CRC Handbook of Laboratory Model Systems for Microbial Ecosystems

Volume I

Editor

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PREFACE

Interactions between microbes and their environment define the study of microbial ecology. The small size of individual microbes and the great range and variety of structures found in the physical world mean that most microbial habitats are spatially and temporally heterogeneous. Such complex systems can be studied successfully *in situ* or they can be investigated in the laboratory. If the latter, they may be incorporated into microcosms or studied in model systems. Microcosms retain most of the complexity of the natural system while models are subsets (more analogues than homologues) of the ecosystem from which they derive. The model aims to investigate a few properties of the natural system while other aspects are either held constant or omitted from the system or simply ignored.

In this book I have tried to collect some of the experimental model systems that are used to investigate microbial behavior in nature. These include homogeneous culture systems like the chemostat which has itself proved such a powerful research tool over the last three or four decades. Linked homogeneous fermenters include the multistage chemostat, which can be used to investigate stratified systems like aquatic sediments, and the gradostat whose bidirectionality means that opposing solute gradients can be studied under steady-state conditions. Bidirectional systems are also available for studying interactions between pure cultures located in different compartments linked only by solute diffusion across a permeable membrane.

Five chapters relate to the attachment and growth of organisms to solid surfaces. These chapters cover attachment and growth itself, as well as the development and employment of microbial film fermenters to investigate such disparate habitats as wastewater treatment systems and dental plaque.

The use of agar as a gelling agent allows the development of model ecosystems where molecular diffusion is the only solute transfer mechanism. These systems are discussed, as is the use of gel-stabilized solute gradient plate methods to investigate the habitat domains of different microbial species.

Packed column reactors are useful model tools for studying systems where there is a unidirectional flow of solutes downward through the structure. These can be good models of soil ecosystems. The latter have been investigated using many other microcosms and models, and these are also discussed.

Other topics covered include specific devices such as the Perfil'ev convectional flow technique, the bacterial colony, and motility in bacteria.

I have deliberately included a discussion of the application of mathematical models to natural ecosystems, because there is a close link between experimental and numerical modeling. Both approaches aim to simplify natural systems by identifying key fundamental processes that dominate the operation of the natural process. The chapter on bacterial motility bridges the gap between experimental and numerical models, and the chapter by Tett and Droop clearly links numerical modeling with experimental investigations.

I hope that the approaches discussed here will be of value both to laboratory-based microbial physiologists and to ecologists who feel that there is some virtue in simplifying systems which can throw light on key processes that operate in nature.

I would like to express my gratitude to all the contributors to both volumes. Also to my wife Lee, and my children Ross, Joshua, and Joanna who might have seen more of me over the period when this book was gestating.

THE EDITOR

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Chapter 1

INTRODUCTION

Julian W. T. Wimpenny

This book is devoted to a description of some of the laboratory model systems which can usefully be applied to investigations of microbial growth and interactions where they are most at home, in their natural habitats.

It is accepted from the start that natural ecosystems are nearly always extraordinarily complex genotypically, structurally, and dynamically. It is for this reason that I and all the contributors to this volume advocate the judicious use of model systems, which, if chosen wisely and if their limitations are constantly born in mind, can throw light on the operation of microbes in the natural world.

MODELS AND MICROCOSMS

First we must discuss terminology. The terms "microcosm" and "model system" are sometimes used incautiously to signify the same thing. It is clear however that the two are conceptually quite different. Parkes[1] suggests that the term microcosm defines " . . . a laboratory system which attempts to actually simulate as far as possible the conditions prevailing in the environment or part of the environment under study". As is pointed out by Burns (Volume II, Chapter 3), the term microcosm can be totally misinterpreted to mean "site of microbial activity". There may, however, be a consensus among other microbiologists, in which a microcosm possesses some or all of the following properties:

1. *Origin.* Microcosms derive from natural ecosystems.
2. *Isolation.* The microcosm, whatever its origin, is physically enclosed and no longer in contact with the natural ecosystems.
3. *Size.* Though variable in size, microcosms tend to be compact subsets of the natural system from which they came.
4. *Genotypic heterogeneity.* With some exceptions, most microcosm work uses natural mixed cultures of microorganisms.
5. *Spatial heterogeneity.* Although spatial heterogeneity is not in any way implied by the term microcosm, virtually all of the latter retain this property to some degree.
6. *Temporal heterogeneity.* The majority of microcosms are closed or partially closed systems, where time-dependent changes in the physical, chemical, and biological properties of the system are seen.

At first sight, a microcosm seems just to be a convenience, a piece of natural ecology tamed and brought into the laboratory without changing anything within it.

The main point in favor of the microcosm is that environmental factors (light, temperature, chemistry, etc.) can be manipulated, prolonging, for example, certain seasons or simplifying systems, perhaps eliminating diurnal variations.

It should be emphasized that however good the intention is to accurately reproduce the natural ecosystem, the microcosm is almost certain to suffer as a result of scale changes. As an "island" community isolated from the original system, there may be successional changes in species composition and concommitant changes in physical chemistry aggravated by the presence of the container boundary. For instance, if the latter is glass and the system is illuminated, photosynthetic species will proliferate. A good example of such a microcosm

is the Winogradsky column[2] which is derived from a sediment ecosystem. Sediment and water are placed over a source of carbon (usually cellulose) and a mixture of calcium sulfate and calcium carbonate in a glass vessel. If the container is kept in normal daylight, colored photosynthetic species develop as a series of pigmented bands. Clearly they mark *vertical* heterogeneity. On the other hand, by their presence and by the input of light energy to these zones, they contribute another level of heterogeneity at right angles to the vertical stratification already present in the sediment. This secondary heterogeneity is of course absent from the natural sediment, since the latter is never illuminated. These wall effects plus the addition of cellulose, gypsum, and chalk to the system mean that the Winogradsky column is no longer homologous with the sediment from which it originated. So is it microcosm or model? It is apparent that the Parkes definition cannot help here. The Winogradsky column obviously does not aim to simulate as exactly as possible the natural sediment. It does start from the natural system but equally it manipulates it. Perhaps a better definition which embraces these feelings is

A microcosm is a laboratory subset of the natural system from which it originates but from which it also evolves.

Such a brief definition suggests that if we establish a sample of a natural microbial ecosystem in the laboratory, it should still be termed a microcosm even if it is altered spatially or in terms of its physical chemistry. Microcosms allow a systematic examination of the responses of natural communities to environmental manipulation.

The model, on the other hand, is always an abstraction. The model never aims to reproduce the entire system in the laboratory. It always seeks to examine the properties of a part of the system ignoring or holding constant all the remaining factors. There may be any number of models investigating particular properties of the system. Some of these may be extremely simple. To grow a pure culture of a sulfate-reducing bacterium in a chemostat is to create a restricted model of one component of a sedimentary ecosystem. Such a model is perfectly valid if its limitations (spatial and temporal homogeneity, the absence of other species, the absence of different phases, and so on) are accepted.

Another model of a sedimentary system might recognize the importance of molecular diffusion and species diversity. The sulfate reducer could be incorporated into a gel stabilized system together with a sulfide oxidizing species. The two operating together in counter gradients of oxygen and a carbon source could carry out the chemical reactions of the sulfur cycle found in the upper levels of natural sediment ecosystems.

The model could be made even more sophisticated. Specific mineral particles and a greater range of substrates and species could be incorporated into the gel. The model system becomes closer to the natural ecosystem with each level of sophistication. At some point of course one could conceivably say "the model is the system . . . !" This is probably not the main virtue of models, however. The modeler may well consider that to travel hopefully is better than to arrive. To understand completely a particular ecosystem is probably less important than to discern rules of behavior which apply at a fundamental level to many different ecosystems. The model, if designed well, can by virtue of abstraction and simplification offer this possibility.

The difference between model and microcosm can, for some people, epitomize the difference between an essentially reductionist approach to understanding ecology on the one hand, and a holistic approach on the other. The reductionist believes that understanding the simplified subunits allows one to predict the properties of system from which they derived. Thus, if the properties of pure cultures of bacteria are totally understood, then their behavior in a natural community and hence the behavior of the community itself can be confidently predicted. The holist, on the other hand, believes that breaking a system down into component

parts loses part of its veracity. A holist philosophy considers that the whole is greater than the sum of its component parts.

It is likely that both approaches are valid, essential even, to the prosecution of experimental science. Clearly, higher levels of complexity bring the need for new rules, new properties, new types of order. After all, a complete understanding of the properties of bricks does not help to comprehend the rules of design or architecture needed to construct a Victorian railway station! On the other hand, systems can be too complicated to comprehend as they stand. It must then be right to take a reductionist stance, at least at first, if this gives a clear understanding of some part of the natural system. If the model is very simple, the information too will be simple. Perhaps if we understand some of the properties of bricks, for example their mechanical strength, we can at least predict how tall we can build our railway station before it collapses. In the same way, understanding the biochemical behavior of a single species can give some insight into the way in which it could behave in a complex community. Once the simple model has been deployed and all the relevant information collated, it is time to sophisticate the model. The next level of complexity can now be investigated and comprehended. This is not really a reductionist approach at all. Simplification merely establishes a platform upon which higher levels of sophistication can rest more securely.

Ought reductionism be distinguished from the normal scientific processes of analysis and synthesis? The reductionist studies elements of a system and is then content to predict the behavior of the system as a whole. The "normal" scientist carefully dissects the whole system before reassembling increasingly more complex subsets of it. Pursued to its logical conclusion, even this distinction fades and reductionism is seen as just one extreme of the normal processes of analysis and synthesis.

In the meantime, right at the other end of this scale, the holist is investigating the system just as it stands. Some of his findings must inevitably be descriptive and may not be easy to interpret at the most basic level; however the holist performs the vital function of searching for and documenting the emergent properties of the complete system. It seems clear that holism and the sort of "reductionism" described here move together and at some point lead to a unified interpretation of the complete system which will satisfy all sides.

Holism and reductionism are not in the end very useful terms, since they simply represent two extreme attitudes to science. The advocate of one believes in starting simple and only increasing the complexity of a problem when it is necessary to do this, while the advocate of the other can only comprehend the entire system and is motivated to study this from an aesthetic sense of unity and even beauty that the system as a whole displays. Finally, science as a process is flexible enough to embrace all sorts of approaches and can accomodate, and indeed gain from, the instincts of a wide range of people so long as they are honestly dedicated to discovering the truth about natural phenomena.

I hope that what I have said so far goes part of the way to justify the use of model systems. I would like next to discuss briefly some of the properties of natural microbial ecosystems which seem to be worth incorporating in simpler models.

HOMOGENEITY VS. HETEROGENEITY

I have emphasized[3,4] that microbiology has pursued the "homogeneity paradigm" for too long. Thus, the dictum that a mono culture in a stirred fermenter, preferably operating as a chemostat, is really all that is necessary to understand all the properties of microorganisms, seems to be a gross simplification. This is not in any way to deny the enormous importance of homogeneous systems and the chemostat in particular to studies in microbial ecology. The excellent chapter by Gottschal and Dijkhuisen in this volume (Chapter 2) illustrates both the power of the technique and the areas in microbial ecology where continuous culture has played such an important part. If the chemostat could answer all the questions concerning

microbial behavior in their natural environments, however, there really would be little need for a book of this sort.

To recognize the need for more elaborate model systems is to accept a conceptual shift away from homogeneity to the heterogeneous systems that seem to predominate in nature. This conceptual shift is not merely to accept the fact implicit in the term "heterogeneity" that physico-chemical conditions alter as a function of position at a given time and of time at a given position, but it is to recognize and to accept instinctively the extreme importance of the space *outside* a cell to the growth and physiological behavior of that cell. However subtle and intricate and intrinsically fascinating are the ordered chemical reactions that go on within the cell envelope, they are dominated and modulated by the chemistry and physics of the regions surrounding it, yet this area has received only the scantiest attention in recent decades compared to the volumes published on the physiology and molecular biology of microbes.

A few of the factors affecting cell behavior include the distribution and flux of substrates needed for growth, the prevailing physical conditions of temperature, pressure, pH value, water potential ionic strength, etc., the presence of different phases and their physical behavior, the presence of inhibitory solutes, the presence of other prokaryotic and eukaryotic organisms, and the range of interactions that each exhibits. In microbiology, especially that of prokaryotic organisms, the business of living revolves around the distribution of solute molecules in a narrow aqueous layer surrounding the cytoplasmic membrane of the organism.

Once the importance of spatial heterogeneity is recognized, one is forced to accept the need for simple laboratory systems which incorporate heterogeneity factors. In the next section, I will briefly discuss a few spatially heterogeneous ecosystems, hopefully to illustrate just where laboratory models may have a useful part to play. This list is not intended to be exhaustive.

SOME SPATIALLY HETEROGENEOUS ECOSYSTEMS

Aquatic Systems

In general, water bodies have little spatial heterogeneity and many therefore behave like homogeneous fermenter systems. The oceans, seas, and large inland water bodies fall into this category at least at a "local" level. There are large-scale differences in chemistry that should be mentioned: for example, deep ocean troughts, some littoral regions, etc., can have differences in temperature, salinity, organic load, dissolved oxygen tension, and so on, while light penetration is a function of both of depth and of the clarity of the water. Related to gradients in light energy and spectrum is photosynthetic activity, which will in turn modulate the oxygen content of the waters. It may be that the ecology of such water bodies is best modeled in the laboratory by single-stage continuous culture systems running at very low concentrations of substrate. This is because although physico-chemical gradients may be present, they are extremely shallow, and this implies that interactions which depend on such gradients will be negligible in importance.

Rapidly flowing water bodies, including streams and "clean" shallow rivers, are also well mixed and substantially homogeneous. However, their ecology as a whole is often heterogeneous, since water flow is adjacent to solid surfaces which can support a substantial sessile population or to sediments which may be rich in microbial flora.

It should also be stressed that freely suspended individual cells are a comparative rarity in even these substantially homogeneous environments. This is because the populations present are usually associated with particulate matter forming rafts containing groups of cells. The propensity of microbes to associate with surfaces is now well recognized and raises interesting questions regarding the distribution of solutes near phase boundaries.

Of considerable interest in terms of spatial order are stratified water bodies. Water may

become stratified for a number of reasons. Commonest by far is the annual cycle of thermal stratification seen in many lake systems. Here, the upper layers of the water become heated in the summer months, and because they are now less dense than the lower layers, little vertical mixing takes place. The lower layers are now deprived of any significant atmospheric gas exchange and, depending on the organic load in the water, can often become anoxic. In such a lake system the most interesting region from the microbial ecologist's point of view is the interface between the two zones. This region shows the steepest change in temperature (thermocline) and often the steepest gradients in chemical species (chemocline). Such a region is normally the site of greatest microbial activities where anaerobic and aerobic species interact by the diffusive transfer of substrates and products.

During the warm summer months, the lower colder layers of these lakes slowly heat up. In the autumn, however, when the weather becomes chillier, the upper layers cool relatively quickly. At length, the system becomes unstable leading to convective "turnover" of the water masses and the anaerobic lower layers now rise to the surface. This mixing represents a catastrophic change in chemistry and hence in microbial populations in the lake.

Much more beautiful in their spatial differentiation are the permanently stratified water bodies. These meromictic lakes and inland seas are formed in general by dense salt water lying beneath a layer of lighter fresher water.

There are several good examples of intensively studied meromictic water bodies. They include the Black Sea which has been the focus of extensive investigations by Russian microbiologists (reviewed by Kriss[6]) and a Transcaucasian lake, Lake Gek Gel', both of which show beautiful patterns of stratification which are spatially very extensive. The Black Sea, for example, is more than 2000 m deep and the point at which sulfide is first detected is between 120 and 200 m below the surface. This point roughly marks the start of the anaerobic regions of the water body. Lake Gek Gel' was studied intensively by several workers. Sorokin[7] has reported patterns of growth and chemistry in this lake as a function of depth. The stability of the system allows the generation of organized solute gradients and the growth of a whole range of physiological types each at its own optimum position in gradients of light, oxygen, sulfur, carbon, nitrogen, and iron compounds. This lake, which is about 70 m deep, together with the Black Sea, pose interesting questions concerning solute transport mechanisms. These permanently stratified water bodies are assumed not to show much vertical exchange of material by mixing, however, molecular diffusion is too slow a process to account for the activities in the various growth regions. Possible mechanisms promoting exchange are the inflow of water from rivers, from surface and subterranean streams, and from shore line run-off; a "fall out" of dead cells and other organic matter from the surface to the bottom of the lake; the ascent of gas bubbles from the sediment at the bottom of the system to the surface, and the mixing activities of macroscopic organisms especially in the aerated surface regions. In the case of the Black Sea, some mixing may be due to the inflow of more-saline-dense water from the Sea of Marmara.

Such gradient systems may be established on a far smaller scale in laboratory microcosms. Kriss[6] cites the work of Egunov, who in 1895 established laboratory systems from estuarine, lake, and Black Sea sediments. In the overlying water, he noticed the development of a thin film of microorganisms which separated a sulfide-rich lower layer from an upper layer in which no sulfide could be detected. Egunov suggested that such a bacterial plate ought also to be present in the Black Sea, however according to Kriss, such a densely packed layer of organisms could not be found. Layering is seen in other lake systems. Thus a narrow band of anaerobic photosynthetic bacteria appears just below the aerobic-anaerobic interface in the waters of Lake Faro (Messina).[8] This phenomenon could also be seen in laboratory microcosm experiments. Suckow and Schwartz[9] incorporated Baltic seawater above a sulfide-generating mud in an aquarium. A bacterial plate which was, after several months, 10 mm thick formed between the aerobic and anaerobic layers and contained pigmented photosynthetic bacteria.

The use of the laboratory system in investigating such spatially extensive systems must be commended if only because it can speed up processes which, in stably stratified water bodies, are almost certainly very slow. It would be interesting to establish such a lake ecosystem in a gel-stabilized laboratory model system in which transport was by molecular diffusion alone. The resolution of such models can be extremely high, and it ought to be possible to map growth zones and chemistry with some precision, and from there to construct a numerical model of behavior of the natural system. The open models discussed in Chapters 3 and 4 (this volume) may also be useful in modeling the dynamic behavior of such systems.

Water, an essentially homogeneous medium, shows heterogeneity only when vertical stratification due to thermal or salinity gradients can occur. Heterogeneity is essentially one dimensional from the top to the bottom of the system, and because it is in this sense rather simple, it can be macroscopically obvious and at the same time beautiful. Ways are suggested to model such systems in the laboratory with a view to speeding up what are extremely slow processes.

Liquid Plus Solid Phase Systems

The addition of a solid phase to aquatic systems adds considerably to its complexity and to its potential for heterogeneity. One ecosystem which has been investigated extensively in the field is the sedimentary ecosystem. Such systems are found at the base of all water bodies including streams, rivers and estuaries, ponds, lakes, inland seas, and oceans. The solid phase of sediments consists of three separate types of component: detrital material derived directly or indirectly from geological erosion, biogenic material which is formed by biological activities, and authigenic components which are formed within the sediment itself. Sediments show several orders of heterogeneity. They are always vertically stratified with the surface, if it is aerobic at all, containing measurable oxygen over the first few millimeters to centimeters. A secondary spherical heterogeneity has been reported. Thus in the aerobic superficial layers, small foci of anaerobiosis due to the activities of sulfate-reducing bacteria on organic debris can be seen.[10] In the upper layer, the burrowing habits of oligochete and chironomid worms can lead to cylindrical burrows in the sediment whose walls are aerated. The additional surface area available for oxygen uptake has been estimated by Fry.[11] A medium population of benthic invertebrates, for example, in a Welsh reservoir, more than doubles oxygen uptake; however, a dense population in the River Thames can increase it by a staggering 116 times! The upper layers of many sediments, depending on their position, can be perturbed not only by the activities of animals but by hydraulic factors including water currents, tides, and breaking waves. Jones has stressed that horizontal heterogeneity (zonation) is also apparent in sediment systems usually around the shore line. This is therefore quantitatively more important the smaller the body of water. In fresh water lakes, the littoral regions can be overlayed with shallow warm aerated water, while the profundal zones during thermal stratification will be covered with cool oxygen-depleted water. Significant differences in metabolic activity result. The last level of heterogeneity of importance in sediments must be at the microscopic level and concerns the growth of organisms in the sediment itself. Here, the nature of microcolony development and the physical chemistry of available surfaces plays an important role.

The scale of the different classes of heterogeneity must now be mentioned. It has already been stated that molecular diffusion is an essentially slow process in liquid phases, slower still where solid particles increase the tortuosity of solute transport. Apart from bioturbation and hydraulic processes, molecular diffusion is the most significant force for solute transfer in sediments. The shorter the diffusion path from sources to sinks, the faster the reactions that can take place. Thus the upper aerobic layer of sediments is a zone of intense metabolic activity, while shallower gradients in the deeper levels mean slower rates of reaction.

Many of the reactions in sediments could profitably be examined using some of the spatially

heterogeneous model systems described in this book. One line would be to emulate some of the possible interactions in a gel stabilized model, and it would be fruitful to investigate the effect of added mineral particles to such a system.

Three Phases Systems: Soils

The addition of another phase to the two-phase system already discussed significantly increases the opportunities for spatial heterogeneity, and soil ecosystems are perhaps the most complex to understand and hence to investigate using model systems.

Soil consists of three phases: gas, liquid, and solid. The gas phase is generally very similar to air in composition; however, it may be depleted in oxygen and enriched in any or all of the following: carbon dioxide, nitrogen oxides, ethylene, and hydrogen sulfide, etc. The water phase is generally rainwater which has picked up solutes as it travels through the soil. This phase moves in one direction on the whole; that is downwards. Percolation is the major solute vector in soil, though local distribution is by capillarity and by molecular diffusion. When the surface layers become dry, water may move in the reverse direction by capillarity. The solid phase is highly heterogeneous and very complex . . . ! Soil contains two main classes of minerals: the rock-pebble-sand family and the clay family. The former provide a relatively small and chemically unimportant surface area; however, they do contribute significantly to the porosity and texture of the soil. Clay particles, on the other hand, are generally colloids having a vast surface area which is physically and chemically reactive, and these play an important part in the overal biology of the system. Organic constituents are divided into nonliving and living. The former consist of biological material in various stages of decomposition, especially the more recalcitrant compounds like lignin and cellulose, chitin, humic and fulvic acids. The living fraction ranges from multicellular eukaryotic animals and plants (especially roots of the latter), and a vast range of unicellular eukaryotic and prokaryotic species. Products from living cells and the cells themselves contribute structure to the system. Thus, fungal hyphae, roots and root hairs, and microbial polysaccharides all help to form and hold together soil crumbs.

Heterogeneity exists in a number of main areas each of which is characterized by differences in scale and hence in biological reactivity. Vertical stratification is usual but much more variable than seen in the solid-liquid system. Scale here is of the order of centimeters and meters. Stratification is dominated by water relationships. A dry porous soil will provide many channels for gaseous diffusion and exchange, so that deeper levels will remain aerobic. Heavy rain or flooding will fill soil pores, gradually blocking off diffusion pathways so that the lower levels will become anaerobic. Deliberate flooding of rice paddy fields has been followed by microbiologists who have noted the fairly predictable change from aerobic to predominantly anaerobic biology. Vertical stratification is not just a question of oxygen relationships. In reality, far more important are the gradients in organic matter and in the living soil flora and fauna which are at their richest near the surface and gradually fall off with depth.

The soil crumb represents another level of heterogeneity. Here the system has an irregular spherical geometry. The crumb consists of mineral components and organic matter held together by roots, mycelia, and by cell capsule components, usually polysaccharides. Bacterial growth is usually as isolated microcolonies associated with clay colloids or organic matter. Once more, depending on prevailing water constraints, the crumb may be saturated even though there may be paths between crumbs allowing gas diffusion deep into the soil profile. If the crumbs are water saturated, their centers may be anaerobic while outer layers may be well aerated. The juxtaposition of aerobic and anaerobic "spaces" allows interesting chemical interactions to take place. These will be discussed later. A third level of heterogeneity is at the boundary between the microcolony and water in contact with it, and there is a final zone of interest at clay mineral interfaces where cation exchange activities associated with the clay lattices lead to steep pH gradients over a few nanometers.

Besides these "structural" levels of heterogeneity, the activities of plant and animals in the soil play a vitally important part in the distribution of substrates and products. Plant roots are so important here that we recognize an entire ecosystem, the rhizosphere, adjacent to them. Because of transfer of nutrients from the root, the microbial population in the rhizosphere is usually much higher than elsewhere. In flooded systems like paddy fields, plants can translocate oxygen into the root associated microflora. Root commensals contribute to the fertility of the soil by the fixation of gaseous nitrogen. The soil fauna, on the other hand, are responsible for physically turning soil over and aerating especially the heavier denser soils. They also play their own part in the energy flow of soil systems through consumption and turnover of other organisms.

For a full understanding of the soil ecosystem, each level of heterogeneity needs to be carefully investigated. Once more it seems likely that carefully selected experimental models ought to play an important part in this work. A model with many of the properties of soil is the percolating column discussed in detail in Chapter 2, Volume II, by Prosser and Bazin. It is perhaps interesting that systems discussed by these authors range from microcosm to model in relatively small steps. Thus some of the earlier work has been carried out on soil columns which must be defined as microcosm, while later work by Prosser and his colleagues, among others, has reduced the complexity in the system by using glass beads and restricted pure cultures of bacteria. It is easy to see that a large number of possible models could bridge the gap between microcosm and model in such systems. Both Caldwell and Fowler (Chapters 6 and 7, this volume) have considered surface attachment and growth, and of course such work is directly relevant to the growth of microcolonies on mineral constituents in soil. Diffusion-linked model systems ought to be employed to look at interactions possible in soil crumbs whose centers have become anaerobic since aerobic-anaerobic interfaces allow important biochemical pathways and cycles to take place. Finally, Burns discusses the soil ecosystem in some detail and illustrates some applications of models and microcosms to such systems (Volume II, Chapter 3). It is clear that there is scope for the application of relevant laboratory models in this area too.

Microbial Film

A family of microbial ecosystems are found attached to solid surfaces which are exposed to aqueous solutions containing nutrients. These microbial films are ubiquitous and have an important economic role besides their obvious interest ecologically. Characklis[12] has emphasized the problems that microbial film can cause. Examples range from the colonization of boat hulls, leading to fouling by larger organisms; films causing corrosion of marine steel or concrete installations; growth in water pipes leading to reduced flow, blockages, infection with pathogenic bacteria like *Legionella* species; or a reduction in heat conduction properties. Microbial films can be the bane of the fermentation technologist's life if they develop in a fermentation system. They can form enormously thick growths on agitator turbines and on baffles and so on in the fermenter, obviously interfering with the dynamics of the whole process.

Other films are associated with animals, including human surfaces. Thus dental caries is entirely correlated with the presence of a bacterial film of dental plaque which will always grow on teeth given the chance. Microbial film is not always bad, however. Most effluent treatment plants encourage the growth of microbial films, for example, on aerobic or anaerobic filter systems and on rotating disc aerators which are all used to recycle organic pollutants. Films of *Acetobacter* covering beech twigs or chips in the "quick" vinegar process or as a film at the air-liquid interface in the traditional Orleans wine vinegar process.

Besides films of economic importance, however, microbial films and slimes associate with numerous other surfaces. Most solid-liquid interfaces can become coated with microbes which tend to attach to a thin layer of adsorbed macromolecules, which quickly bind to any

"clean" surface immersed in natural aquatic systems. Films are found on the gastric mucosa and internal epithelial linings of many animals. While many oral organisms attach to dental enamel, others "prefer" the check and tongue epithelial cells, for example. Films are formed on most surfaces immersed in any of the natural water systems. These surfaces are not simply mineral or wooden structures, but the surfaces of plant stems and leaves and of aquatic animals. Often, a thin film of biological origin can be found in the "neuston" at air-water interfaces. It can be shown that this film can grow and thicken if adequate nutrients are present. Microbes develop on terrestrrial surfaces, too. Thus the phylloplane is a habitat on the surface of leaves, which shows a succession of organisms throughout the growing season. Sometimes these proliferate enough to form a coherent film. A corresponding region around plant roots, the rhizoplane, leads to a cylindrical film-like proliferation of microbes using root exudates as nutrients. There is even a region around germinating seeds called the spermosphere which has some of the characteristics of a biofilm.

Research into biofilm has predictably followed two main routes: first, how to eliminate it from sensitive equipment/areas, and second, how to control its formation and activity where it performs a useful function. Microbiologists have become increasingly interested not so much in the film itself but in mechanisms for the attachment of microbes to surfaces. Really very little is known about the structure and physiological functioning of microbial films, and it is at this point that model film fermenters will play an increasingly important part. The ubiquity of microbial films makes any research into this area of fundamental importance. Each film is normally composed not just of a single species, but of a group of different genotypes each having some part to play in the overall behavior of the structure. It is of interest to find out what comprises a minimal structure that is capable of quasi steady-state growth. Biofilms usually become thick enough that certain solutes, in particular oxygen, become exhausted before the base of the film is reached. This region is therefore a suitable "space" for the proliferation of anaerobic species, so long as sufficient nutrents are available to them. The close juxtaposition of aerobic and anaerobic spaces allows all sorts of interactions to take place. For example, in films where anaerobic corrosion due to sulfate-reducing bacteria takes place, sulfide formed in the anaerobic regions can be reoxidized in the aerobic surface layers by sulfide-oxidizing bacteria (Hamilton[13]). A laboratory model film fermenter is badly needed that can allow the growth and maintenance of a highly reproducible microbial film. Such fermenters are described by Characklis (Chapter 8, this volume) and by Peters and Wimpenny (Chapter 9, this volume) and for dental plaque (a specific film important to human well being) by Tatevossian (Chapter 10) in this book.

TEMPORAL HETEROGENEITY

Succession is a direct result of environmental physical chemistry changing at a point in real space. Given a wide range of genotypes at the location in question, the habitat will be appropriate for maximal growth of a particular species or group of interacting species. This will be the point from which successional changes can start. The physical chemistry of the point in question now alters. If external environmental changes occur the succession is said to be *allogenic;* if conditions change due to the activities of the community itself, then the succession is *autogenic.*

There are numerous examples of successional changes. Indeed, the latter are more the rule than the exception in natural ecosystems. The "classical" examples include the changes in population in haystacks or in compost heaps which result from a rise in temperature. The populations can change from predominantly mesophilic to thermophilic types such as *Bacillus stearothermophilus* and the thermoactinomyces. Successional changes may be caused by pH fluctuations. The silage pit and fermentations producing sauerkraut and the Korean "kim-chee" are of this type. Successions in the gut population of newly born mammals have also

been studied intensively. Breast-fed infants often support large populations of *Bifidobacterium* species at first. These are later replaced by the more typical gut populations of the adult.

A particularly interesting example is surface colonization leading to microbial film formation. The topic is reviewed by Fletcher and Marshall[14] whose account leads to the following conclusions: a clean surface is initially coated with a conditioning film of organic molecules. In natural aquatic systems, small copiotrophic species then attach. The latter are almost always small, Gram-negative, rod-shaped bacteria including pseudomonads, flavobacteria, and achromobacteria. Secondary colonizers then appear such as *Caulobacter, Hyphomicrobium,* and *Saprospira.* The diversity of attached species increases with time, and electron microscopy may reveal the presence of numerus eukaryotic organisms including fungi, diatoms, and protozoa. Successions in human dental plaque film are also documented (Tatevossian, Chapter 10, this volume).

Seasonal changes can lead to allogenic successions. The latter have been discussed for the phyto- and zooplanktonic populations of lakes and ponds, for example, by Cairns in 1982.[15]

If the spatial and temporal heterogenity of the physicochemical environment represent the stage, its sets and effects, then microbes and their interrelationships are the players.

GENOTYPIC HETEROGENEITY AND MICROBIAL INTERACTIONS

There is little point in citing examples of genotypic heterogeneity in nature since almost every conceivable habitat is populated by a wide range of different classes of organism. More interesting are the possible interactions between species. Interactions can be discussed and subdivided on a number of different criteria. Thus the best known classifications based mainly on nutrition can be found in almost any textbook of microbial ecology and will not be summarized here. Another way to consider microbial interactions is based on their space relationships. Thus we can distinguish same-space from different-space interactions. Same-space interactions can proceed in a homogeneous environment and may be investigated in stirred fermentation systems, more particularly continuous flow devices. Such interactions give rise to the term "consortium" being applied to a steady state culture of organisms interacting to form a stable community. Consortia may be simple two-membered communities engaging perhaps in cross feeding, or they may be more complex communities such as the Dalapon consortium which involved seven different species. Such communities are discussed in more detail by Gotschall and Dijkhuisen (Chapter 2) in this volume.

Another important group of same-space interactions are the syntrophic associations in which the metabolism of two organisms are tightly coupled by the transfer of nutrients between them. Hydrogen scavenging, leading to an alteration in the available free energy relationships of certain organisms, is perhaps the classic syntrophic system, most clearly seen in the *Methanobacillus omelianskii* association.[16] The latter originally isolated as a single species was later shown to consist of two species, a methanogen and the so called "S" organism. The "S" organism oxidized ethanol to acetic acid and hydrogen, a reaction which only became thermodynamically probable once the hydrogen concentration was reduced to a very low level by the methanogen. Other syntrophic associations between photosynthetic bacteria and sulfate-reducing species are just as tightly coupled. Looser associations are also common, especially in anaerobic environments where hydrogen scavenging is an important function for the community as a whole.

Different-space systems offer other opportunities for profitable interactions. The most common interface between physico-chemically different spaces are at the boundary between aerobiosis and anaerobiosis. This is because oxygen is both a rapidly metabolized substrate and a very sparingly soluble molecule in aqueous media. Aerobic species rapidly remove