Method for Comparing Concentrations of the Open-Air Factor

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It is shown how concentrations of the open-air factor may be compared from day to day; this has not been possible previously.

The open-air factor (OAF) is one or more pollutants formed probably from the interaction of ozone and olefins (3-7) and at extremely low concentrations can kill microorganisms when suspended as aerosols (1, 4-7, 9). The concentration of OAF varies considerably from day to day, and it may be a mixture which varies in composition; however, at present no sufficiently sensitive chemical technique exists for the identification and measurement of the concentration of OAF. It is the purpose of this paper to describe a method whereby the concentration of OAF can be estimated from day to day by using viable decay of microorganisms as a criterion for OAF concentration.

As for the mechanism for the toxic action of oxygen (2), it is supposed that OAF combines reversibly with a carrier, Y,

\[ \text{OAF} + Y \xrightarrow{k_+} \text{YOAF} \]  

and that this complex then reacts with an acceptor, B, to give a second complex, i.e.,

\[ \text{YOAF} + B \xrightarrow{k_-} \text{BOAF} + Y \]  

The main difference between the above mechanism and that for oxygen is that here reaction 1 is a slowly established equilibrium reaction, whereas for oxygen-induced death a very rapid equilibrium is established. This difference produces a drastic change in the shape of the decay curves for the two death mechanisms, namely, an "induction period" before the onset of OAF-induced viable decay which is not observed in oxygen-induced viable decay (2).

From equations 1 and 2,

\[ \frac{d[YOAF]}{dt} = k_+ [Y][OAF] \]  

\[ -k_- [YOAF] - k[Y][OAF][B] \]

\[ \frac{-d[B]}{dt} = k[B][YOAF] \]  

In practice it was found that numerical integration of equations 3 and 4 for \( B = [B]_0 \), \( [YOAF] = 0 \), \( [Y] = [Y]_0 \) at time \( t = 0 \), and \( d[OAF]/dt = 0 \) gave very similar values for \( [B] \) as a function of time as when the term \( k[YOAF][B] \) is neglected. Further validity for this approximation can be obtained under steady state conditions when \( d[YOAF]/dt = 0 \).

Analytical integration of equations 3 and 4, neglecting the above term, and use of the relationship between \( [B] \) and viability as previously deduced, namely:

\[ \ln V_i = K_1 [B] - K_1 [B]_0 + \ln 100, \]

\[ \ln V_i = K_1 [B]_0 \exp \left( (\alpha \beta - \frac{\alpha}{\beta} (e^{-\beta t} - 1)) \right) \]

\[ -K_1 [B]_0 + \ln 100 \]

where:

\[ \alpha = \frac{k_+ [Y]_0 [OAF]}{k_- [OAF] + k_+} \]

\[ \beta = k_+ [OAF] + k_- \]

\[ [B]_0 = \text{initial concentration of acceptor B} \]

\[ [Y]_0 = \text{initial concentration of carrier Y} \]

\[ [OAF] = \text{concentration of OAF}, \text{which is assumed to remain constant} \]

\[ V_i = \text{viability} \text{ (%)} \]

\[ K_1, k_+ \text{, and } k_- \text{ = constant, and} \]

\[ t = \text{time (h)} \]

An OAF-induced decay curve (typical of the data available and corrected for any viable loss occurring under similar conditions but in the absence of OAF [21]) for Escherichia coli commune was analyzed as before (2), but with equation 5, arbitrarily assigning a value of 10 to the pertaining OAF concentration. The follow-
ing values of the constants were obtained: $k_1 [B]_o = 16.44$ (unitless), $k_2 [Y]_o = 1.37$ (h$^{-1}$), $k_3 = 0.02$, $k_4 = 0.88$ (both arbitrary units).

With these values for the constants, a family of decay curves (Fig. 1) was calculated from equation 5 for other values of OAF concentration. Comparison of other experimental decay curves (obtained with *E. coli commune* grown, etc., under similar conditions) with those in Fig. 1 enabled a value of OAF concentration to be assigned for such decay curves. Hence, the concentration of OAF may now be compared on a scale giving relative concentrations. Comparison of OAF concentrations so derived showed that usually values from 2 to 15 were obtained (i.e., a seven- to eightfold change in OAF concentration), whereas a value close to 10 was obtained on most occasions. Previously, it has not been possible to give any estimate for the day-to-day variation in OAF concentration.

By using the ventilation technique for Hood (submitted for publication), it was possible to ensure that the above method does apply in practice. Samples of a culture of *E. coli commune* were exposed concurrently on microthreads (8) to open air and to open air diluted with known amounts of clean air, and viability was measured as a function of time. These experimentally derived decay curves were compared with those in Fig. 1, and acceptable agreement was usually obtained (Fig. 2).

The method described in this paper also has been used successfully for coliphage T1 and T7, and Semliki Forest virus exposed to OAF, i.e., the same mechanism (equations 1 and 2) can account for loss of viability (induced by OAF) of at least some bacterial and viral strains. However, the species *Y* and *B* need not be the same biological moieties in all life forms affected by OAF.

OAF probably occurs in the parts-per-hundred-million range (5), and therefore it may be difficult to detect it or to determine its concentration other than by using viable decay of microorganisms, e.g., long-path infrared studies failed to indicate the nature of OAF. However, if an absolute method is developed, then the

![Figure 1](image-url)

**Fig. 1.** Viability (%) as a function of OAF concentration and time, calculated by equation 5 (see text).

![Figure 2](image-url)

**Fig. 2.** Viability (%) as a function of different dilutions of open air and time compared to theoretical curves as given in Fig. 1. Symbols: ——, experimental curves; ----, theoretical curves taken from Fig. 1. Percent open air values of 100, 75, and 50 have OAF values of 10.7 (100%), 8.2 (77%), and 4.8 (45%), respectively. Average experimental variation for replicate experiments = ±25% of percent viability value and ±10% of percent open air value for values <100%.
scale proposed here can be converted to absolute units.

The method described in this paper seems likely to be applicable to the study of other pollutants which induce viable decay.

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