Short Communication

A Simple Carbon Dioxide Injection System for Photosynthetic Studies1,2

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ABSTRACT

A simple carbon dioxide injection system has been developed for the maintenance of CO2 concentrations in semiclosed cuvette systems suitable for photosynthesis and gaseous pollutant studies. The device injects small volumes of pure carbon dioxide into the cuvette in response to a signal from an infrared gas analyzer.

During our studies on the metabolism of carbon monoxide by plants, it was necessary to design a plant chamber that over several hours would maintain a constant environment with regard to light intensity, relative humidity, temperature, and CO2 concentration. Since radioactive carbon monoxide was to be used in the system and, since maintenance of a constant CO2 level was considered to be critical, a semiclosed system seemed the most appropriate for these studies. A major drawback of such systems is the need for expensive valves, flow meters, and pressure regulators. In addition, to attain satisfactory accuracy, most semiclosed systems integrate CO2 uptake with time (1, 2, 6). This reduces their sensitivity to short term changes in the photosynthetic rate (7).

The semiclosed system (Fig. 1) described here circumvents those drawbacks through the injection of small volumes of pure CO2 so that pressure changes as a consequence of the CO2 addition are minimized. It is also capable of measuring small transient changes in photosynthesis, yet is simple and inexpensive. The major components used are a Liston-Becker 15A infrared gas analyzer; a Leeds-Northrup Speedomax recorder; a 10.8-liter Plexiglas chamber; and the newly developed amplification-injection device connected to two Asco 8262 C1 solenoids (12 mm orifice) and a 0 to 10 p.s.i. pressure regulator.

The CO2 concentration within the cuvette is maintained at any desired level by injecting a known amount of pure CO2 whenever the amplitude of the IRGA signal falls below the preset output. Transistor T1 acts as a signal amplifier for the signal from the IRGA (2.5 V at 5 ma at full scale deflection), and T4 acts as a switch between the 6-v power supply and relay B. When the CO2 level in the cuvette reaches this critical point, the amplified IRGA output has decreased sufficiently to switch T4 and thus deactivate relay B. This causes the circuit to the rear solenoid S, to be broken and the current instead flows to S, the front solenoid. The rear solenoid closes immediately upon deactivation. After a 2-sec lag in the activation of relay C1, caused by delay relay A1, the front solenoid opens and the CO2 held under pressure is released into the gas stream. The increased CO2 concentration in the gas-sampling line causes the IRGA signal to rise, which in turn reactivates relay B. The flow of current is thereby switched back from solenoid S1, which closes immediately, to the circuit controlling solenoid S2. The opening of S2 is delayed by a similar delay system, A2 and C2. When S2 opens, CO2 can again enter the injection tube and the cycle is set to be repeated.

The delay circuits serve to regulate the interval between the opening and closing of the solenoids, as well as insulating them from any chattering of relay B caused by a very slowly falling signal. This precaution ensures more equal injection size and avoids gas breakthroughs by allowing the back solenoid to close fully before the front one opens.

The output from the Liston-Becker 15A IRGA was not immediately adaptable to the available relays. The amplification circuit shown allows the IRGA output to be modified so that it is capable of triggering relay B.

In employing this system, the amount of CO2 injected in each burst could be varied by either increasing the pressure of gas or the size of the injection tube. We found that for our work the 32 mm × 1.6 mm injection tube was satisfactory. This allowed for injections sized from 1.5 to 85 μmoles of CO2 by varying the pressure from 0.25 to 5.0 p.s.i. (Fig. 2). The cycling time per injection was 15 sec. This meant that photosynthetic rates between 0.060 and 20.400 mmolcs of CO2/hr could easily be measured. This also allowed each individual run to last from minutes to several hours because, unlike many continual flow systems (1, 2, 6), the quantity of gas introduced was identical to the amount of CO2 taken up by the plant.

The size of the injection should be adjusted at the beginning of a run to the smallest injection volume that will compensate for the photosynthetic rate of the plant within the limits of the response time of the system. This permits a larger number of injections per unit time and, therefore, reduces error associated with machine variations and electronic drift. When the system is set for the smallest sized injection, the actual concentration within the cuvette will vary the least. In our system, an injection size of 20.8 μmoles allowed uptake measurements of up to 3.744 mmolcs/hr. By installing the injection system just

1 This work was supported by Grant FS-NE 03 of Northeastern Forest Experiment Station of the United States Forest Service, through the Pinchot Institute for Environmental Studies.
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3 Abbreviation: IRGA: infrared gas analyzer.
Fig. 1. System layout and circuit diagram for CO₂ injection system.

Fig. 2. Relationship between pressure, the amount of CO₂ per injection, and the chamber concentration change as the result of one injection.

Table 1. Photosynthesis by a P. deltoides Branch at 22 C and 380 Microeinsteins Meter⁻² Sec⁻¹

<table>
<thead>
<tr>
<th>No.</th>
<th>Peaks per 30 Min</th>
<th>Net CO₂ Uptake mmoles per 30 min</th>
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<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>1.290</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>1.310</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>1.290</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>1.269</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>1.269</td>
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<tr>
<td>6</td>
<td>63</td>
<td>1.310</td>
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<tr>
<td>7</td>
<td>63</td>
<td>1.310</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>1.269</td>
</tr>
<tr>
<td>9</td>
<td>62</td>
<td>1.290</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>1.290</td>
</tr>
</tbody>
</table>

before the IRGA, maximum signal deflection was obtained, yet the actual CO₂ concentration within the cuvette varied less than 4.5 µl/l between injections.

The steady state CO₂ level within the chamber can also be regulated using this system. At any given setting of the variable resistor R, which sets the point where the injection occurs, the injection system is triggered by a set output from the IRGA. The gain on the IRGA can then be adjusted so that this output amperage represents any concentration desired in the cuvette.

By increasing the gain the set output is attained at lower CO₂.
concentrations, therefore, lowering the steady state CO₂ concentration in the cuvette. Conversely, by decreasing the gain a higher CO₂ level can be maintained in the cuvette. In addition, the ambient concentration of CO₂ in the cuvette can also be adjusted anywhere within the range of the two CO₂ concentrations for which an IRGA is calibrated for a given experiment by changing the setting of the variable resistor R. This changes the output amperage which effects activation of the injection system. This system, therefore, allows assimilation rates to be measured quickly and accurately over a broad range of ambient CO₂ concentrations should the need arise.

In our system, the injection of the CO₂ just prior to the entry point of the gas analyzer ensured that very small injections of CO₂ would produce an IRGA output of sufficient magnitude to recycle the injection system. It also could be used for immediate detection of leaky solenoids or inconsistent injection size. The IRGA output as plotted on a recorder, appears as a succession of peaks, each representing an injection. Alternately, the output can be recorded by putting an event marker in series with one of the relays in the system. However, when the results are graphed, the defects described above are more apparent, and changes in the photosynthetic rate are more immediately evident, appearing as variable spacing between the peaks.

Table I shows the results of 10 consecutive 0.5-hr assays of photosynthesis by a branch of Populus deltoides and demonstrates the consistency and accuracy of the injection system. In the above experiment, 28.89 ml of 100% CO₂ were added per 0.5 hr to maintain the CO₂ concentration at 325 μl/l. The O₂ produced by photosynthesis resulted in a calculated 0.22% increase in the O₂ concentration within the chamber. This change in O₂ concentration should be taken into account when designing experiments where small changes in O₂ concentration are of significance. In gas-tight systems, provisions must be made for the release of any pressure build-up as a result of net O₂ production (2), or when CO₂-air mixtures are employed to reduce the amount of CO₂ injected into systems of smaller volume.

The merits of the system described here are that it is easily and cheaply constructed. All electronic components are commonly available and their tolerance requirements are not critical. The amplification-injection system, though simple, has a high degree and range of flexibility and should prove valuable for other applications where the use of a semiclosed system is deemed necessary.

**LITERATURE CITED**