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Measurement of Steady-State Growth Rates of a Thermophilic Alga Directly in Nature

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Steady-state growth rates of thermophilic algae can be determined directly in nature in the flowing waters of a hot spring channel by measuring the rate of loss of algal cells when the channel is darkened. The half time of the loss rate in the steady state is identical to the generation time. We studied the unicellular blue-green alga *Synechococcus* in Yellowstone National Park. Temperature and flow rate remained relatively constant throughout the experiment. Quantitative cores were taken and homogenized; the algal cells were then counted by use of a Petroff-Hausser counting chamber. After ascertaining that the algal population was in a steady state, the channel was darkened in such a way that neither flow rate nor temperature was altered. The population began to decrease within 1 day; the loss rate was exponential with time for 2 to 3 decades. Half times were then calculated from these loss rates. The growth rates found were considerably lower than those for the same organism in laboratory culture. The results suggest that in nature the organism may be an obligate phototroph. In two cases, after the algal populations decreased to an undetectable level, the dark covers were removed and the rate of recolonization was measured. The kinetics of recolonization were different from the kinetics of washout.

One of the most important parameters of a microbial ecosystem is the growth rate of the organisms present, since this value gives an index of turnover time, and hence, indirectly, of the rate of metabolism of the ecosystem (1). Although extensive data are available on growth rates of microorganisms in laboratory culture, there is no reason to believe that growth rates even approximately similar will prevail in nature. The difficulties of measuring growth rates in nature have been discussed briefly (1, 2). One method has been to use tritiated thymidine autoradiography (3); however, this procedure is limited to those cases where it is known that the organism is able to assimilate thymidine. In this paper, a technique of a more general nature is presented. It is most easily applied to steady-state situations, although a steady state is not required for the method to be effective. The simplest type of steady-state system is a homogeneous single-stage chemostat (8). In such a device, the generation time of the culture is a function of the replacement rate of the medium, which is determined by the volume of fluid in the vessel and the flow rate. Given a chemostat in operation under steady-state conditions but with an unknown flow rate (and hence an unknown generation time), in principle, we can still calculate the generation time if we decrease to zero the concentration of the limiting nutrient in the inflow. Further growth cannot occur because of the lack of nutrient—only washout occurs. We can measure the rate of washout by obtaining cell counts on samples at periodic intervals. The rate of loss of cells will be exponential with time because this rate follows the same kinetics as any dilution process. The time required for the population to be reduced to half its initial level is then equivalent to the washout rate or replacement rate. Because the generation time is proportional to flow rate, it can thus be calculated.

Although in this example growth was stopped by eliminating an essential nutrient, other methods of stopping growth could have been used. For instance, if we were studying an obligately phototrophic organism, growth could be stopped by eliminating the light. The experiments presented in this paper employ such a photosynthetic system to measure growth rate of a thermophilic alga directly in nature.

The effluent channels of many hot springs in Yellowstone National Park provide flow rate and temperature characteristics that are relatively constant over extended periods of time (4). At the higher temperatures, the only photosynthetic

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organism is *Synechococcus*, a unicellular blue-green alga, which grows in mats along the bottom of the channels. Because it is a unicellular organism, quantitation by cell counting was relatively simple. Steady-state growth occurs, as indicated by the small amount of daily or long-term variation revealed by our quantitative studies during a 2-year period. However, when the channel was darkened, the algae decreased in number and eventually disappeared completely. At the high temperatures involved, animal grazers were absent (5); thus, the main reason for this disappearance was probably the washing away of the algal cells in the flowing water after they had loosened from the bottom of the channel. In the steady state, the washout rate was equivalent to the generation time; thus, the latter parameter could then be calculated. This particular system was analogous to that described by Herbert (8) as a heterogeneous single-stage chemostat.

MATERIALS AND METHODS

Study area. Studies were carried out on two hot springs in the Lower Geyser Basin of Yellowstone National Park, Wyoming. One of these, Mushroom Spring (Fig. 1), was described in detail previously (5). This spring has a large pool and a single outflow through a narrow channel; the pool temperature was about 73 C, and the temperature of the water in the channel ranged from 71 C at the source to 25 C at the foot. The other was a nearby unnamed spring, known to us as Grassland Spring. This spring has no pool, and the source arises directly in the bottom of the channel [a rheotherm according to the terminology of Schwabe (11)], at a temperature of 82 to 83 C. The uppermost temperature for algal growth is from 73 to 75 C in these springs; thus, the algae are seen throughout the length of the channel in Mushroom Spring, but in Grassland Spring algae are seen only when the water has cooled to about 73 to 75 C. In Mushroom Spring the channel has a hard siliceous bottom, whereas Grassland Spring has a clay bottom. The water in all areas of the channels used for our study had approximately laminar flow characteristics, resulting in relatively flat homogenous algal development over regions of at least 100 cm². Permanent stations were established at suitable locations along the channels (5). There was no shading of the regions of the channels studied during the day. The water column over the algae was about 2 cm thick; therefore, the organisms received essentially maximal sunlight, which at midday was about 1.3 g cal per cm² per min.

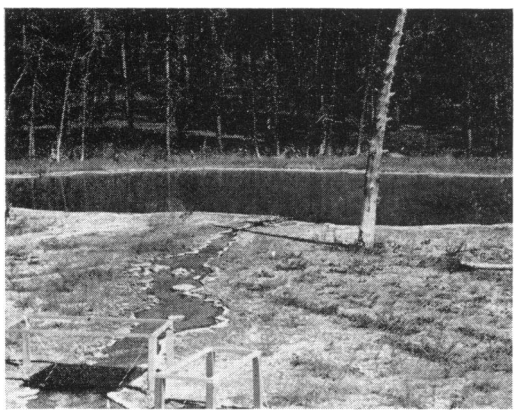

FIG. 1. *Mushroom Spring, Lower Geyser Basin, Yellowstone National Park. Station II is in the foreground and Station I is at the point where the effluent leaves the main pool. Photographed on 28 August 1967.*

Sampling and cell counting. Quantitative cores of algal material were removed with a no. 3 brass cork borer (0.28 cm²); duplicate cores were then acquired from separate parts of the coring region (no two cores were taken more closely together than 3 cm). The cores were placed in vials, and the volume was brought to 5 ml with spring water containing 4% formaldehyde. The cores were homogenized (usually on the same day they were secured) with a Teflon homogenizer in a polypropylene centrifuge tube, (Brock and Brock, Limnol. Oceanog., *in press*). A 1-ml amount of homogenate was removed for cell counting, the remainder was centrifuged and the pellet was then suspended in 4 ml of acetone for chlorophyll assay (Brock and Brock, Limnol. Oceanog., *in press*). Although all cores were assayed for chlorophyll, these results will not be given here because chlorophyll content bears no simple relationship to cell number. Cell counting was performed with a Petroff-Hausser counting chamber by use of phase microscopy (40 objective, 12.5 ocular). The alga was easily recognized and was readily distinguished from filamentous bacteria and debris (Fig. 2). Chains of cells were never seen, although occasionally two cells remained attached after cell division. When a furrow was seen separating the cells, they were counted as two. All counts were corrected to the volume of the original homogenate. In most cases, at least 100 cells were counted for each sample. When the cell count was low, the counting chamber was filled several times. At very low cell densities, this practice could not be followed and smaller numbers of cells were counted. All counts were duplicated, and at each sampling duplicate cores were taken. Counts are presented as the average number per milliliter of homogenate.

Environmental measurements. Temperatures were measured with a thermistor (Yellow Springs Instrument Co., Yellow Springs, Ohio) and checked occasionally with a conventional mercury thermometer. Some chemical analyses of Mushroom Spring water were described previously (5).

Darkening procedure. After samples were tested at the various stations for several days to establish the steady-state levels, the areas were darkened in a way which did not impede water flow. Opaque black plastic

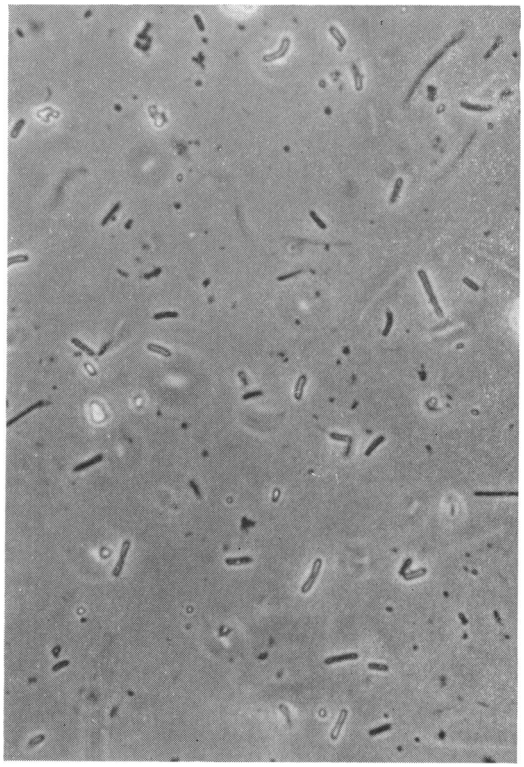

FIG. 2. *Phase-contrast photomicrograph of the homogenate of a core taken at Station II, Mushroom Spring. Synechococcus cells are the predominant organisms; also shown are a few filamentous bacteria and particles of silica.* × *445.*

sheeting (10 mils thick) wrapped around plastic-coated welded fencing and fastened with black plastic tape gave a stiff flat form, about 45 × 60 cm. This form was suspended by a nylon rope from a wooden frame which straddled the stream (Fig. 3). Cores were removed only from the central region under the plastic (usually within an area of 100 cm^2) where total darkness existed. The total darkness of this central area was verified by measuring $^{14}CO_2$ incorporation into algal mats (Brock and Brock, Limnol. Oceanog., *in press*). The rate of incorporation was the same in the transparent vials as in the opaque vials; both rates were less than those for similar cores exposed to full sunlight.

RESULTS

The initial darkening ocurred at about 1800 hr, Mountain Daylight Time, so that the initial darkening period corresponded with the normal night-time period. Cores were then secured at about 1200 and 1800 hr for the next 2 days, at daily intervals through several more days, and at less frequent intervals throughout the next month. Figure 4 summarizes the data obtained for the

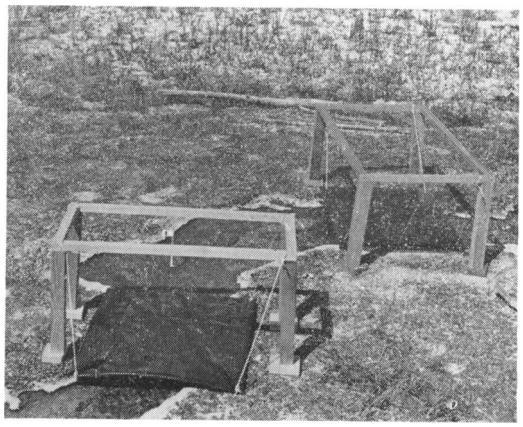

FIG. 3. *View of the procedure for darkening the channel. Station II, Mushroom Spring. The frame in the foreground is for the darkening experiment; that in the background contains neutral-density glass for an unrelated series of experiments.*

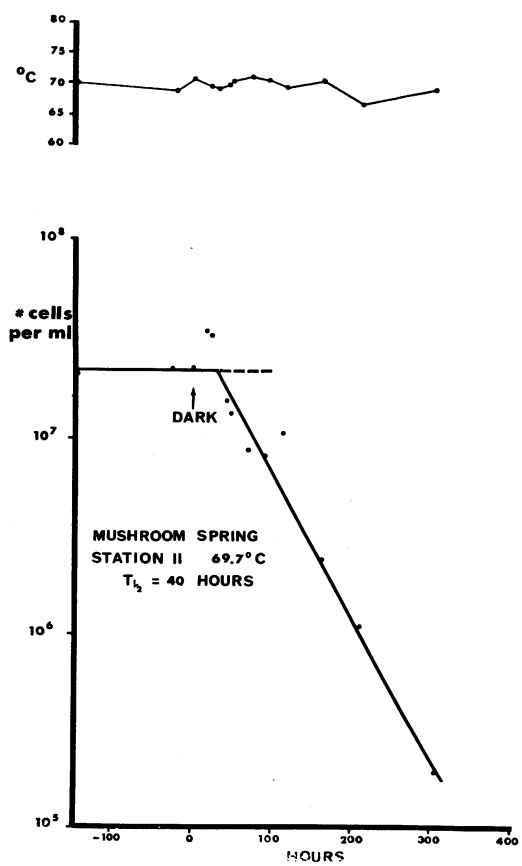

FIG. 4. *Loss rate at Station II, Mushroom Spring. Day zero, 15 August 1967.*

area (Station II) at 69.7 C shown in the photographs. The rate of loss of cells was exponential with time. The half time of the loss rate was 40 hr, and the generation time was 40 hr (if we assume that cell division can occur in both light and darkness). Within 1 week no more algae were visible and only bacteria remained. Figure 5 depicts the appearance of Station II after 2 weeks of darkness.

Data of a similar nature for a station at Grassland Spring (72.3 C) are presented in Fig. 6. Again, an exponential rate of cell loss was observed, with a half time of 22 hr. Similar experiments were conducted at five other stations; in all cases the loss rate followed an exponential function, although each station showed a different half time.

For two stations (after the loss of algal numbers appeared complete), the covers were removed and the rate of recovery was measured. After a delay, there was an exponential increase in cell numbers, followed by a continued slower increase. In neither case did the population density return to its original steady-state level during our observations. Presumably, the later stages of the recovery phase involved a gradual slow return to the original population density because even during the recovery period there was probably some washout, and thus the recovery rate can never represent the actual growth rate in the steady state.

DISCUSSION

This technique was a simple means of measuring growth rates of algae directly in nature. It should be applicable to any situation where steady-state conditions prevail, although the precise experimental design will naturally depend on the nature of the habitat.

FIG. 5. *Appearance of the area under the dark cover at Station II, Mushroom Spring, on 28 August 1967 after 13 days of darkening.*

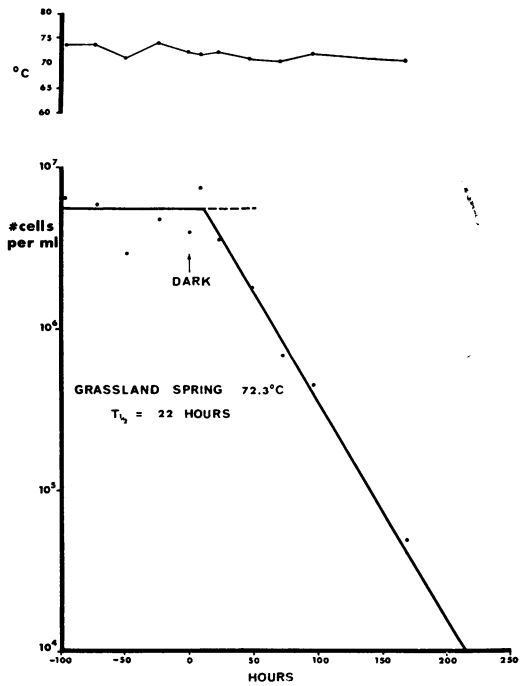

FIG. 6. *Loss rate at 72.3 C station, Grassland Spring. Day zero, 19 July 1967.*

An important requisite for this technique to be used is that the organism of interest must be recognizable microscopically in some specific way. Therefore, it is most useful for algae, protozoa, and the larger or morphologically distinct bacteria.

This technique could also be used to calculate the growth rate of heterotrophic organisms, if some means were available to inhibit their growth without affecting other organisms of the ecosystem. With bacteria, this might involve the addition of an antibiotic before the quantitative sampling.

The technique may also be applied to organisms not in the steady state, although with considerably more difficulty. In a habitat where cell numbers of an organism were increasing with time, there would still be losses occurring through settling, grazing, or washout (7); but growth would be occurring at rates greater than those needed to counterbalance losses. Under these conditions, loss rates should be measured by a technique similar to the one previously described for samples tested at various times. If the loss rate at each time were added to the observed rate of increase at this time, it would be

possible to arrive at a value for the actual growth rate.

Recently, there has been speculation whether algae in nature can grow heterotrophically (10; A. L. S. Munro and T. D. Brock, J. Gen. Microbiol., *in press*). At least in our study, such heterotrophic growth probably did not occur because the elimination of light alone led to the reduction of the algal population to undetectable levels. But this observation does not rule out very slow heterotrophic growth, at rates too low for the organism to maintain itself in the flowing water.

The growth rates calculated here were considerably slower than those obtained with *Synechococcus* cultures growing in enriched media in the laboratory (6, 9). This result emphasizes the point that the growth rate in nature is usually less than that observed under favorable laboratory conditions.

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