Transfer of Ethylene from Natural Gas during Aseptic Technique: A Possible Source of Error in Acetylene Reduction Experiments

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INTRODUCTION

A project student in our laboratory obtained inexplicable results whilst using the acetylene reduction method (Stewart *et al.*, 1967; Hardy *et al.*, 1968). All her data showed consistently high values for ethylene when a range of values had been expected. It was noted that during this experiment, which required transfer of cultures using aseptic procedures, she was using a blue, but partially vented, Bunsen flame. When the experiment was repeated using a fully vented Bunsen flame during aseptic transfer, the predicted range of data was obtained. This led to an investigation into the transfer of ethylene during aseptic procedures and the implications for acetylene reduction assays.

METHODS

The tops were removed from Bijou bottles (7 cm³, with tops drilled to expose rubber liners to facilitate transfer of gas with a syringe) and the necks of the bottles were heated in a Bunsen flame using a standard aseptic procedure. The tops were immediately replaced. During exposure to the flame the bottles were held at 45° to avoid spillage of contents and to prevent abnormal amounts of gas entering the bottles.

Ethylene was measured by injecting 1 cm³ of the atmosphere from the bottle into a Varian Aerograph Series 1400 gas chromatograph. The column (2.7 m × 2.5 mm diam.) was packed with Poropak R and run at 50 °C with nitrogen as the carrier gas at 30 cm³ min⁻¹. The presence of ethylene derived from the Bunsen flame was confirmed by comparing its retention time on the column of the gas chromatograph with that for pure ethylene and by enhancement of its peak with the pure gas.

RESULTS

Aseptic procedures were carried out with Bijou bottles containing 1 cm³ sterile medium. Different Bunsen flames were used ranging from yellow, with the air vent closed, to blue, with the air vent fully open. The results (Table 1) show that ethylene is transferred during aseptic technique if the air vent is less than 50 % open and that the longer the time the neck of the vessel is in the flame the more ethylene is transferred. The time taken for standard aseptic technique will vary with the operator but the author takes between 1.5 and 2 s.

DISCUSSION

Workers using aseptic techniques involving the use of Bunsen-burners burning natural gas and who do not wish to expose cultures to large doses of ethylene should use fully vented Bunsen flames since quiet, blue flames contain ethylene. The effects of ethylene on plant growth are well known (Abeles, 1973), but the transfer of ethylene during aseptic procedures may not be fully recognized and the implications for work with micro-organisms and tissue cultures sensitive to ethylene are obvious.

Table 1. *Effects of flaming time on the transfer of ethylene to Bijou bottles during aseptic procedure using a range of Bunsen flames*

Bunsen flame (colour)	Air vent (% open)	Time in flame (s)	Ethylene	
			($\mu\text{g}/\text{bottle}$)	(p.p.m.)
Unlit	Closed	1-2	0	0
Yellow	Closed	1-2	3.78	630
Blue	10	1-2	1.38	230
Blue	10	5	3.06	510
Blue	25	1-2	0.92	152
Blue	25	5	1.98	330
Blue	50	1-2	0	0
Blue	50	5	0.35	59
Blue	75	1-2	0	0
Blue	75	5	Trace	Trace
Blue	100	1-2	0	0
Blue	100	5	0	0

There are occasions when the transfer of ethylene from Bunsen flames can interfere with acetylene reduction experiments. In long-term growth experiments, when samples of a culture are regularly taken and tested for acetylene reduction, the culture must be maintained free of contaminating bacteria from the atmosphere. Therefore, although the exposure time to acetylene may be short and not require the use of aseptic procedures, such techniques are frequently employed because they are being used for transferring material from the growing culture. Thus the culture is repeatedly dosed with ethylene and the bottle used for the acetylene assay has ethylene trapped in it. In the experiment quoted in the Introduction the student used a partially vented blue flame and inadvertently added 19.8 μg ethylene to each of the vessels to be used for acetylene reduction assays. This masked the ethylene formed as a result of acetylene reduction.

Ethylene transferred during aseptic procedures is only a major problem if the containers are immediately sealed. Normally cultures are grown aerobically or anaerobically in which case any ethylene is lost due to diffusion into the atmosphere from aerobic cultures or flushed out during the provision of anaerobic conditions for anaerobic cultures.

The amounts of ethylene transferred differed from day to day. Such variations were attributed to differences in the composition of natural gas.

Where aseptic techniques are used during acetylene reduction experiments, appropriate controls should be carried out which take into account both the time taken and the number of aseptic transfers used.

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