Determination of growth of anaerobic fungi on soluble and cellulosic substrates using a pressure transducer

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A pressure transducer was used to determine the accumulation of fermentation gases during growth of Neocallimastix hurleyensis and Caecomyces communis on soluble (glucose) and particulate (cellulose and wheat straw) carbon sources. The anaerobic fungi were grown in submerged batch culture in gas-tight bottles using conventional anaerobic techniques. As the fermentation progressed, fermentation gases accumulated in the head-space of culture bottles causing an increase in the head-space gas pressure. Gas was measured and released using a pressure transducer connected to a digital readout voltmeter and gas-tight syringe assembly. By repeating this gas measurement-release procedure at regular intervals during the fermentation, and summation of the calculated (regression corrected) gas volumes, gas accumulation profiles were constructed. For cultures grown on glucose, this technique enabled the growth of anaerobic fungi to be evaluated without destructive sampling of the fungal culture. The resultant gas accumulation profiles were related to glucose loss and biomass accumulation and could be used to determine specific growth rates, doubling times and fermentation gas yields. For cultures grown on cellulose and wheat straw, measurement of gas accumulation enabled growth phases and the course of the fermentation to be easily monitored. The results obtained establish the pressure transducer as an instrument for rapid, precise and reproducible determination of the growth of anaerobic fungi on soluble and particulate substrates.

Keywords: Neocallimastix hurleyensis, Caecomyces communis, anaerobic fungi, rumen fungi, pressure transducer

INTRODUCTION

Optical density procedures have been used for many years as the method of choice for rapid and non-destructive determination of the growth of bacteria cultured on soluble substrates in submerged culture. However, where it is necessary to determine mycelial growth or the growth of micro-organisms on particulate substrates, optical density methods are often inappropriate. This is because of the spatial heterogeneity of mycelial biomass and/or the interference caused by the light scattering properties of the substrate upon which the micro-organisms are growing. This is a problem of considerable significance in microbial ecology where it is often the norm for micro-organisms to grow on or at the expense of particulate substrates.

The fermentation of cellulosic substrates by anaerobic fungi is usually determined in batch culture by gravimetric measurements of dry matter loss, or by quantification of one or more of the soluble or gaseous end-products of fermentation (Akin et al., 1983; Lowe et al., 1987a, b; Mountfort et al., 1982; Mountfort & Asher, 1983). On the whole these techniques are time-consuming and involve destructive sampling of the culture under investigation, thereby requiring many replicate cultures in time-course studies. The gravimetric procedures tend to be insensitive making it difficult to measure earlier stages of growth where weight changes are small. Moreover, measurements of substrate dry matter loss are often compromised, particularly towards the end of the fermentation, by a build up of adherent microbial biomass on the extensively degraded surfaces.
An absence of simple easy-to-use, non-destructive procedures for determination of microbial growth on particulate substrates has meant that important research on the kinetics of degradation of plant cell walls has often been restricted to a study of their digestion end-points. As an alternative to the gravimetric procedures, gas accumulation in anaerobic cultures has been measured using chromatographic, manometric, volumetric or automated pressure sensing instruments (McBee, 1953; El-Shazly & Hungate, 1965; Menke et al., 1979; Taya et al., 1980; Beaubien et al., 1988; Mountfort & Asher, 1983; Pell & Schofield, 1993). Such studies, in which gas accumulation is used as a measure of carbon metabolism, are of particular interest in relation to the energy value of ruminant feedstuffs and in the digestion of anaerobic wastes and biogas accumulation (Taya et al., 1980; Beaubien et al., 1988; Menke & Steingass, 1988; Beuvink & Spoelstra, 1992; Pell & Schofield, 1993). Apart from the syringe barrel procedure described by Menke et al. (1979) and the use of hydrogen gas by the gas chromatographic procedure of Mountfort & Asher (1983), the techniques employed are generally designed to work with small sample numbers and the equipment involved can be complex and (for the computer-assisted procedures) expensive. Although the procedure of Menke et al. (1979) is relatively simple and inexpensive and can cope with larger numbers of samples, it cannot be operated aseptically.

The following report outlines the use of a new technique to follow the growth of anaerobic micro-organisms in submerged batch cultures on soluble and particulate substrates. The procedure is presented in the context of anaerobic fungal research, where we consider it essential to have a rapid and easily reproducible method for resolution of the course of the fermentation, particularly in relation to the digestion of plant cell walls. The procedure uses a pressure transducer to measure and release the fermentation gases produced by anaerobic fungi grown in batch culture on soluble (glucose) and particulate (cellulose and wheat straw) substrates. The technique has application in other areas of microbiology, microbial ecology and herbivore nutrition where studies are concerned with the growth of anaerobic micro-organisms on or at the expense of particulate substrates and surfaces.

**METHODS**

**Micro-organisms and culture.** The anaerobic fungus, *Neoalvimastix hurleyensis*, was isolated from the rumen of sheep by Lowe et al. (1985) and classified by Webb & Theodorou (1991). *Casomyces communis*, also from sheep, was isolated at INRA (CR de Clermont-Ferrand, Theix, France) and kindly provided by Professor Michel Fevre.

 Cultures of *N. hurleyensis* were maintained at 39 °C on wheat straw (10 g l⁻¹, milled to pass through a 1 mm dry mesh screen) in medium C, a complex liquid medium containing rumen fluid (Davies et al., 1993), or defined medium B, a liquid medium without rumen fluid (Lowe et al., 1985). *C. communis* was maintained as above, except that 45 g glucose l⁻¹ (25 mM) served as the carbon source. Cultures were incubated without agitation and subcultured every 5 d.

For experiments, anaerobic fungi were grown in medium B on 3.6 g glucose l⁻¹ (20 mM), 3.8 g cellulose l⁻¹ (Solka Floc grade bw 40, Brown Company), or 8.8 g extensively washed wheat straw l⁻¹ (milled as above). All cultures were grown under an atmosphere of 100% CO₂ in gas-tight serum bottles (Phase Separations Ltd; nominally of ca. 160 ml capacity, but retailed as 125 ml bottles) sealed with butyl rubber stoppers and aluminium crimp seals (Belco Glass). Prior to each experiment, the fungus was transferred (10% v/v, inoculum concentration) for up to three successive subcultures in medium containing the same carbon source as that to be used experimentally.

Glucose-grown cultures of *N. hurleyensis* were incubated in the presence of Vermiculite (2 g per bottle; Silvapearl, Sinclair Horticulture and Leisure Ltd). This technique provided an inert particulate matrix for fungal attachment and prevented the fungus from colonizing the glass walls of the culture bottle. It also facilitated determination of the weight of surface-bound fungal biomass in the glucose-grown cultures. Control cultures were identical to experimental cultures, except that they contained 10 ml sterile distilled water in place of the fungal inoculum; these were used to adjust for any weight change in the Vermiculite during the incubation.

**The pressure transducer procedure.** This technique was first described by Theodorou et al. (1994) for nutrition research. A detachable pressure transducer and LED digital read-out voltmeter (Bailey & Mackey Ltd) were used to measure the head-space gas pressure of fermenting cultures. The voltmeter was calibrated by the manufacturer to read units of pressure (p.s.i.) and was assembled and housed (by IGER electronics personnel) in a moulded plastic case (200 x 145 x 75 mm, length x depth x height; RS Components). The pressure transducer was originally designed for continuous in-line monitoring of gas pressure and had a range of 0–15 p.s.i., with an accuracy of ± 0.1%. For use in gas accumulation studies, the transducer was modified such that it could be connected to the inlet of a disposable Luer-lock three-way stopcock (Robinet three-way stopcock; Laboratoires Pharmaceutiques) and disposable membrane filtration unit (0.22 μm pore-size; Gelman Sciences). One of the two outlets of the stopcock was connected to a disposable hypodermic syringe needle (23 gauge x 1.5 inch), the second outlet was connected to a disposable plastic syringe of 5, 10, 20 or 60 ml capacity (Sterillin), according to the quantity of gas to be measured.

Bottles were warmed to the incubation temperature of 39 °C and the head-space gas pressure in each bottle was adjusted to ambient pressure prior to and just after inoculation with the anaerobic fungus. Gas pressure in the head-space was read from the LED display unit after insertion of the hypodermic syringe needle through the butyl rubber stopper (Fig. 1). The corresponding gas volume was then determined by recording the volume of gas displaced into the syringe barrel on withdrawal of the syringe plunger until the head-space gas pressure returned to ambient pressure, as indicated by a zero reading on the display unit; both pressure and volume were recorded at 39 °C. Following measurement of pressure and volume, the transducer assembly was withdrawn from the bottle closure, gas in the syringe barrel discarded and the bottle returned to the incubator until the next reading. The time taken for determination of pressures and volumes was relatively short, amounting to not more than 10–15 s per bottle. Only a few bottles were removed from the incubator at a time and thus it was assumed that the temperature (and volume) of the head-space gas remained unaltered during the measuring period. In more recent work, experiments were conducted in a 39 °C water bath or in an incubation room to avoid any problems associated with a reduction in temperature of the head-space gas.
Pressures and volumes were recorded in this way, using three or five replicate cultures at 3–24 h intervals during the fermentation period. Readings were more frequent from 20 to 60 h after inoculation (after the lag phase) when head-space gas pressures increased rapidly, requiring use of the larger syringes for gas collection. During the later part of the fermentation, when relatively small amounts of gas were produced, gas volumes were measured less frequently, using the smaller syringes. It is to be noted that volume readings are subject to variation in relation to barometric pressure. This is of consequence for comparisons of gas accumulation profiles determined at sites of different elevation, or at the same elevation where measuring small volumes of gas during slow gas accumulation. In these situations, correction factors, given by the ratio of site pressure to standard sea-level pressure, must be used to scale the volume measurements.

**Dry matter determination.** The weight of fungal biomass produced in glucose-grown cultures with Vermiculite, or the residual dry matter of cultures grown on cellulose or wheat straw, were determined by washing the entire culture (culture supernatant plus Vermiculite, or particulate substrate plus attached fungal biomass) with distilled water through pre-weighed filter crucibles (Sintaglass porosity 1 crucibles, 70 ml capacity; Gallenkamp) using vacuum filtration. Vermiculite, or particulate residues with their associated fungal biomass, were freeze-dried to constant weights. For *C. communis* cultures grown on glucose, fungal biomass was harvested by centrifugation, washing procedure and freeze-dried to constant weight as above.

**Glucose analysis.** Culture supernatants (10 ml) were collected for determination of glucose concentration and stored at −20 °C until required for analysis. Trinder reagent (Sigma diagnostics No. 315) was used to measure glucose concentration. This method relies upon the enzymic production of gluconic acid and hydrogen peroxide, the latter compound being analysed colorimetrically using a discrete analyser (Lab-systems) via a reaction involving peroxidase to produce a quinainmine dye.

**Data processing and statistics.** A PC-compatible spreadsheet program (Quattro-Pro, Version 2.1; Borland International Inc.) was used for data processing. This software includes a linear regression facility and was used to calculate regression-corrected values for gas volumes and rates of gas accumulation from semi-logarithmic plots. A Micro Vax 3600 computer (Digital Equipment Corp.) with Genstat 5 (1987) or MLP (Ross, 1987) was used for statistical analysis.

**RESULTS**

**Performance of the pressure transducer, conversion of head-space gas pressure to fermentation gas volume and construction of gas accumulation profiles**

When the mean pressure readings from each set of cultures were plotted against their corresponding mean gas volumes, the linear relationship shown in Fig. 2 was obtained. These results show that pressures and volumes, as determined by the pressure transducer, were linearly related over the pressure range tested with a typical regression coefficient, $r^2$, of 0.98. Essentially similar relationships were obtained when pressure readings from individual bottles were plotted against corresponding gas volumes. Comparable results were obtained in numerous other experiments (not reported herein) where different, but predictable and equally reproducible relationships were obtained from bottles of different sizes and/or containing different volumes of liquid and/or head-space gas. In the current study, where fermentations were conducted in 100 ml medium in 160 ml bottles, a common expression:

$$V = XP + I$$

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**Fig. 1.** The pressure transducer assembly and digital display unit for measurement of head-space gas pressure and volume. With the stopcock open between the transducer and syringe needle, via the membrane filter, the syringe needle was inserted through the bottle closure and the head-space gas pressure recorded on the digital display. The stopcock was then rotated through 180° to open the link to the syringe barrel and plunger, and the plunger withdrawn from the barrel until the head-space gas pressure returned to ambient pressure as indicated by a reading of zero on the display unit. The pressure transducer assembly was then withdrawn from the bottle, the volume of gas collected in the barrel was recorded, the gas discarded and the bottle returned to the incubator until the next reading.

**Fig. 2.** Typical relationship between head-space gas pressure and gas volume. The line of best fit was obtained from linear regression analysis of data within the range of 0–7 p.s.i. ($r^2 = 0.97$).
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of glucose of fungal thalli. Cultures were inoculated with 10 ml inoculum and 2% (w/w) Vermiculite as an inert substrate for attachment and incubated at 39°C for up to 674 h without agitation.

Specific growth rates ($\mu$), doubling times ($T_d$) and regression coefficients ($r^2$) were determined from cultures in the exponential phase of growth, from 23 to 84.5 h after inoculation, using data from linear regression analysis. The solid and dotted lines in (b) represent the lines of best fit from gas accumulation and gravimetric measurements, respectively.

where $V$ = volume, $P$ = pressure, $X$ = slope and $I$ = intercept value (bias correction factor), could be used to describe the relationship between volume and pressure for all sets of data derived from bottles of similar size and with the same liquid and head-space volumes. However, to correct for small differences between bottles, caused by small variations in medium volume associated with the dispensing of liquids and/or small variations in manufactured bottle size, regression equations for individual bottles (with bias correction) were used in all gas accumulation studies to predict gas volumes from their experimentally determined gas pressures.

Gas accumulation profiles were determined by summation of the regression-corrected gas volumes from each set of replicate culture bottles after subtraction of gas which accumulated in the corresponding set of control cultures, incubated without substrate.

**Growth of N. hurleyensis and C. communis on defined medium B containing glucose as the carbon source**

Growth of N. hurleyensis on defined medium B containing 3.6 g glucose l$^{-1}$ (20 mM) is shown in Fig. 3(a). A semi-logarithmic plot of the gravimetric and gas accumulation data (Fig. 3b) showed that the fungus grew exponentially from 23 to 84.5 h after inoculation. Linear regression analysis of the dry weight data from the exponential phase of growth gave a specific growth rate for biomass ($\mu_b$) of 0.056 h$^{-1}$ ($\pm 0.004; r^2 = 0.97$) and a biomass doubling time ($T_d$) of 12.4 h. Analysis of the gas accumulation data gave a specific growth rate for gas accumulation ($\mu_g$) of 0.055 h$^{-1}$ ($\pm 0.001; r^2 = 0.99$) and $T_d$ of 12.6 h. Both biomass and biogas indicators showed that the culture entered stationary phase ca. 84 h after inoculation (Fig. 3b), but subsequent autolysis of the fungus, as indicated by a decrease in dry weight, was not shown by the gas accumulation data (Fig. 3b). A maximum of 120 mg biomass per culture was produced prior to autolysis, and the mean gas volume in stationary phase cultures was 156.9 ml (Fig. 3a, b). Glucose utilization during the exponential phase of growth was inversely related to both biomass and gas volume, with cultures entering stationary phase on depletion of the carbon source (Fig. 3a). Linear regression analysis of a semi-logarithmic plot of the glucose loss data (Fig. 3b) showed that the fractional rate of loss of glucose from the culture was 0.061 h$^{-1}$ ($\pm 0.009; r^2 = 0.87$), equivalent to a $T_d$ for glucose loss (glucose half-life) of 11.3 h.

During exponential growth, but not during autolysis, highly significant correlations were observed between glucose loss and culture dry weight ($r^2 = 0.94$), between glucose loss and culture gas accumulation ($r^2 = 0.86$) and between biomass dry weight and culture gas accumulation ($r^2 = 0.90$) (Fig. 4a-c, respectively). From the lines of best fit shown in Fig. 4, regression coefficients ($r^2$) of 372 mg biomass (g glucose)$^{-1}$ and 389 ml gas produced (g glucose)$^{-1}$ were determined. Consequently, during exponential growth on glucose, there was a 1:1 relationship between gas accumulation (ml) and the production of fungal biomass (mg).

Similar results (not shown) were obtained for C. communis when grown on defined medium B containing glucose. For this species, $\mu$ values of 0.092 and 0.094 h$^{-1}$, and biomass $T_d$ values of 7.5 and 7.4 h were obtained from dry weight and gas accumulation data, respectively.

**Growth of N. hurleyensis on defined medium B containing cellulose or wheat straw as the carbon source**

Growth of N. hurleyensis on defined medium B containing 3.6 g cellulose l$^{-1}$ (in the form of Solka Floc) is shown in Fig. 5. From the semi-logarithmic plot for gas accumulation and linear regression analysis, it was determined...
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Culture dry wt (mg)

100 200 300 400

Glucose loss (mg)

100 200 300 400

Fig. 5. Semi-logarithmic plot of gas production from N. hurleyensis grown on defined medium B containing 3.6 g cellulose l⁻¹ as the carbon source. Cultures were inoculated with 10 ml inoculum and incubated at 39 °C for up to 340 h without agitation. Specific growth rates (μ), doubling times (T₅₀) and regression coefficients (r²) were determined from cultures in exponential phase, from 22 to 107 h after inoculation, using data obtained from linear regression analysis.

that the fungus grew exponentially from 31 to 151 h after inoculation, with a μₛ of 0.026 h⁻¹ (± 0.001; r² = 0.99) and a T₅₀ for gas accumulation of 26.7 h. Growth on this form of cellulose was relatively slow and accompanied by a lengthy deceleration phase resulting in a mean cumulative gas volume in stationary phase (from 220 to 320 h after inoculation) of 163 ml gas. Gravimetric determination of culture residue at the end of the fermentation period showed that the cellulose was extensively digested, giving a final dry matter loss of 89.5% (± 0.18).

Growth of N. hurleyensis on defined Medium B containing 8.8 g wheat straw l⁻¹ is shown in Fig. 6. From the semi-logarithmic plot of gas accumulation and linear regression analysis, it was determined that the fungus grew exponentially from 33.5 to 57.5 h after inoculation, with a μₛ of 0.080 h⁻¹ (± 0.006; r² = 0.99) and T₅₀ for gas accumulation of 8.7 h. Growth on wheat straw resulted in a mean cumulative gas volume in stationary phase (from 120 to 195 h after inoculation) of 195.5 ml. Gravimetric determination of culture residue at the end of the fermentation period showed that the wheat straw was digested to give a final dry matter loss of 49.1% (± 0.6).

Based upon the assumption that the 1:1 biomass/biogas ratio for growth of N. hurleyensis on glucose also held for growth on cellulose, the amount of fungal biomass produced during the cellulose fermentation was 0.163 g. However, given that 89.5% of the cellulose was digested during the fermentation, substantially less fungal biomass (i.e. up to a maximum of 0.04 g dry matter) was associated

Fig. 4. Linear correlations of (a) gas accumulation and culture dry weight, (b) glucose utilization and culture dry weight, and (c) glucose utilization and gas accumulation, during exponential growth of N. hurleyensis on defined medium B containing 3.6 g glucose l⁻¹. See legend to Fig. 3 for details of batch culture.
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Fig. 6. Semi-logarithmic plot of gas accumulation from *N. hurleyensis* grown on defined medium B containing 8.8 g extensively washed wheat straw l⁻¹ as the carbon source. Cultures were inoculated with 10 ml inoculum and incubated at 39 °C for up to 200 h without agitation. Specific growth rates (μ), doubling times (T_d) and regression coefficients (r²) were determined from cultures in exponential phase, from 33 to 57.5 h after inoculation, using data obtained from linear regression analysis.

with the cellulosic residue at the end of the fermentation period. This finding suggests that the fungus was extensively autolysed by the end of the fermentation period and that fungal biomass made a negligible contribution to the weight of the fermentation residue, although it may have contributed to gas accumulation following autolysis.

**DISCUSSION**

Many factors contribute to the generation of gas by fermenting cultures, including the type(s) of microorganisms involved, the rate of growth, the concentration and nature of the growth-limiting nutrient(s), the buffering system/capacity of the medium and the fermentation stoichiometry. These factors have not been examined in detail here, although some have been the subject of other studies (Beuvink & Spoelstra, 1992; Theodorou *et al.*, 1994). The main objective of the current work was to validate the use of a simple pressure transducer procedure for rapid determination of growth of anaerobic fungi cultured on soluble and particulate substrates.

Axenic cultures of anaerobic fungi were grown on a medium which contains predominantly bicarbonate but also phosphate buffering systems. Under these conditions, gas will be produced from biomass growth, but as a consequence of two different processes: (1) the formation of gaseous end-products (CO₂ and H₂) via fermentable substrates; and (2) the release of CO₂ from bicarbonate buffer as a consequence of neutralization of organic acids (formic, acetic, lactic acids, etc.) produced via fermentation. A small amount of gas is also likely to contribute to the gas pool from the recycling of moribund microorganisms particularly during the latter incubation period. Thus, although it is relatively easy to measure gas accumulation with a pressure transducer, the interactions which ultimately determine the amount of gas produced, via fermentation end-products, buffering systems and recycling phenomena, are quite complex. Nevertheless, for anaerobic fungi growing on glucose, there was a 1:1 agreement between specific growth rates as determined by dry weight and gas accumulation measurements.

There are numerous methods for direct estimation of microbial biomass, namely protein, lipids, lipid phosphorous, nucleic acids, chitin, etc. Except for chitin, none of these methods can be used to follow the growth of anaerobic fungi on particulate plant biomass and indeed this is the kind of situation where the present technique will be most useful. Moreover, none of these accepted methods for biomass estimation provide an instantaneous measurement of growth. This is likely to be of significance in transducer studies where one wishes to assess growth both rapidly and non-destructively, in response to toxins or stimulants for example.

There have been many studies to show that growth and fermentation of anaerobic fungi are linked. For example, Lowe *et al.* (1987a, b) showed that the fermentation end-products, formate and acetate, could be used as a convenient indicator of microbial growth, and Mountfort & Asher (1983) demonstrated that hydrogen yield reflected growth yield and that hydrogen production could be used as a convenient estimate of microbial growth. In our studies we have found it imperative to vent culture bottles particularly during the rapid gas accumulation phase. This avoids a build up of gaseous components in the head-space which ultimately reduces the rate of gas accumulation of the culture. This effect is likely to be related to the removal of hydrogen as was described for co-cultures of anaerobic fungi and rumen methanogens. In the absence of methanogenic bacteria, growth and activity of the anaerobic fungus was reduced and hydrogen rather than methane accumulated in the gas phase (Bauchop & Mountfort, 1981; Bernalier *et al.*, 1990, 1991; Marvin-Sikkema *et al.*, 1990). This result appears similar to that described for *Ruminococcus albus* and *Methanobrevibacter ruminantium* (Wolin, 1975). Use of hydrogen by the methanogen results in a lower hydrogen partial pressure, thereby allowing re-oxidation of co-factors such as NADH, reduced during glycolysis. As a consequence of this inter-species hydrogen transfer, the metabolism of the primary organism favours the production of electron-sink products at the expense of less reduced compounds. Such a shift in metabolism will lead to an increase in energy (ATP) production for the primary organism, thus explaining the increase in biomass, biomass activity and products noted in some studies. Physical removal of hydrogen from anaerobic fungi, by frequently venting cultures, may have the same effect as its removal via inter-species hydrogen transfer, thereby
accounting for the observed decreases in gas accumulation and activities in non-vented as compared to vented cultures. We have measured gas composition in the headspace during fungal fermentations and most is composed of a mixture of CO₂ and H₂. At this stage in our research, however, we are unclear about the partition of gas between direct production via fermentation end-products (CO₂ and H₂) and indirectly (CO₂) as a consequence of medium acidification. Autolysis in anaerobic fungal cultures is likely to produce only a small quantity of gas, presumably during the latter incubation period.

With a pressure transducer, it is possible to obtain an entire growth curve from a single culture bottle (i.e. the method is non-destructive). Unlike gravimetric determinations, where it is often difficult to obtain sufficient microbial biomass (or particulate substrate loss) for quantitative analysis at the early stages of incubation, such information is relatively easy to obtain from gas accumulation data. The technique is straightforward and relatively inexpensive, exhibiting a high degree of precision and reproducibility. When considered as a procedure for quasi-continuous measurement of mycelial growth in submerged culture, perhaps this is the most significant characteristic of the technique. Although we have demonstrated the potential of the pressure transducer procedure by observing growth of anaerobic fungi on soluble and cellulosic substrates, the technique could make a contribution to the study of surface colonizing micro-organisms generally. For example, the pressure transducer technique could be used to screen for better plant cell-wall degrading organisms and/or to determine the effect of modification of the cellulase system on the kinetics of degradation of plant biomass. To this end, the pressure transducer procedure has already been used to screen numerous anaerobic fungal isolates for their ability to degrade plant cell-walls (unpublished results of authors). The technique has also been used in preliminary studies to determine the rate and extent of digestion of modified (transgenic) plant cell-walls and of laboratory-grown plant cell cultures.

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