

SOURCES OF VARIATION AND VARIABILITY IN MEASURING AND
ADJUSTING CONTROLLED ATMOSPHERES

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Introduction

The Orsat type gas analyser, which has been used for many years for analyses of oxygen and carbon dioxide is being replaced by electronic instrumentation which can be used, not only to monitor, but also to control atmospheric composition. Although electronic instrumentation has been available for some time (6, 10), the reliability of instrument function and accuracy of measurement were often questionable. Nevertheless such instruments were often used by researchers who required daily or more frequent analyses of many chambers and commercial operators who sought relief from the tedium of using Orsat analyzers. Solid state, reliable electronics, minaturization of components, microprocessor control, and electrically controllable, mechanical equipment for lowering the oxygen level and/or scrubbing carbon dioxide have led to the development of completely automatic control of CA storage atmospheres (5,11,13). As well some computer systems could be used to control refrigeration equipment (24), and possibly even be linked to grading or inventory systems. The demand for automatic control has been furthered by the increasing number of CA rooms, the high cost of labor, and the need for accurate and precise analyses of levels in low-oxygen storage systems.

In the following report we review sources of variation in some equipment used for analyses of oxygen and carbon dioxide with emphasis on the analyzers not the control systems. The list of analyzers is not meant to be complete, nor is the theory of operation, which can be

found in instrument manuals or text books, described.

Jameson (11) briefly described analysers used in the United Kingdom. The theory and application of several types of oxygen analyzers have been reviewed by Tipping (22), and described for carbon dioxide analysers in the manuals provided with the instruments (2). For Orsat-type analyzers the best procedures for operation are often available as grower service releases or other unpublished material. Basic texts on gas chromatography are available (17) and one is particular for gas analyses (21).

We also examine the variation in atmosphere of some commercial CA rooms and interpret that variation in terms of possible biological significance. Jameson (11) previously indicated the large variation in an atmosphere may have an influence not apparent by inspecting the mean values.

General sources of error (variation) in gas analyses.

1. Inaccurate size of the gas sample used for analyses (Orsat or gas chromatograph).
2. Variable moisture content.
3. Variable pressure during sampling and calibration.
4. Sample not representative of storage atmosphere because of incorrect sampling techniques.
5. Leaks in the sampling line(s) and manifold which should be equipped with positive action valves.
6. Operator errors in obtaining a sample or in measuring the level of water in the burette (Orsat) or in estimating the reading on a dial or recorder trace in other instruments. In many cases scales are graduated only to the nearest 0.2, and in some cases even 0.5, %. Although operators may estimate to the nearest 0.1% such estimations incorrectly magnify instrument sensitivity. Non-linear scales such as on the infrared analyzer are particular sources of error.
7. Incorrect calibrations Oxygen and carbon dioxide analysers used for CA analyses require calibrating at 2 points on the scale with suitable calibration gases. It is usual to use a gas with zero concentration of the gas being measured to set the analyser zero and a gas with a known quantity of the measured gas for full scale calibration.

Any inert gas which is guaranteed to be oxygen free can be used for setting the zero of oxygen analysers. Nitrogen is the usual gas but care must be taken to specify the oxygen-free grade. It is convenient to use a mixture of nitrogen and carbon dioxide for setting the zero as this gas can then also be used for setting the carbon dioxide analyzer. Because oxygen-free gas is analysed at the parts per million level by the manufacturer, errors due to

the zero gas are small. Any error in zero setting is, however, important as this becomes an absolute error throughout the analyser measuring range.

An inert gas which is free of carbon dioxide can be used for zeroing the carbon dioxide analyser. Bottled nitrogen is the obvious choice for this as it is readily available. Atmospheric air is commonly used for setting the zero of carbon dioxide analyzers but care should be taken. Typical atmospheric air contains approximately 340 ppm carbon dioxide but the level may vary between 300 and 400 ppm depending on local conditions. These levels are not sufficient to cause significant errors in calibration. Errors can however be caused if the calibration air is obtained close to the outlets of storage rooms or scrubbers where the carbon dioxide concentration will be higher.

It is normal practice to calibrate the full scale of an oxygen analyser with a linear response with atmospheric air. The concentration of dry air is given as 21.0% (26) and 20.95% (23). This is considered to be a very stable level but some small variations will occur due to moisture content. The effective reading on an analyser will vary with barometric pressure and altitude but this is because the analyser reading is the "partial pressure" of the gas. The percentage oxygen is independent of pressure, and calibration at 21% automatically compensates for the pressure effect on all other reading within the analyser range. Any calibration errors at 21% are reduced proportionally to the reading so that a 0.5% oxygen error at 21% is reduced to 0.05% at 2.1% oxygen. A calibrated gas mixture which is certified can also be used for setting the full scale of an oxygen analyser but this would normally give a less accurate result than using atmospheric air. Very expensive gravimetric gas mixtures should be used if a more accurate calibration is required. The use of a certified oxygen calibration gas at the low end of the scale will indicate if there has been an error in the zero setting which could result in an absolute error throughout the range of the scale as indicated previously.

A gas mixture with a known quantity of carbon dioxide is required for setting the up-scale calibration of an infrared carbon dioxide analyser. This mixture can be carbon dioxide in air or nitrogen. If nitrogen is used as the background gas this mixture can also be used for setting the zero of the oxygen analyser.

8. Instrument Drift. An analyser calibrated against known gases and assuming good linearity and resolution will provide an accurate analysis at the instant of calibration. If, however, continuous measurement or automatic control is required then changes in calibration with time must be considered. The 2 major effects on

analysers are temperature and barometric pressure. Ambient temperature will affect all types of analysis. For automatic oxygen control at levels from 2 to 1% oxygen it is essential that the oxygen analyser be equipped with a heater and temperature controller to ensure stability of the measurement. Temperature control is also beneficial to a carbon dioxide analyser but generally accuracy of measurement of carbon dioxide is not as important as that of oxygen, particularly in a low-oxygen CA. Because both oxygen and carbon dioxide analysers measure the absolute amount of the measured gas present in the sample rather than the percentage of the total, the readings will vary in proportion to the absolute pressure and will therefore respond to changes in atmospheric pressure. Again, because it is a partial pressure effect the absolute change will be directly proportional to the reading. A typical change in barometric pressure is 4% (990 - 1030 mb) and this will change the reading at 21% by 0.84% oxygen, at 2% oxygen by 0.08% oxygen and of course at zero oxygen, pressure will have no effect.

Particular sources of variation in instruments

Orsat:

1. Improper sample size resulting from improper zeroing.
2. Improper reading of the burette.
3. Improper initial or final adjustment of liquid levels in pipettes.
4. Insufficient time in, or number of passes through the absorption pipettes. If carbon dioxide is not completely "absorbed" the residual carbon dioxide will be measured as oxygen.
5. "Absorbing" reagents not strong enough. This is a particular problem with the oxygen reagent.
6. If the gas sample is considerably warmer or colder than the analyzer the change in gas volume during analyses will result in an incorrect reading.

Fry gas analyzer:

This analyzer (9), like the Orsat, is based on selective absorption of oxygen and carbon dioxide by reagents. This analyzer might be of interest to researchers who do not have funds to buy other analyzers, but it is unlikely to be used by more affluent researchers or commercial CA operators. The sources of variation are similar to those for the Orsat but with the added problem that the size of the sample is limited to a few ml and ideally to 1 ml. The small size of the gas sample required is advantageous but a gas chromatograph provides more versatility of analyses and possibility of recording measurements.

Gas Chromatograph

1. The non-linear response of the thermal conductivity detector usually used for carbon dioxide analyses may be a source of error (17). The flame ionization detector with a linear response can be used if the carbon dioxide is converted to methane (25). However 'methanizers' are only suitable for measuring low concentrations of carbon dioxide such as those found in respiration studies, rather than the high concentrations found in CA storages.
2. Separating oxygen and carbon dioxide normally requires a 2-column system and possibly special plumbing of the detector (8).
3. Argon and oxygen are usually not separated by columns used for oxygen analyses. Chen et al (7) have described a method of compensating for argon content without using sub-ambient column temperature. The separation of argon may not appear important but the percentage of argon in air approaches 1% (23,26) with obvious implications when measuring atmospheres in low-oxygen CA.
4. The volume of the sample may vary with the operator unless a sampling valve is used.
5. The recorder which simplifies record keeping, may reduce such error, but interpretation of the recorder trace may be an additional source of variation (18).
6. Changing attenuation to keep the recorder response on scale will offset the baseline if the instrument and recorder are not zeroed correctly and use of a high attenuation will reduce sensitivity. An electronic integrator will solve these problems, but these expensive devices are hardly suitable for commercial CA operators.
7. Moisture accumulating in the column may reduce its effectiveness in separating the gases. However a drying column can be located upstream of the separatory column (8). If the moisture is removed the percentage levels of oxygen and carbon dioxide will be slightly different than in the moist sample from the storage.

Infrared analyzer for carbon dioxide:

1. Errors caused by non-linear response, and a non-linear read-out (2), can be reduced by linearizing the output.
2. Pressure fluctuation caused by barometric changes or pump surges will cause variation in readings.
3. Moisture in the sample can be somewhat of a problem although in most analyzers the wavelength is specific for carbon dioxide.
4. Change in ambient level of carbon dioxide in the "light path" will cause fluctuations in readings if the system is not enclosed. In some analyzers with an open light path a small canister of carbon dioxide absorbent is used to reduce this problem. If the ambient concentration of carbon dioxide is high, as it might be close to CA rooms, the canister will require frequent changing. A dual-beam

instrument will be compensated to a great extent for the change in ambient level of carbon dioxide but not entirely so (15), because the response to carbon dioxide from reference and measurement cells may be in different locations on the non-linear response curves of the 2 cells.

Paramagnetic oxygen analyzer

This analyzer is almost specific for oxygen with only 3 gases, and these not found in storage atmospheres, which could interfere with oxygen readings (22). Although the detecting system is complicated, and in instruments from some manufacturers is delicate, both portable and stationary instrument are available. This instrument is adapted to analyses of flowing gases. The response is linear over the entire range.

1. The analyzer is sensitive to pressure changes originating from the sample pump or barometric fluctuations. Although it is possible to compensate the case for barometric changes (3) a better method is to use a dual-sensor instrument (20) in the ratio mode (22). However such compensation is not necessary for measurements of CA atmospheres. As indicated previously in the text and by Tipping (22) barometric pressure changes could influence readings at 21% by 0.84% oxygen, but at usually measured levels (2 to 3%) the error will amount to only approximately 0.1% oxygen.
2. There is some response to rate of gas flow through the detecting cell and very high flows may injure the sensor movement. In some instruments there is a bypass system to allow rapid clearing of the sample line but with only a portion of the sample entering the sensor.
3. Although water vapor will not cause direct errors in measurement water condensing in the cell may cause damage. If water vapor is removed from the sample the measured oxygen level will be slightly higher than what was actually present in the "undried" sample (22).

Polarographic oxygen analyzer

1. The Teflon membrane (4) through which the oxygen diffuses is sometimes difficult to install. Some in the current models require replacement of the complete sensing element.
2. Tipping (22) indicates that carbon dioxide, because of its acidic properties, should be scrubbed from the sample. If the carbon dioxide concentrations are similar in all samples, including the calibration gas) there should not be a problem - if the levels vary in the samples there could be some error.

Variations in measured oxygen and carbon dioxide levels in CA rooms.

In many jurisdictions apples can legally be called CA only if the

oxygen content is held below a legal maximum (usually 5%). This is understandable in a legal sense but in a biological sense recommended conditions should be more restrictive. In Figure 1, for instance, the desired and average oxygen and carbon dioxide levels were very similar but the variation were considerable. The data in Figures 2 and 3 represent readings for 2 successive 30-day periods in the same low-oxygen storage. For scrubbing carbon dioxide lime was not placed inside the room as is usual for low-oxygen CA, but in an exterior lime scrubber. Although the carbon dioxide level was higher than normally "recommended" the level was controlled with little fluctuation. This small variation is a characteristic of the use of a lime scrubber. Although the mean oxygen concentrations in both figures were reasonably close to the desired of 1% there was considerable measured fluctuation particularly in Figure 2.

However the real fluctuation in gas concentrations may be greater than the measured daily readings indicate. If corrective measures are performed manually by turning on an air pump to raise the oxygen level it is likely that the duration of pump operation will be estimated to bring the to raise the oxygen concentration to the desired level at the time of analyses the next day (Fig. 4A). Data in Figure 5 illustrate the actual readings in a pilot-sized room (14). Although the mean readings were close to the desired (1.0% oxygen) the lowest reading was 0.6% and the highest after adding air was 1.3%.

If, the CA room is sufficiently tight that oxygen consumed in respiration is not replaced through air leakage into the room, to have the reading correct for the next day the oxygen concentration must be adjusted somewhat higher than that desired. The average of the daily readings then does not truly represent the average concentration, which in this case is above the average reading. Similarly, changes may occur through generator use (Fig. 4B) or scrubber action (Fig. 4C). In some cases the oxygen concentration will increase with carbon dioxide scrubber use because of infiltration of air through the scrubber. It must be expected that removal of carbon dioxide by scrubbing, unless it occurs very slowly as it does with use of a lime scrubber, will result in a lowered pressure within the room. Normally entry of air during pressure equilibration will result in fluctuations in both oxygen and carbon dioxide level. It is possible to compensate for the lowered pressure caused by scrubbing and to reduce infiltration with air by adding nitrogen gas to reduce the negative pressure in the CA room.

Variation in atmosphere composition may be small when computerized monitoring and control is used (Fig. 6,7). In these cases only the oxygen level was under computer control but the variation in carbon dioxide was also relatively small. Although the oxygen calibration varied considerably because of barometric pressure changes (Fig. 6) (no data on barometric pressures) the room readings

changed little. As indicated before, if the instrument calibration is adjusted incorrectly, such as by incorrect adjustment of the zero because of the linear response of the paramagnetic oxygen analyzer, all readings will be in error by the same absolute amount. However, in this case the errors were not due to incorrect calibration, but to the changes in pressures. Thus the large daily fluctuation in measured oxygen concentration (Fig. 6A), reflecting barometric changes, was associated with only small fluctuations in measured oxygen concentrations in the 2.0 (Fig. 6B) and 1.0% (Fig. 7) ranges and in the effectiveness of the control of oxygen concentrations.

Conclusions

Because the benefit as well as potential for injury may be determined by the accumulated time in a particular atmosphere, interpretation of CA logs, which usually provide only one daily reading, should be done with some caution.

Of particular concern would be the periodic operation of a generator to maintain a measured concentration of 1% oxygen. This could be achieved with a few long or many brief periods of operation. Obviously the latter would give more precise control of the oxygen level but may not be as energy or cost efficient. If automatic control (5, 11) is used it is possible to use a series of incremental corrective measures so that the concentrations will be maintained close to that desired. However, satisfactory control can be achieved by daily measurement and adjustment of atmospheres. The amount of variation would undoubtedly be reduced by several readings each day and frequent but smaller adjustments to the atmospheres. Research and commercial experience indicate that a low, safe concentration of oxygen is necessary for the long-term storage of apples. However research is needed to minimize the injury by oxygen concentration which are too low, as well as reduce the time at concentrations which are too high to be effective.

At 2 to 3% oxygen, up to 5% carbon dioxide effectively delays apple fruit softening at 2 to 3°C, the temperature required necessary to control chilling injury such as brown core (19). At 1.0 to 1.5% oxygen moderate carbon dioxide concentrations of 2.5% apparently have had little effect on the firmness of apples. However, there was an increase in the incidence of "core flush" of Cox's Orange Pippin when the carbon dioxide concentration was raised levels from 0.1 to 3.0% (12), and elevated concentrations of carbon dioxide may cause core injury in Idared and McIntosh. Overall there is less concern about specific concentrations of carbon dioxide than there is for proper oxygen concentrations. However the interaction of oxygen and carbon dioxide levels is not entirely clear (1, 16).

Critical to atmospheric analysis are an appropriate sampling

system and the proper knowledge of the analytical method. Although not discussed in this paper, the measurement and control of temperature, relative humidity, and ethylene concentrations are also necessary to understanding the effects of CA.

Acknowledgement

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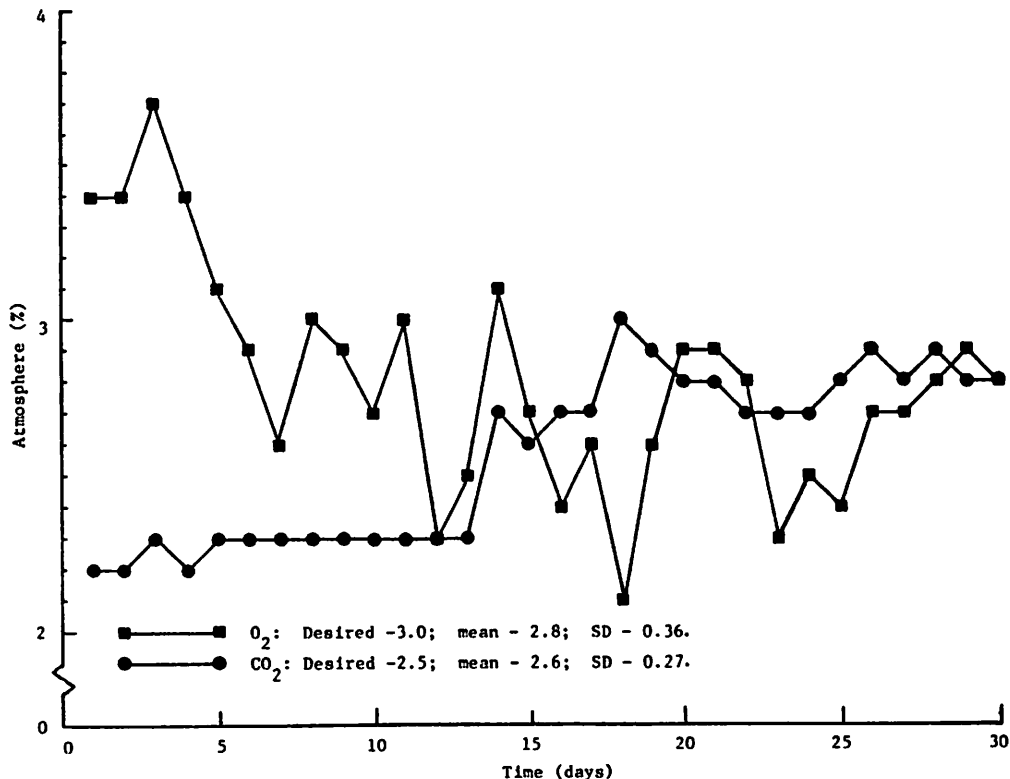


Fig. 1. Variation over a 30-day period in measured atmospheric composition of a small commercial CA room - McIntosh at 3°C, Suizer scrubber, Arcat generator.

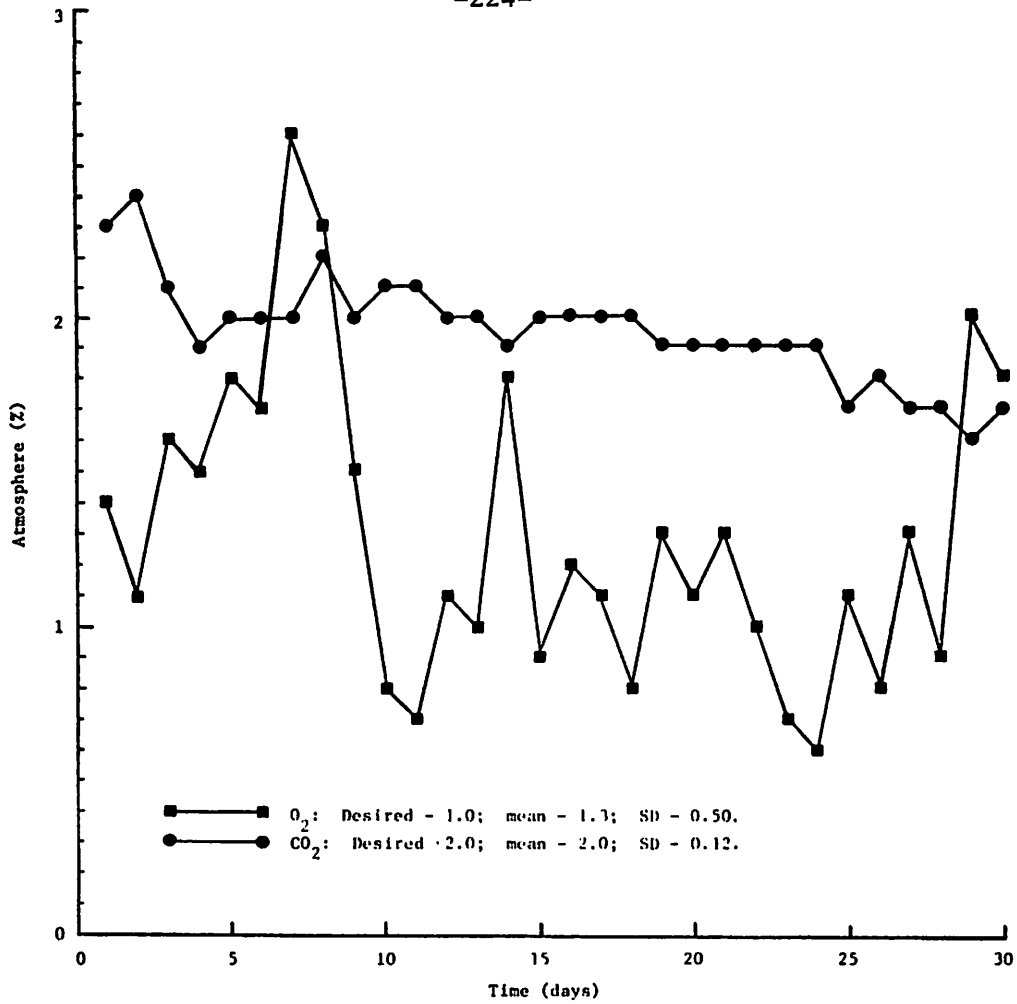


Fig. 2. Variation over a 30-day period in measured atmospheric composition of a commercial CA room - McIntosh at 3°C, lime scrubber, Arcat generator.

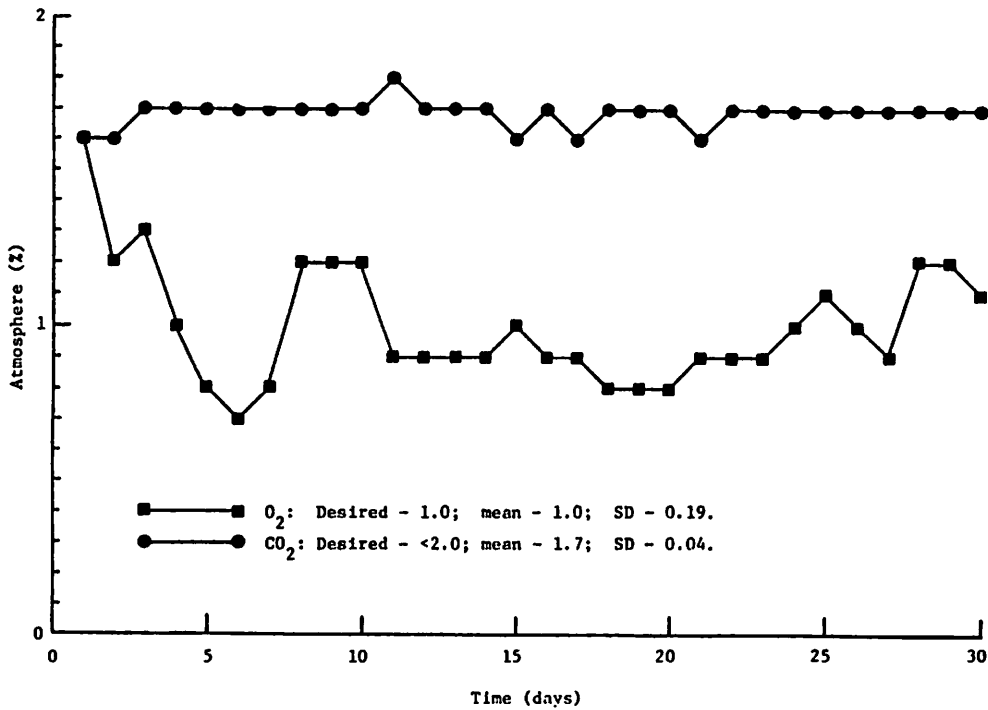


Fig. 3. Variation over a 30-day period in measured atmospheric composition of a commercial CA room - McIntosh at 3°C, lime scrubber, Arcat generator.

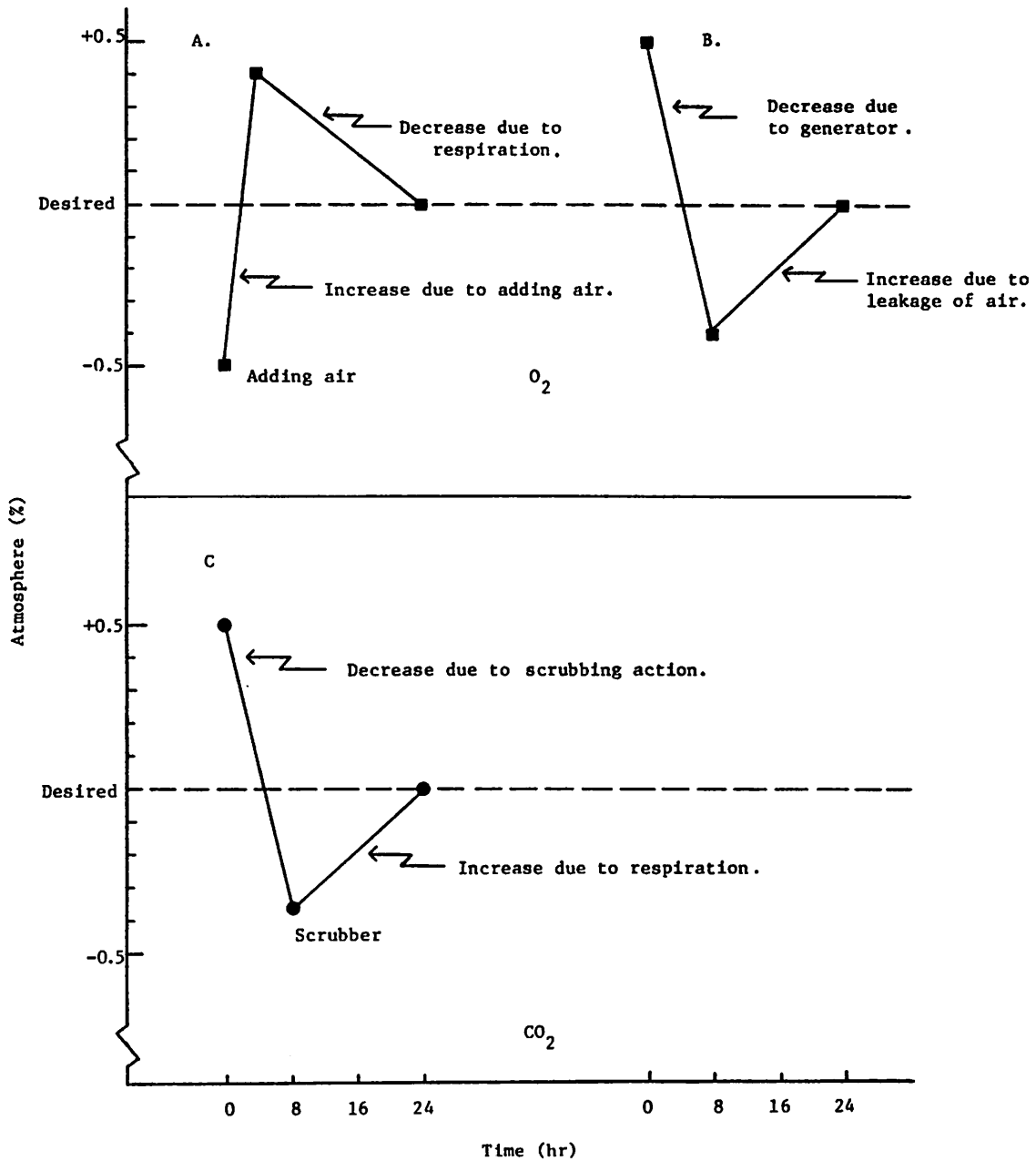


Fig. 4. Estimated possible variation in CA atmospheres with corrective procedures which by the next day's readings appear to be "perfect".

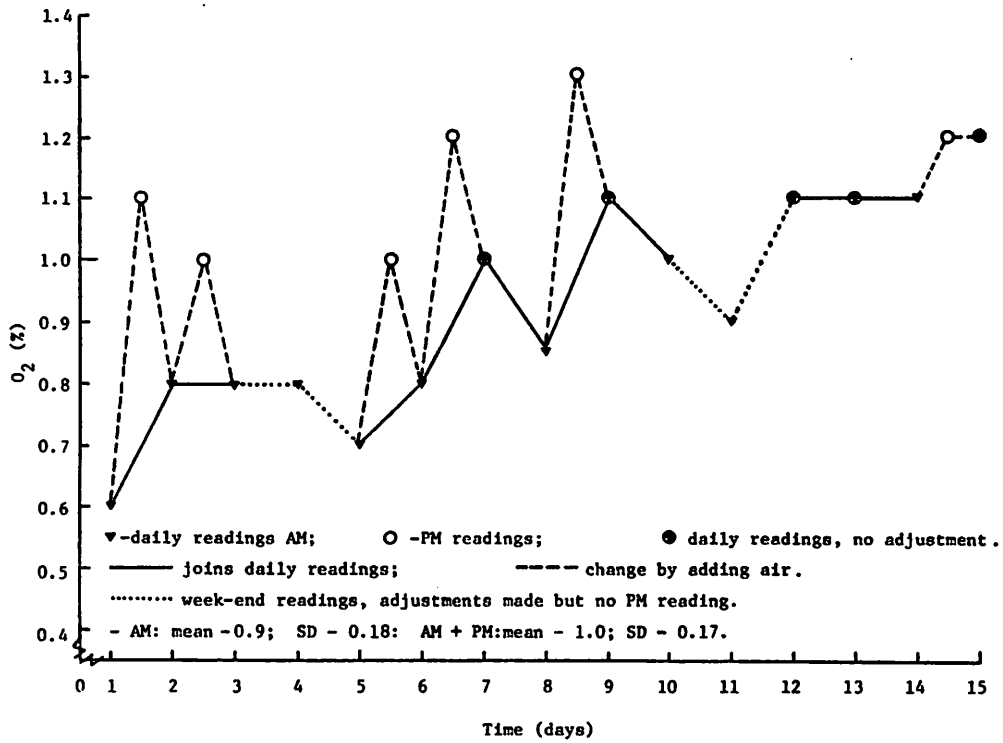


Fig. 5. Variation over a 15-day period in O_2 levels, including morning readings before adjustment and afternoon readings after adding air, in a pilot-sized CA room.

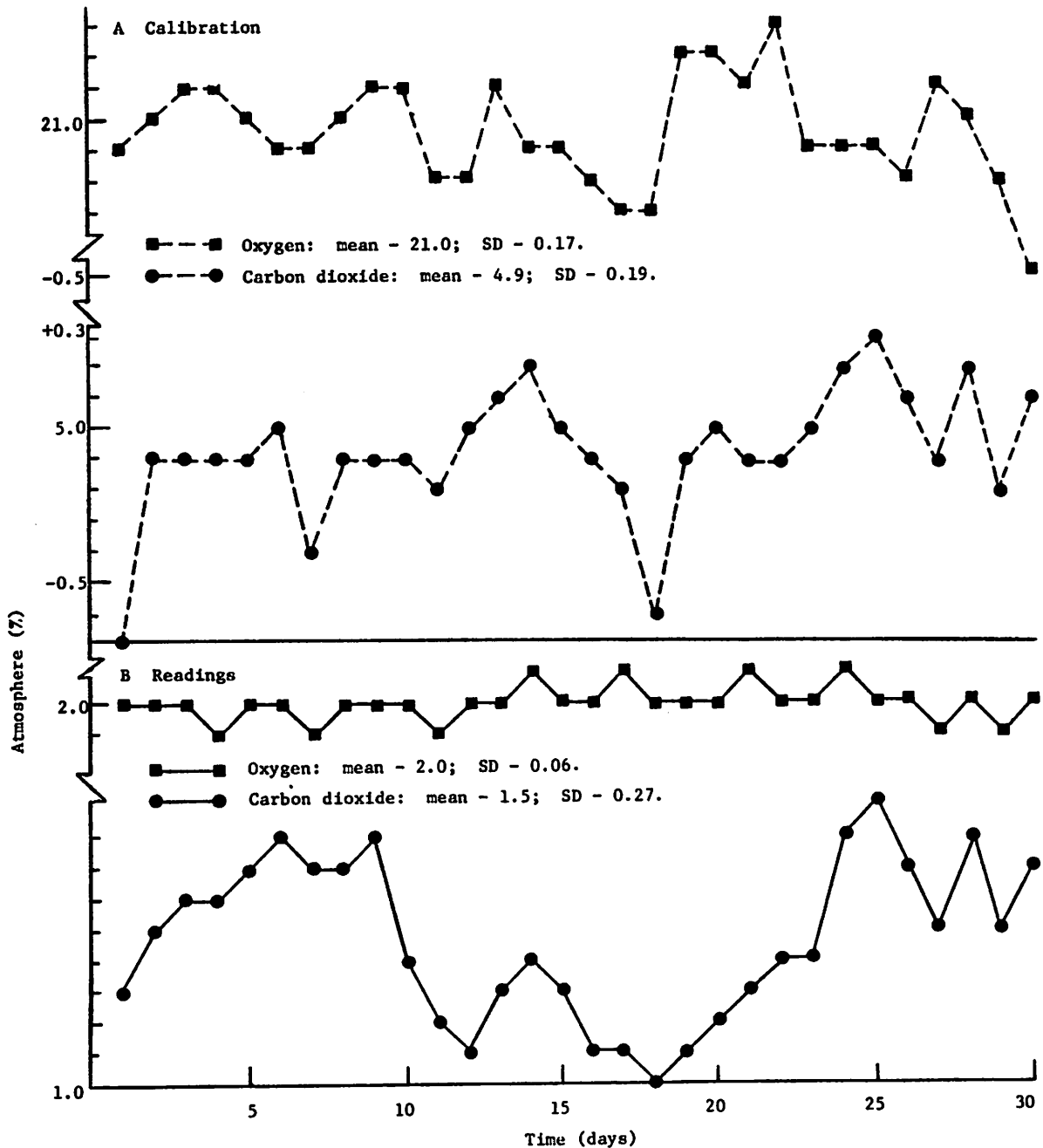


Fig. 6. Variation in unadjusted calibration readings (A), and variation over a 30-day period in atmospheric composition (B) of a commercial CA-room - Cox's Orange Pippin apples at °C, charcoal scrubber, Bishop oxygen control system (5, 13). All readings taken in early morning before manual calibration adjustments.

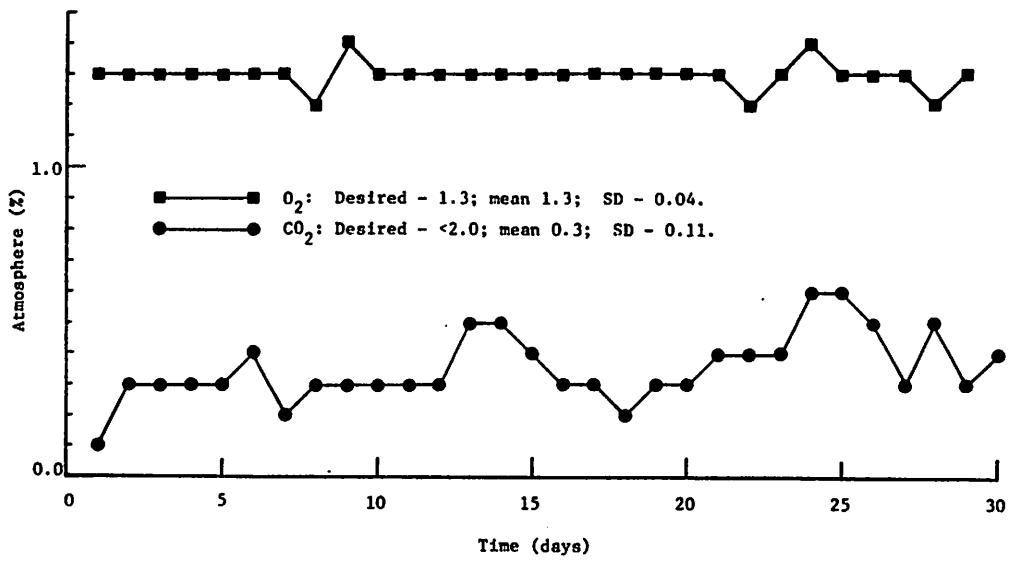


Fig. 7. Variation over a 30-day period in atmospheric composition of a commercial CA room - Cox's Orange Pippin apples at 3-4°C, charcoal scrubber plus lime in room; Bishop oxygen control system (5,13) readings taken in early morning before manual calibration adjustments. For variation in unadjusted calibration readings (see Fig. 6 A).