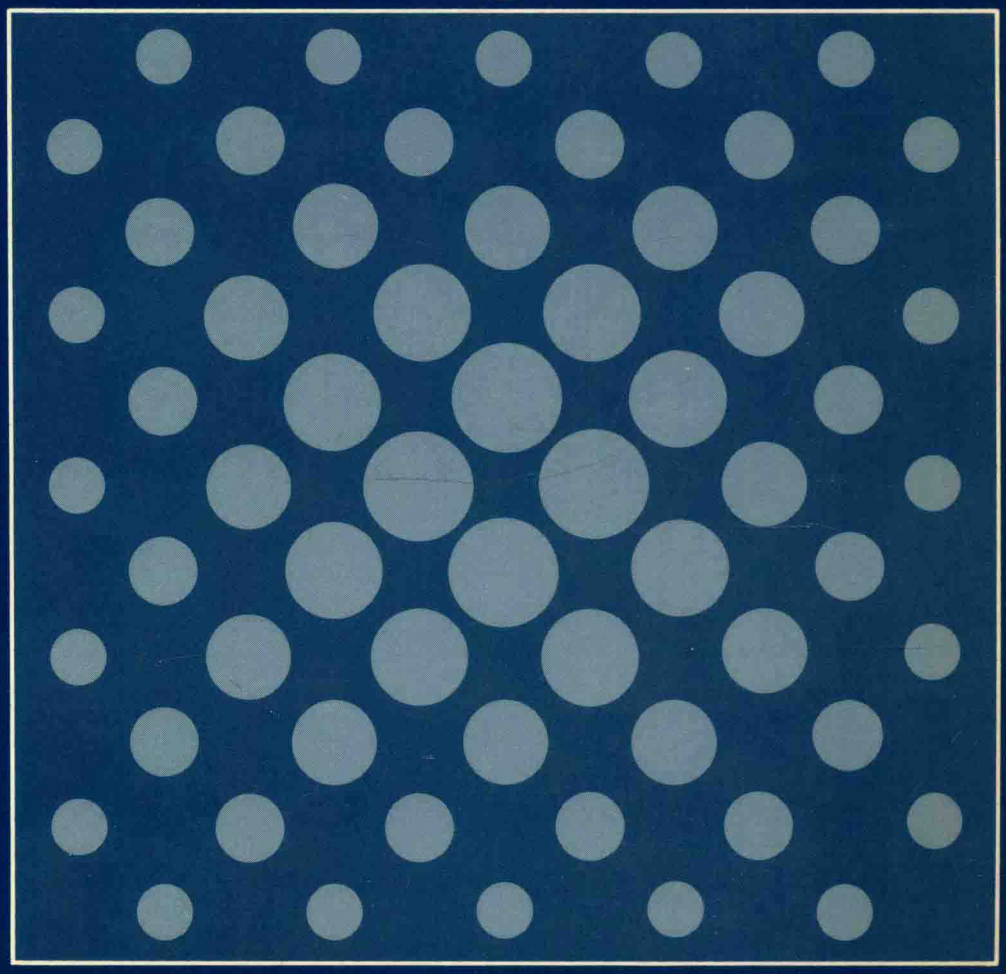
BIOTOL

BIOTECHNOLOGY BY OPEN LEARNING

In Vitro Cultivation of Micro-organisms



BUTTERWORTH-HEINEMANN

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In Vitro Cultivation of Micro-organisms

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Sources

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The Biotol Project

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This series of books has been developed through a collaboration between the Open universiteit of the Netherlands and Thames Polytechnic to provide a whole library of advanced level flexible learning materials including books, computer and video programmes. The series will be of particular value to those working in the chemical, pharmaceutical, health care, food and drinks, agriculture, and environmental, manufacturing and service industries. These industries will be increasingly faced with training problems as the use of biologically based techniques replaces or enhances chemical ones or indeed allows the development of products previously impossible.

The BIOTOL books may be studied privately, but specifically they provide a cost-effective major resource for in-house company training and are the basis for a wider range of courses (open, distance or traditional) from universities which, with practical and tutorial support, lead to recognised qualifications. There is a developing network of institutions throughout Europe to offer tutorial and practical support and courses based on BIOTOL both for those newly entering the field of biotechnology and for graduates looking for more advanced training. BIOTOL is for any one wishing to know about and use the principles and techniques of modern biotechnology whether they are technicians needing further education, new graduates wishing to extend their knowledge, mature staff faced with changing work or a new career, managers unfamiliar with the new technology or those returning to work after a career break.

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Project Manager: Dr J.W. James

How to use an open learning text

An open learning text presents to you a very carefully thought out programme of study to achieve stated learning objectives, just as a lecturer does. Rather than just listening to a lecture once, and trying to make notes at the same time, you can with a BIOTOL text study it at your own pace, go back over bits you are unsure about and study wherever you choose. Of great importance are the self assessment questions (SAQs) which challenge your understanding and progress and the responses which provide some help if you have had difficulty. These SAQs are carefully thought out to check that you are indeed achieving the set objectives and therefore are a very important part of your study. Every so often in the text you will find the symbol Π , our open door to learning, which indicates an activity for you to do. You will probably find that this participation is a great help to learning so it is important not to skip it.

Whilst you can, as a open learner, study where and when you want, do try to find a place where you can work without disturbance. Most students aim to study a certain number of hours each day or each weekend. If you decide to study for several hours at once, take short breaks of five to ten minutes regularly as it helps to maintain a higher level of overall concentration.

Before you begin a detailed reading of the text, familiarise yourself with the general layout of the material. Have a look at the contents of the various chapters and flip through the pages to get a general impression of the way the subject is dealt with. Forget the old taboo of not writing in books. There is room for your comments, notes and answers; use it and make the book your own personal study record for future revision and reference.

At intervals you will find a summary and list of objectives. The summary will emphasise the important points covered by the material that you have read and the objectives will give you a check list of the things you should then be able to achieve. There are notes in the left hand margin, to help orientate you and emphasise new and important messages.

BIOTOL will be used by universities, polytechnics and colleges as well as industrial training organisations and professional bodies. The texts will form a basis for flexible courses of all types leading to certificates, diplomas and degrees often through credit accumulation and transfer arrangements. In future there will be additional resources available including videos and computer based training programmes.

Preface

To many, the term biotechnology conjures up a picture of large volume reactors in which cultures of micro-organisms are used to make products such as wine, beer and antibiotics. Of course, biotechnology is much more diverse than this, but the common misconception reflects the fact that the use of micro-organisms dominates many biotechnological endeavours. There are several reasons for this. The fast growth rates of micro-organisms and the associated high rates of metabolism coupled to their metabolic diversity ensure that micro-organisms are often the agents of choice. These features together with the relative ease by which they may be genetically manipulated and increasingly detailed knowledge of their metabolism provides further impetus for their systematic exploitation to produce an enormous array of metabolites and enzymes. Opportunities to use micro-organisms to deal with domestic and industrial waste and contributors to agriculture are being increasingly grasped.

Within the context of the BIOTOL series, the text dealing with the *in vitro* cultivation of micro-organisms needs no further justification. The ability to cultivate micro-organisms underpins so many biotechnological activities ranging from the small volumes enclosed in the genetic manipulation of plant and animal systems to the enormous capacity of many large volume processes. This text aims to provide the essential knowledge of the core processes involved in the cultivation of micro-organism irrespective of the organism or the scale of the operation. Discussion of the *in vitro* cultivation of cells from higher plants and animals has been specifically excluded. Although cultivation of these cells has much in common with the cultivation of micro-organisms, they do display some important differences and two BIOTOL texts have been produced to cover these important groups ('In vitro Culitvation of Plant Cells' and 'In vitro Cultivation of Animals Cells').

A key feature of any process involving micro-organisms is the need for containment. Some micro-organisms are pathogenic and need to be prevented from either entering an industrial process or for that matter, leaving a process to infect workers or the community at large. Many processes need to be conducted with pure cultures, contamination by undesirable strains leads to poorer, unpredictable productivity. This the need to prevent the unwanted transfer of micro-organisms is essential. Thus aspect of *in vitro* cultivation of micro-organisms is introduced in the first chapter and is a recurrent theme within the text.

The major part of the text deals with the nutrition of micro-organisms with particular emphasis on media design, the evaluation and characterisation of growth and the influence and control of the physical and chemical parameters which influence microbial performance in culture. A chapter is devoted to discussion of the cultivation of viruses, especially bacteriophages. The importance of bacteriophages in contemporary biotechnology lies in their use as genetic vectors and they also offer some potential as antibacterial agents for some bacterial infections. The final chapter deals with the chemical agents that may be used to control microbial growth. Disinfection and disinfection policies are key components of good microbiological practice at both laboratory and manufacturing scales of operation. Good microbiological practice is in its turn, essential to good laboratory and good manufacturing practice.

Although a discussion of growth in batch and continuous culture is included, this discussion focusses predominantly on the biological and scientific issues involved. For those seeking to expand their knowledge into the engineering issues of *in vitro* cultivation of micro-organisms, we recommend the BIOTOL series of technology texts, especially 'Bioprocess Technology: Modelling and Transport Phenomena'; 'Operational Modes of Bioreactors' and 'Bioreactor Design and Product Yield'.

The authors are to be congratulated on their synthesis of a sound and logical development of this topic. The quality and relevance of the technical material they have used are matched by their ability to design interactive activities within the text which aid the reader towards a full understanding of the issues under discussion. We encourage readers to take full advantage of these opportunities.

Scientific and Course Advisors: Dr M.C.E. van Dam-Mieras
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Contents

Introduction to microbial growth and cultivation

Introduction to microbial growth and cultivation

1.1 Introduction

Microbiology is a very specialised area within the science of biology and the strategies and methods required to grow micro-organisms are very different to those required for growth of, for example plants. The chapters of this text are structured and ordered such that the reader can follow the theme of growth of mainly unicellular organisms which divide regularly by asexual means forming two daughter cells from each parent cell. The major exception to this system is the group of viruses and they will be treated separately.

We will not include discussion of animal and plant cells in culture within this test. Although cultivation techniques for these systems have much in common with the cultivation of micro-organisms, they also have many special features. A description of the cultivation of plant and animal cells is given in two other BIOTOL texts: 'In vitro Cultivation of Plant Cells' and 'In vitro Cultivation of Animal Cells'.

It is appropriate at this stage to introduce a few words of caution. Throughout the book we will mention the fact that micro-organisms, can particularly in high numbers, be dangerous and should always be treated with caution and great respect. This text will provide you with insight into the theoretical and practical aspects of microbial cell culture which you could then employ under supervision at your place of work, if suitable, or in an appropriate laboratory under qualified supervision. The text is not intended to be a 'do-it-yourself' manual encouraging you to experiment in, for example your own home. To do so could be both dangerous and quite unwise.

1.1.1 The structure of the text

Chapter 1 introduces the microbial world and gives you an overview of the history of microbiology followed by a discussion of appropriate current safety legislation and recommendations.

Chapter 2 gives an overview of the chemical composition of the cell leading on to the nutritional requirements of different types of micro-organisms and thus the design of laboratory media. The types of media, culturing conditions and sterilisation methods are discussed and you are encouraged to investigate the strategy behind selecting suitable source(s) of micro-organisms and obtaining pure cultures.

Chapter 3 describes the estimation of biomass, by cell numbers, weight or volume. Several methods of measurement are examined and a critical evaluation of the advantages, disadvantages and uses of each method are described.

Chapter 4 examines the growth of micro-organisms in the various forms of batch culture and introduces you into the energetics of growth.

Chapter 5 discusses the environmental factors which influence growth indicating how we predict the effect on growth that changes in environmental parameters would bring

about. Finally in this chapter we explain how these factors may be manipulated to our advantage.

Chapter 6 examines growth of micro-organisms in continuous culture. This system is compared to batch cultures and suitable treatment of the topic involves discussion and derivation of the mathematical relationships of various parameters and terms relevant to microbial cell cultivation. Industrial uses and applications of continuous culture are also discussed.

Chapter 7 examines the influence of and the control of selected factors which affect growth in a chemostat, for example pH, temperature and oxygen concentration.

Chapter 8 deals exclusively with viruses beginning with a brief history of the science of virology and followed by an examination of viral structure and classification. The forms of viral replication are discussed with particular emphasis on bacterial viruses (bacteriophages) and the methods available for production of large quantities of viruses in the laboratory. Methods available for estimating viral numbers are also described and evaluated.

In the final chapter, the chemical control of growth is examined. The distinction between antiseptic, disinfectant, metabolic inhibitor and antibiotics is explained and the kinetics of microbial death is described.

1.2 The composition and characteristics of the microbial world

definition of microbiology

A useful, working definition of microbiology is the study of organisms too small to be seen by the naked eye. The human eye can clearly see objects which are 1 mm or more in size but objects which are of the order of 0.2 mm or less cannot be resolved. Thus we could defines microbiology as the study of organisms (or more commonly - micro-organisms) which are less than 1 mm in size. We will see in later discussion that this is not a strict definition and in practice this definition is not a rigid one.

The millimetre or mm is one thousandth of a meter but within microbiology it is easier to denote length using a millionth (10^{-6}) metre which is more commonly known as a micrometre (μm) or micron (μ). The length of a typical bacterium as we shall see is commonly about 2 μm.

It has been known for a long time that all living organisms are composed of one or more fundamental units called cells. Thus if we were looking at any organism at the individual cell level the study could, in one sense, be termed microbiology. However, we usually reserve the term for living systems in which the whole organism, that is the entity which can and normally exists independently, is too small to be seen by the unaided eye.

Π Write a list of the groups of living organisms which normally exist independently as unicells?

Some of the major groups which you may have considered include the bacteria, fungi, algae and protozoa. Possible your answer would have included viruses; we will refer to these again shortly.

The terms microbiology and micro-organisms are really very ill defines and have no taxonomic significance. From the organisms listed above, the bacteria, protozoa and viruses can exist independently as single units though the first two sometimes purposely produce aggregates. Fungi and algae, however, vary from small, simple unicellular structures through to multicellular structures, for example mushrooms and seaweed. The study of mushrooms several centimetres high and of seaweed several metres long is nor strictly speaking microbiology, although because of their relationship with unicellular forms they are frequently included in courses on microbiology.

To allow us to investigate the characteristics of micro-organisms it is necessary to return to the concept of the cell. The ability to successfully exist independently - the concept of unicellularity - is an important one not displayed by higher organisms. Unicellular organisms, sometimes called 'unicells', are generally relatively simple cells having a high degree of adaptability. Early theories on classification or the assigning of living systems into groups suggested that all living matter was either 'plant' or 'animal'. As our knowledge of microbiology increased during the last century micro-organisms were themselves assigned to plants or animals largely on the basis of the presence of motility (indicating an animal cell) or photosynthesis (indicating a plant cell). From the beginning of this century, however, it became apparent that there were many micro-organisms which could not fit into one of the two categories above. For example, there are non-motile, non-photosynthetic protozoa and also motile, photosynthetic protozoa.

prokaryotic cells

eukaryotic cells

Around 1950 the development of the electron microscope led to the discovery that there are two basic types of living cell. One type, which is a relatively simple structure always lacking a true membrane-bound nucleus has been termed a prokaryotic cell. The second type of cell is more complex, generally larger and always has a membrane bound nucleus and is termed a eukaryotic cell. This primary division is extremely useful to us because there are no exception in that all living cells are very definitely either prokaryotic or eukaryotic. Knowledge of this tells us immediately quite a lot about the properties of the cell. Prokaryotic cells include bacteria and blue-green algae (now more usually referred to as cyanobacteria) whereas the fungi, algae, protozoa, plants and animals are all, without exception, eukaryotes. At this stage the possibility of dividing living systems into three groups or kingdoms (animals, plants and prokaryotes) was suggested. This division helped to remove the difficulty of assigning bacteria to either plant or animal kingdoms but still did not resolve the dilemma of where to place the protozoa like those mentioned earlier.

five kingdoms

The currently favoured system is the five kingdom approach. In this system the proposed kingdoms are Monera (prokaryotes), Fungi, Animals, Plantae and Protista. The major characteristics of each are shown in Table 1.1. The real difficulty of this system is to find a precise definition of the Protista.

A study of Table 1.1 indicates that the microbiologist can therefore study examples of three of the kingdoms, Monera, Fungi and Protista.

Kingdom	Primary characteristics	Example
Monera or Prokaryotae	prokaryotic cells	bacteria and blue-green algae (cyanobacteria)
Fungi	eukaryotic cells - mycelial, usually walled and septate, multinucleate	higher fungi
Animalia	eukaryotic cells - multicelluar, wall-less, aerobic, capable of complex differentiation, primarily ingestive nutrition	animals
Plantae	eukaryotic cells - multicelluar, walled, aerobic, usually differentiated, primarily photoautotrophic nutrition	plants
Protista	eukaryotic cells - ingestive or photoautotrophic	simple algae, protozoa, simple (lower) fungi

Table 1.1 The primary characteristics used to assign organisms to one of the five kingdoms.

Archaebacteria

It should be pointed our here that as our knowledge increases the grouping of organisms even at this broad level changes. As we currently learn more of a rather specialised group of bacteria called *Archaebacteria,* some researchers suggest that these should be placed separately from other living systems. Briefly they are prokaryotic in structure but have cell chemistry unlike all other living systems. Examples of this group are the halophiles which will be mentioned later in the text.

Π Which major group have we not mentioned in Table 1.1?

viruses

The answer is the viruses. Viruses are acellular and by definition non-living. However, due to their intimate relationship with living cells and their profound effect on our lives and environment we study them within the context of biology. Because they are very small we study them within microbiology. Due to their uniqueness they have to be treated independently and their cultivation and enumeration will be studied in a separate chapter in this text.

In summary the microbiologist largely studies free-living, unicellular organisms which are virtually always too small to be seen by eye. The organisms, collectively termed micro-organisms, to be studies include all of the bacteria, all of the blue-green algae, fungi, algae and protozoa. In addition, the acellular entities called viruses are a part of microbiology.

divisions of microbiology

As we shall see in Section 1.4, micro-organisms are incredibly diverse and occur in virtually all habitats throughout the world. The diversity of the subject means that microbiologists tend to specialise in one of a number of specific areas. We can for example identify microbial geneticists or biochemists or physiologists or we can identify specialists that focus on specific groups, for example bacteriologists, mycologists (the study of fungi) or protozoologists. This text which focuses onto the cultivation of micro-organisms underpins all of these specialities.