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The design and use of aerated microcosms in mineralization studies

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Abstract

The need for aeration of microcosms during mineralization of ^{14}C-labeled compounds in high oxygen demand environments was assessed using active compost-soil mixtures as the model system. Rapid mineralization of ^{14}C-hexadecane occurred in continuously aerated microcosms while no mineralization occurred in unaerated microcosms. Daily flushing with air also yielded no mineralization. Mineralization of ^{14}C-glucose was much less dependent on aeration. The alkaline solution volume and number of CO_2 traps required for continuous aeration were calculated and tested experimentally.

Introduction

Bioremediation is often the least expensive means of contaminated site remediation. Its progress is monitored by standard chemical analyses of soil and groundwater samples from the site undergoing bioremediation. Although chemical analysis of soil samples may show a reduction in the level of contaminants in the polluted soils, additional tests are required to determine whether these compounds are truly undergoing mineralization to CO_2 and H_2O (Kästner and Mahro 1996). Standard sealed microcosms containing an internal alkaline trap to capture $^{14}CO_2$ are widely used to assess mineralization rates in soils. Unfortunately, there are occasions when such a simple approach may produce misleading data. In particular, if organic matter is added to stimulate biological activity, the rate of oxygen uptake may increase greatly leading to anaerobiosis in the microcosms. An extreme example of this is composting. Composting of soil has been shown to increase the rate of biodegradation of a variety of pollutants (McFarland et al. 1992; Freeman and Harris 1995; Michel et al. 1995; Beaudin et al. 1996; Beaudin et al. in press). In this process, organic amendments such as straw and manure are mixed with the soil and stacked in windrows or treated in a reactor. Immature active compost-soil mixtures have a very high oxygen demand and also produce large amounts of CO_2. Some authors have tried to satisfy the oxygen demand in microcosms by periodic flushing with air or pure oxygen (Rasiah et al. 1991; McFarland et al. 1992). However, since O_2 consumption rates can reach 100 mmol h^{-1} (kg of dry compost)$^{-1}$ during composting (Bach et al. 1984; Beaudin et al. 1996), a simple calculation will show that oxygen limitation occurs quickly even after pure oxygen has been added. Many researchers are aware of this issue and have used continuously aerated devices (Pritchard and Bourquin 1984; Kästner and Mahro 1996; Rosenbrock et al. 1997) but even this approach will produce inaccurate results when not all of the metabolic CO_2 is trapped. Even when the amount of alkaline solution is sufficient to capture the large quantities of CO_2 produced, mass transfer in the traps may be ineffective due to inadequate contact between the gas and the alkaline solution. The objective of the present work was to design a small inexpensive continuously aerated microcosm apparatus and to predict and verify its performance in terms of the provision of O_2 and the capture of the metabolically-derived radioactive CO_2. It was tested with radiolabeled glucose as suggested by Harrison et al. (1971) and applied to degradation

of hexadecane in compost-soil matrices as a model process.

Materials and methods

Preparation of the compost

A batch reactor having a total volume of 11 L and equipped with a heating jacket was used to prepare the compost-soil matrix. Heat loss through the reactor walls was calculated based on the temperature difference between its inner and outer surfaces. The heating jacket was activated depending on the calculated heat loss value. Using this temperature control strategy and aerating with 1 L/min of moist air, an autoregulated temperature profile was obtained which consisted of a mesophilic, a thermophilic and a curing stage.

For all compost-soil matrices the initial composition was 640 g of a silty soil contaminated with aliphatic hydrocarbons (TPH = 40 000 mg/kg) and PAH (630 mg/kg), 250 g maple leaves, 750 g alfalfa and 80 g (w/w) $CaCO_3$. The thermophilic stage compost-soil matrix was obtained after 23 hours (temperature and CO_2 production rate at sampling 51°C and 83 mmol $kg^{-1}h^{-1}$ respectively). The mesophilic stage-compost matrix was obtained after 22 hours (temperature and CO_2 production rate at sampling 31°C and 65 mmol $kg^{-1}h^{-1}$ respectively).

Microcosm apparatus and experimental

The apparatus used for microcosm experiments requiring continuous aeration or flushing is shown in Figure 1. For experiments with no aeration or flushing (standard sealed microcosms), the same 125 ml serum bottles and septa were used but with an internal trap (12 × 75 mm culture tube) containing 6 ml of a 2.5 M KOH solution. Each microcosm contained 10 g of compost (moisture content about 50% (w/w)). One set of experiments used thermophilic phase compost-soil spiked with glucose (0.046 μCi of ^{14}C-glucose (6.1 mCi/mmol, ICN, Radiochemical Division, Irvine, California) and 2.2 mg non-radioactive glucose per microcosm). Microcosms (duplicates) were incubated at either ambient temperature or 55°C and were either aerated continuously or flushed (for 10 min after 3.25h, 20 min after 19h, and then daily for 20 min). Four external CO_2 traps with 4 ml 2.5 M KOH each were connected to each microcosm. Abiotic controls contained 0.4% (w/dry weight) NaN_3 applied as an aqueous solution. At the end of the experiment, 50 ml water was added to each microcosm and by addition of HCl the pH was lowered to 2. All of the gas generated during the acidification step was passed through the alkaline traps. The radioactive CO_2 released during and after acidification of the compost-soil matrix at the end of experimentation with ^{14}C-glucose accounted for less than 3% of the total CO_2 production. Another set of experiments used mesophilic phase compost-soil spiked with 0.046 μCi of ^{14}C-hexadecane (2.6 mCi/mmol, Sigma-Aldrich Canada Ltd, Oakville, Ont). and 2 mg unlabelled n-hexadecane. Microcosms (triplicates) were incubated at ambient temperature and were aerated continuously. Standard sealed microcosms were set up in parallel as were abiotic controls (0.4% of NaN_3, duplicates). The air flow rate for each aerated microcosm was about 40 ml/min (1 bar, 21°C).

Analyses

The 2.5 M KOH solution of the traps was exchanged periodically and replaced by fresh KOH solution. Ten ml of scintillation cocktail (OptiPhase 'HiSafe' 3, Fisher Chemicals) was added to the sampled KOH solution and mixed vigorously. Radioactivity levels were determined with a liquid scintillation counter (model LSC 1409, Wallac Scintillation Products).

Results and discussion

The necessity for continuous aeration

Samples from the thermophilic phase of batch soil composting were placed in microcosms, spiked with ^{14}C-glucose, and incubated at room temperature or at 55°C. After 3.25 h at ambient temperature, there was 17% more mineralization when the microcosms were continuously aerated (Figure 2a). This difference became less pronounced as the experiment progressed probably due to decreased O_2 demand. Thus, although continuous aeration was initially required to avoid oxygen limitation, there was little significant difference in the cumulative glucose mineralization data whether the microcosms were continuously aerated or flushed daily. However, at 55°C, the difference was more pronounced. When continuously aerated, glucose was immediately and rapidly mineralized, whereas under periodic flushing conditions, significant mineralization only began after 8 days (Figure 2b). Glucose may be degraded either aerobically or fermentatively. It is possible that the mesophilic glucose-degrading

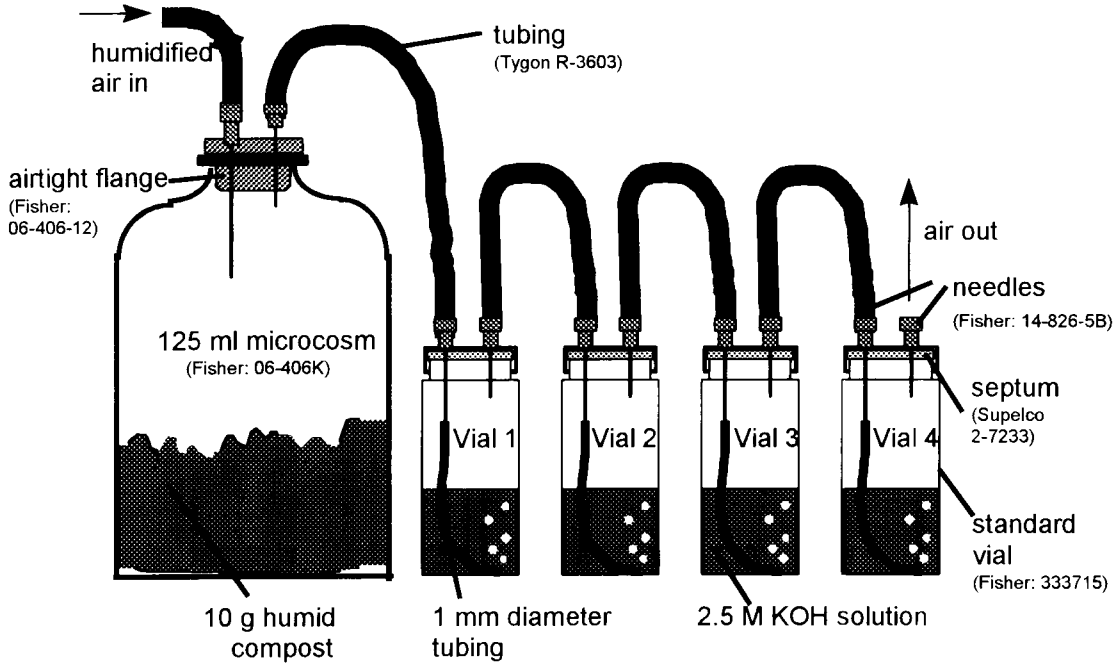

Figure 1. Experimental apparatus used for tests with continuous aeration and periodic flushing. Each external CO_2 trap contained 4 ml KOH solution corresponding to an exchange height of 1 cm. The source and catalogue number of materials are shown in brackets.

population was composed of numerous facultative anaerobes while under thermophilic conditions a period of adaptation was required to enrich for organisms that could function anaerobically.

The metabolism of hydrocarbons such as n-hexadecane has never been shown to proceed fermentatively but requires an electron acceptor such as molecular oxygen or nitrate. No significant mineralization occured in the standard sealed microcosms when mesophilic stage compost-soil was spiked with ^{14}C-hexadecane (Figure 3). The initial oxygen content (21% v/v) as well as the small amount that may have leaked into these microcosms during sampling of the internal CO_2 trap was insufficient. After 30 days the standard sealed microcosms were flushed daily for 5 min with about 40 ml/min of humidified air but still no hexadecane mineralization occured. Only after subjecting the microcosms to continuous aeration (day 37) was a positive response obtained (Figure 3). The mineralization rate decreased significantly when aeration was again diminished to daily flushing (data not shown). Hence, continuous aeration is necessary to correctly assess aerobic mineralization rates in actively respiring matrices such as thermophilic-stage compost.

Design of CO_2 traps

Care must be taken in the design of continuously aerated microcoms due to the large amount of unlabelled CO_2 produced and the increased gas flow rate. The amount of KOH required to trap all of the CO_2 produced in our system was about 7.2 mmol/day (for 5 g dry compost per microcosm). Since ambient air also contains some CO_2(40 ml/min, 0.03% CO_2), an additional 0.7 mmol/day of CO_2 must be considered. Since two moles of KOH are required to react with each mole of CO_2, the minimum amount of KOH necessary should have been 15.8 mmol/day, or 6.3 ml of a 2.5 M KOH aqueous solution per day. The contribution of radiolabeled CO_2 would only be 10^{-4} mmol and can thus be neglected.

Insufficent mass transfer in the alkaline trap can lead to incomplete CO_2 capture even when a sufficient amount of alkaline solution is present. Standard microcosms use an internal trap (Millette et al. 1995) where mass transfer of CO_2 into the alkaline solution only occurs at the static gas-liquid interface. Use of an external trap where the gas bubbles pass through the alkaline solution allows for a greatly increased mass transfer rate. At low CO_2 concentrations and assuming a fast reaction in the liquid phase, transport from the bulk gas phase to the gas-liquid-interface repres-

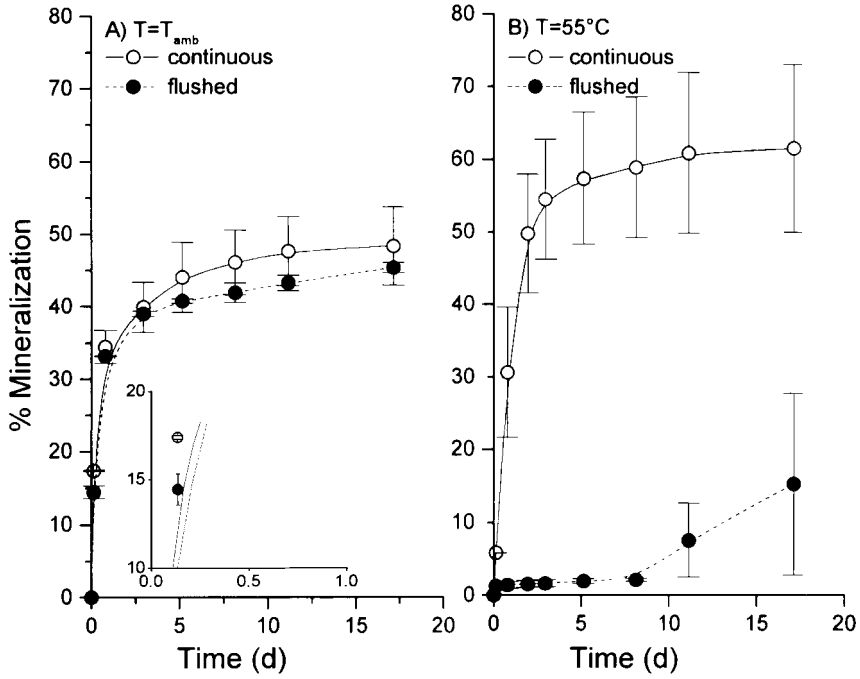

Figure 2. Comparison of the effects continuous aeration and periodic flushing on the mineralization of glucose in thermophilic-phase compost-soil incubated at ambient temperature (A) or at 55°C (B).

ents the transport resistance (Deckwer 1991) and the absorption rate (R_A) becomes:

$$R_A = k_s C_g$$

where the mass transfer coefficient (k_s) depends on the rising velocity of the bubble in the KOH trap, on the bubble diameter and on the diffusivity of CO_2 in air. The residence time corresponding to absorption of 99% of initial CO_2 in the bubble is:

$$\tau_{99} = \frac{\ln 100 d_b}{6 k_s}$$

With an inlet diameter of 1 mm and a viscosity of 1.24 cp (corrected for 2.5 M KOH), the bubble size diameter (d_b) should be 3.6 mm (Perry 1973). Bubbles of this diameter rise with a velocity of about 23 cm/s (Sherwood et al. 1975), and k_s is in the range of 2 to 5 cm/s (McCabe et al. 1993). With the above rising bubble velocity and residence time, the minimum height required is 3.0 cm. This corresponds to 3 standard scintillation vials (inner diameter 2.2 cm) in series, each filled with 4 ml KOH since this volume of KOH solution gives a liquid level of about 1 cm in each vial.

Verification of the alkaline trap efficiency

Experiments were conducted with thermophilic phase active compost-soil to verify the aforementioned design parameters. Four vials, each filled with 4 ml of solution, were used (one more than the minimum 99% absorption height). The results confirmed the calculations since more than 99% of total radioactivity was captured in the first three CO_2 traps (Table 1). The measured values for the transfer coefficient k_s at ambient temperature as well as at 55°C were in the range of 1.9 to 3.3 cm/s which is consistent with values found in the literature (Sherwood et al. 1975; McCabe et al. 1993). Although sufficient KOH was present in the first trap to capture all the CO_2 generated, significant amounts were found in the second and third traps (Table 1) demonstrating the predicted exponential absorption behavior. Thus, even when KOH is in excess, the CO_2 can only be trapped if sufficient contact with the aqueous (KOH) phase is allowed.

In summary, insufficient aeration of microcosms may significantly affect mineralization data. To assess aeration requirements for a microcosm, the oxygen uptake rate should be predicted, or preferably, measured. This value can be used to calculate the time for the oxygen concentration in the headspace to fall to an

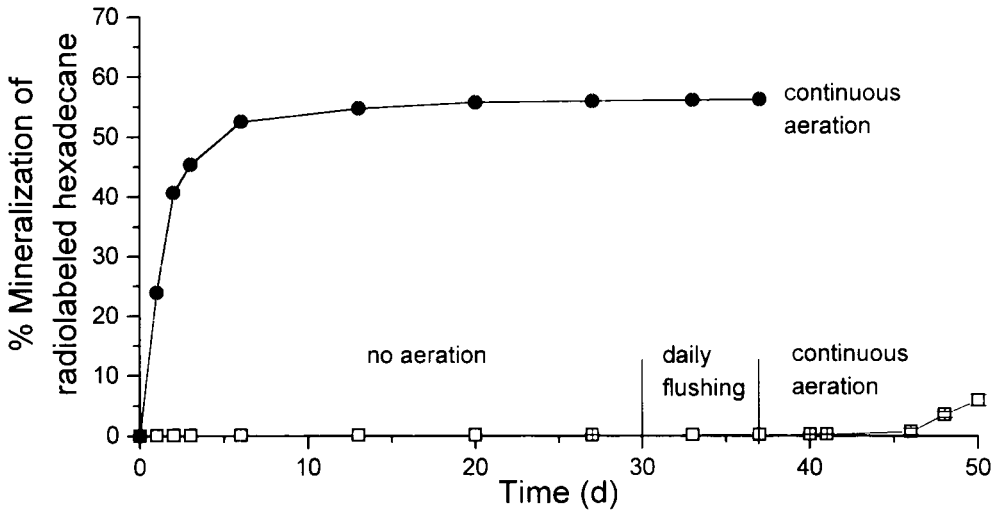

Figure 3. The effect of aeration on the mineralization of hexadecane in mesophilic-phase compost-soil.

Table 1. Radioactivity values measured after 19 h in the four CO_2 traps connected in series to aerated microcosms incubated at either ambient temperature or 55°C

Trap no.	Measured radioactivity (dpm)	
	ambient temperature	55°C
1	15,489	15,042
2	1,656	680
3	107	77
4	16	7
	k_s = 3.3 cm/s	k_s = 3.6 cm/s

unacceptable value, below which major physiological changes may be expected to begin to take place in the culture. This should be taken as the maximum time between flushing. Whether flushing is adequate or if continuously aerated microcosms are required, both the amount of alkaline solution and the mass transfer of CO_2 into the traps must be considered. The sample calculation shown in the trap design section can serve as a model to determine conditions for complete absorption (i.e., at least 99%) of the radioactive CO_2.

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