An Apparatus to Produce Gas Mixtures with Controlled CO₂, O₂, and Water Vapor Concentrations

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ABSTRACT

An apparatus to produce continuous gas mixtures for use in measurements of plant gas exchange is described. A wide range of CO₂ and water vapor concentrations can be provided and O₂ concentration can be varied from 0 to 21%. Changes in the concentrations of the components are accomplished conveniently, rapidly, and independently. With occasional adjustments, CO₂ and O₂ concentrations can be maintained to within ± 1%, and ± 0.1%, respectively. Dew point of the gas mixture can be maintained to within ± 0.05 C.

Measurements of CO₂ exchange and transpiration by plants using the flow-through or open chamber technique often require the preparation of air mixtures in which CO₂ and water vapor concentrations are precisely controlled. In such experiments, it is also sometimes desirable to alter the O₂ concentration, particularly to levels below that of normal air.

Systems to produce air mixtures with controlled CO₂ and water vapor concentrations have been described (2–4) but none of these permits the convenient regulation of O₂ concentration. Oxygen concentration has been controlled by using single premixed gases (5) or by blending together two prepared gas mixtures differing in O₂ concentration but equal in CO₂ concentration (1). These methods have obvious disadvantages, particularly where various CO₂ concentrations are required.

In addition to lacking a convenient means of controlling O₂ concentration, the systems previously described rely on cumbersome liquid overflow valves and manometers for the control and determination of gas flow rates. We found that these systems lacked the necessary combination of precision control and convenience of operation necessary to permit rapid measurements of gas exchange.

Described here is an apparatus to produce gas mixtures with a wide range of controlled CO₂ and water vapor concentrations and O₂ concentrations between 0 and 21%. Changes in gas composition can be made rapidly and the concentration of any component can be altered without significantly influencing the concentrations of the other components. The method of regulating O₂ concentration eliminates the requirement for prepared gas mixtures.

MATERIALS AND METHODS

A schematic representation of the apparatus appears in Figure 1. Oxygen concentration is controlled by diluting air with N₂. Room air is pulled through an opening in container C1 by pump P. If an O₂ concentration below that of air is desired, compressed N₂ is metered into another opening in C1 via regulator R1a, valve V1, and flowmeter F1. As N₂ flow rate is increased, the amount of room air entering C1 decreases proportionally as a result of increased pressure in C1. Oxygen concentration is, therefore, inversely related to N₂ flow rate.

The gas stream leaving pump P passes through pressure regulator R2, a unidirectional check valve V2, and 40% KOH CO₂ scrubbers, S. A glass wool trap Tr blocks the passage of any air-borne droplets of KOH solution.

Water vapor concentration is controlled by passing the gas stream through humidifier H and then through condenser Cd which is immersed in a refrigerated, temperature controlled bath.

The gas stream passes through valve V3, flowmeter F2, and a “T” at the junction of the CO₂-free and CO₂ streams. Carbon dioxide concentration is controlled by regulating the rate of flow of CO₂ into this junction. A compressed mixture of 10% CO₂ in N₂ is metered through pressure regulator R1b, flow regulator R3, flowmeter F3, and a short length of capillary tubing Cₚ.

The final gas mixture enters container C2. From C2 a small pump pulls a continuous sample for analysis of CO₂, O₂, and water vapor concentrations. A larger pump delivers the gas mixture to the leaf chamber at the desired flow rate. The remaining gas escapes through flowmeter F₄.

Metal tubing is required between R1b and Cₚ in the CO₂ injection circuit due to high pressure, low flow rate, and large CO₂ diffusion gradient. Metal tubing is preferable throughout the remainder of the system. Leak-free connections are essential.

Some of the components were constructed in the laboratory, and some were purchased commercially. The two containers were made of acrylic tubing, and tube fittings were cemented in place. Approximate volume of each is 150 ml. The room air opening in C1 consists of a 15-cm length of tubing with a 1-cm i.d.

Carbon dioxide scrubbing is accomplished in two 40% KOH columns arranged in parallel. Within each of the two columns are immersed two large polyethylene gas dispersion tubes. Each column was constructed of acrylic tubing with an inside diameter of approximately 10 cm and a length of 50 cm. This cylinder was cemented to an acrylic base. A glass liner, made by closing one end of a piece of large glass tubing, rests inside the acrylic cylinder. A removable acrylic top, containing appropriate tube fittings, clamps onto the acrylic cylinder and

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1 Contribution from the Purdue University Agricultural Experiment Station, Lafayette, Ind. 47907. Journal Paper No. 5025. Research was supported in part by a grant from the Crop Improvement Council, National Soybean Processors Association.
seals the column with the aid of a rubber O-ring. The columns are filled to approximately two-thirds volume with the KOH solution.

The humidifier is a flask with a diameter of approximately 15 cm, fitted with entrance and exit tubes. The entry tube extends downward to 1 cm above the water level. The condenser consists of four coiled 3.6-m lengths of 4.75-mm (o.d.) copper tubing arranged in parallel. All four tubes lead into a common copper trap with a volume of approximately 100 ml. The gas stream exits this trap through another tube. An additional tube, which is normally capped, extends to the bottom of the trap and permits periodic removal of condensate. The entire condenser, including the trap, is submerged in the bath.

The following components were purchased commercially:


Measurements of O₂ concentration were made with a Beckman Instruments (Fullerton, Calif.) Model C2 oxygen analyzer, range 0 to 21% O₂, with flow-through cell. Carbon dioxide concentration was measured with a Beckman model 215A infrared CO₂ analyzer with two 34-cm flow-through cells, operated in a differential mode in conjunction with a suppressed zero recorder to provide a resolution of 1 μl/l. The gas stream was dried over anhydrous CaSO₄ before passing through the O₂ and CO₂ analyzers. Water vapor concentration was measured with a Vap-Air (Vapor Corp., Chicago, Ill.) Model 84 dew point hygrometer.

**RESULTS AND DISCUSSION**

With the components indicated, the apparatus is capable of delivering to the leaf chamber up to 10 l/min of the mixed gas. The purpose of the leak through flowmeter F4 (Fig. 1) is to maintain a relatively constant pressure in container Ct2 when altering leaf chamber flow rate. This is required in order to maintain a constant balance of flows in the CO₂-free and CO₂ circuits. A positive reading on flowmeter F4 assures that external air is not being drawn into container Ct2.
The apparatus was not designed for the purpose of calibrating the gas analyzing instruments. The flowmeters serve primarily as an aid in obtaining a close approximation of the desired gas composition. Final flow adjustments are based on instrument readings. Adjustments in \( N_2 \) and \( CO_2 \) flow rates elicit a complete response in approximately 1 min. Changes in water vapor concentration are less rapid and depend primarily upon the speed with which the bath temperature surrounding the condenser reaches the desired level. For this reason, it is desirable to use a bath with small volume and high heat exchange capacity.

As a \( CO_2 \) source, 10% \( CO_2 \) in \( N_2 \) was chosen in preference to pure \( CO_2 \). The higher flow rates appropriate with this dilution are more easily measured and controlled. Capillary \( Cp \) in the \( CO_2 \) injection circuit stabilizes the float in flowmeter F3.

The apparatus is capable of producing a gas stream with a range in \( O_2 \) concentration from near 0% to that of the ambient air (normally about 21%). Range in \( CO_2 \) concentration is from near 0 to over 600 \( \mu l/l \). The actual upper limit of \( CO_2 \) concentration is unknown because the \( CO_2 \) analyzer used to make the measurements has an upper limit of 600 \( \mu l/l \). The lower limit of water vapor concentration, in terms of dew point temperature, is 0 \( C \), the point at which icing occurs in the condenser. The upper limit of water vapor concentration is determined by the efficiency of the humidifier. The purpose of the humidifier is to assure that the dew point of the gas stream entering the condenser is above the condenser temperature. At room temperature, our humidifier will raise the humidity of very dry air or \( N_2 \) to a dew point of approximately 16 \( C \). A higher humidity, if desired, could presumably be conveniently obtained by heating the water in the humidifier.

After a 0.5-hr “warm-up” period, variation in \( CO_2 \) concentration during a 4-hr period of continuous operation, without manual adjustments, normally does not exceed \( \pm 3 \mu l/l \). With occasional adjustments in \( CO_2 \) flow rate, \( CO_2 \) concentration can be maintained to within \( \pm 1 \mu l/l \) of the desired concentration. Without adjustments, \( O_2 \) concentration and dew point temperature, over a 4-hr period, normally vary less than \( \pm 0.2\% \) and \( \pm 0.1 \ C \), respectively. With occasional adjustments in \( N_2 \) flow rate and bath temperature, the magnitude of these deviations is easily halved. Nearly all deviations occur as gradual, long term drifts. An adjustment in the level of a gaseous component, over the entire range, has no significant effect on the levels of the remaining components.

The tolerance in predicting \( CO_2 \) concentration from flowmeter F3 is approximately \( \pm 0.1 \mu l/l \). Therefore, in attempting to reach a specified \( CO_2 \) concentration, it is necessary to make additional adjustments in flow regulator R3 based on \( CO_2 \) analyzer readings. To obtain a desired \( CO_2 \) concentration within a tolerance of \( \pm 1 \mu l/l \) normally requires approximately 15 min. However, in normal practice, the concentration of \( CO_2 \) exiting the leaf chamber is of more importance than the entering concentration. Exit concentration is determined partly by the uncontrolled rate of \( CO_2 \) exchange by the leaf. Therefore, the time required to reach a specified exit \( CO_2 \) concentration cannot be appreciably reduced by decreasing the tolerance in predicting the \( CO_2 \) concentration of the manufactured gas stream. The desired entry \( CO_2 \) concentration is, in effect, unknown.

LITERATURE CITED