

ELEVATED MERISTEMATIC RESPIRATION IN PLANT ROOT CULTURES: IMPLICATIONS TO REACTOR DESIGN*

DIVAKAR RAMAKRISHNAN AND WAYNE R. CURTIS

Department of Chemical Engineering and Biotechnology Institute, The Pennsylvania State University, University Park, PA 16802

Key Words: Plant Tissue Culture, Root Respiration, Biological Oxygen Demand, Meristem, Mass Transfer, Reactor Design

Introduction

Root cultures (especially *Agrobacterium* transformed roots) have been shown to have a biosynthetic capability for producing plant-derived chemicals, such as dyes, pesticides and pharmaceuticals^{2, 4}. They can also serve as an artificial seed system, for the clonal propagation of genetically screened / transgenic plants¹⁶. The market demand for root-derived phyto-chemicals and root-based artificial seed propagation systems is quite large and fiercely competitive in terms of alternative technologies; therefore, to achieve commercial potential roots need to be grown to high tissue densities in large-scale industrial bioreactors³.

A fundamental challenge of aerobic culture is supplying adequate oxygen (due to oxygen's low solubility in liquid nutrient medium). For roots, this design constraint is amplified by the resistance to bulk fluid flow within the root matrix. Therefore, unlike conventional biocatalytic systems, gas-liquid interfacial mass transfer is not necessarily the predominant design consideration because convective liquid mixing can become the dominant mass transfer resistance^{6, 13, 19}.

Despite a difference in the relative contribution of mass transfer resistance, the design objective of meeting the biological oxygen demand of root tissue is the same as any other aerobic biocatalytic system. However, in carrying out these characterizations of oxygen demand, a potentially critical aspect of root respiration has been overlooked. Most reports have estimated oxygen mass transfer requirements of root cultures on the basis of a volume averaged biological oxygen demand^{6, 7}. While this may be a very reasonable approach for suspended cell systems, it may not be applicable to the mass-transfer demand of plant root cultures, where respiration can vary along the root axis. Classic studies on plant root respiration (based on excised roots from plants and intact roots from seedlings) have repeatedly shown that biological oxygen demand is localized at root meristems by as much as 2-10 times the demand observed in bulk root tissue^{1, 9, 10}. The extent of localization has been shown to be dependent on the growth

rate and the particular plant species.

In this paper, we demonstrate elevated respiration rates in the meristematic region of *Agrobacterium* transformed root cultures of *Hyoscyamus muticus*, and show that such a localization is consistent with the hydrodynamic flow requirements others have shown necessary to overcome oxygen mass transfer limitation in root cultures.

Root Culture System

Agrobacterium transformed root cultures of *Hyoscyamus muticus* were used in the respiration experiments, since abundant data on reactor growth performance with these cultures is available^{3, 11, 15}. Cultures were grown on a gyratory shaker (2 inch stroke at 80 rpm and 25°C) and maintained on B5 medium by serial subculture every two weeks into 50 mL of medium in 125 mL flasks. Intact root segments with growing tips (in the logarithmic growth phase: 7 to 10 days old) were used in the respiration experiments.

Experimental System

A schematic of the experimental set-up is shown in Fig. 1A. The system comprises a Biological Oxygen Monitor (Model No: 5300 YSI INC., Yellow Springs, OH) with a Micro-D.O. probe (Clark-type dissolved oxygen polarographic probe) inside a 600 micro-liter cell with a magnetic stirrer. The micro-cell is enclosed in an aluminum housing through which a heat transfer fluid is circulated, to maintain constant temperature (25°C). The lid of the micro-cell was replaced with a modified Plexiglas cover with an aperture (1 mm diameter) for inserting different lengths of root tip into the micro-cell. The choice of the aperture diameter was based on microscopic measurements of the average root diameter of *Hyoscyamus muticus*. Silicone grease (used for vacuum sealing applications) was used to ensure a leak-free connection between the micro-cell and the Plexiglas lid (Fig. 1A). Leak-free operation was verified by steady D.O. readings in the micro-cell with degassed medium, in the absence of a root.

* Received on March 6, 1995. Correspondence concerning this article should be addressed to Wayne R. Curtis.

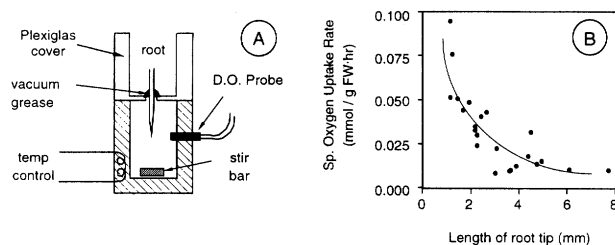


Fig. 1 Localization of respiration in transformed root cultures of *Hyoscyamus muticus*. (A) Schematic of experimental set-up of root segment in micro-cell. (B) Scatter plot showing specific oxygen uptake rate of intact root tips of different lengths (based on total length from apex)

Procedure

An aseptically grown root tip was removed from a 7–10 day old culture flask and carefully inserted into the aperture of the Plexiglas lid. Sterile air-saturated B5 medium (with 20 g/L sucrose) was then introduced into a previously leak tested, sanitized (with 5% Clorox solution) and rinsed micro-cell. This was immediately followed by the placement of the Plexiglas lid with the root tip segment. The magnetic stirrer (3mm × 8mm) was set to a speed of 1400 RPM to minimize external mass transfer resistances around the root segment and the D.O. probe's membrane. After allowing the system to equilibrate to 25°C, for a period of 5 minutes, the decrease in the dissolved oxygen in the micro-cell was monitored over the next 10 minutes. The root tip segment protruding into the micro-cell was then excised and subjected to fresh weight and geometric measurements. Length and diameter measurements were made microscopically. This procedure was repeated with about 25 root tips inserted to different depths within the cell.

Results and Discussion

Elevated meristematic respiration

Figure 1B shows the specific oxygen uptake rate as a function of root tip length. The data clearly demonstrates elevated respiration at the root meristem (by almost an order of magnitude). This corresponds to the region extending up to 1mm from the apex of the root tip. The “physiological” basis for this observation could be due to increased metabolic activity due to cell division or due to a high dry cell density (on a flesh volume basis) very near the root apex^{5, 8, 18}.

Implications to mass transfer analysis of root reactors

Results presented above, clearly indicate that the specific oxygen uptake rate is significantly higher towards the root meristem, as has been observed in “real” plant roots. The observed variations in respiration rate indicate that there are corresponding differences in mass transfer rates required to meet localized biological oxygen demand along the root axis. A ten-fold elevation in respiration would require mass transfer rates significantly higher than would be necessary for a uniformly distributed oxygen

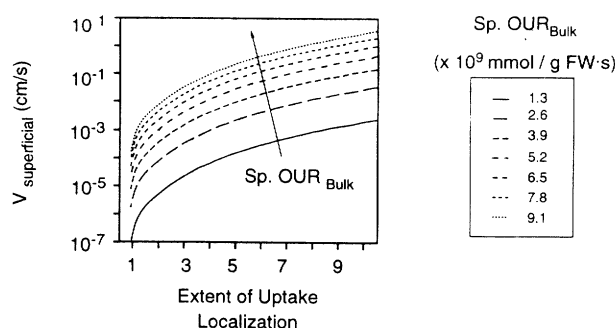


Fig. 2 Theoretical prediction of minimum liquid superficial velocities required to meet the BOD of root cultures which display different bulk and localized oxygen uptake rates ($D_r = 0.05 \text{ cm}^2/\text{s}$; $\epsilon = 0.8$). The x-axis gives the ratio of meristematic respiration to bulk respiration rate.

demand. Evidence to support this contention can be found from experimental measurements of root culture oxygen consumption under defined (liquid submerged) hydrodynamic conditions. A well defined hydrodynamic environment for root culture growth is the forced-convective liquid flow, where externally aerated/oxygenated liquid nutrient medium is pumped through a growing root bed¹⁴. The mass transfer process in such a hydrodynamic condition can be described by the following equation:

$$\overline{OUR} \cdot \left(\frac{V_r}{S_r} \cdot \rho_r \right) = k_L \cdot (C_{bulk} - C_{surf})_{mean} \quad (1)$$

where \overline{OUR} is the specific oxygen uptake rate, k_L is the mass transfer coefficient (cm/s); $(C_{bulk} - C_{surf})_{mean}$ is the mean driving force in terms of dissolved oxygen concentration at the bulk (C_{bulk}) and the surface (C_{surf}) ($= 0.5 C_{sat}$ ^{12, 19}). The most appropriate correlation for prediction of mass transfer coefficient, k_L , in a root bed would be fluid flow past a packed bed of cylinders¹⁷, as given below:

$$\frac{k_L}{v_s} \cdot Sc^{2/3} = \begin{cases} 3.8155 \cdot \left(\frac{N_{Re,p}}{1-\epsilon} \right)^{-0.7313} & \text{for } N_{Re,p} / (1-\epsilon) < 20 \\ 1.6218 \cdot \left(\frac{N_{Re,p}}{1-\epsilon} \right)^{-0.4447} & \text{for } N_{Re,p} / (1-\epsilon) > 20 \end{cases} \quad (2)$$

Eqs. (1) and (2) permit a calculation of the minimum liquid velocities required to provide mass transfer rates that meet root biological oxygen demand (Fig. 2). If oxygen requirements are localized, the bulk mass transfer rates must be high enough to meet the maximum local respiration rate. From this figure, we can see that the superficial velocity required to overcome oxygen mass transfer limitations increases by an order of magnitude with a corresponding increase in oxygen uptake localization. In a recent report on the growth of transformed roots under a convective flow environment⁶, it was experimentally shown that the root bed of *Beta vulgaris* (void fraction = 0.8846, tissue average BOD = $1.3 \times 10^{-8} \text{ mol O}_2/\text{g FW}\cdot\text{s}$)

was oxygen mass transfer limited at liquid flow rates as high as 0.59 cm/s. The corresponding theoretical estimate of the minimum superficial velocity required to overcome mass transfer limitation reveals that a flow rate in the range of about 0.003-0.19 cm/s would be sufficient to supply the bulk-tissue averaged oxygen demand (based on a typical root diameter of 0.03-0.05 cm¹⁴⁾). This finding implies that the actual root oxygen demand is higher than estimates based on bulk oxygen demand - consistent with elevated meristematic respiration. Another recent publication on the oxygen mass transfer requirements of root cultures has dealt with the experimental determination of minimum internal convective flows through root clumps¹⁹⁾. Although the internal flow profiles through root clumps are less defined, a minimum internal liquid superficial velocity was estimated on the basis of streamline flow through the center of the root clump^{13, 19)}. A theoretical prediction of the minimum internal superficial velocities (based on bulk oxygen demand) can be similarly obtained using Eqs. (1) and (2). Such a theoretical prediction (with clump densities of 9.1 & 14.4 g DW/L; $D_r \approx 0.055$ cm: personal communication, P. Doran) was found to be significantly lower than the experimentally reported minimum internal superficial velocities (e.g. $v_{theo} = 8.4 \times 10^{-5}$ cm/s, $v_{exp} = 0.39$ cm/s-for a clump density of 9.1 g DW/L). However, an estimate of the internal superficial velocity on the basis of a localized oxygen uptake (by an order of magnitude) compares well with the experimentally reported values (e.g. $v_{theo} = 0.44$ cm/s, $v_{exp} = 0.39$ cm/s-for a clump density of 9.1 g DW/L). This analytical comparison of experimental and theoretical estimates of minimum superficial velocities required to overcome mass transfer limitations further supports our conclusion that, the mass transfer requirements of root cultures should be estimated on the basis of maximum localized oxygen uptake and not on the bulk tissue average oxygen uptake.

Acknowledgments

We would like to thank William Reed and Mark Signs of the Bioprocessing Resource Center (PSU) for use of the Micro-Biological Oxygen Monitor. We would also like to thank Edgard Carvalho, Don Lucas, Kai Nielsen and Kathy Peters. This work was supported by the National Science Foundation (Grant No. BCS-9110288).

Nomenclature

BOD	: biological oxygen demand	[mmol/g FW·s]
C_{bulk}	: dissolved oxygen concentration in bulk liquid	[mmol/L]
C_{surf}	: dissolved oxygen concentration at root surface	[mmol/L]
$D.O.$: dissolved oxygen	[%saturation]
D_r	: diameter of root segment	[cm]

FW	: fresh weight	[g]
k_L	: mass transfer coefficient	[cm/s]
$N_{Re, p}$: particle Reynolds number ($D_r \rho v_s / \mu$)	[dimensionless]
\overline{OUR}	: specific oxygen uptake rate	[mmol/g FW·s]
Sc	: Schmidt number ($\mu / \rho D$)	[dimensionless]
V_r / S_r	: ratio of volume to surface area of root segment	[cm]
v_{exp}	: experimentally observed minimum liquid superficial velocity	[cm/s]
v_s	: superficial liquid velocity	[cm/s]
v_{theo}	: theoretically observed minimum liquid superficial velocity	[cm/s]
ρ_r	: density of root tissue	[g/cc]
ϵ	: void fraction of root bed	[dimensionless]

References

- 1) Berry, L.J. and W.E. Jr. Norris: *Biochim. Biophys. Acta*, **3**, 593-605 (1949)
- 2) Charlwood, B.V., K.A. Charlwood and J. Molina-Torres: "Secondary Products from Plant Tissue Culture (Charlwood, B.V. and M.J.C. Rhodes, Eds.)" p. 167-200, Clarendon Press, Oxford, England (1991)
- 3) Curtis, W.R.: *Current Opinion in Biotechnology*, **4** (2), 205-210 (1993)
- 4) Flores, H.E. and W.R. Curtis: "Biochemical Engineering VII (Pederson H., R. Mutharasan, D. Di Biasio, Eds.)" p. 188-209, The New York Academy of Sciences, New York, USA (1992)
- 5) Goddard, D.R. and W.L. Bonner: "Plant Physiology: A treatise, Vol. IA (Steward, F.C., Ed.)" p. 209-312, Academic Press, New York, USA (1960)
- 6) Kino-Oka, M., M. Taya and S. Tone: "Better Living through Innovative Biochemical Engineering (Teo, W.K., M.G.S. Yap and S.K.W. Oh, Eds.)", Proc. Third Asia-Pacific Biochem. Eng. Conf., Singapore (1994)
- 7) Kondo, O., H. Honda, M. Taya and T. Kobayashi: *Appl. Microbiol. Biotechnol.*, **32**, 291-294 (1989)
- 8) Lambers, H., A. van der Werf and H. Konings: "Plant Roots-The Hidden Half (Waisel, Y., A. Eshel and U. Kafkafi, Eds.)", p. 229-263, Marcel Dekker, New York, USA (1991)
- 9) Luxmoore, R.J., L.H. Stolzy and J. Letey: *Agronomy J.*, **162**, 322-324 (1970)
- 10) Machlis, L.: *Am. J. Bot.*, **31**, 281-282 (1944)
- 11) McKelvey, S.A., J.A. Gehrig, K.A. Hollar and W. R. Curtis: *Biotech. Prog.*, **9**(3), 317-322 (1993)
- 12) Payne, G.F., V. Bringi, C. Prince and M.L. Shuler: "Plant Cell and Tissue Culture in Liquid Systems", Hanser Publishers, Munich, Germany (1991)
- 13) Prince, C.L., V. Bringi and M. L. Shuler: *Biotech. Prog.*, **7**, 195-199 (1991)
- 14) Ramakrishnan, D. and W. R. Curtis: "Studies in Plant Science, 4: Advances in Plant Biotechnology (Ryu, D.D.Y. and S. Furusaki, Eds.)", p. 281-305, Elsevier, Amsterdam, The Netherlands (1994)
- 15) Ramakrishnan, D., J. Salim and W. R. Curtis: *Biotech. Tech.*, **8** (9), 639-644 (1994)
- 16) Uozumi, N. and T. Kobayashi: "Studies in Plant Science, 4: Advances in Plant Biotechnology (Ryu, D.D.Y. and S. Furusaki, Eds.)", p. 307-338, Elsevier, Amsterdam, The Netherlands (1994)
- 17) Upadhyay, S.N. and G. Tripathi: *J. Chem. Eng. Data*, **20** (1), 20-26 (1975)
- 18) Yemm, E.W.: "Plant Physiology: A Treatise, Vol. VIA (Steward, F.C., Ed.)", p. 231-310, Academic Press, New York, USA (1965)
- 19) Yu, S. and P. M. Doran: *Biotech. Bioeng.*, **44**, 880-887 (1994)