Method for Measuring Mineralization in Lake Sediments

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A method is described for measuring the mineralization of an organic solute (14C-glucose) by the heterotrophic indigenous bacteria in lake sediments. Since there is no suitable procedure for the determination of in situ microbial activities in sediments, the procedure described is probably the best devised so far and may serve as a base for a more definitive procedure.

The surface sediments of aquatic ecosystems are the boundary between a circulating dynamic medium primarily dominated by properties of water and its solutes and a structurally more stable medium with properties much like soil. This boundary area is the site of intense microbial activity in many natural waters. A great variety of bacteria can be isolated from sediments, and they are present in high concentrations, but only limited information can be obtained by culturing these bacteria. Methods are needed that give information on biological and chemical processes promoted by the whole community of microorganisms in the sediment environment.

One important function of sediment microorganisms is their role in mineralizing organic matter. For measuring the response of sediment microorganisms to low concentrations of specific organic solutes, 14C-labeled compounds are an obvious choice. This paper is a report on the application of 14C-labeled glucose and acetate to lake sediments as a means of obtaining data on the ability of microorganism communities in the mineralization of those compounds.

MATERIALS AND METHODS

Principles applied. Measurements of organic solute uptake in marine and fresh waters have demonstrated the presence of microbial populations with transport capabilities that are extremely effective at the low substrate concentrations found in such habitats (6, 7, 11, 12). In most fresh waters, and in estuarine and coastal marine areas, these populations often (but not always) show a response of transport rate to substrate concentrations that can be analyzed according to Michaelis-Menten kinetics. The kinetics agree well with those found for transport by pure cultures of bacteria (1, 4, 8). However, samples from open ocean areas show this kind of response only after being exposed to a given substrate for several hours or longer, and often do not begin to show utilization of a substrate even after several days of enrichment contact. A delayed response may be due to the need for an increase in the size of the natural population before their activities can be detected or to the need for the induction of transport systems in the existing microorganisms, or to a combination of these. More definitive data are needed to clarify this problem. The present paper deals only with samples not exposed to the substrates prior to making the measurement.

Until recently, measurements of solute uptake by natural microorganism populations dealt only with substrate retained in the cells; any respired substrate was not detected. However, Hobbie and Crawford (3) developed a method that corrects for respired substrate by measuring the 14CO2 given off during incubation. This information combined with uptake, as measured by substrate retained in cells filtered on membrane filters after incubation, gives total uptake. Their results indicate that with or without the respiration correction, uptake follows Michaelis-Menten kinetics.

If uniformly labeled substrate is used, the data obtained from respiration measurements can be treated independently and will allow calculation of several parameters for mineralization of the substrate by natural populations. Data from Klamath Lake, Ore., for several substrates have indicated that mineralization is proportionate to total uptake, and total uptake usually follows Michaelis-Menten kinetics when measured over a range of substrate concentrations. These findings, to be published elsewhere, are then in good agreement with those of Hobbie and Crawford (3). The adapted Lineweaver-Burk equation derived by Wright and Hobbie (11) is employed to give mineralization turnover time and maximal velocity.

This equation is \( t/f = (K + S)/V_{\text{max}} + A/V_{\text{max}} \) where \( t \) is incubation time (in hours), \( f \) is the fraction of available substrate mineralized, \( K \) is a constant related to uptake, \( V_{\text{max}} \) is the maximal velocity for mineralization (which would apply when transport is at maximal velocity), \( S \) is the natural substrate
concentration (unknown), and \( A \) is the concentration of added substrate. Mineralization is measured at several concentrations of \( A \), each of which generates a different \( t/f \), and when \( t/f \) is plotted against \( A \) a straight line should result. Extrapolation back to the \( y \) intercept of the line, where \( A = 0 \), gives the turnover time for mineralization at the natural substrate concentration. The inverse of the slope of the line is a measure of \( V_{\text{max}} \).

Experimental procedures. \(^{14} \)C-labeled substrate was added in equal amounts to a series of small serum bottles (we used 0.1 \( \mu \)Ci per sample in 50-ml capacity serum bottles; see Fig. 1). Then unlabeled substrate was added at several concentrations, followed by sterile distilled water so that the total water volume per sample was 2 ml. A large number of bottles can be prepared in this way and, if desired, can be stored frozen or chilled for transportation to the field.

Sediment samples were obtained with an Ekman dredge and stored at 5 C. A few hours before making the measurement, sediment was allowed to warm to natural temperature while being stirred gently with a magnetic stirring bar. Dry weight was determined by oven-drying replicate samples for 24 hr at 100 C and comparing weight with measured volume. Working at natural temperatures, we added replicate 5-ml portions of slurried sediment (approximately 350 mg, dry weight) to each prepared serum bottle and mixed them with the substrate, with careful control of the time of addition. We normally used four samples for each concentration of added substrate. One of each set of four samples was a blank to which was added 1.0 ml of 1 N \( \text{H}_2\text{SO}_4 \) just prior to addition of the sediment. Each bottle was capped immediately after addition of the sediment with a serum cap pierced with a small plastic rod and cup assembly (Kontes Glass Co., Vineland, N.J.; K-882320). The cup, which was suspended in the air space over the sediment slurry, contained an accordion-folded piece of filter paper (Whatman no. 1, 2.5 by 5 cm). Mineralization was allowed to proceed for 5 min, and then 1.0 ml of 1 N \( \text{H}_2\text{SO}_4 \) was added through the serum cap to kill the microorganisms and release all dissolved \(^{14} \)CO\(_2\). After 2 hr, 0.15 ml of \( \beta \)-phenylethylamine was added to the filter paper through the serum cap. The \( \beta \)-phenylethylamine completely adsorbed the \(^{14} \)CO\(_2\) within 1 hr, and after this time had elapsed the bottles were opened, and the filter paper was removed and placed in a scintillation vial containing 2,5-diphenyloxazole and 1,4-bis-2-(4-methyl-5 phenyloxazolyl)-benzene in toluene. Quenching was corrected with the channels ratio method, and data were corrected for blank activity and applied as in the equation above to give \( t/f \), which was plotted against added substrate concentration (labeled plus unlabeled).

RESULTS AND DISCUSSION

The method was applied to sediment obtained with an Ekman dredge from Upper Klamath Lake, Ore. Klamath Lake is shallow (mean depth, 2.7 meters), and the bottom is often mixed by wind action. The sediment is a gray and flocculent clay-base material that gives an appearance and texture quite similar to activated sludge. The plate counts on the sediment showed a range of \( 10^7 \) to \( 10^8 \) casein- and starch-hydrolyzing bacteria per g (dry weight).

Results for glucose mineralization are shown in Fig. 2. Turnover time and \( V_{\text{max}} \) obtained from

![FIG. 1. Serum bottle apparatus used.](image-url)
Michaelis-Menten kinetics. The most important requirements are (i) linearity of response over time, (ii) the absence of induction phenomena, and (iii) little change of substrate concentration over the incubation period. We found mineralization in the sediment to be so rapid that concentrations routinely used for measuring planktonic mineralization were much too low. To establish the useful concentration range, the time course of mineralization was measured over several orders of magnitude. The results, as can be seen in Fig. 3 and 4, showed several interesting phenomena. At the lowest concentrations, glucose was mineralized so rapidly that the available glucose was quickly depleted. At the highest concentrations, there seemed to be so much glucose present that induction of additional transport and mineralization enzymes might be occurring in a remarkably short period of time.

The intermediate ranges of 1 to 5 µg of unlabeled glucose per 5 ml of sediment were judged suitable for the kinetic study, and were used suc-

Fig. 3. Effect of increased amounts of unlabeled glucose on the mineralization of labeled glucose. Time of incubation is the variable. Each reaction vessel contained 2 ml of substrate (various concentrations) and 5 ml of slurried sediment (ca. 350 mg, dry weight; see Materials and Methods).

this measurement were 2.25 hr and 2.4 µg of glucose per g (dry weight) per hr. Converting this to wet volume on the basis of a dry weight content of 7% gave a V_max of 0.17 µg of glucose per ml of sediment per hr. The same parameters measured from Klamath Lake water on the same day were 220 hr for turnover time and a maximal velocity of 2.6 \times 10^{-8} \mu g of glucose per ml per hr. By this comparison, the top square centimeter of sediment was capable of mineralizing 24 times as much glucose as the overlying square centimeter column of lake water, based on a mean depth of 2.7 meters. The short turnover time indicates that such biologically active substrates as glucose could not accumulate in lake sediments. This is in agreement with the findings of Vallentyne and Whittaker (9) of extremely low glucose concentrations, in the order of 5 µg/liter of interstitial water in lake sediment.

It is essential, in measuring what are hoped to be natural parameters of mineralization, to establish a range of concentration of added substrate which fulfills the requirements of using
Mineralization in sediments successfully in all subsequent measurements, with a 5-min incubation time. These data clearly show the inadequacies of measuring mineralization at one concentration of glucose.

Another substrate we applied to Klamath Lake sediments was acetate. Time-course and concentration studies indicated the need to work at even higher concentrations than for glucose. The results of one kinetic measurement are shown in Fig. 5. Turnover time was rapid (0.75 hr), compared with the mineralization turnover time of 250 hr from the overlying water on the same date. When working with acetate, higher than normal blank values have consistently been obtained, both from sediment and water samples. Although we have not investigated this phenomenon, it is probably attributable to absorption of volatilized $^{14}$C-acetate by the $\beta$-phenylethylamine.

Other methods for measuring mineralization have been suggested in which $^{14}$C-labeled substrate is used (5, 10). However, these methods involve measurements at only one substrate concentration. Our results have shown that a range of concentrations should be employed to avoid the accelerating mineralization we have attributed to induction, or deceleration of measured mineralization due to exhaustion of the substrate. Of course, the kinetic approach by definition implies the use of a range of concentrations. Wood and Hobbie (personal communication) used a kinetic approach to measure uptake by sediment bacteria, and if they were to use uniformly labeled substrate their approach would also give mineralization turnover and maximal velocity. However, they added 1 ml of sediment to 99 ml of filtered water, and thus changed the physical character of the habitat. It could even be argued that the present method is too drastic a technique to measure a "natural" process, and this may be especially serious with sediments more solidly structured than those of Klamath Lake. Where disturbance of the sediment is considered serious, the technique of obtaining small core samples might be applicable, and labeled substrate could be added only to the material from the mud-water interface (2). Use of the mud-water interface would represent the most important portion sampled because it contains most of the organic matter sedimented from the water above as well as the greatest microbial population. Other parameters should also be considered, such as the state of oxidation of the sediment. All the work reported here was performed on well-stirred sediment.

These early results have enabled us to compare the mineralization activity of sediment with that of the overlying water. For glucose and acetate, microbial activity of the sediment is several orders of magnitude greater than that of the overlying water. Other substrates available with uniform $^{14}$C labeling may be applied with this technique. If the naturally occurring substrate can be measured independently, the kinetic approach allows calculation of actual mineralization rates. The complexity of the kinetic approach is unfortunate, but there is probably no other way, at present, to obtain the desired information. This approach has already proven to be a powerful tool in the study of in situ planktonic bacterial activity, and, with proper cautions, it may also prove quite useful in efforts to learn what bacteria are doing in the sediments.

There is, at present, no suitable procedure for determining the actual in situ activities of microorganisms in the sediment. The procedure presented here is, we believe, the best currently available method, and it may serve as the foundation for a truly definitive procedure.
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