

This Week's Citation Classic

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Hobbie J E & Crawford C C. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. *Limnol. Oceanogr.* 14:528-32, 1969. [Dept. Zoology, North Carolina State Univ., Raleigh, NC]

A method is described for using the uptake kinetics of organic compounds as an assay of activity of planktonic microbes. Parts-per-billion concentrations of ^{14}C -labeled amino acids, sugars, or fatty acids are incubated with plankton for 0.5-2.0 hours and the incorporation of ^{14}C and the release of $^{14}\text{CO}_2$ is measured. [The *SCI*[®] indicates that this paper has been cited over 140 times since 1969.]

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"The problems of measuring rates of microbial activity in the plankton of lakes and oceans are formidable. Obvious methods such as looking at the release of CO_2 or the uptake of O_2 do not work because the changes are so slow that days or weeks of incubation are needed. If the microbes are concentrated by filtration or even placed in a bottle for more than a few hours then communities and rates change.

"Richard Wright and I, young postdoctoral fellows at Uppsala, started out by looking at algal uptake of low levels of sugars and organic acids. We had to modify the kinetic approach of Parsons and Strickland¹ for the mixed populations of lakes and for the parts-per-billion levels of added substrates but it soon became obvious that bacteria were really doing the uptake in lakes. More important, the kinetic parameters of V_{max} , K , and turnover of substrate gave us a handle on the seasonal changes of bacterial activity caused by temperature, release of organics by algae, and input from rivers.

"Despite my interest in microbes, the department of zoology at North Carolina State University hired me and soon a graduate student, Claude Crawford, and I began to look at microbes in estuaries, oceans, and a nearby cow pond.

"At that time, the kinetic approach only measured the incorporation of ^{14}C into the microbes; the respired $^{14}\text{CO}_2$ was lost. Our first attempts to improve the method used the incredibly laborious ion chamber technique.² Then Don Smith, a mammalian physiologist, gave a job interview seminar and we were excited and chagrined to learn of a quick and easy method (Kontes even made the glassware). The key was absorption of the $^{14}\text{CO}_2$ on a wick soaked with a basic amine and detection of the ^{14}C with liquid scintillation. We quickly adapted this method from rat uteri to plankton and published the paper described here. Crawford was able to run hundreds of samples a day in his study of amino acid cycling in an estuary.³

"Our paper is cited today in part because it presents to ecologists a simple, rapid technique that is still the easiest way to measure the production of $^{14}\text{CO}_2$. It is also cited because the data given on the patterns of percent of respiration of amino acids, glucose, and acetate in the cow pond are the same as later found in all waters, even in the Sargasso Sea. Finally, the timing of the paper was right as liquid scintillation had just replaced planchette counters, proportional counters, and ion chambers for measuring ^{14}C and nothing better has come along. Today, a number of ecologists have had biochemical and microbiological training so new techniques from many fields are quickly tested for ecological applications. One group of techniques that measure bacterial growth by incorporation of isotopes into nucleic acids will probably soon give us the best method for microbial activity in the plankton. Then we can begin on the next question of controls of microbes and microbial activity in nature."

1. Parsons T R & Strickland J D H. On the production of particulate organic carbon by heterotrophic processes in seawater. *Deep Sea Res.* 8:211-22, 1962.
2. Hobbie J E, Crawford C C & Webb K L. Amino acid flux in an estuary. *Science* 159:1463-4, 1968.
3. Crawford C C, Hobbie J E & Webb K L. The utilization of dissolved free amino acids by estuarine microorganisms. *Ecology* 55:551-63, 1974.