Physical and Morphological Constraints on Transport in Nodules

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ABSTRACT

For active nodule nitrogen fixation, O₂, N₂, and carbohydrate must be transported throughout the nodule. No quantitative analysis of these transport processes in the nodules has been presented. By invoking several simplifying assumptions, a second-order differential equation for the various gradients and concentrations in the nodule was solved. Even though the nodule can only be approximated in this analysis, it indicates clearly that intercellular gas spaces must exist in nodules for adequate O₂ distribution. To preserve low O₂ concentrations and protect the nitrogenase, these gas spaces cannot be in direct contact with the ambient atmosphere. It is hypothesized that a gas barrier exists in the cortical region of the nodule to limit O₂ diffusion. This barrier would not substantially inhibit N₂ transport. Carbohydrate transport from the vascular tissue via diffusion in the liquid phase can adequately accommodate the requirements within the nodule.

To sustain symbiotic N fixation, there are a number of physical processes involved in transporting materials about an intact nodule. Each of them may place a limit on high N-fixation rates. One process which has been given some attention is the transport of O₂ in the intact nodule.

The significance of O₂, of course, is the requirement that bacteroids have for O₂ to sustain N₂ fixation. Nitrogenase is readily inactivated by O₂ so that very low concentrations must be preserved around the enzyme. This paradoxical situation appears to be resolved by the presence of leghemoglobin which has a very high affinity for O₂ and can apparently make O₂ available to the bacteroids even at very low O₂ concentrations (3).

Tjepkema and Yocum (15) measured O₂ concentration in soybean nodules with microelectrodes. They found a substantial drop in O₂ concentration at the inner layer of the nodule cortex and a uniformly low O₂ level in the bacteroidal volume of the nodule. Sprent (14) and Bergeresen and Goodchild (2) found, in anatomical studies of soybean nodules, that there were gas-filled, intercellular spaces which were apparently continuous from the nodule surface to the central volume. On the nodule surface, Pankhurst and Sprent (9) found lenticals, which they concluded allowed gas to readily enter the nodule under turgid conditions.

No quantitative analysis of the O₂ transport process in nodules has been made to elucidate the significance of these observations. Of course, the complex geometry and biochemistry of nodules make an exact mathematical analysis impossible. Here, we solve a second-order differential equation to approximate the O₂ gradients and concentrations which may exist in nodules. Even though several simplifying assumptions are required, this analysis confirms that the intercellular air spaces are essential for gas transport in nodules and that a barrier to O₂ diffusion apparently must exist in the nodules.

Further, the solution of the second-order diffusion equation also allows an analysis of the problem of carbohydrate transport in nodules. Since vascular tissue forms a spherical shell around the bacteroidal volume, carbohydrate as a substrate must diffuse from the nodule perimeter throughout the nodule. The diffusion of carbohydrate may or may not limit N₂ fixation.

MATERIALS AND METHODS

Estimation of Flows. We will approach the quantification of the flows from a realistic agronomic goal of a nitrogen fixation rate of 2 kg N ha⁻¹ day⁻¹ (13). We will consider a soybean crop with a plant population of 2 × 10⁶ plants ha⁻¹ and with 100 nodules/plant. Each nodule then will be assumed to fix nitrogen at a rate of about 1 × 10⁻⁹ g N s⁻¹. This number will be used to estimate first the required flow of glucose and then of O₂.

Glucose is required in the nitrogen fixation process as a source of energy and carbon skeletons and as a substrate for sustaining maintenance respiration in the nodule. On the basis of the theoretical considerations of Penning de Vries (10, 11), it is possible to construct the following reaction for the fixation of 1 g N:

\[ 15.5 \text{ g glucose} + 6 \text{ g O}_2 + 1 \text{ g N}_2 \rightarrow 7 \text{ g amino acids} + 10.7 \text{ g CO}_2 + 4.8 \text{ g H}_2\text{O} \]

Experimentally, Ryle et al. (12) measured the CO₂ loss from nodules of soybean, cowpea, and clover. Expressing this loss/g nitrogen fixed, full-grown, nonsenesed nodules were found to lose 3 to 4 g CO₂/g N fixed. Converting this ratio to CO₂ loss yields 4 × 44/12 = 14.7 g CO₂ evolved, or 4.0 g more than derived above. This excess CO₂ loss can be attributed to maintenance respiration and represents an additional glucose requirement of about 2.7 g. Approximately 18 g glucose is used by the nodule during its fixation of 1 g N or, to meet the agronomic goals, about 18 × 10⁻⁹ g glucose s⁻¹ needs to be imported by a nodule.

A similar approach can be used to estimate the O₂ requirement. From the reaction above, 6 g O₂ is required in fixation itself and maintenance respiration requires 2.9 g O₂. Consequently, approximately 8.9 g O₂ is required during the fixation of 1 g N, and the agronomic goal requires 8.9 × 10⁻⁹ g O₂ s⁻¹ to be obtained by a nodule from the surrounding atmosphere.

Model. A second-order differential equation can be used to define material transport in the nodule after invoking several simplifying assumptions. It is assumed that the nodule is a uniform, homogeneous sphere because this geometry reasonably approximates many nodules and it allows an analytic solution. The biochemical events of N₂ fixation are assumed to be reasonably
represented by a Michaelis-Menten expression. The differential equation is
\[ \frac{D}{r^2} \frac{d}{dr} \left( r^2 \frac{dS}{dr} \right) = \frac{V_m S}{S + K_m} \] (1)

where \( D = \) diffusion coefficient (cm\(^2\)/s), \( r = \) distance from center of sphere (cm), \( S = \) substrate concentration (g/cm\(^3\)), \( V_m = \) maximum biochemical velocity (g/cm\(^3\)/s), and \( K_m = \) Michaelis-Menten constant (g/cm\(^3\)). To solve equation 1, the additional assumption will be made that the substrate concentration everywhere in the nodule is much greater than \( K_m \) (\( S \gg K_m \)). Therefore, equation 1 reduces to
\[ \frac{D}{r^2} \frac{d}{dr} \left( r^2 \frac{dS}{dr} \right) = \frac{V_m S}{S} \] (2)

Equation 2 is readily solved for substrate concentration by defining the boundary conditions. At the surface of the sphere (\( r = R \)), the substrate concentration is defined (\( S_0 \)). The second boundary condition is obtained by assuming an inner, concentric sphere containing senesced material (\( r = R_e \)). Since no fixation is occurring in the inner sphere at its surface there is no net movement of substrate, \( \left( \frac{dS}{dr} \right)_{r=R_e} = 0 \). Solving for \( S \):
\[ S = S_0 - \frac{V_m}{6D} \left( R_0^3 - r^3 \right) \] (3)

Since the flux/unit surface area (\( \psi \)) is given by \( D \left( \frac{dS}{dr} \right)_{r=R} \), the total flux (\( \psi \)) is
\[ \psi = \frac{V_m R}{3} \left( 1 - \frac{R_e^3}{R^3} \right) \] (4)

Finally, by multiplying \( \psi \) by the sphere surface area (4\( \pi R^2 \)), the total volume
\[ \phi = \frac{4}{3} \pi R^2 V_m \left( 1 - \frac{R_e^3}{R^3} \right) \] (5)

Equation 5, of course, confirms that the total flux is equivalent to the volumetric consumption rate (\( V_m \)) multiplied by the total volume.

Of course, if the nodule contains no senesced material, then \( R_e \rightarrow 0 \) and equation 3, 4, and 5 are further simplified. It is also evident from these equations that \( R_e \) can be a substantial fraction of \( R \) before any appreciable reduction in flux will result. Due to the third power ratio of \( R_e/R \), which reflects the volumetric ratio, \( R_e \) must approach nearly half the radius of the nodule before the flux is reduced 10\%. For convenience in the following discussion, \( R_e \) will be assumed to be negligible.

RESULTS AND DISCUSSION

O\(_2\) Transport. A basic assumption employed in the solution of equation 1 is that the substrate concentration, in this case \( O_2 \), is much greater than the Michaelis-Menten constant. For \( O_2 \), hemoglobin is assumed to initially react with \( O_2 \) and its \( K_m = 0.02 \) has been found to be very low (16). Therefore, under many conditions of diffusion the assumption of \( S \gg K_m \) would be met.

If simple diffusion of \( O_2 \) through liquid is the main mode of transport, the distance into the nodule at which anaerobic conditions exist can be estimated from equation 3 by solving for \( r \) where \( S = 0 \). To make this calculation estimates of \( D, R, S_0, \) and \( V_m \) are required. The nodule diameter will be assumed to be 0.3 cm, so \( R = 0.15 \) cm. The value of the diffusion coefficient (\( D \)) is assumed equal to that of \( O_2 \) in H\(_2\)O or 1.8 \( \times 10^{-9} \) cm\(^2\)/s. The estimate of \( S_0 \) will be that for \( O_2 \) in equilibrium between the atmosphere and the outer shell of H\(_2\)O. Therefore, \( S_0 \) is assumed to be the atmospheric concentration of \( O_2 (0.27 \times 10^{-3} \text{ g/cm}^3) \) multiplied by its solubility (0.033 at 20 C), or about 9 \( \times 10^{-5} \) g/cm\(^3\). The \( O_2 \) flow/nodule was previously estimated as 8.9 \( \times 10^{-5} \) g s\(^{-1}\). Using equation 5, \( V_m \) is estimated to be 6.3 \( \times 10^{-3} \) g cm\(^{-3}\) s\(^{-1}\).

Solving equation 3 for the distance into the nodule where conditions become anaerobic, only an outer shell of 52 \( \mu \)m thickness would be aerated. This, of course, would be an unacceptable assumption because it would mean that the entire central volume of the nodule was anaerobic and \( N_2 \) fixation would be impossible. To obtain a more satisfactory solution, the variables would have to be changed by orders of magnitude. The estimates of \( R, D, \) and \( S_0 \) are reasonably good and have limited flexibility. The estimate of \( V_m \) would have to be decreased by a factor 15 to permit \( O_2 \) to reach the center of the nodule, but then only 0.13 kg N ha\(^{-1}\) day\(^{-1}\) can be fixed.

The only resolution of the above dilemma seems to be a revision in the assumption that \( O_2 \) diffuses only through liquid. Since Pankhurst and Sprent (8, 9) observed lenticels on the nodule surface and Sprent (14) and Bergersen and Goodchild (2) observed continuous air spaces inside nodules, these air passages may allow considerable gaseous diffusion. Bergersen and Goodchild estimated that the air spaces occupied 2.5 to 5\% of the nodule volume. The value of \( D \) for \( O_2 \) in air is 4 orders of magnitude greater than in H\(_2\)O, or 0.18 cm\(^2\)/s. Assuming an "effective" \( D \) roughly proportional to the fraction of air spaces, we estimate \( D \) for a nodule with air spaces to be about 5 \( \times 10^{-9} \) cm\(^2\)/s. If the air spaces are continuous within the nodule, this new estimate of \( D \) results in a decrease of \( O_2 \) concentration in air from the nodule surface to its center of about 5 \( \times 10^{-9} \) g/cm\(^3\), which is a negligible portion of the atmospheric concentration of 0.27 \( \times 10^{-3} \) g/cm\(^3\). Therefore, the air spaces seem adequate and crucial to having \( O_2 \) transported throughout the bacteroidal volume of the nodule.

Since the air spaces provide adequate "ventilation" for \( O_2 \), equation 3 can next be used to solve for the \( O_2 \) concentration in the bacteroid-containing cells. In this case, it is clear that \( D \) must be for \( O_2 \) in liquid. Assuming the radius of the bacteroid-containing cell is 20 \( \mu \)m, the \( O_2 \) concentration at the center of the cell is calculated to be about 99.5\% of that at the cell surface. Consequently, this situation would lead to possible \( O_2 \) inactivation of nitrogenase.

A barrier to \( O_2 \) diffusion seems to be required to minimize the \( O_2 \) concentration in the bacteroid-containing volume. Fraser (6) observed a common endodermis in the cortex of nodules which she suggested would restrict gaseous diffusion. Tjeppkema and Youcum (15) suggested, from their measurements of \( O_2 \) concentration in the nodule, the existence of a barrier to \( O_2 \) transport in the inner layer of the nodule cortex. Analysis of nodule gas-exchange data also led Pankhurst and Sprent (8) to hypothesize at the diffusion barrier. Simply imposing a H\(_2\)O shell around the bacteroid-containing volume would effectively provide such a barrier. Assuming that most of the \( O_2 \) concentration drop occurs at this barrier and the flux density of \( O_2 \) (\( \psi \)) is 3.1 \( \times 10^{-7} \) g/cm\(^2\)/s, then the permeability coefficient (\( k_{O_2} \)) for \( O_2 \) for this barrier would need to be about 1.3 \( \times 10^{-4} \) cm/s. The thickness of a H\(_2\)O-layer barrier then must be about 45 \( \mu \)m. This thickness for a barrier is consistent with the concept of a continuous shell of cells completely surrounding the central volume of the nodule.

Another consideration in relation to gas transport is the potential inhibition of \( N_2 \) transport imposed by this barrier. The concentration of \( N_2 \) in the ambient atmosphere is 4 times greater than that of \( O_2 \), but its solubility in H\(_2\)O is only half of \( O_2 \). However, the flux density of \( N_2 \) is expected to be less than 15\% of \( O_2 \). For the same thickness of H\(_2\)O shell, the concentration of \( N_2 \) inside the barrier would be more than 96\% ambient. Consequently, this barrier would impose no serious limitation to \( N_2 \) transport.

It seems that anatomically nodules are very well designed to...
accommodate the dual requirement of low, but uniform, O₂ requirement throughout the bacteroidal volume. The proposed barrier at the inner cortex apparently would need to be only one or two cells thick to result in a low O₂ concentration. The intercellular air spaces would allow O₂ at the lowered concentration to be transported rapidly inside the nodule. Increases in ambient O₂ concentration up to 0.4 atm would raise the O₂ levels in the bacteroidal volume and allow greater N₂ fixation rates. The data of Bergeresen (1) suggest that, under this elevated O₂ concentration, the concentration of O₂ in the central volume of the nodule may be 0.02 to 0.03 atm. Further increases in O₂ concentration potentially inhibit the activity of nitrogenase. These increases in O₂ concentration would, however, also stimulate the respiratory activity of the mitochondria in the central volume of the nodule. Consequently, a rather complicated response to changes in O₂ concentration by intact nodule systems may result (1, 5). Thus, the barrier at the inner cortex apparently would need to be only one to two cells thick to result in a low O₂ concentration up to 0.4 atm. Further increases in O₂ concentration would apparently be adequate as a barrier to O₂. The resulting low O₂ concentrations around the bacteroids would, of course, demand the presence of an O₂ scavenger, such as leghemoglobin, to provide the O₂ required for high N₂ fixation rates (4).

**CONCLUSIONS**

This analysis indicates that the physical constraints to carbohydrate transport involved in maintaining a functioning nodule are not significant. Only a relatively small gradient in sucrose expressed as osmotic potential between the vascular shell of the nodule and its interior is required for adequate transport by diffusional processes.

On the other hand, the analysis of gaseous transport in nodules points to several important conclusions. First, it seems impossible for N₂ fixation to be sustained at high rates if N₂ and O₂ must be transported only by diffusion through the liquid. We conclude that the air spaces observed in soybean nodules by Sprent (14) and by Bergeresen and Goodchild (2) are critical for adequate ventilation of nodules fixing N₂ at a relatively high rate. The continuity and abundance of the observed air spaces are adequate to allow diffusional transport of these gases. We hypothesize that such air spaces exist in other species that exhibit high N₂ fixation rates. Second, to maintain near-anaerobic conditions around the bacteroid-containing cells, a barrier to O₂ diffusion is seemingly required in the nodule. The barrier at the inner cortex proposed by Fraser (6), Tjepkema and Yocum (15), and Pankhurst and Sprent (8) could result in the required low O₂ concentrations in the bacteroidal volume. A continuous shell of H₂O only about 45 μm thick would apparently be adequate as a barrier to O₂. The resulting low O₂ concentrations around the bacteroids would, of course, demand the presence of an O₂ scavenger, such as leghemoglobin, to provide the O₂ required for high N₂ fixation rates (4).

**LITERATURE CITED**

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