Direct Measurement of the Penetration of Ruminal Bacteria into the Lumina of Maize Sclerenchyma

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Introduction

Matrix interactions among cell wall polymers are generally believed to be the major factor limiting forage digestion by ruminal microorganisms. Research from several laboratories over the last fifteen years has suggested that the architecture of certain plant cell types may also be an important determinant of digestibility. Electron microscopic evidence suggests that plant cell walls are digested from the interior (luminal) face, apparently because of the recalcitrance of the highly lignified middle lamellae that separate the outer face of the cell walls from one another. Consequently, the ability of fibrolytic microbes to reach the interior of the plant cell—a process thought to occur primarily by diffusion—may be particularly important in long, narrow cell types such as sclerenchyma, whose cells may have lengths up to 1 mm, but luminal diameters of only a few μm. Theoretical calculations of Wilson and Mertens (1995) suggest that a bacterium of 1 mm diameter will require 4.3 days to penetrate halfway down a sclerenchyma cell of 0.5 mm length. However, direct measurements of this penetration have not been made, owing to the lack of a suitable method. The purpose of this study was to develop a suitable method for measurement of this penetration.

Theory and Methods

In principle, the penetration of cells into a porous or capillary-like structure may be determined by a solute exclusion method. This technique (Fig. 1) was originally developed by Stone and Scallan in 1968 to characterize the pore structure of wood pulps. The method uses a series of macromolecular probes (each having its own diameter) as "feeler gauges" to determine the pore diameter and total pore volume of wetted biomass material. Probes having diameters smaller than the diameters of the pores will enter the pores by diffusion, resulting in the displacement of pore water and consequent dilution of the probe solution, which can be measured by optical methods (light scattering or optical rotation). Materials that have proven successful as probes include dextrans and polyethylene glycols. For this study the method was modifed to use cells of the ruminal cellulolytic bacterium *Fibrobacter succinogenes* S85 as the probe.

The original solute exclusion technique is an equilibrium method in which the diffusion of probes into the pores continues until the concentration of the probe in the inaccessible water reaches a constant value (i.e., when influx into the pores equals efflux out of the pores). However, in this study the method was modified to measure the rate of probe dilution with time. This was possible because the bacterial cells (~ 1μm diameter) are much larger than macromolecular probes and thus will diffuse into the lumina only (not pores within the plant cell wall), and at a rate that will be much slower and thus useful for kinetic studies. One of the required characteristics of the probes is that they do not physically or chemically interact with the biomass

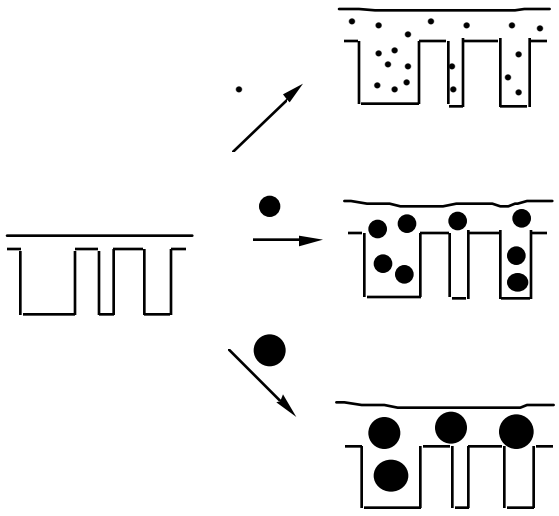

Figure 1. Schematic of the solute exclusion (Stone and Scallan 1968) technique for measuring the pore structure of wood pulps. The displacement of water resulting from diffusive entry of a molecular probe causes measurable decrease in probe concentration in the bulk liquid phase. The measurements are performed with multiple subsamples using a variety of different probes of known molecular size. Application of the data to a series of equations permits estimation of the sizes of the entire population of pores.

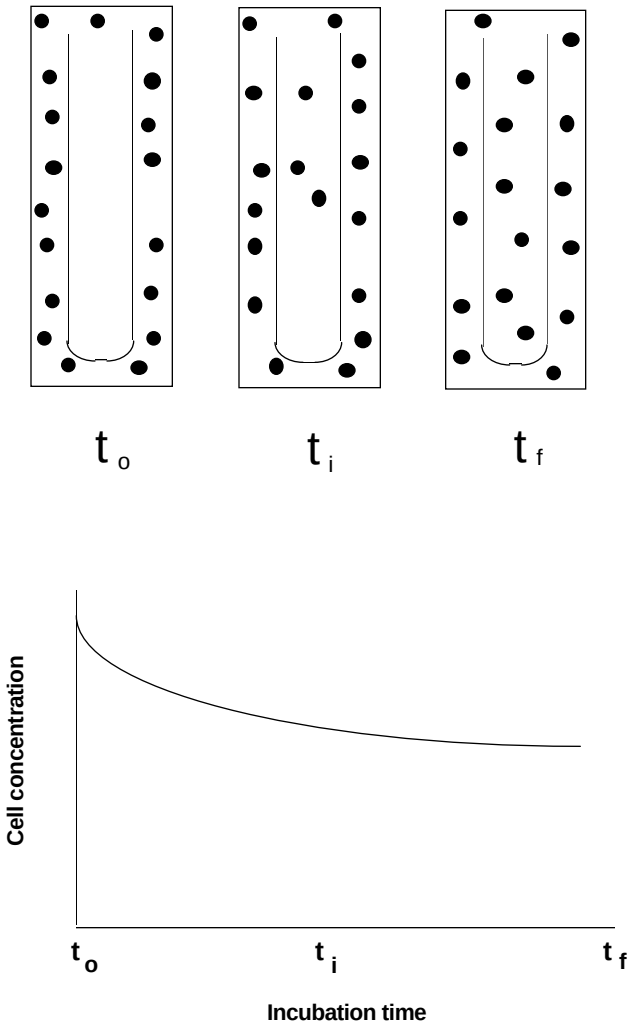

Figure 2. Modification of the solute exclusion technique to measure the rate of entry of bacterial cells into the lumina of isolated forage cell types. Top panel: Bacterial cells (illustrated as dark spheres) are allowed to displace water in the lumina of sclerenchyma cells (indicated by the open tube-like structures, not drawn to linear scale). Bottom panel: The kinetics of dilution of the original cell suspension can be related to the rate of entry of the bacterial cells.

substrate. This characteristic would appear to exclude the use of cells of the ruminal cellulolytic bacteria as probes because they avidly bind to cellulose. Fortunately, this difficulty can be overcome by conducting the experiments in the presence of methylcellulose, a soluble cellulose ether that prevents binding of cells to the cellulosic substrate. Addition of formaldehyde to the culture as a fixative prevents both an artifactual increase in cell density that would result from growth, and artifactual decrease in cell density that would result from lysis of starving cells.

Dried maize sclerenchyma (25 mg, isolated by the method of Grabber and Jung) was equilibrated in MCF solution (0.1% methylcellulose / 1% formaldehyde (v/v) in water) for 48 h, after which the solution was withdrawn with a Pasteur pipette. The wetted sclerenchyma was then resuspended in 3.00 mL of MCF containing freshly harvested cells of the ruminal cellulolytic bacterium *Fibrobacter succinogenes* S85 (A_{525} ~ 0.35). Vials were incubated with gentle (60 rpm) orbital shaking. At various intervals, 1.00 mL samples of solution were withdrawn from the vials, taking care not to disturb the rapidly settling sclerenchyma, and the absorbance of the solutions measured at 525 nm. Samples were returned to the vials after each absorbance reading.

Results

Typical results are shown in Fig. 3. Equilibration of cell density (the indicator of penetration of cells into the sclerenchyma) reaches equilibrium by about 40 h. This penetration, while somewhat faster than that predicted by Mertens and Wilson (1995), nevertheless represents over half of the retention time of large particles in the rumen. Thus, it appears that penetration of nonmotile fibrolytic bacteria into the lumina of sclerenchyma is slow and is a limitation to digestion in the rumen.

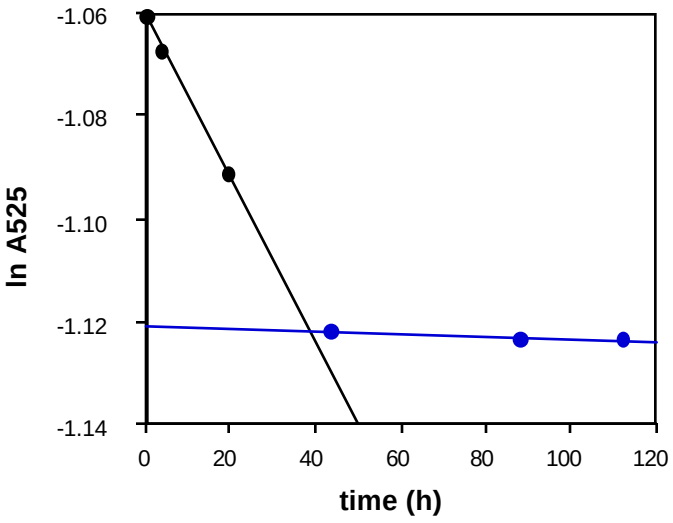

Figure 3. Diffusion of F. succinogenes S85 cells into maize sclerenchyma tissue. The time course is plotted as two lines corresponding to an initial first-order rate of dilution (r^2 = 0.999) and a plateau region where dilution reaches equilibrium (r^2 = 0.768).

Conclusion

Direct measurement of the rate of diffusion of bacterial cells into sclerenchyma tissue is possible via modification of the solute exclusion technique. These measurements indicate that the diffusion process is a potential limiting factor in the digestion of sclerenchyma tissue.

References

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