mass transfer coefficient (h\(^{-1}\)). \( C_x \) is the dissolved oxygen concentration at equilibrium with the gas phase (mmol l\(^{-1}\)) and \( C_L \) is the actual dissolved oxygen concentration (mmol l\(^{-1}\)) in the culture medium. \( K_L a \) is frequently used to measure the efficiency of oxygen transfer in a bioreactor. Oxygen solubility increases with decreasing temperature; the dissolved oxygen concentration for 100% air saturated water at sea level is 8.6 mg O\(_2\) /L at 25\(^\circ\)C. The oxygen mass transfer coefficient is strongly affected by agitation speed, air flow rate and design of a bioreactor. In general,

\[ K_L a = k \left( \frac{P_2}{V_R} \right)^{0.4} \left( V_s \right)^{0.5} \left( N_s \right)^{0.5} \]  

Where, \( P_2 \) is the power required to aerate the bioreactor, \( V_R \) is the volume of the bioreactor, \( V_s \) is the air flow rate, \( N \) is the agitation speed. Note that the mass transfer coefficient increases with agitation speed and/or air flow rate. Most of the bioreactors designed are capable to agitate (mixing) and aerate the medium simultaneously. In some cases, such as in airlift bioreactor [10] to increase the dissolve oxygen concentration, only aeration is used. In such case, \( N \) can be counted as zero. Many bioreactors have been designed with liquid medium circulation system with the aim to improve the oxygen transport. There are usually two different mechanisms of transporting oxygen throughout the bioreactor, one is mixing and the other one is circulation. [see Curtis and Tuerk in this volume]. As described by Curtis and Tuerk, liquid circulation is a measure of how fast a fluid element gets from one side of the bioreactor to the other. Whereas, mixing means, how quickly a fluid element can be dispersed throughout the