NATIVE AQUATIC BACTERIA: ENUMERATION, ACTIVITY, AND ECOLOGY

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Foreword

The symposium on Native Aquatic Bacteria: Enumeration, Activity, and Ecology was held 25 June 1977 at Minneapolis, Minn. Sponsored by the American Society for Testing and Materials' Committee D-19 on Water, the symposium was arranged by Section D19.24.02 on Native Aquatic Bacteria, of Subcommittee D19.24 on Water Microbiology. J. W. Costerton, University of Calgary, and R. R. Colwell, University of Maryland, presided as cochairmen of the symposium and also served as editors of this publication.

Related ASTM Publications

Manual on Water, STP 442A (1978), $28.50, 04-442010-16

Estimating the Hazard of Chemical Substances to Aquatic Life, STP 657 (1978), $19.50, 04-657000-16

Sulfur Bacteria, STP 650 (1979), $15.00, 04-650000-16

Aquatic Toxicology, STP 667 (1979), $37.75, 04-667000-16

A Note of Appreciation to Reviewers

This publication is made possible by the authors and, also, the unheralded efforts of the reviewers. This body of technical experts whose dedication, sacrifice of time and effort, and collective wisdom in reviewing the papers must be acknowledged. The quality level of ASTM publications is a direct function of their respected opinions. On behalf of ASTM we acknowledge with appreciation their contribution.

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Introduction

This special technical publication has evolved from a symposium organized by Section D19.24.02 (Native Aquatic Bacteria) of Subcommittee D19.24 (Water Microbiology) of Committee D-19 on Water of the American Society for Testing and Materials. This symposium, entitled "Native Aquatic Bacteria: Enumeration, Activity, and Ecology," was held in Minneapolis in June of 1977, and it was organized as a broad discussion and evaluation of the methods currently used for studying aquatic bacteria. Detailed technical examination of the methods was carefully eschewed, and the speakers were commissioned to "sketch in" the method itself and then to discuss the accuracy of the results obtained and to evaluate the usefulness of the data in a variety of different possible applications. It is fair to state that each of the methods discussed in this book is widely conceded to have one or more flaws that make it somewhat imperfect, and so a broad comparative discussion was deemed useful so that users of these methods could be fully cognizant of the particular strengths and weaknesses of each method and the limitations of the data generated thereby.

This broad discussion of the theoretical basis and the practical problems of methods currently used in the field of aquatic microbiology is comprehensive and is intended to be useful to the reader in his evaluation and subsequent choice of methods for the study of an individual system. As an example, most service companies that seek to control the bacterial fouling of industrial cooling installations use plate counts and adenosine triphosphate determinations of the flowing water to assess the degree of fouling. This special technical publication will allow workers in this area to compare these methods with others designed to yield the same information (for example, epifluorescence and the *Limulus* lipopolysaccharide assay) and to evaluate these methods in terms of accuracy and the usefulness of the data. Because aquatic systems differ in important respects, these broad and candid discussions will allow the reader to evaluate available methods in relation to his own system, which may be peculiar in some important respect (for example, a high proportion of submerged surfaces to the water volume, or the presence of algae and invertebrates) that will have a very profound influence on the method to be chosen.

A recent ASM (American Society for Microbiology) conference on aquatic microbial ecology discussed the adequacy of current methods in this field in the important tasks of assessing environmental impacts on aquatic bacterial populations and of assessing environmental quality by using bacterial indicators. These wide-ranging discussions, summaries of which will be published by ASM in *Microbiology 1980*, tended to examine the degree of accuracy of methods and the completeness of the description of processes within aquatic bacterial populations that could be expected by agencies interested in environmental impact assessment. It is the intention of Section D19.24.02 on Native Aquatic Bacteria of ASTM Subcommittee D19.24 on Water Microbiology to initiate the examination and detailed assessment of specific methods whose accuracy and usefulness are generally conceded (for example, enumeration by epifluorescence) and to evolve standard techniques for the application of these methods to studies of aquatic bacteria.

J. W. Costerton

University of Calgary, Calgary, Alberta, Canada; symposium cochairman and coeditor.

Direct Enumeration of Aquatic Bacteria

Introduction

Methods for the direct enumeration of aquatic bacteria fall into two broad groups. One group of methods seeks to visualize the bacterial cells, to count a limited number of them, and to extrapolate from these data the number on an area of submerged surface or in a volume of water. The simplicity and directness of these methods makes them inherently attractive, but even their most enthusiastic proponents admit that they fail to distinguish between live and dead bacteria, and their accuracy depends, therefore, on the extent to which dead bacterial cells persist in the aquatic environment. Death is as difficult to define in bacteria as in other biological forms, but enumeration is facilitated by the fact that moribund bacteria tend to lyse, because of their content of cell wall amidases, and, therefore, unlike fungi, bacteria do not produce a large residuum of dead cellular material. Recent demonstrations that ±80 percent of the bacteria counted in an aquatic environment by epifluorescence were "alive" in that they could take up radiolabelled organic substrates, as determined by autoradiography, support this contention that most of the morphologically distinct bacterial cells in the aquatic environment are physiologically active, and, thus, these data support direct enumeration by microscopy.

The second group of direct enumeration methods is based on the "replicon" concept that a live bacterial cell, when transferred to a suitable medium and diluted sufficiently, will produce a single colony. These methods easily distinguish living from dead bacteria, but they tend massively to underestimate aquatic bacterial populations because the media most commonly used do not support the growth of all the bacteria present and because bacteria in a slime-enclosed clump (microcolony) will produce only one colony even though 100 cells were originally present. Because the bacterial population of aquatic environments is often composed of many different physiological types of bacteria, including some that are killed by high levels of nutrients, a small number of media cannot support the growth of the whole bacterial population, and, thus, total numbers are very difficult to obtain by these methods. These methods are, however, very useful in enumerating the bacteria of a specific physiological group (for example, sulphate-reducing bacteria) by the use of a selective medium. The problem of clumping is especially vexing in the enumeration of bacteria attached to floating

particles or to submerged surfaces because these bacteria live in a fibrous matrix, and each clump is an effective replicon; a manipulation that simply broke up the matrix to produce individual bacteria would provide the illusion of a distinct bacterial proliferation. Thus, methods for the direct enumeration of bacteria in aquatic environments must be chosen with some care and some knowledge of the nature and distribution of the species that comprise the population to be enumerated.

J. W. Costerton

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Which Populations of Aquatic Bacteria Should We Enumerate?

REFERENCE: Costerton, J. W. and Geesey, G. G., **"Which Populations of Aquatic Bacteria Should We Enumerate?,"** *Native Aquatic Bacteria: Enumeration, Activity, and Ecology, ASTM STP 695,* J. W. Costerton and R. R. Colwell, Eds., American Society for Testing and Materials, 1979, pp. 7–18.

ABSTRACT: Bacteria in aquatic systems comprise at least four distinct populations. The planktonic population floats in the water column with the particle-associated population, while the epilithic and epiphytic populations are attached to submerged surfaces, and the sediment population occupies the water-filled spaces of the sediment. Samples taken from the water column include the planktonic and particle-associated bacteria, and core samples include the sediment population, but special techniques are required to sample the epilithic and epiphytic organisms. In all bacterial populations, except the planktonic, the natural adhesion of the cells to surfaces and to each other produces adherent microcolonies whose cohesion presents special problems in bacterial enumeration.

Since we have described freshwater systems in which epilithic bacterial populations are numerically dominant, and since large sediment populations are found in most aquatic systems, it is imperative that bacterial enumeration studies recognize each of these distinct populations. The authors examine the methods available for the enumeration of the bacteria in each distinct population and suggest a holistic approach to bacterial enumeration that can be applied to any aquatic system.

KEY WORDS: aquatic bacteria, sampling, enumeration, microcolony, adhesion, epiphytic bacteria, epilithic bacteria, planktonic bacteria, sediment bacteria, lipopolysaccharide (LPS), adenosine triphosphate (ATP), epifluorescence, most-probable-number (MPN) techniques, plate count

In this volume the authors will examine the various techniques available for the enumeration of bacterial cells and for the quantitation of bacterial biomass in aquatic environments. These techniques include direct counting of specifically stained bacterial cells, for example, epifluorescence and immunofluorescence; indirect counting by dilution and growth techniques, for example, plate counts and most-probable-number (MPN) determinations; and chemical methods that

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seek to determine bacterial biomass by the measurement of a unique bacterial component, for example, lipopolysaccharide (LPS) or muramic acid, in a mixed population, or of a common cellular component, for example, adenosine triphosphate (ATP), in a bacterial population. Our purpose is to compare the accuracy of these techniques and to assess the usefulness of the data yielded by each. But, before we begin this comparative exercise, perhaps we should reflect briefly on the kinds of samples that should be assessed by these techniques.

Since Henrici's pioneering work [*1*][3] it has been known that certain aquatic bacteria preferentially colonize submerged surfaces, and subsequent work [*2–12*] has shown that these epilithic and epiphytic bacteria produce large adherent populations on these submerged surfaces. Furthermore, analyses of planktonic bacteria in aquatic systems have shown that many of these floating cells are, in fact, attached to small particles [*13–15*]. It is now clear that the bacteria in aquatic environments constitute at least four distinct populations (Fig. 1) — free-floating planktonic bacteria, bacteria adhering to suspended particles, bacteria attached to submerged solid supports (epiphytic and epilithic forms), and sediment bacteria. The relative importance of these four populations is partly a matter of geometry in that the first two are preeminent in the ocean and in large deep lakes, while the latter two are very significant in rivers and in shallow water bodies.

Description of Four Distinct Aquatic Bacterial Populations

The truly planktonic bacteria constitute the simplest case in bacterial enumeration since they occur singly and thus are relatively evenly distributed (Fig. 2) on the filters used to concentrate them for counting, and they each give rise to a colony or to growth when diluted out for plating or for MPN determination. Serious losses in planktonic bacterial counts have been noted when samples were allowed to stand before enumeration [*16*] because many of these free-floating bacteria are attracted to, and adhere to, surfaces of the vessel in which they are contained.

On the other hand, those bacteria that adhere to suspended particles pose serious problems in enumeration because many bacteria may adhere to the same particle to form a "microcolony." The individual cells are difficult to count directly, and each microcolony constitutes only a single reproductive unit in plating [*17*] and MPN techniques. In many systems these heavily colonized particles may really be a part of the sediment that has been temporarily suspended in the water column by wave action or other disturbance. Chemical methods of enumeration (LPS, ATP, muramic acid, etc.), on the other hand, "see" each individual cell in these microcolonies and may, therefore, provide a more accurate estimate of this particle-associated population.

Samples taken for enumeration usually contain only these first two populations of bacteria and omit epilithic, epiphytic, and sediment bacteria. Direct examina-

[3]The italic numbers in brackets refer to the list of references appended to this paper.

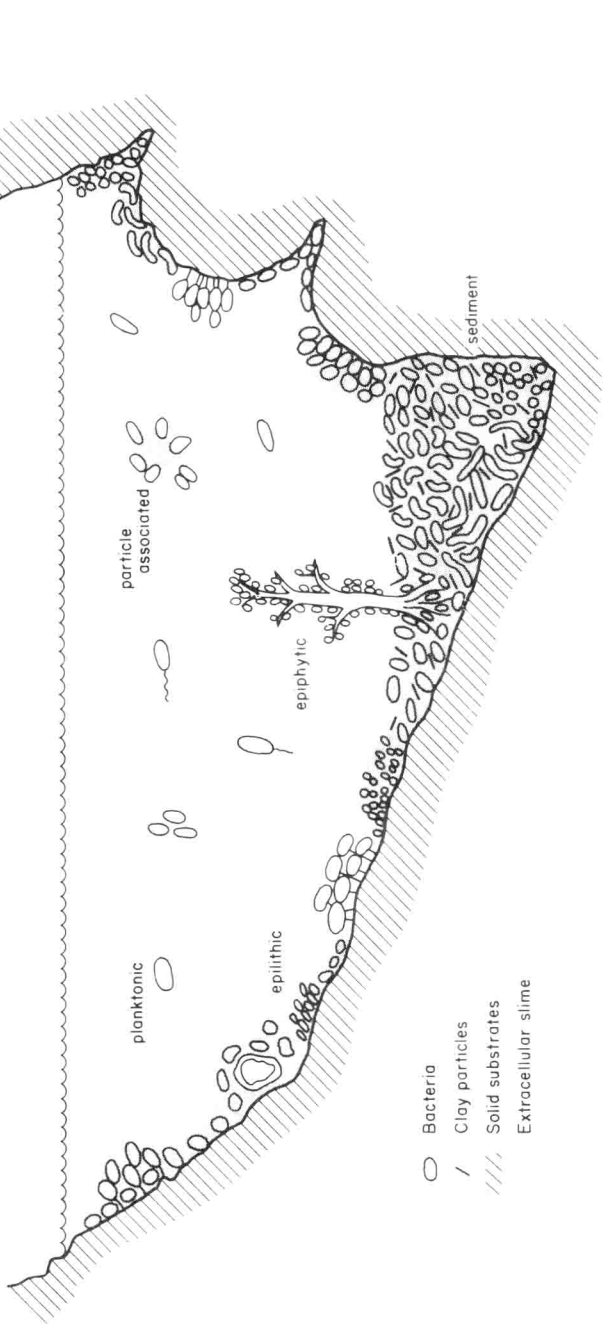

FIG. 1—*Diagram of the relative locations of four distinct bacterial populations in aquatic environments.*