

differences in stereochemistry occur within a homologous series, related to chain-length, reflecting different sources of the higher and lower homologues. The stereochemical purity of a constituent is often lower than in the alkan-2-ols, reflecting differences in source and in biochemical processes as outlined above. The main features are outlined in Table 1.

The topics reviewed above give an indication of the complexity of sedimentary lipids in terms of the wide variety of compound classes, their chemical state in the sediments, the source organisms and biochemical processes involved in the early stages of diagenesis.

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GROWTH RATES OF BACTERIA IN RIVERS

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Ecosystems in general and rivers especially are not fixed in a particular state, but are continuously changing; sometimes rapidly, at other times imperceptibly, but never remaining exactly the same. Therefore, if we are to understand an ecosystem, we must observe and measure the way in which it is changing. One of the most important changes in the biota is brought about by growth, and this is just as relevant to the bacteria as it is to the more obvious components of river systems such as the fish. However, unlike fish, bacterial cells do not increase in size throughout their lives; hence we are concerned here with the growth of the population and not of individuals. Bacterial populations in rivers, as in most other environments, are numerically large; e.g. in the River Frome, Dorset, there are approximately 50 000 bacteria per ml (Baker & Farr 1977), but because bacteria are so small (of the order of 10^{-6} m long) their total biomass is also small.

In theory the determination of population growth is simply a matter of counting the entire population at time A and again at time B. The growth, which can be positive or negative, is then the difference between the two counts and can be called a growth rate when expressed per unit time. The difficulty of estimating growth arises because changes in the populations between time A and time B may arise from factors other than growth; for example grazing, wash out, recruitment from outside, etc. The problem is to isolate growth from all these other changes. Nevertheless it is relatively easy to count the numbers of bacteria per unit volume of samples of river water and observe how these change with time. There are wide fluctuations from week to week, but over relatively long periods (years) the average population in the River Frome remains more or less constant (Baker & Farr 1977). In other words the net change over a year in the numbers of bacteria suspended in the river is zero, but in the shorter term (hours or days) positive growth rates can be determined. Three different approaches will now be described.

In order to limit the changes in the bacterial population to those due to growth, one can isolate a portion of water, e.g. in a flask, and determine the population changes within it. However, over a period of days, the conditions such as temperature, aeration and nutrient concentration within the flask will differ markedly from those in the river, and the bacterial population will change accordingly. Thus the growth rates of the bacterial population in the flask bear little or no resemblance to those occurring at the same time in the river. The desirability of isolating river water from extraneous influences, while retaining its essential characteristic of continuous flow, is common to many problems of lotic ecology, and hence a large

recirculating experimental channel has been built (illustrated in *43rd Annual Report*). It consists of a race-track-shaped tank made of glass-reinforced plastic, 53 m in circumference and with a trapezoidal cross-section. It is filled to a depth of 70 cm with water which is circulated by an Archimedean screw pump at a velocity of approximately 0.25 m s^{-1} , similar to the current velocity of the R. Frome. Borehole water is used, because it forms 74% of the discharge of the R. Frome (Paolillo 1969) and is virtually free of all living organisms (bacterial concentration $< 5 \text{ ml}^{-1}$).

The circulating channel thus provided conditions much closer to those of the river than a simple flask, but it was still different in two obvious ways: (i) there was no sediment initially present; (ii) the channel was kept covered to prevent aerial contamination by birds and to inhibit the growth of algae. Under these conditions the bacterial population was periodically sampled and its size estimated by the viable or plate count technique using casein/peptone/starch agar (Jones, 1977). The number of bacteria rose steadily for about ten days and similar results were obtained when the experiment was repeated, as shown in Fig. 1. The lines are computed regressions based on the assumption that the bacteria grew logarithmically. This is a reasonable assumption, given that the most common form of bacterial multiplication is by binary fission, and it results in a good fit of the lines to the data. Thus the correlation coefficients were 0.96 and 0.99. The slopes of the lines are directly related to the growth rates of the bacterial populations and an analysis of covariance shows that the two slopes are not significantly different from each other. Growth rates of bacteria are commonly expressed as the time taken for the population to double, and the computed common slope for these experiments gives a doubling time of 4.2 h.

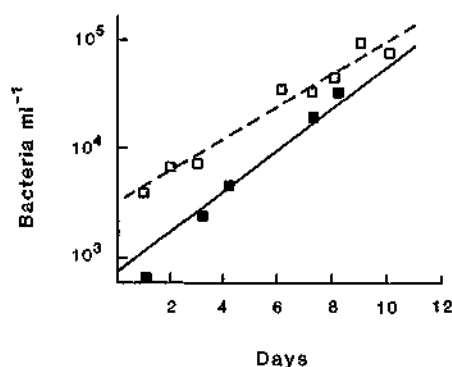


FIG. 1. Semi-logarithmic regression of bacterial numbers against time in the Waterston recirculating channel. Each point is the mean of approximately ten determinations.

After ten days the bacterial population stopped increasing and oscillated around abundances typical of those in rivers (Ladle et al. 1977). The recirculating channel thus resembled a giant 'batch culture' of a natural bacterial population with bore-hole water as the medium. Nevertheless, as mentioned earlier, the recirculating channel differed greatly from a natural river in certain respects, and there was clearly a need to devise a different method which was applicable to natural as well as experimental situations. This second method approaches the determination of growth rates not by counting the number of bacteria at different times, but by determining the rate at which a metabolite is taken up by the population as a whole. It is therefore sometimes called a process-orientated method as opposed to organism-orientated methods.

In the past the process which has often been examined is the incorporation of dissolved organic carbon compounds (DOC) into the bacterial biomass. Relatively simple organic compounds are usually used, such as acetate or glucose, labelled with radioactive carbon (¹⁴C). Stated simply, the theory assumes that the rate of uptake of the radioactive compound is proportional to the activity of the population before the compound was added, provided that the incubation time is of the order of hours. Two of the drawbacks to the ¹⁴C method are that (a) the radioactive material is probably quite different to the natural carbon substrate and (b) the addition of radioactive DOC may substantially affect the total concentration of available DOC. Hence this method might lead to erroneous results.

One possible way to overcome these drawbacks is to use radioactive sulphur, in the form of sulphate (³⁵SO₄), instead of ¹⁴C (Campbell & Baker 1978a). Sulphur compounds (as in the R. Frome), is obtained from sulphate. Furthermore, sulphate is an essential element and, in the absence of reduced sulphur is naturally present in excess and the addition of radioactive sulphate is most unlikely to affect the growth rate of the bacteria. The method consists of adding a known quantity of ³⁵SO₄ to a fixed volume of river water and incubating it in the dark at river temperature for a few hours. The river water is then passed through a fine filter to catch the bacteria. The bacteria are washed and the amount of radioactivity they have accumulated during the incubation period is determined.

Some problems associated with this technique have been discussed by Campbell & Baker (1978a, b), but essentially it has to be assumed that the quantity of sulphate incorporated into the bacterial cells has a fixed relationship with the amount of carbon incorporated, and hence is directly proportional to the bacterial growth rate. The sulphate uptake rate varies during the year, but a value of $5 \text{ ng S l}^{-1}\text{h}^{-1}$ seems about average outside the periods of high algal activity. In order to turn this figure into a bacterial doubling time, we need to know the size of the bacterial population ($1.5 \times 10^5 \text{ ml}^{-1}$) and the dry weight of an average bacterium ($2 \times 10^{-13} \text{ g}$). This last figure is subject to considerable error. However if

we make these assumptions then the bacterial doubling time, as calculated from the sulphate uptake rate, is 42 h (Hossell & Baker, 1979a). The fact that it is exactly the same as the figure obtained from the recirculating channel is purely fortuitous, but the similarity is encouraging.

Both of the above methods are concerned with bacteria in suspension, but in a river many bacteria are attached to surfaces such as plant leaves and neither method can be easily adapted to this situation. Any surface in a river is continually bombarded by bacteria carried by the current. Some of these bacteria stick to the surface while many 'bounce' off again. Four possible fates await those organisms which attach themselves: they might multiply and form a colony; they might be eaten by a grazer; they might detach themselves again; or, lastly, they might remain there apparently doing nothing. These four possibilities were considered by Hossell & Baker (1979a) with respect to the surface of the common duckweed *Lemna minor*.

Duckweed is a small, floating, disc-shaped plant a few millimetres in diameter. It is commonly found in still water; in rivers it occurs amongst the emergent vegetation and in the slow eddies, particularly during the summer. Bacteria on the surface of duckweed can be observed directly by staining them with phenolic aniline blue (Hossell & Baker 1979b). Thus the numbers of bacteria per unit area of duckweed surface can easily be counted. Reproduction in the duckweed is mainly asexual and new plants appear every few days. These new plants have no bacteria on them initially and, by counting the bacteria on plants of known ages, the gradual colonization of the plant can be quantified. The results of such an experiment are illustrated in Fig. 2.

It would be possible to use linear regression analysis directly on the data in Fig. 2, as was used in Fig. 1, but the resulting rate of increase would not be equivalent to the *in situ* growth rate of the bacteria which, as stated above, is the sum of four components. First let us deal with the two increasing components, namely attachment and growth. Once more we can assume that growth is logarithmic and we can further reasonably assume that an approximately constant number of bacteria attach per unit time, i.e. the attachment rate is arithmetic in character. From these two assumptions it is possible to write a theoretical equation describing the mathematical form which an increase in number due to these two components alone would have (Hossell & Baker 1979a):

$$N_t = (N_{t-1} e^r) + A$$

where r is the growth rate of the bacteria, N_t is the number of bacteria cm^{-2} on plants aged t days and A is the attachment rate. Non-linear regression analysis was employed to obtain the best value of A for an arbitrary value of r and the experimentally determined values of N_t and N_{t-1} . The line of best fit (shown in Fig. 2) results in an attachment rate of $5.7 \times 10^5 \text{ cm}^{-2} \text{ day}^{-1}$ and an apparent doubling time of 164 h.

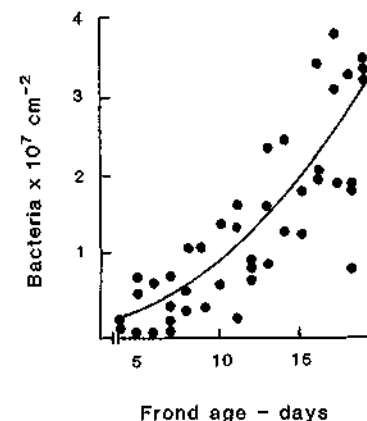


FIG. 2. Mean population density of bacteria on duckweed plants of known age with the computed curvilinear regression. Each point is the average of twenty counts. (After Hossell & Baker, 1979a: *Freshwat. Biol.* 9, 319-27).

This apparent doubling time does not take into account either detachment or grazing, both of which would reduce it. Detachment rates have been determined in the laboratory, but not in the river. In the laboratory the assumption was made that bacterial growth in one hour on a duckweed surface, or in a beaker of river water, was negligible. Then the number of bacteria detaching from a known surface area of duckweed was determined by removing the plants after one hour from a beaker of previously sterile river water. The plants were placed in a similar beaker of sterile river water and the bacteria in the first beaker were counted. This process was repeated many times, and for the first ten hours the arithmetic detachment rate was approximately constant. When this detachment rate is added to the apparent rate of increase, a corrected doubling time of 61 h results (Hossell & Baker 1979a). No suitable method has so far been found to estimate the losses due to predation and so this corrected doubling time is still an underestimate.

Bacterial growth rates are directly proportional to the prevailing temperature and thus in a river they would be expected to be lower in winter than in summer. The water temperatures during the recirculating channel experiments and ^{35}S -uptake investigations were approximately the same. On the other hand the temperature of the duckweed plants on the surface of relatively still water during summer is likely to have been substantially higher. Nevertheless, bacterial growth rate due to the higher temperature is likely to be increased by, at most, a factor of two.

At the present time there is a considerable interest in the productivity of ecosystems. The prediction of bacterial production rates in the R. Frome

from data so few as are presented here must be largely speculative. However, bacterial secondary production in the water column has been estimated from the ^{35}S experiments, and Campbell & Baker (1978a) suggest a range of 6–13 mg C m $^{-3}$ d $^{-1}$.

The three methods described above for determining bacterial growth rates in rivers are open to quite serious criticisms, as are the methods used by other workers. Because of their fundamental importance to microbial ecology, however, it is well worth trying to obtain estimates of such growth rates, even though the methods are known to be inadequate. It should be noted that all three estimates are close together and hence despite their weaknesses they lend strength to each other.

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THE RESEARCH PROGRAMME: LIST OF PROJECTS AND REPORTS

The List of Projects and Reports given below is in the same form as in last year's Annual Report. In it the Association's research programme has been divided into projects and each project has been given a reference number. As old projects are completed the numbers are omitted from the list and new projects are given new numbers. However, in some projects the emphasis changes as the work progresses, so that over the course of time a project may become rather different from what it was as originally conceived. In such a case the title may change, but the number remains the same. All new projects at the River Laboratory are using numbers from 100, while those from the Windermere Laboratory are filling the gap up to 99.

The list of Projects includes all those worked on during the year 1 April 1980 to 31 March 1981 and a few which are planned to start in April 1981. The names of staff and the reports are for the work done between April 1980 and March 1981.

The information is listed in the following format:

- (a) Project number and title.
- (b) Personnel. The project leader's name is italicized; assistants and collaborators follow. In a few cases there are two or more project leaders, especially where there is division into sub-projects. RS = Research Student. SS = Sandwich Student. VS = Vacation Student. WEEP = Work Experience on Employers' Premises personnel.
- (c) Sub-projects where appropriate.
- (d) Report for the period April 1980 to March 1981.
- (e) Publication numbers (as listed on pages 88–104).
- (f) The cost. This is the cost of direct salaries and is expressed as a percentage of all the research staff salary costs. It does not include any allowance for workshop, administration, library or similar general expenditure. It may be used as an indication of the size of the project, but it must be remembered that projects involving the more senior staff members cost more.
- (g) Customer: where the project is part of a research contract the customer is given. 'Part' is used where only part of the project is contracted.
- (h) Liaison with: lists the organizations outside the Association with which there are special links, either in the research or in provision of facilities etc.
- (i) Use of: here are mentioned expensive or unusual facilities or apparatus which are used for the project.