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SHORT COMMUNICATION

Effect of oxygen supply on cell growth and saponin production in bioreactor cultures of *Panax ginseng*

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Summary

The effects of oxygen supply within the range 20.8–50% (using pure oxygen and air), on cell cultures of *Panax ginseng* were investigated in a balloon-type bubble bioreactor (5 L capacity, containing 4 L Murashige and Skoog medium, supplemented with 7.0 mg L^{-1} indolebutyric acid, 0.5 mg L^{-1} kinetin and 30 g L^{-1} sucrose). A 40% oxygen supply was found to be optimal for the production of both cell mass and saponin yielding values of 12.8 g (DW) L^{-1}, 4.5 mg (g DW)$^{-1}$ on day 25, respectively. Low (20.8%, 30%) and high (50%) oxygen concentration supplies were unfavorable to cell growth and saponin accumulation. The results indicate that oxygen supplementation to bioreactor-based ginseng cultures was beneficial for biomass accumulation and saponin production.

Introduction

Ginseng (*Panax ginseng* C.A. Meyer), a member of Araliaceae family, is traditionally considered to be one of the most potent medicinal plants. Ginsenosides (saponins) are regarded as the most important active components in ginseng roots and are attributed with cardio-protective, immunomodulatory, anti-fatigue, and hepato-protective physiological and pharmacological effects (Tang and Eisenbrand, 1992).

In recent years, plant cells have been cultured in bioreactors for production of secondary metabolites, including pharmaceuticals, pigments and other chemicals; considerable progress has been made in stimulating formation and accumulation of

Abbreviations: EC, electrical conductivity; HPLC, high-performance liquid chromatography; MS, Murashige and Skoog medium; SOUR, specific oxygen uptake rate

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secondary metabolites (Rao and Ravishankar, 2002). Growth and accumulation of secondary metabolites in bioreactors is influenced by various physical and chemical factors, including gaseous composition. In particular, oxygen supply has been shown to significantly affect secondary metabolite formation in cell cultures (e.g. Gao and Lee, 1992; Han and Zhong, 2003; Schlatmann et al., 1994). Gas exchange between the gas and liquid phases is especially important in the context of scale-up of plant cell cultures. In bioreactors, forced aeration is needed to supply oxygen and to improve fluid mixing. However, it may also lead to the removal of some known (e.g. CO_2, ethylene) or unknown gaseous components. It has been suggested that such gaseous metabolites are important for cell growth and/or synthesis of secondary metabolites (Gao and Lee, 1992; Schlatmann et al., 1994).

In the present study, ginseng cells were cultured in balloon-type bubble bioreactors and interactions between oxygen supply, cell growth, and saponin production were investigated. The significance of oxygen supply during bioreactor culture was revealed. This study is of relevance in the application of ginseng cell cultures to the large-scale production of saponin.

Materials and methods

Cell suspensions of *P. ginseng* were maintained in Murashige and Skoog (MS) medium, supplemented with 2.0 mg L^{-1} α-naphthalene acetic acid (NAA), 0.1 mg L^{-1} kinetin, and 30 g L^{-1} sucrose. The medium pH was adjusted to 5.8 before autoclaving. Cell cultures were grown in 300 mL Erlenmeyer flasks with a working volume of 100 mL and were maintained on a rotary shaker at 105 rpm in the dark at 25 °C. Cells were maintained by subculturing (6% inoculum) into fresh medium, every 15 days.

Cell cultures were also established in a 5-L capacity balloon-type bubble bioreactor containing 4 L of MS medium (working volume) supplemented with 7.0 mg L^{-1} indolebutyric acid (IBA), 0.5 mg L^{-1} kinetin and 30 g L^{-1} sucrose. Sixty grams cell fresh weight per liter was added as inoculum. In the balloon-type bubble bioreactor, a sintered-glass sparger was used for aeration. The airflow rate was maintained at 0.1 vvm using a flow meter (Dwyer Inc., IN, USA). To investigate the effects of different levels of oxygen on biomass and saponin production, the inlet air was mixed with oxygen to yield oxygen concentrations of 20.8% (control), 30%, 40%, and 50%. A schematic diagram of the experimental system is shown in Fig. 1. The cultivation temperature in the bioreactor was controlled at 25 °C and the vessel was maintained in the dark. Three identical cultivation vessels were operated under each set of conditions investigated, and the cultivation data shown represent average values with standard deviations. The bioreactor cultures were maintained for up to 30 days.

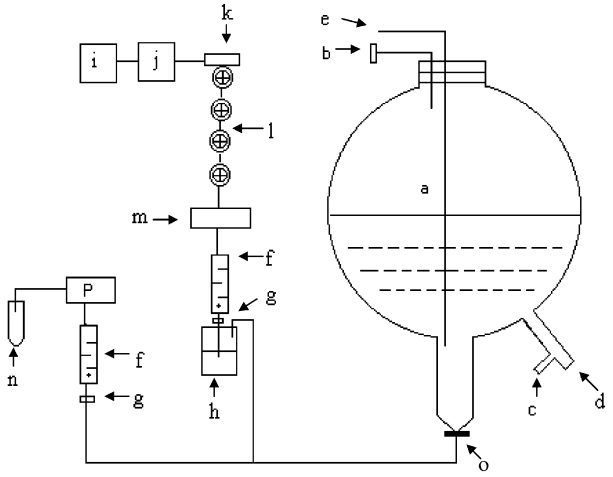

Figure 1. Schematic diagram of the balloon-type bubble bioreactor culture system: a – balloon-type bubble reactor, b – air vent, c – inoculum port (5 mm diameter), d – sampling port (10 mm diameter), e – medium-exchange port, f – air flow meter, g – membrane filter, h – water column, i – air compressor, j – air reservoir, k – air cooler, l – filter system, m – air dryer, n – oxygen tank, o – sparger, p – pressure gauge.

A 30 mL suspension sample was taken from each bioreactor every 5 days. The cell suspensions were filtered and washed several times with distilled water for the measurement of cell (fresh and dry) weights. The culture supernatants were analyzed for residual sugar using HPLC, following the analytical procedures described by Zhang and Zhong (1997). Specific oxygen uptake rate (SOUR) measurement was performed as described by Pan et al. (2000). The supernatant conductivity was determined using a conductivity meter (Model LF-54, WTW GmbH, Wielhalm, Germany).

Extraction and analysis of saponin were undertaken using the method of Yu et al. (2002). The saponin fraction was analyzed using HPLC (Waters 2690 separation module; Waters 996 photodiode array detector; Waters millennium 2010 chromatography manager) with Altec Platinum C_{18} column (particle size 1.5 μm, 33 mm $\times$ 7 mm), eluting with water/acetonitrile at 3:1 (v/v) for 10 min, then at 63:37 (v/v) for 25 min, with a flow rate of 1.2 mL min^{-1}. Saponin reference standards were obtained from Chromadex Inc., USA. Total saponin