Journal of Industrial Microbiology & Biotechnology (1999) 22, 44–47

http://www.stockton-press.co.uk/jim

Shear stress effects on plant cell suspension cultures in a rotating wall vessel bioreactor

X Sun and JC Linden

Department of Chemical and Bioresource Engineering, Colorado State University, Fort Collins, CO 80523, USA

A rotating wall vessel, designed for growth of mammalian cells under microgravity, was used to study shear effects on *Taxus cuspidata* plant suspension cell cultures. Shear stress, as quantified by defined shear fields of Couette viscometers, improved specific cell growth rates and was detrimental to volumetric product formation rates.

Keywords: RWV; shear; Taxol®; *Taxus*; secondary metabolite; plant cell culture

Introduction

Plant cell culture has promise as a source of a wide range of phytochemicals [12]. Large-scale cell culture production has in part been limited by the large size, rigid cell wall and extensive vacuole of plant cells, which render them sensitive to shear stress [18,20]. Shear stresses are generated by the impeller and bursting gas bubbles in stirred tank bioreactors [8,15,17]. At an average shear rate of 30 s^{-1} in such bioreactors, Zhong *et al* [25] found high intracellular secondary metabolite content and reduced specific growth rate. Toshiyo *et al* [21] cultured plant cells in a reactor under different impeller rotation speeds and compared the results with those of shaken flasks. Differences suggested hydrodynamic stress may affect physiological processes such as metabolism, cell growth and cell viability.

Most studies of plant cell shear sensitivity have been conducted in stirred tank reactors in which impeller types, dimensions and speeds were varied. Complex fluid dynamics in the turbulent regime make it very difficult to analyze quantitatively the shear conditions to which the cultured cells are subjected. Plant cells in immediate contact with impellers and reactor walls are more often damaged by mechanical forces and pressure changes than by shear stress [2]. Gas bubble-rising and bubble-bursting are also potential sources of cell damage [9]. Foaming and cell flotation are other common problems associated with plant cell cultures in aerated bioreactors [24].

Quantitative analysis of effects on plant cell physiology under defined shear conditions would be beneficial. A bioreactor called the rotating wall vessel (RWV) allows such analyses under laminar flow conditions because fluid hydrodynamics of Couette viscometers are approximated. Our objective was to investigate quantitatively shear stress effects on growth of cultured cells in suspension and Taxol® production.

Correspondence: Dr JC Linden, Department of Chemical and Bioresource Engineering, Colorado State University, Fort Collins, CO 80523, USA
Received 5 January 1998; accepted 8 December 1998

Materials and methods

The cell line *Taxus cuspidata* P991A2, was kindly provided by Dr DW Gibson (Plant, Soil and Nutrition Laboratory of the USDA Agricultural Research Service, Ithaca, NY, USA). The growth medium was according to Gibson *et al* [6]. Cells were subcultured every 2 weeks in 125-ml Erlenmeyer flasks; 10 ml inoculum was added to 40 ml of fresh medium. The culture flasks were capped with 28 mm i.d. Belco silicone closures and agitated at 125 rpm in a New Brunswick incubator shaker at 25°C in the dark.

The rotating wall vessel (RWV) was designed and built by Synthecon, Houston, TX, USA. The RWV was originally developed by NASA for microgravity cell culture applications in mammalian cell and tissue cultures [7,10,13,19]. No published report of plant cell cultures in the RWV has been found. The plant cell suspension totally filled the 110-ml working volume of the RWV that lay in the annular chamber between concentric outer and inner cylinders (Figure 1). Oxygen from a pressurized cylinder flowed through the inner cylinder at one atmosphere. A gas-permeable membrane covered the outer surface of the inner cylinder and served as the oxygen exchanger to the culture medium, which provided sufficient mass transfer such that the cultures were never under oxygen limitation (data not shown). The cylinders were arranged horizontally rather than vertically as in traditional Couette viscometers. The culture medium, cells and cell aggregates rotated with the vessel and did not collide with the vessel wall or any other damaging objects. Fluid dynamic shear stress, imposed by differential rotation rates of the inner and outer cylinders, was the only physical factor affecting cell behavior; other destructive forces were minimized because the system had no impellers, air bubbles or agitators. The plant cells established a uniform fluid suspension orbit within the horizontally rotating culture vessel in a laminar flow regime that is characteristic of Couette viscometers [1,22].

With the custom model of RWV used in this study, the two concentric cylinders were able to rotate independently at different velocities and directions; these were controlled through a stepper motor controller card (Cybermation, Fullerton, CA, USA). Newtonian rheology, as predicted for plant suspension cell cultures containing 4 g L^{-1} dry cell

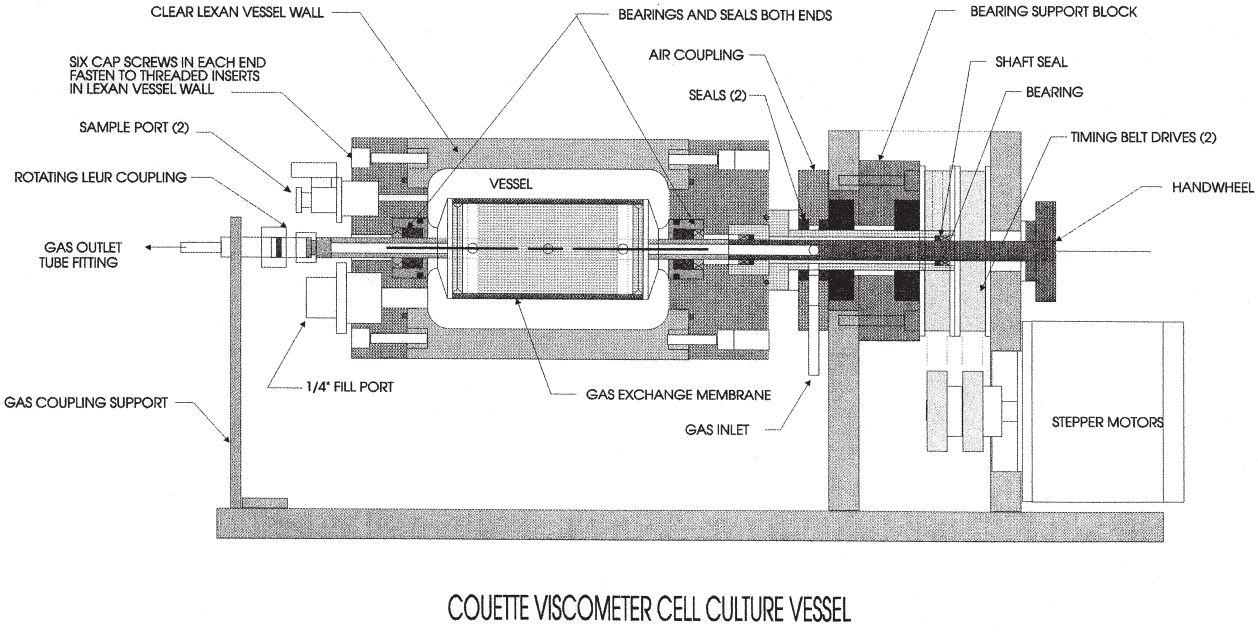

Figure 1 Schematic representation of the rotating wall vessel (RWV) system for studying shear stress effects on suspension plant cell cultures of *Taxus cuspidata*. The rotation speeds of the two concentric cylinders were computer controlled.

weight [3], was verified experimentally (data not shown). The shear rate and shear stress were readily calculated at the given operating conditions. The zero shear stress condition was obtained with rotation of both cylinders in the same direction at identical velocities. Differential rates of 10, 25, 40 and 50 rpm produced respective average shear stress values of 2.3×10^{-3}, 5.6×10^{-3}, 8.9×10^{-3} and 11.1×10^{-3} N m^{-2} with respective average shear rates of 2.1, 5.2, 8.3 and 10.4 s^{-1}. Each condition was studied only once.

For every experiment, RWV inoculation was of 33% (v/v) using 2-week-old cultures of *T. cuspidata* cells. Each batch culture lasted for 2 weeks. The RWV was maintained in an incubator at 25°C in the dark. A 5-ml sample was taken every 2 days from the RWV. Simultaneously, the same amount of fresh medium was added to the reactor, so as to allow no gas bubbles into the culture medium.

Plant cells in the samples were filtered through 47 mm diameter, 0.45 μm pore size cellulose nitrate filters (Sartorious, Edgewood, NY, USA) under vacuum and washed three times with distilled water. The cells were collected in aluminum weighing pans, which were previously dried and weighed. The aluminum pans with plant cells were dried at 70°C for 25 h and weighed again. Taxol® concentration in the filtrate was analyzed according to the method of Ketchum and Gibson [11] using standards provided by Hauser Chemical, Boulder, CO, USA.

Results

Batch culture experiments with *T. cuspidata* P991 were conducted for 14 days in the RWV at five different shear rates. Typical cell growth curves of this cell line in shake flasks are compared with those at a shear stress of 2.3×10^{-3} N m^{-2} in the RWV in Figure 2. The semi-log plots of the growth data indicate exponential growth during at least 12 days of the experiment; the fact that growth slowed after day 12 is especially obvious in linear plots (data not shown), but during the log phase, specific growth rates of 0.10 day^{-1} were obtained under these conditions.

Specific growth rates of the culture increased with shear stress to approximately 0.19 day^{-1}, which was obtained at 11.1×10^{-3} N m^{-2} shear stress (Figure 3). Under higher shear stress (8.9×10^{-3} N m^{-2} and 11.1×10^{-3} N m^{-2}), the cultures exhibited a decreased lag phase and increased specific growth rate but decreased time span of exponential growth (data not shown). At lower shear stress values, for example at 0, 2.3×10^{-3} N m^{-2} and 5.6×10^{-3} N m^{-2}, cells grew well and demonstrated growth characteristics similar to those in flask cultures. Measured dissolved oxygen concentrations of 6–14 μl L^{-1} were similar in all experiments (data not shown).

The extracellular Taxol® concentration in *T. cuspidata* cultures was a function of culture time and shear stress. Taxol production kinetics at shear stress 2.3×10^{-3} N m^{-2}

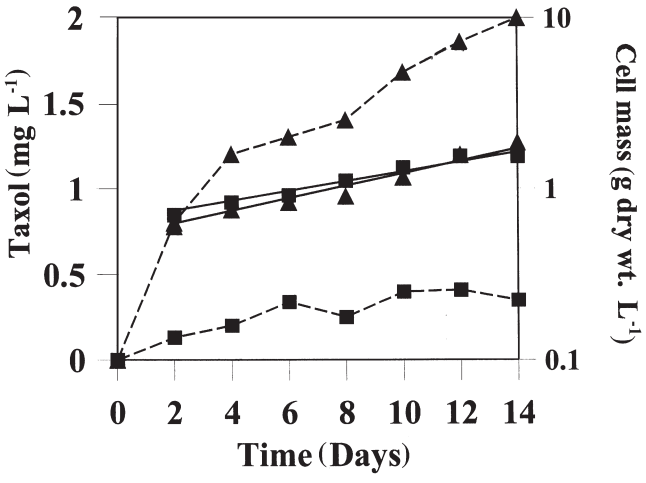

Figure 2 Cell growth and Taxol® accumulation kinetics of *Taxus cuspidata* suspension cultures at a shear stress level of 2.3×10^{-3} N m^{-2} in shake flask culture (■) and RWV with oxygen as gas component (▲). The semi-log scale on the right-hand axis and solid lines represent cell growth. Dashed lines represent Taxol® accumulation.

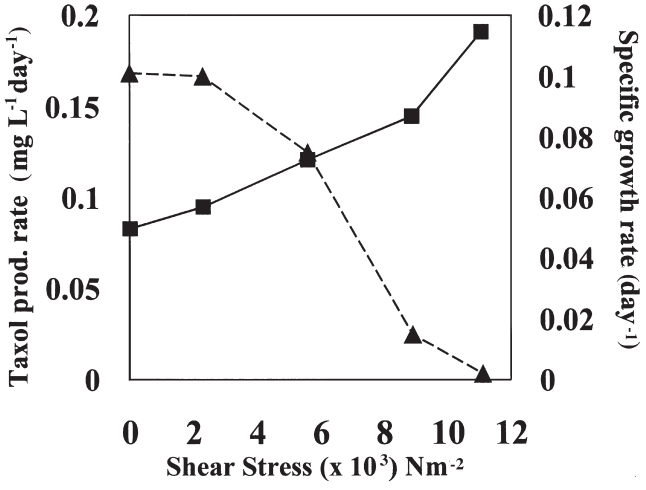

Figure 3 Specific Taxol® productivities and specific growth rates of *Taxus cuspidata* suspension cultures in the RWV operated under different shear stress levels; specific growth rate (■, solid lines) and volumetric Taxol® productivity (▲, dashed lines).

in the RWV as well as those of shake flask cultures are presented in Figure 2. Relatively little Taxol® was produced in the shake flask cultures compared to the RWV.

In Figure 3, volumetric taxol production rates are plotted as a function of shear stress values. Relatively little Taxol® was found at 11.1×10^{-3} N m^{-2} shear levels. Taxol® production rates were greater at low shear conditions and decreased with increasing shear stress. The highest Taxol® concentration obtained from this relatively poor producing cell line was about 2 mg L^{-1} on day 14 in the culture at 2.3×10^{-3} N m^{-2} shear stress.

Discussion

The results show that secondary metabolite production can be optimized with respect to shear stress. Taxol® production was suppressed under high shear conditions, at which cells may have been vitally damaged in the later stages of the culture period. Under zero shear conditions Taxol® productivity was equally as great as at moderate shear stress conditions.

Compared with shear experiments on other plant cell cultures, it was evident that this *T. cuspidata* cell line was relatively sensitive to hydrodynamic shear stress; the greatest average shear rate studied here was 10.4 s^{-1}. For example, the specific growth rates and maximum cell concentrations of batch cultures of *Perilla frutescens* were not affected by shear stress until the average shear rate was approximately 30 s^{-1} [25]. Cells grew well under average shear rate 64 s^{-1} in batch cultures of *Cinchona robusta* [23]. Under high shear conditions, plant cells appear unable to carry out normal cellular functions and eventually lose viability.

The extent to which cells possess this ability after subjection to a hydrodynamic load constitutes their tolerance to shear stress. This depends on cell lines, subculture history and other physiological conditions [18]. Cells have been shown to repair small lesions in the cell wall by deposition of polysaccharide [14] and recover from the inflicted damage. Results of other plant cell shear-sensitivity studies have been obtained by exposing plant cells to high shear rates for short times. Dunlop *et al* [5] showed a hierarchy of shear sensitivity from regrowth ability at a subtle level to aggregate breakup and lysis at a gross level. Mitochondrial activity, secondary metabolism and cellular enzyme levels show important physiological damage. There are no reports on cellular responses during the process of cell recovery from the unfavorable shear condition once the condition is released or relaxed.

The mechanism by which plant cells identify shear stress forces and convert them to electrophysiological and biochemical responses remains unclear. In animal cells, it has been observed that some molecules in the membrane can be activated by physical displacement (conformational change) or indirectly by mass transfer gradients (which change ligand-receptor interactions) [16]. Membrane structures called mechanotransductors include ion channels, G-protein linked receptors, tyrosine kinase receptors, and integrins [4]. Stimulation of mechanoreceptors generates biochemical cascades of responses at the cytoplasmic face of the cell membrane by secondary messengers, activation of protein kinases followed by activation of cytosolic transcription factors and regulation of gene transcription in the nucleus [16].

Acknowledgements

The authors acknowledge the contribution of Professor EH Dunlop, now at the University of Adelaide, Australia, for participating in the concept of the study. The support of the National Cancer Institute under project No. NIH 5 RO1 CA 55138–04, the Colorado Institute for Research in Biotechnology and the Colorado State University Agricultural Experiment Station Project 347 is acknowledged. A preliminary report of this work was presented at the RAFT II meetings in San Diego, CA in November 1997.

References

1 Bird RB, WE Stewart and NE Lightfoot. 1960. Transport Phenomena. John Wiley & Sons, New York.
2 Bronnenmeier R and H Markl. 1982. Hydrodynamic stress capacity of microorganisms. Biotechnol Bioeng 24: 553–578.
3 Curtis WR and AH Emery. 1993. Plant cell suspension culture rheology. Biotechnol Bioeng 42: 520–526.
4 Davies PF. 1995. Flow-mediated endothelial mechanotransduction. Physiol Rev 75: 519–560.
5 Dunlop EH, PK Namedy and MZ Rosenberg. 1994. Effects of fluid shear forces on plant cell suspensions. Chem Eng Sci 49: 2263–2276.
6 Gibson DW, REB Ketchum, NC Vance and AA Christen. 1993. Initiation and growth of plant cell lines of *Taxus brevifolia* (Pacific yew). Plant Cell Rep 12: 479–482.
7 Goodwin TJ, TC Prewett, DA Wolf and GF Spaulding. 1993. Reduced shear stress: a major component in the ability of mammalian tissues to form three dimensional assemblies in simulated microgravity. J Cell Biochem 51: 301–311.
8 Hooker BS, JM Lee and A Gynheung. 1989. Response of plant tissue culture to a high shear environment. Enzyme Microb Technol 11: 484–490.
9 Hua JM, LE Erickson, TY Yin and LA Glasgow. 1993. A review of the effects of shear and interfacial phenomena on cell viability. Crit Rev Biotechnol 13: 305–328.
10 Jessup JM, TJ Goodwin and GF Spaulding. 1993. Prospects for use of microgravity-based bioreactors to study three-dimensional host-tumor interactions in human neoplasis. J Cell Biochem 51: 290–300.
11 Ketchum REB and DM Gibson. 1995. A novel method of isolating taxanes from cell suspension cultures of yew (*Taxus* spp). J Liquid Chromato 18: 1093–1111.
12 Kutney JP. 1993. Plant cell culture combined with chemistry: a powerful route to complex natural products. J Am Chem Soc 26: 559–566.
13 Lewis M, DM Moriarit and PS Campbell. 1993. Use of microgravity bioreactors for development of an *in vitro* rat salivary gland cell culture model. J Cell Biochem 61: 265–273.
14 Maijer JJ, HJGT Hoopen, KCAM Luyben and KR Libbenga. 1993. Effects of hydrodynamic stress on cultured plant cells: a literature survey. Enzyme Microb Technol 15: 234–238.
15 Martin SM. 1980. Mass culture systems for plant cell suspensions. In: Plant Tissue Culture as a Source of Biochemicals (Staba EJ, ed), pp 149–166, CRC Press, Boca Raton.
16 Papadaki M and SG Eskin. 1997. Effects of fluid shear stress on gene regulation of vascular cells. Biotechnol Prog 13: 209–221.
17 Scragg AH, EJ Allah and F Leckie. 1988. Effects of shear on the viability of plant cell suspensions. Enzyme Microb Technol 10: 361–367.
18 Scragg AH. 1992. Bioreactor for the mass cultivation of plant cells. In: Comprehensive Biotechnology, Plant Biotechnology (Moo-Young M, ed), Vol 2, pp 45–62, Pergamon Press, Oxford.
19 Spaulding GF. 1993. Advances in cellular construction. J Cell Biochem 51: 249–257.
20 Tanaka H, H Semba, T Jitsufuchi and H Harada. 1988. The effects of physical stress on plant cells in suspension cultures. Biotechnol Lett 10: 485–490.
21 Toshiyo TT, M Seki and S Furusaki. 1994. Hydrodynamic damage of cultured cells of *Carthamus tinctorius* in a stirred tank reactor. J Chem Eng Jpn 27: 466–471.
22 Tsao YD, TJ Goodwin, DA Wolf and GF Spaulding. 1992. Responses of gravity level variations on the NASA/JSC bioreactor system. The Physiologist 35: 49–50.
23 Wanger F and H Vogelmann. 1977. Cultivation of plant tissue cultures in bioreactors and formation of secondary metabolites. In: Plant Tissue Culture and its Biotechnological Application (Barz W, E Reinhard and MH Zenk, eds), pp 245–252, Springer-Verlag, Berlin.
24 Wongsamuth R and PM Doran. 1994. Foaming and cell flotation in suspended plant cell culture and effects of chemical antifoams. Biotechnol Bioeng 44: 481–488.
25 Zhong JJ, K Fujiyama, T Seki and T Yoshida. 1994. A quantitative analysis of shear effects on cell suspension and cell culture of *Perilla frutescens* in bioreactors. Biotechnol Bioeng 44: 649–654.