A Sensitive Technique for the Rapid Measurement of Carbon Dioxide Concentrations

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ABSTRACT

A method has been developed to measure concentrations of CO₂ in gases rapidly. A gas sample is injected into a flowing carrier gas that passes through an infrared CO₂ analyzer. A strip chart recorded peak response is obtained which is proportional to the CO₂ concentration. A resolution of better than 2 microlitres of CO₂ per litre of gas was obtained. Seven to 10 seconds were required for sample analysis once the sample was obtained. Sorghum bicolor plant respiration was determined at different temperatures by measuring CO₂ using this system and by using a conventional system. The correlation between techniques was 0.996, and about the same variation occurred within each method. This technique greatly increased the efficiency of the infrared CO₂ analyzer in our laboratory for use in plant respiration and photosynthetic studies.

The concentration of CO₂ in gases is frequently measured in environmental and biological studies. Both respiration and photosynthesis can be monitored by measuring CO₂ exchange. Because analysis of a large number of samples is often necessary, the method for measurement should be rapid and sensitive. The IR gas analyzer is the instrument most commonly used to measure CO₂ concentrations. Flow-through systems which monitor CO₂ exchange of plants or plant parts enclosed in a chamber usually depend on the detection of a 10 to 30 μl/l CO₂/l concentration differential between input and exhaust air (3, 7-9, 12). The CO₂ differentials can be related to net photosynthesis or respiration. CO₂ concentrations of the air surrounding photosynthesizing plants can also be monitored and a concentration level maintained by automatically injecting CO₂. The amount of CO₂ added is equal to net photosynthesis (5, 11). In the field, photosynthesis may be determined aerodynamically by determining the CO₂ gradients within the crop canopy (4, 6, 10).

In many photosynthetic studies determination of CO₂ concentrations requires an IR gas analyzer to monitor continuously a single CO₂ exchange chamber, or several chambers may be monitored consecutively with a sample switching device at the analyzer while continually purging the sampling lines. After switching, 1 to several min equilibration time is usually necessary to stabilize the analyzer and obtain a reliable reading (13).

A method for measuring CO₂ in small volumes of gas has been described which was used in a working range generally between 1,000 and 15,000 μl/l CO₂ (1). With proper calibration, CO₂ concentrations of samples as low as 30 μl/l were measured.

In this paper a technique is described for rapidly measuring CO₂ concentrations in a range of 2 to 400 μl/l. Preliminary results concerning this technique have been previously reported (2).

MATERIALS AND METHODS

The basic system consisted of an IR gas analyzer, mv recorder, flow meter, and drying columns (Fig. 1). Tygon tubing was used to connect the system. The tubing lengths from the tee connector to the IR gas analyzer were the same to balance line resistance. The flow rate of the carrier gas (usually N₂) was adjusted to approximately 0.6 l/min. At this flow, a narrow based peak was recorded on the strip chart recorder and only measurement of peak heights was necessary to obtain the CO₂ concentration of the injected gas sample. A 10-ml or less sample was injected with a syringe through a short section of surgical tubing in the sample line. The gas sample passed through the drying column for water removal and then through the IR gas analyzer where the CO₂ was detected with the instrument response being traced by the mv recorder. Total time between injections was approximately 7 to 10 sec. The recorder was adjusted so that the analyzer's signal output gave near a full scale recorder deflection upon injection of the maximum CO₂ concentration that was expected to be measured in a particular series of samples.

Glass syringes (such as B-D multitit, 10 ml) with the plunger coated with mineral oil and the needles stopped with Teflon caps were preferred for taking samples.

Respiration at different temperatures was determined on grain sorghum (Sorghum bicolor [L.] Moench) plants enclosed in plastic chambers in a growth chamber. Air was pumped through the chambers and the CO₂ differential between the inlet and exhaust ports was monitored with a differential IR gas analyzer. Three replications at each temperature were used. A 10-ml sample of air was taken with a syringe from both inlet and outlet port and the CO₂ differential calculated by our method. Respiration rates of the plants were determined on a dry weight basis for each method. The analysis of variance, correlation between the methods, and the per cent coefficient of variation were calculated.

RESULTS AND DISCUSSION

Different CO₂ concentrations were obtained by injecting volumes of gas of 10 ml or less from a 455 μl/l CO₂ standard. For example, 2 ml represented 91 μl/l, 4 ml represented 182 μl/l up to 10 ml which represented 455 μl/l CO₂. A linear relationship existed between CO₂ concentration and analyzer/recorder re-

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2 Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.
sponses (Fig. 2). To check the validity of using sample fractions for constructing a standard curve, 10 ml of commercially prepared standards of 190, 310, and 455 µl CO₂/l were injected. A plot of the response of the commercial standards is also shown in Figure 2. The values of these standards fell on the response line with variation from the standard curve well within the tolerance of the analysis. Thus, fractions of a standard gas can be used for low concentrations which would reduce the need for stocking of a number of costly commercially prepared gas standards.

The precision of consecutive 6-ml injections of the standard gas is shown in Figure 3. The maximum difference between peak heights was 1%, and the average difference was 0.5% of full scale recorder response. A gas flow rate through the system of approximately 0.6 l/min minimized the influence of the injection rate; however, the injection procedure should be standardized.

Sensitivity could be increased by adjusting the strip chart recorder (subtracting voltage) to give an incipient mark where the minimum concentration to be used was injected and then expanding the scale for maximum response when the highest concentration to be used was injected. Figure 4 shows the results of calibrating the system for operation within the range of approximately 200 to 325 µl CO₂/l. In this case each division represented 1.62 µl CO₂/l which was a 3-fold increase in sensitivity when compared to the calibration in Figure 2.

Respiration of sorghum plants in relation to temperature was determined by measuring CO₂ using the syringe sampling technique or by continuous monitoring with an IR analyzer. Results are shown in Table I. Statistically there was no difference between the methods. The correlation between the techniques was 0.996. The coefficient of variation within each method was 21% for the conventional technique and 26% for the syringe sampling technique. The determination of CO₂ concentrations by our method was as reliable as directly measuring CO₂ with an IR analyzer.

The importance of this technique for measuring CO₂ concentra-
tions is its utility. CO₂ concentrations can be measured rapidly by our method. Measurement of CO₂ concentrations by this procedure is suitable for adaptation to most of the systems used for measuring photosynthesis and respiration. A particularly important advantage of our method is the release of the IR analyzer from a system in which it is an integral part, thus increasing the research potential of the instrument. A single IR gas analyzer was used to measure CO₂ concentrations of three different experiments on net photosynthetic rates on the same day. This included a field experiment which was 8 km from the laboratory where the IR CO₂ analyzer was located. Our procedure for measuring CO₂ concentration is the basis for development of a new portable method for measuring photosynthesis in the field (12). A paper will follow on the details of this method. This technique for measuring CO₂ also has potential for use in measurement of CO₂ in air pollution monitoring, decarboxylation enzyme reactions, CO₂ compensation points of plants, and many other processes involving CO₂ use or production.

LITERATURE CITED
1. ATKINS CA, JS FATE 1977 An IRGA technique to measure CO₂ content of small volumes of gas from the internal atmospheres of plant organs. Photosynthetica 11: 214-216
2. CLEGGE MD, CY SULLIVAN 1975 A rapid method for measuring carbon dioxide concentrations. Agron Abstr p 70
4. GILBERT TJ 1971 Carbon dioxide profiles and apparent diffusivities in corn fields at night. Agric Meteorol 8: 51-57

Table I. Plant respiration at different temperatures as determined by continuous monitoring of CO₂ differentially with an infrared analyzer or by using the syringe sampling system.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Respiration (mg CO₂/g dry wt·hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional</td>
</tr>
<tr>
<td>25</td>
<td>2.15</td>
</tr>
<tr>
<td>26</td>
<td>1.03</td>
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<tr>
<td>Coefficient of variation (%)</td>
<td>21</td>
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</tbody>
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Table II. Observations of vertical gradients and concentration.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Respiration (mg CO₂/g dry wt·hr)</th>
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