METHODS IN BIOTECHNOLOGY

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Contents

Summary

The outstanding advances in biotechnology over the last 50 years have been underpinned by the development and application of a range of "biotechnological methods," from the development of fermentation technology in the 1940s and 1950s, through to the development of genetic engineering techniques in the late 1970s, to the more recently developed methods of protein-engineering and DNA amplification by the polymerase chain reaction.

Some of these methods, and the potential consequences of their application have caught the media's imagination and therefore have received widespread attention. Many will know that methods have been developed which enable us to genetically manipulate

plants and animals. These genetic engineering methods, and many other less well-publicized methods, can be seen as the "tools" of biotechnology. This topic article aims to give an overview of how these tools are used in the development and operation of new biotechnology processes, with the focus given to fermentation processes, since genetic engineering methods were covered under the previous topic, Fundamentals of Biotechnology. Questions of how new biotechnologists find out about, use and report on the methods used in their research are also addressed.

1. Methods in biotechnology: A Topic in its Own Right

Since the advent of modern biotechnology, biology has been passing through a golden age, with great opportunities to study, understand and control fundamental life processes in benefit of humankind. Although biotechnology may be a relatively new term, there is a long history of the use of biological processes in the manufacture of products, ranging from the ancient process of alcohol fermentation to the somewhat more recent production of antibiotics and antibodies.

The rapid pace of advances in current times is achieved on the basis of understanding of the life processes involved. Biotechnology brings together subjects such as microbiology, engineering, agriculture, genetics and biochemistry, in a combined effort to increase production of commercially interesting products or to solve environmental problems. Advances in this field rely on the development of methods in both research and application of biotechnological processes.

Various methods are employed at the different steps of a biotechnology process: upstream processing (inoculum and substrate preparation), bioconversion (by microorganisms, plants or animals or parts thereof), and downstream processing (products recovery, waste disposal). This chapter will give to the apprentice of biotechnology an overview of where methods are used in each of these steps, either to check the performance of the process (i.e. fractionation, identification, quantification methods) or to convert materials into a different form (i.e. enzymatic or chemical reactions, genetic transformations). It also gives an insight into how methods are found, used and reported in biotechnology.

The other sections under the theme "Biotechnology" talk about biotechnology from the perspectives "What is biotechnology?", "What products and services can biotechnology offer us, especially in terms of sustainable development?" and "What is the relationship between biotechnology and society?". The present topic, "Methods in Biotechnology" addresses biotechnology from a different perspective.

It attempts to answer the question "How do we do biotechnology?" or more specifically "What are the technical procedures that we use in biotechnology, and how do we use them in conjunction to develop and operate biotechnological processes?". The aim is not simply to give a list of methods, but rather to show that the development or operation of a biotechnological process requires the rational selection and coordinated use of a suite of methods originating from biological, physical, chemical and engineering sciences.

As defined in the Section *Fundamentals of Biotechnology*, biotechnology involves the use of microorganisms, plants or animals or parts thereof for the production of useful compounds or to carry out useful processes. As such, biotechnology involves processes and products such as waste treatment (see also - *Environmental Biotechnology*), the production of commodity chemicals (see - *Industrial Biotechnology*), therapeutic compounds, vaccines and antibodies by microbial fermentation or cell culture techniques (see - *Medical Biotechnology*), and the production and raising of transgenic microbes, animals and plants (see - *Agricultural Biotechnology*).

These products arise out of basic research in a wide range of sciences such as biochemistry, microbiology, immunology, and plant and animal sciences. It is impossible in the limited space available to give a complete overview of all the methods involved in these various biotechnological applications. Due to the central importance of fermentation and cell culture in the majority of biotechnological products and processes, especially those related to sustainable development, the current topic focuses on the methods used at various stages in the development of new cultivation processes, and during the routine operation of a cultivation process once developed.

Advances in biotechnology have always been based on the development of new methods. In traditional processes developed centuries ago, such as the production of wine, beer, bread, and various other fermented foods and beverages, these methods were developed over long periods of experience, in an essentially “trial and error” process with the aim to “conserve and reproduce successful recipes”, but with essentially no understanding about how the process worked.

Modern biotechnology began with the use of scientific methods to characterize these traditional processes, such as done by Pasteur in the context of wine in the second half of the 1800s and, with the development of pure culture techniques around the same time, the basis for fermentation processes was established.

The pace has increased over the last 50 years or so, and again the advances have been based on the development of new methods. The improvement of fermentation methods in conjunction with penicillin production during the 1940s laid the foundation for the establishment of many new fermentation processes. In the 1950s and 1960s random mutation and selection procedures were used to produce high-producing strains for many fermentations.

The development of genetic engineering techniques (see - *Methods in Gene Engineering*) in the 1970s laid the foundation for an explosion in biotechnology applications, for example, enabling the production of human proteins by microorganisms.

More recently still, to name one example, the polymerase chain reaction (PCR) was developed, in which a single desired gene sequence can be targeted amongst an abundance of other genes and can be amplified exponentially into large quantities.

Note that the idea of repeatedly amplifying DNA was first published in 1971 by Kleppe and coworkers, but was exploited commercially only a decade later by the Cetus

Corporation. This technique has become a basic tool for the molecular biologist, and it is still continuing to open up new applications of biotechnology.

This topic overview "Methods in Biotechnology" is organized as follows. Firstly, it defines what we mean by "a method".

Then it gives a broad overview of typical fermentation-based biotechnological processes, showing how suites of methods are employed at all stages of development and operation. Finally, it addresses the question of how biotechnologists learn, use and report methods.

The articles which follow this topic overview give greater details of what the methods involve and the principles on which they are based. However, they do not attempt to provide sufficient detail to enable the reader to use the methods, since the amount of space that this would require is far beyond what is available.

In fact, there are a number of handbooks and journals dedicated to providing descriptions and evaluations of methods. As pointed out below, biotechnologists need the information skills to identify and locate these methods, and then to evaluate the suitability of the methods for their particular purpose.

This topic is written for the novice in biotechnology. We envisage that you as the reader may be:

- A student undertaking a diploma or bachelor's degree who intends to follow a career in biotechnology, but for whom the whole of biotechnology is a new experience. In this case, this Methods in Biotechnology topic overview will help you to see how the methods you are learning in various theoretical and practical classes will become a suite of tools which you will call upon in a coordinated way during the development of new biotechnology processes and the operation of existing biotechnology processes.
- A postgraduate student in the early stages of a biotechnology-related research project. Typically such a project will address some aspect of the development of a new biotechnological process, although it is also possible that it will address some problematic, poorly performing or poorly understood aspect of an operating process. At this stage of your research career, you have relatively little experience in how a research project is carried out. This topic overview, in addition to giving basic information about a range of methods which might be relevant to the research project, will also show how finding, using and reporting methods is a key activity in the work of a biotechnologist.
- A more experienced scientist or engineer, without experience in biotechnology, who becomes involved in a biotechnology project. In this case you will have a good grasp of the methods in your own area, but not in the area of biotechnology. This topic gives a brief overview, enabling you to move quickly to more detailed descriptions of the methods relevant to you.
- Anyone else who, while lacking an in-depth knowledge and experience of biotechnology, has an interest in the question "How is biotechnology done?".

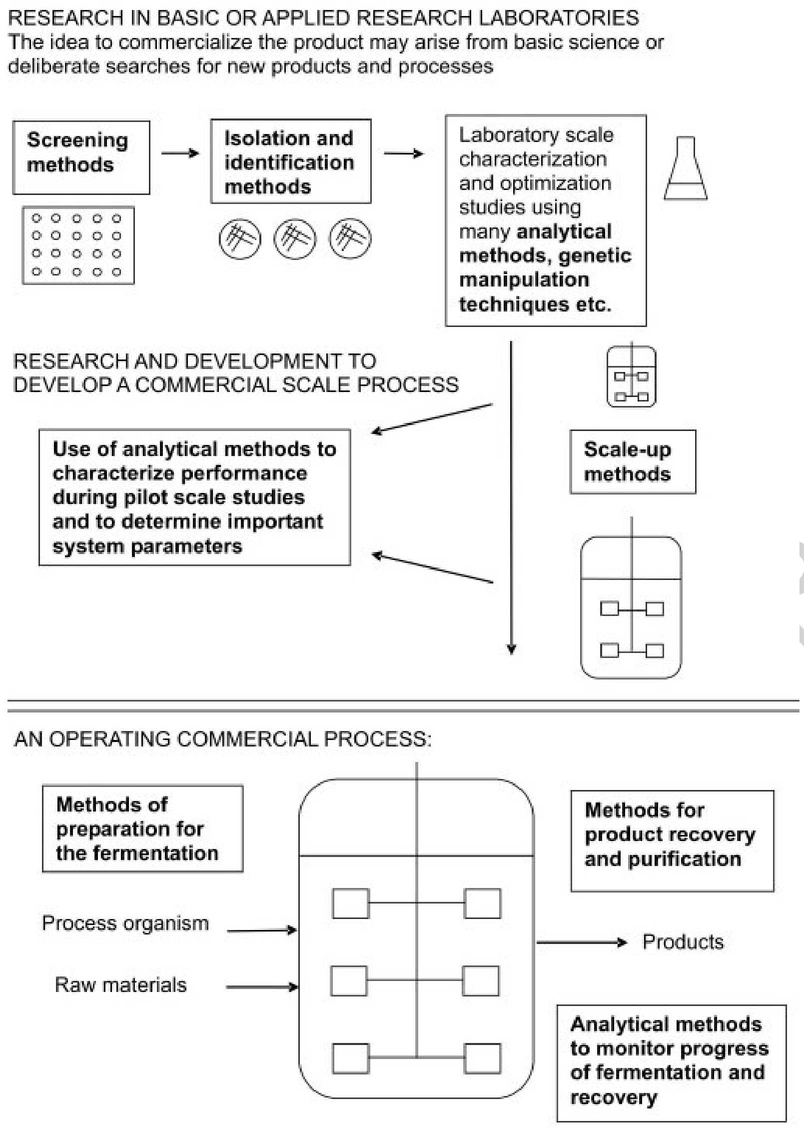

Figure 1: Fermentation biotechnology can be thought of as being comprised of two phases: the development of new processes and the routine operation of processes once developed. Both of these phases involve the application of various methods, more details of which are shown in Figures 4 and 5.

2. What is a Method?

In this topic, we define a method as a deliberate and systematic series of manipulations designed to achieve a particular aim. Furthermore, the label "method" suggests that the series of manipulations can be used in a range of related systems and therefore that the method can be recorded as a set of instructions (see Figure 2), allowing its use by anyone who considers it appropriate, although some modifications may be required if the system is sufficiently different from that for which the method was originally developed.

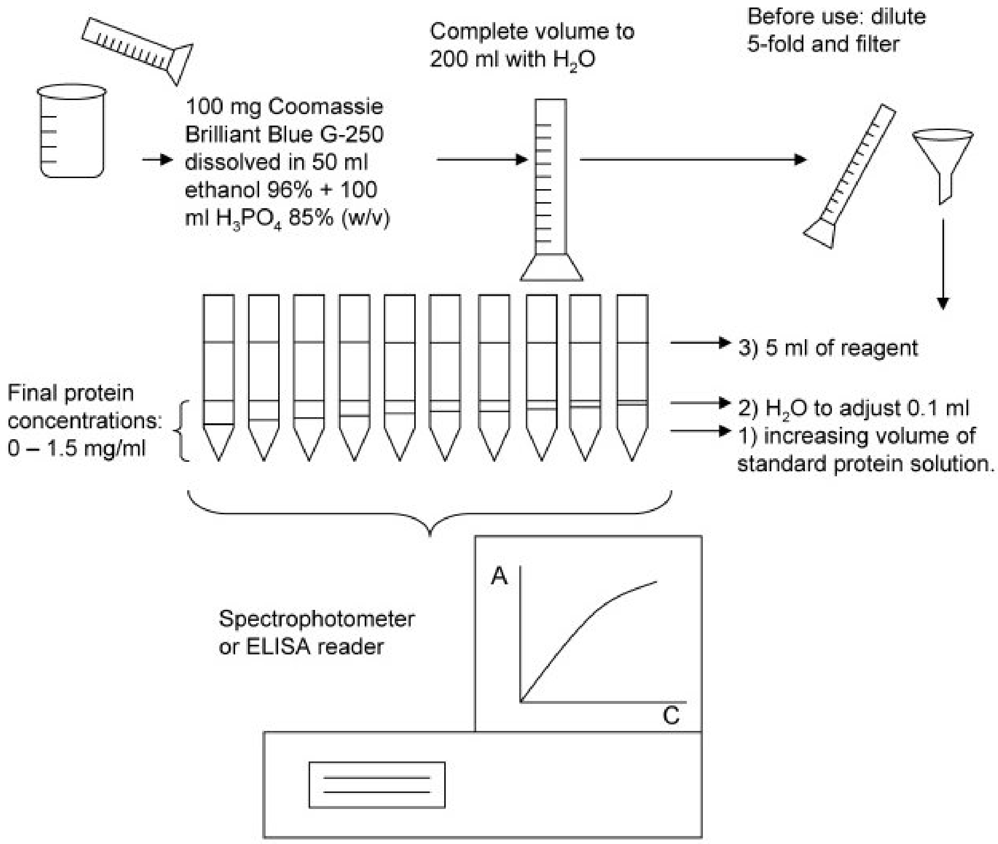

Figure 2: The level of a method that first comes to mind when a scientist hears the word "method". The method comprises a set of instructions which enable the measurement of protein concentrations.

To illustrate this point, consider a simple method such as a colorimetric method for protein determination based on the chemical reaction between the protein and a compound which produces a colored substance. The intensity of the color can be measured with a spectrophotometer and used as a measure of the concentration of the protein in the sample. This method can be used in a range of different circumstances in biotechnology, among which are the following:

- to determine the concentration of protein during a process for the recovery of an enzyme or a therapeutic protein
- to determine the concentration of protein in an enzyme reaction mixture in order to calculate the specific activity (amount of activity per mg of protein in the reaction mixture)
- to determine the concentration of microbial protein in a fermentation system, using the amount of protein present as an indicator of the amount of microbial biomass present
- to determine the concentration of protein in a substrate such as soybeans, yielding information which may help interpretation of the fermentation data (e.g. the organism probably grew so well because of the high concentration of organic nitrogen available in the protein of the soybean meal that was added to the system)

At the other end of the scale, the “scientific method” can be thought of as a set of general principles that guides how scientific research is done.

Given the difference between a method for protein determination and the scientific method, it is obvious that it is possible to classify methods into a hierarchy, depending on their level of complexity and the number of sub-methods involved in their execution (See Figure 3).

As an analogy, methods at different levels of the hierarchy can be compared to the hierarchy of organization of a community of multicellular organisms, considering individual cells as the basic building blocks. Simple methods can be likened to the individual cells of the organism.

In the same way that individual cells are crucial building blocks of higher level structures, but do not have an independent life, simple methods are crucial, but the individual bits of information provided are of limited use. An experiment that utilizes a range of methods can be likened to an organ.

In the same way that the organ is constructed of many individual cells, giving a structure with a definite role, an experiment uses a range of different methods to provide a definite answer, be it to confirm or negate an hypothesis, to determine a kinetic parameter, or to find an optimal condition for the process (see - *Process optimization strategies for biotechnology products: From discovery to production*).

However, in the same way that an organ is not capable of an independent existence, an individual experiment is usually not sufficient to achieve the aims of the research. A research project can be likened to an organism.

In the same way that the organism is a relatively independent unit comprised of interconnected organs, the research project is a set of experiments that fulfills an overall objective. At a higher level still, science and technology as a whole can be likened to the community in which the organism lives.

The community is made of many organisms, each of which may have quite different characteristics, but all of which have features which identify them unmistakably as living organisms. Furthermore, the members of the community are not totally independent, rather there is a web of interactions.

Likewise, science and technology can be thought of as consisting of many different research projects. Each project is different, but all have a common feature in that they employ what is called the scientific method in their execution, and there are often many similarities in the sub-methods used.

Like the interactions amongst the members of the community, research projects are interrelated, insofar as research projects build on the knowledge base provided by previous and contemporaneous work.

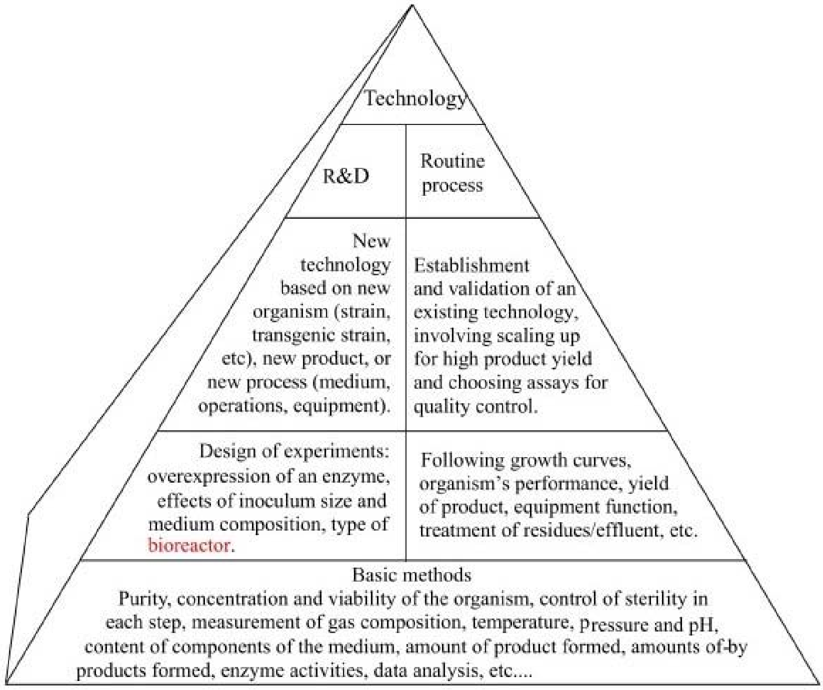

Figure 3: The hierarchy of methods and how they might be applied slightly differently in research and development and routine process operation

Methods may be used in order to achieve a range of different ends in biotechnology, however, it can be useful to think of all methods as having either of two basic ends:

- A method may be used to gain information about the performance of a biotechnological process. This category includes measurements of the concentrations of compounds in a fermentation broth or during product recovery operations, as well as methods to check the identity, viability and kinetic parameters of the biological material utilized. The higher-level method of applying a factorial experimental design in order to identify the optimal conditions for growth also belongs to this category. Identification of the optimum provides information about the conditions under which the process performs best.
- A method may be used to process a material from one form to another. This material could be a chemical compound, converted into a different compound through an enzymatic reaction. Methods for the genetic manipulation can also be seen in this light: an organism lacking a desirable feature is processed to produce an organism which now has that feature.

Of course, when applying methods to process an organism or compound, we often need to use measurement methods to follow the success of our process. Likewise, when applying a method to increase our knowledge about a process, such as in the measurement of a compound in a fermentation broth, we often need to process a compound from one form to another (for example, from a colorless form to a colored derivative which can be measured with a spectrophotometer).

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Biographical Sketches

David Mitchell obtained a Bachelor of Science with honours in Microbiology, and a PhD in Biotechnology, both from The University of Queensland, in Brisbane, Australia. After 9 years as a lecturer in the Department of Chemical Engineering at The University of Queensland, he migrated to Brazil and is currently teaching within the Biochemistry and Molecular Biology Department of the Federal University of Paraná. He has published extensively in the area of solid-state fermentation, where, for the past several years, he has been using mathematical modeling as a tool to understand how microbial growth in solid-state bioreactors is controlled by diffusion processes within particles and heat and mass transfer phenomena within the substrate bed.

Adriana Contin concluded her undergraduate studies in 1990, obtaining a degree of "Industrial Pharmacy" from the Federal University of Parana in Brazil. In the same year and University, she commenced a Master's degree in Botany, with emphasis in Phytochemistry. She obtained her Master of Science in 1993 and started her career as a lecturer in the Faculty of Pharmacy. In 1995 she started her Ph.D. studies on plant cell biotechnology in the Division of Pharmacognosy of the University of Leiden, The Netherlands. In 1999 she received her doctoral degree and restarted lecturing in Brazil. She is currently developing research on plant biotechnology for the production of secondary metabolites.