Plant Physiol. (1984) 75, 582–585
0032-0889/84/75/0582/04/$01.00/0

A Highly Sensitive, Flow Through H_2 Gas Analyzer for Use in Nitrogen Fixation Studies[1]

Received for publication December 20, 1983 and in revised form March 7, 1984

DAVID B. LAYZELL*, GLENN E. WEAGLE, AND DAVID T. CANVIN
Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 4L1

ABSTRACT

Studies of H_2 evolution by N_2 fixing systems are frequently limited by an inability to accurately measure H_2 gas concentrations of less than about 10 microliters per liter. In this study, a H_2 gas analyzer is described which is able to accurately and reproducibly detect up to 100 times lower H_2 concentrations than most thermal conductivity gas chromatographs or other conventional instruments used for the measurement of H_2 gas. This high level of sensitivity (maximum of about 0.02 microliter per liter H_2 per millivolt output) and the ability to continuously monitor H_2 concentration directly in a flowing gas stream, makes this instrument well suited for use in an open gas exchange system.

Since the sensor used in the instrument was also sensitive to other combustible gases, it was necessary to demonstrate that H_2 was the only combustible gas produced by the N_2 fixing system being studied. When an air stream was passed through a pot containing nodulated soybean (*Glycine max* L.) roots, gas chromatographic analysis of the effluent gas stream revealed that H_2 was the only combustible gas present. These results were supported by other studies in which no combustible gases were detected in the effluent gas stream from soybean roots nodulated with USDA 110, a *Rhizobium* strain which displays active uptake hydrogenase activity.

Traditionally, rates of C_2H_2 reduction and H_2 evolution in N_2 fixing legumes have been determined in sealed vessels of known volume in which changes in gas concentration are measured with time. H_2 accumulation in the vessels equivalent to a change of 10 $\mu l\ l^{-1}$ or more, can be detected using thermal conductivity gas chromatography. The closed system, however, does not permit the maintenance of a constant gas composition around the nodules and small leaks or removal of samples can introduce further errors. These latter problems are largely alleviated in open gas exchange systems where gas composition is constant and sampling or minor leaks do not affect pressure or composition of the atmosphere. However, since the gas of interest is not allowed to accumulate within the assay vessel, much higher levels of sensitivity are required for its detection. This is generally not a problem for the measurement of C_2H_2 reduction, as evidenced by recent studies in which open gas exchange systems have been used (3, 5). However, the best current instruments would usually not be sensitive enough to detect the H_2 produced.

In this study, we describe a highly sensitive H_2 analyzer which is capable of continuously monitoring a change of as little as 0.1 $\mu l\ l^{-2}$ of H_2 in a flowing gas stream. The instrument is a modification of a type of gas chromatograph described previously (1) and its high sensitivity makes it well suited for use in an open gas exchange system.

MATERIALS AND METHODS

Electronics. The central components of this analyzer are two gas sensors, (Figaro model No. 812, Southwest Tech. Prod., CA) each containing a heated n-type semiconductor. The semiconductor material absorbs O_2 gas onto its surface and in the presence of a combustible donor gas (*e.g.* H_2), electrons are passed from the donor gas to the O_2. The resultant decrease in semiconductor conductivity is electronically converted to a voltage differential and amplified (for use with a chart recorder).

The electronic circuitry employed here (Fig. 1) was similar to that of Christman and Hamilton (1). Our major modifications were the removal of the display circuitry, and the incorporation of a second gas sensor having a separate zero and gain control. Although the sensors were identical, one was used for the continuous detection of combustible gases in a flowing gas stream (channel A), while the other was used as a gas chromatography detector (channel B), as described below. The channel A sensor was wired such that it could be set up remote (to 12 feet or more) from the instrument. Separate switches controlling the sensor heaters permitted independent heating of each sensor. Previous studies (1; Figaro Gas Sensor, Technical Information) indicated that a heater warm-up period of 30 to 120 min was required to obtain stability in sensory conductance. A linearizing chip (LH0094CD, Honeywell Corp.) was used to provide a linear signal to the chart recorder since a logarithmic relationship existed between the sensor conductance and H_2 concentration. A multiposition selector switch was installed to adjust the degree of linearization required for each sensor. This switch may be replaced with a potentiometer (100 ohm). The 'span' control, allowed adjustment of recorder output at sensor saturation to a maximum of 1.7 v. The data presented in this report were collected with the maximum recorder output adjusted to 500 mv.

Continuous Flow System. The remote, channel A sensor (Fig. 1) was used for the continuous detection of combustible gases in a flowing gas stream. The gases to be analyzed were dried using a magnesium perchlorate column (6 mm × 15 cm glass) and moved past the sensor at a flow rate of 40 to 400 ml/min. The gas sensors used in this instrument were originally constructed to permit gas flow through the center of the sensor casing. However, when used in this way, the sensor response was found to be markedly affected by flow rate of the gas stream being analyzed (1). Better stability with flow rate was achieved at the expense of slightly (about 15%) lower sensitivity by sealing the exit port in the sensor with tape, and directing the sample stream past the sensor rather than through it. Using this design, the response of the analyzer was found to be, at 350 ml/min, 70% of its response at 40 ml/min.

[1] Supported by the natural Science and Engineering Research Council of Canada (D. B. L.), Queen's Advisory Research Committee (D. B. L.) and Agriculture Canada (D. T. C.).

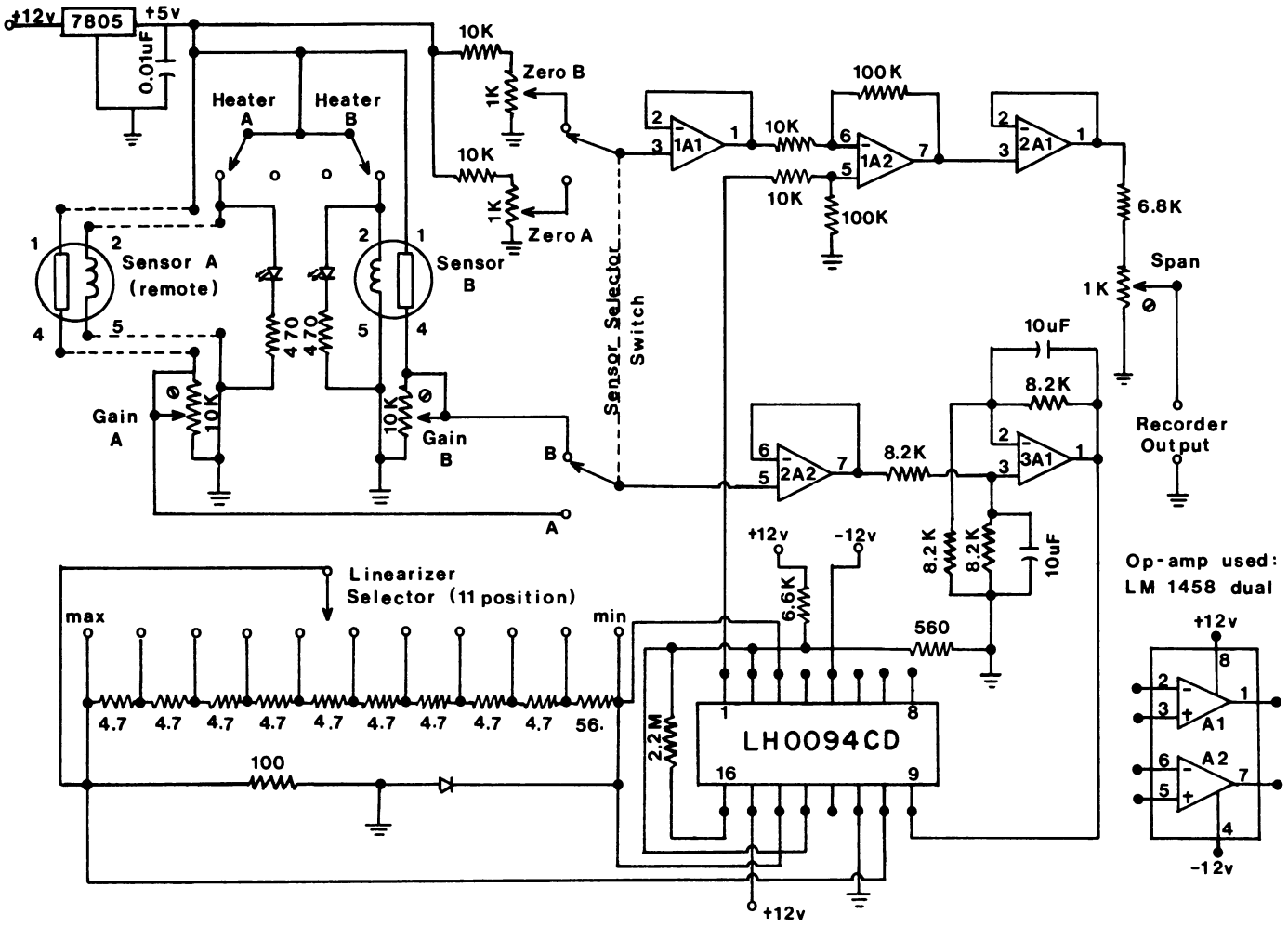

FIG. 1. Schematic of electronic circuit for the H_2 gas analyzer. A standard, regulated ±12-v DC (1 amp) power supply is required. Alternatively, suitable batteries may be used. See text for full description.

Gas Chromatographic System. The second gas sensor (sensor B, Fig. 1) was incorporated in a gas chromatographic system similar to those described previously (1, 2, 4). Dry compressed air was passed (75 ml min^{-1}) by an injection port made from a brass fitting (4) and through a stainless steel chromatography column and magnesium perchlorate drying column (6 mm × 10 cm glass) before reaching the sensor. Two-column packing materials were found to be of use: Porapak N (100–120 mesh) (1/8″ × 2 m stainless steel column, at room temperature) for separation of H_2, C_2H_2, and C_2H_4 and Molecular Sieve 5A (80 to 100 mesh) (1/8″ × 30 cm stainless steel column, at room temperature) for separation of H_2, CH_4, and CO.

RESULTS AND DISCUSSION

Instrument Performance. Calibration of the instrument was carried out using a set of four gas mixing pumps (Woestoff, Calibrated Instruments, Ardsley, NY) to provide various concentrations of H_2 in air. At any single H_2 concentration and instrument setting, continuous monitoring of the flowing gas stream resulted in an instrument output 16 to 50 times greater than that obtained by injection of 1.0 ml samples into a gas chromatographic system (Fig. 2). Through simple adjustment of the instrument gain control, it was possible to vary instrument output from 0.02 to 25 $\mu l\ l^{-1}\ H_2$, mv^{-1} in the continuous flow system, or 1.0 to 250 $\mu l\ l^{-1}\ H_2\ mv^{-1}$ in the gas chromatographic system (1.0 ml samples). Using the continuous flow system, accurate and highly reproducible measurements of 0.1 to 10 $\mu l\ l^{-1}\ H_2$ (instrument gain set at 0.1 $\mu l\ l^{-1}\ mv^{-1}$) were possible.

The greater sensitivity of the instrument when used as a

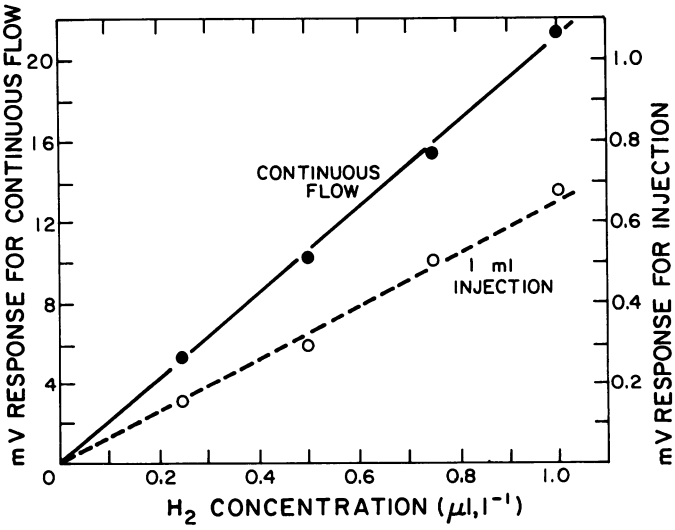

FIG. 2. Response of the analyzer at near-maximal gain settings to H_2 concentration either in a continuously flowing gas stream (●——●) or in an 1.0 ml sample injected into an air stream (75 ml/min) flowing past the sensor (O- - -O).

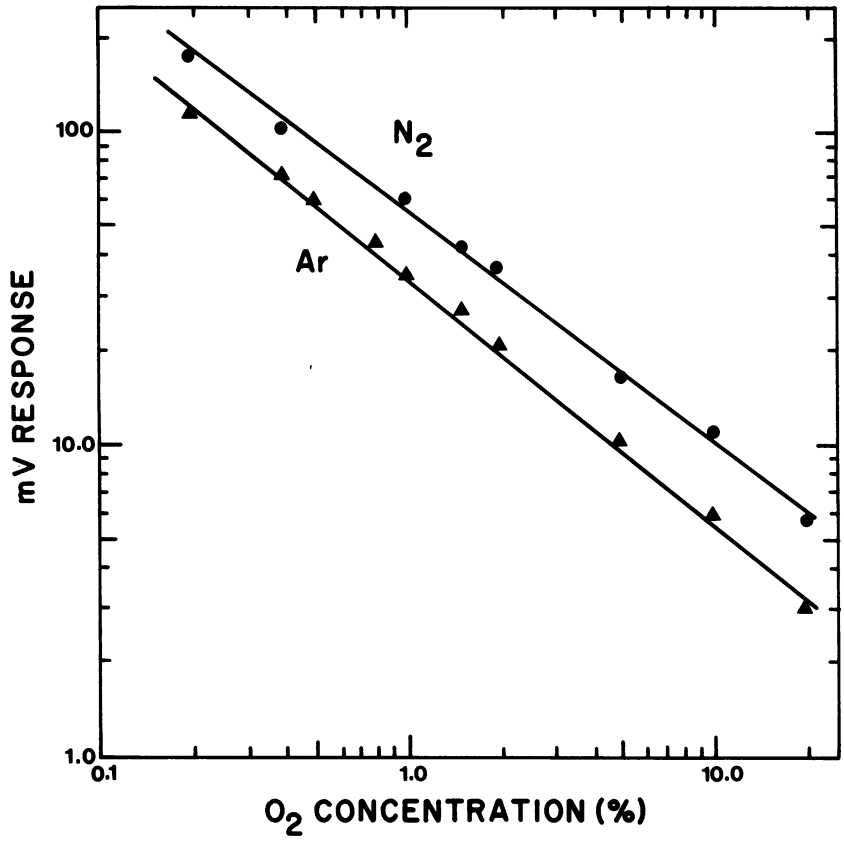

FIG. 3. Effect of O_2 (0.2–20%) concentration on the response of the analyzer to 10.9 μl l^{-1} H_2 in a continuously flowing gas stream containing the balance of gas as either Ar (▲) or N_2 (●).

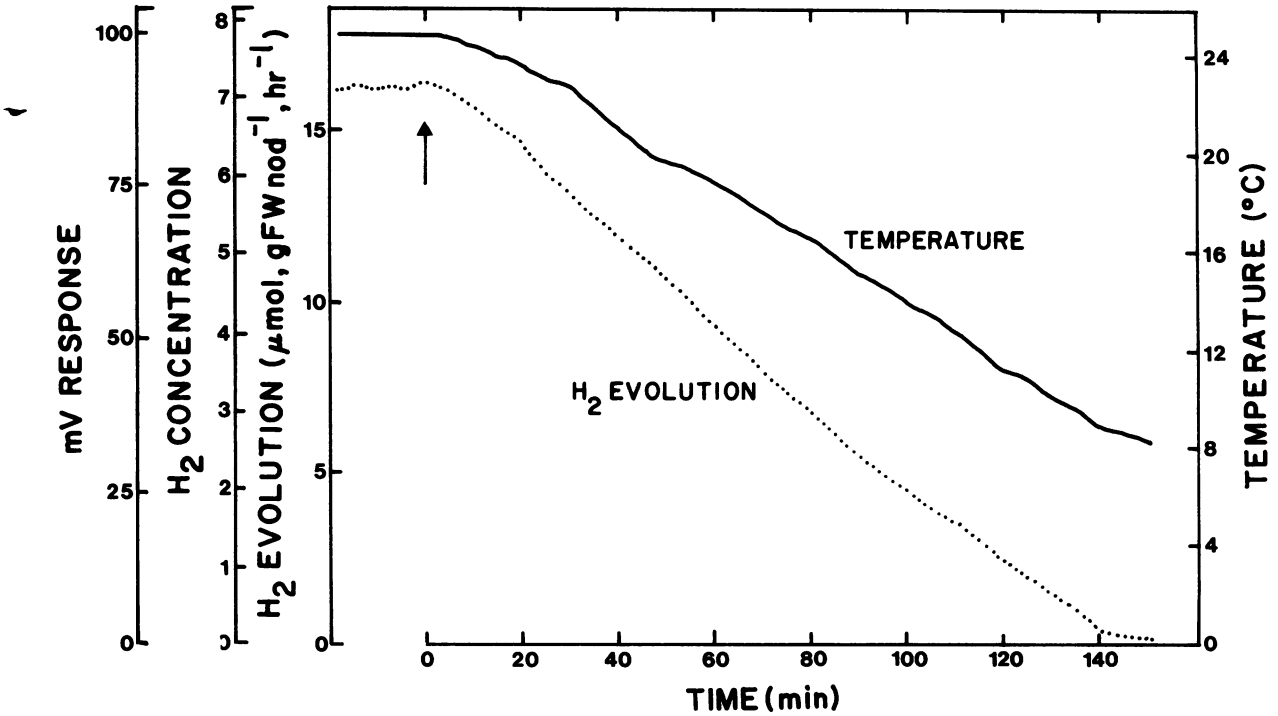

FIG. 4. Time course of H_2 evolution (· · ·) from intact, nodulated soybean roots (0.24 g fresh wt nodules) following transfer (arrow) to conditions where root temperatures (——) were declining. Measurements were made using sensor A (Fig. 1) in a continuously flowing air stream (230 ml min^{-1}) and the results are expressed as the mv response of the instrument, with the corresponding H_2 concentration and H_2 evolution rate.

continuous flow rather than a gas chromatographic analyzer was primarily due to the relatively slow response time of the sensor. Following exposure of the sensor to a change in the concentration of combustible gas, approximately 20 to 120 sec were required to obtain a maximal instrument response. Since the residence time in the presence of the gas chromatographic sensor was small, the response of the analyzer was only a fraction of that obtained in a continuous flowing gas stream. The presence of water vapor in the air increased both the response time and baseline drift. Consequently, replacement of the magnesium perchlorate filter was necessary every 6 to 24 h of operation assuming an air flow rate of about 150 ml/min. Flow rate and

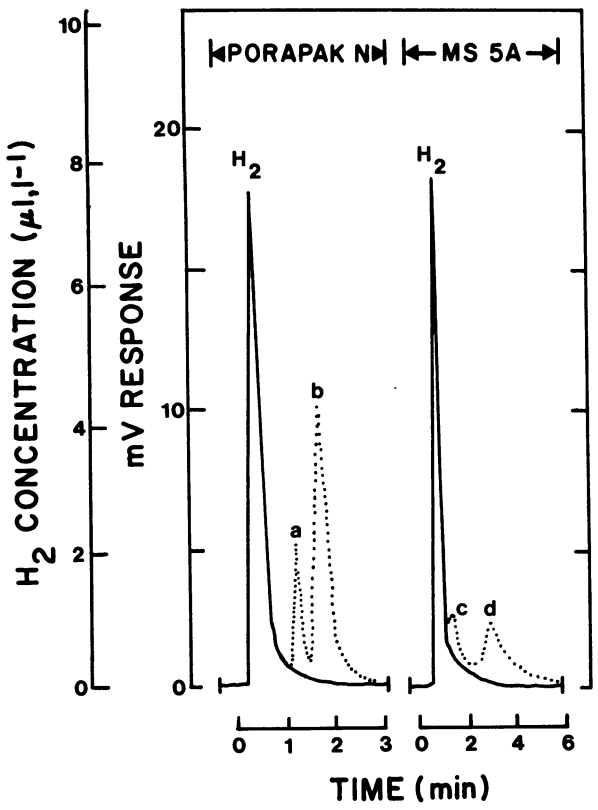

FIG. 5. Gas chromatographic traces of 2.5-ml samples of gases taken from the effluent gas stream of the plant described in Figure 4 with roots held at 25°C. Samples were injected onto columns packed with either MS 5A or Porapak N and were detected on sensor B (Fig. 1) of the analyzer which was adjusted to maximum sensitivity. The dotted lines represent the position and size of 10 μl l^{-2} concentrations of other gases, included ethylene (a), acetylene (b), methane (c), and carbon monoxide (d). Peaks in these regions were not detected in the effluent gas stream, nor were any other peaks detected having retention times of less than 30 min.

O_2 concentration were also important, with flows of less than 509 ml min^{-1} or subambient O_2 concentrations resulting in increased response times. Finally, long term (about 48 to 72 h) exposure of the sensor to low (0 to 50 μl l^{-1}) H_2 concentrations resulted in a gradual lengthening of the response time. This problem was corrected by periodical exposure (10–30 s) of the sensor to high (0.1 to 50%) concentrations of H_2 gas in air.

Effect of Other Gases. The sensors used in this instrument were sensitive to all combustible gases. Similar concentrations of C_2H_2, CH_4, CO, and NH_4 displayed instrument responses 1.27, 1.70, 0.34, and 0.03 times that for H_2 gas, respectively, when measured in a continuous flow system. Since any of these gases would have interfered with the measurement of H_2, it was necessary to determine whether or not they were present within the analytical gas stream. This problem will be discussed in more detail below.

Although the sensor was not able to detect other, noncombustible gases, some of these gases were found to alter the sensitivity of the instrument to H_2 gas. Figure 3 shows the effect of O_2 concentration in either N_2 or argon on the response of the analyzer to a single concentration of H_2 gas. A power-curve relationship was observed such that a 10-fold higher instrument response was obtained under 1% O_2 than under 20% O_2. In addition, when N_2 was the primary constituent of the gas phase, the instrument response was 1.6 times higher than that obtained with the balance of the gas as argon. CO_2 concentrations (0 to 5%) had no apparent effect on the sensitivity of the analyzer to H_2 gas.

Operation in an Open Gas Exchange System. The instrument was employed in an open gas exchange system in which a flowing gas stream of H_2-free air was passed through the root zone of a potted, nodulated legume and subsequently dried, first in an ice water trap and then in a glass column (6 mm × 12 cm) of magnesium perchlorate. These driers were found to be critical in maintaining a stable baseline and quick detector response. The dried gas stream then entered an aluminum or plexiglass detector block, and flowed past gas sensor A (Fig. 1) before venting to the atmosphere.

In a typical application (Fig. 4), the rate of combustible gas production from a nodulated soybean root was monitored first under a constant root temperature (25°C), and then during a temperature drop of about 0.1°C min^{-1}. The mv output of the analyzer continuously declined with temperature reaching a value equivalent to 0 μl l^{-1} H_2 at 8°C.

Since the analyzer was sensitive to a variety of combustible gases, including H_2, CH_4, CO, or C_2H_4, it was necessary to demonstrate that H_2 was the only combustible gas produced by nodulated soybean roots. This was done by removing 1.0 to 5.0 ml samples from the input and effluent gas streams and injecting these into the gas chromatographic system employing detector B (Fig. 1) of the analyzer. The larger samples (2.0 to 5.0 ml) were used to improve the detection limits of the analyzer when used in a gas chromatographic system. In all studies to date, H_2 has been the only gas detected in the effluent gas stream from chambers containing nodulated legume roots (Fig. 5). In addition, the levels of H_2 detected using the gas chromatographic system were in good agreement with the instrument response obtained using the continuous flow sensor. Consequently, it was determined that in the N_2 fixing roots studied, H_2 was the only significant combustible gas produced. This conclusion was strongly supported by the observation that no combustible gases have been detected in the effluent gas streams of pots containing nonnodulated roots or in a soybean symbiosis infected with USDA 110, a strain which lacks H_2 evolution due to the presence of an active uptake hydrogenase (3).

Acknowledgments—We appreciate the assistance of Dr. Scott Edie in providing the data on the effects of O_2 concentration on the response of the analyzer to H_2. Thanks are due to Bob Marjoram and Roy Young (Biomedical Engineering Unit, Queen's University) for construction of the H_2 analyzer and to J. Bollen for typing the manuscript.

LITERATURE CITED

1. CHRISTMAN NT, LH HAMILTON 1982 A new chromatographic instrument for measuring trace concentrations of breath hydrogen. J Chromatogr 229: 259–265
2. HOLFELD HS, CS MALLARD, TA LARUE 1979 Portable gas chromatograph. Plant Soil 52: 595–598
3. LAYZELL DB, P ROCHMAN, DT CANVIN 1984 Low root temperatures and nitrogenase activity in soybean. Can J Bot. In press
4. MALLARD TM, CS MALLARD, HS HOLFELD, TA LARUE 1977 Portable gas chromatograph for the acetylene reduction assay for nitrogenase. Anal Chem 49: 1275–1277
5. WITTY JF, FR MINCHIN, JE SHEEHY 1983 Carbon costs of nitrogenase activity in legume root nodules determined using acetylene and oxygen. J Exp Bot 34: 951–963