MICROBIAL DYNAMIC TRANSFORMATIONS: BASIC CONCEPTS

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Keywords: Closed systems, parameters, variables, differential equation, cost index, gray box models, manual control

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Summary

Biotechnological processes show a complex behavior. The understanding and prediction of their temporal evolution, determination of the parameters that define it, and their control are problems that the biotechnologist often confronts. A recurrent concept during the next sections will be that of *dynamical behavior*. By this, we are referring to a type of temporal behavior, which determines the type of models and mathematical tools to use. Comprehension of this point along with its implications for modeling and control will be the principal goal addressed. Throughout this chapter, we try to define basic concepts as well as properties needed to carry out the analysis of a biotechnological process and eventually design an effective control of the cellular population behavior during controlled conditions of growth.

1. Introduction

Throughout centuries different civilizations have used traditional fermentation (equivalency, traditional biotechnology), i.e., the artisan style to direct the transformation of different substances such as, milk, grape juice, flour wheat triticum, barley and hops... Using this empirical technology they increased the digestibility and/or stability of the perishable products e.g. enlarged the period of preservation. During this time the underline causes of these transformations were unknown (e.g., the fact that the agents causing these processes were microorganisms). Today, microorganisms are used not only for fermentation transformations in the traditional sense, but also as tools in modern biotechnological processes where our ability to introduce genetic modifications enables us to use them for a broader range of applications. For example, in fermentation transformations cells are used as hosts to produce biomolecules of commercial interest. However, the public perception of the fermentation world continues to be that of an empirical area linked to the transference of recipes that grant the quality of natural things to the final product. The reality is different, and because of scientific technological advances, we are under the conditions that allow us to obtain either traditional products or transformation of substances that enable the generation of new products in a highly controlled way. The difference is that now the quality of these products must be guaranteed and maintained. Before reaching this point, there has been a long challenging period for science with a large number of experimental designs and conceptual analysis in the search and identification of the cause-effect patterns enabling to reproduce the observed biological phenomena.

The history of microbiology can be traced back to the development of the first microscope in 1684 by Leeuwenhoek, which allowed the visualization and identification of the different forms of microorganisms. Evidence arose in favor of the thesis on the existence of polymorphic microbial life. This thesis was finally refuted centuries later thanks to the works of Pasteur around 1857 and Koch, the isolation and sterility techniques contributed by Lister in 1878 and Tyndall, and finally the contributions by Winogradsky (1887) who developed a microscopic culture technique.

In fact, once these techniques and experimental methods of culture, sterility, isolation, staining, and microscopic observation were available, the study of pure cultures was possible. These advances allowed the progress of microbiology in the direction of the

monomorphic thesis. The controversy about the polymorphic microbial life was closed and the spontaneous generation theory refuted. This was the origin of the microbiological pure culture philosophy**,** which established the modern fermentation industry, as part of the new biotechnology.

The transformations occurring during fermentation processes are complex phenomena, involving systems composed of a large number of interrelated elements. Some characteristics associated with the concept of complexity are: large variety of relationships among these elements, the hierarchy of these relationships, the operation mode, and the organization of these elements in complex structures. In addition, systems can be classified as, Open systems, characterized by both energy and mass exchanges across the system boundary and Closed systems, which are characterized only by energy exchange.
The discussion above applies both to natural systems (e.g. communities of organisms) as well as artificially created ones (e.g. computers). The concept of complexity generated during 20^{th} century with the emergence of disciplines, such as the general systems theory and systems engineering, which try to analyze complex dynamical phenomena from the mathematical point of view. Thus, because these disciplines make intensive use of the qualitative theory of differential equations, dynamical optimization, etc., they allow qualitatively and quantitatively analyze biotechnological systems, make predictions about them and control them despite the presence of uncertainty and/or partial knowledge.

The mathematical models both encode in their structure our heuristic knowledge about the process and provide new **qualitative** comprehension of the complete process from a biological perspective. This enables us to reason out what would be the process behavior and what action would have to be to accomplish our control objectives, such as fixing a specific growth rate, obtaining a fixed productivity, etc. Mathematical models also imply quantitative information thus allowing not only to describe the general characteristics of behavior but also to predict the temporal evolution of the process. In brief, it is possible to find the factors that influence the final result and consequently control of the process.

Whatever the application or the scale of the process with life cells, if one seeks reproducibility, a good description, and control of the phenomena it is necessary to combine knowledge from the biological sciences, mathematics, and process control engineering.

One of the goals in the chapter is to convey a global picture encompassing the previously mentioned disciplines. In the following sections, we present in an integrated way some basic biotechnological and system engineering concepts, opening the doors to powerful mathematical and engineering tools. We intend the reader to be acquainted with the processes that are involved in both the traditional biotechnology as well as the new biotechnology.

2. Growth and Microbial Kinetics

Microbial kinetics is the study of all dynamic manifestations in the microbial world

such as growth, survival, death, adaptations, mutations, product formation, cellular cycles, and environmental and inter-specific interactions. The examination of each characteristic will require precise premises because life organisms are extremely complex systems. The study of the interactions between the organisms and the environment is traditionally known as ***Microbial physiology*** (see also – *Basic Strategies of Cell Metabolism*). Because of these interactions, processes and/or products of interest are generated, that may be exploited by biotechnology.

Usually, the life manifestations are expressed as instantaneous rates (of growth, of substrate consume, of product generation) in cases where the dynamic characteristic under study show a continuous distribution in the population. Kinetic studies require the perception of subjacent mechanisms of the process through the combination of experimental measurements and a mathematical model.
Thus, the quantitative aspects of microbial growth require an essential tool such as the mathematical model (see also: *Mathematical Modeling in Biotechnology*) (see later *Application to the bioreaction modeling*). The model is the formal representation of our particular system and will be used to define the putative mechanism of the reaction under study.

If this is the situation, ***the model will be useful*** and will enable the comparison between the observed phenomena and the prediction of the model. This permits for the elimination of wrong hypothesis and even the simulation of the possible states of the system under different hypothesis.

Therefore, microbial kinetics tries to explain the microbial world from knowledge of its temporal evolution, proposing general principles and models that describe rates and the mechanisms that play a role during the biological processes.

The growth of a microbial population (see also: *Microbial Cell Culture*) may be modeled using the so called ***Black box models,*** which do not take into account the internal cellular variables or internal factors (the internal cellular processes). These kind of microbial growth models are traditionally defined in terms of the cellular mass increment as a function of mass or cell number.

In this context, the growth phenomena will also depend on the availability and existence in the surrounding environment of the necessary cell nutrients and the capability of its cellular internalization. Also, environmental parameters (temperature, pH, aeration...), must be permissive for the development of our organism (permissive *variables or external factors*). Under these conditions, the cellular division process will be the result of a complex number of regulated steps and mechanisms, which control the flux of the required metabolites for the synthesis of macromolecules, which control the processes of chromosomal replication, and finally the increase in cellular mass (see also: *Cell Thermodynamics and Energy Metabolism*). The timing of these events is regulated and coordinated by the cell and originate a cellular division process that is perfectly ordered.

To carry out the quantitative study of the growth process simple methods have been developed that allow to determinate the populations growth. These methods must take in consideration the reproduction phenomenon of the cells of interest. The prokaryotic

cells reproduce by binary fission, and it is not possible to differentiate a mother cell from its offspring. Due to that, the maximum age of each cell equals the time between to two continuous replications. The reproduction phenomena in eukaryotic organisms are different and we can distinguish between yeasts and filamentous fungi. Yeast duplicate by budding and posses a reproduction cycle with spore generation. Usually the division generates a daughter cell and a mother cell, which are different. Thus, the culture will be heterogeneous with respect to the age and physiological stage. Filamentous fungi are more complex because on the one hand the reproductive cycle of these organisms occurs by sporulation; on the other, they also present growth by hyphae formation. This generates a heterogeneous population both from the physical and from the physiological perspective. All these phenomena can be analyzed from the mathematical point-of-view, although to do this we previously need to know some basic concepts.

3. System and Signals

To introduce the concepts that conform to the nucleus of the theory of modeling and control of dynamic systems, we will use two habitual examples within the biotechnology area.

3.1. Mixture and Homogenization of Compounds

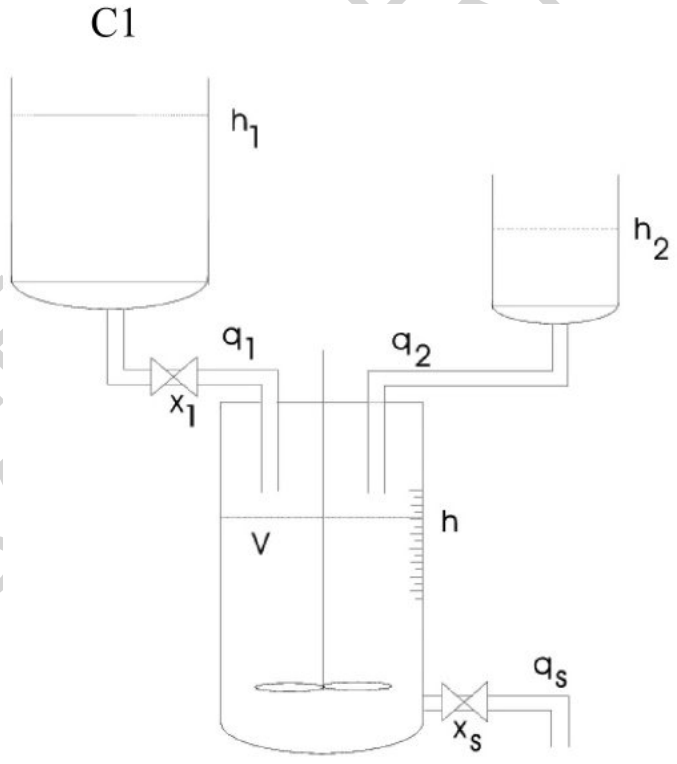

Figure 1: Mixture and homogenization of compounds

Figure 1 shows a system of mixture and homogenization of two compounds, which is composed by two fed deposits C1 and C2 and one for homogenization. Each fed deposit contains a concentration of the compounds A and B, respectively. The input volumetric

flow q_1 from the feed deposit C1 can be modified by manipulation of the opening degree of valve x_1. The flow q_2 from the feed deposit C2 cannot be adjusted. From homogenization deposit is extracted a certain flow q_s can be extracted adjusting x_s (the exit valve), h is the inside level of liquid.

3.2. Batch Fermentation

Figure 2 shows the results from different experiments of a yeast growth in batch mode. During these experiments, the environmental variables (temperature and pH) were maintained constant using controllers **PID (proportional-integral-derivative).**

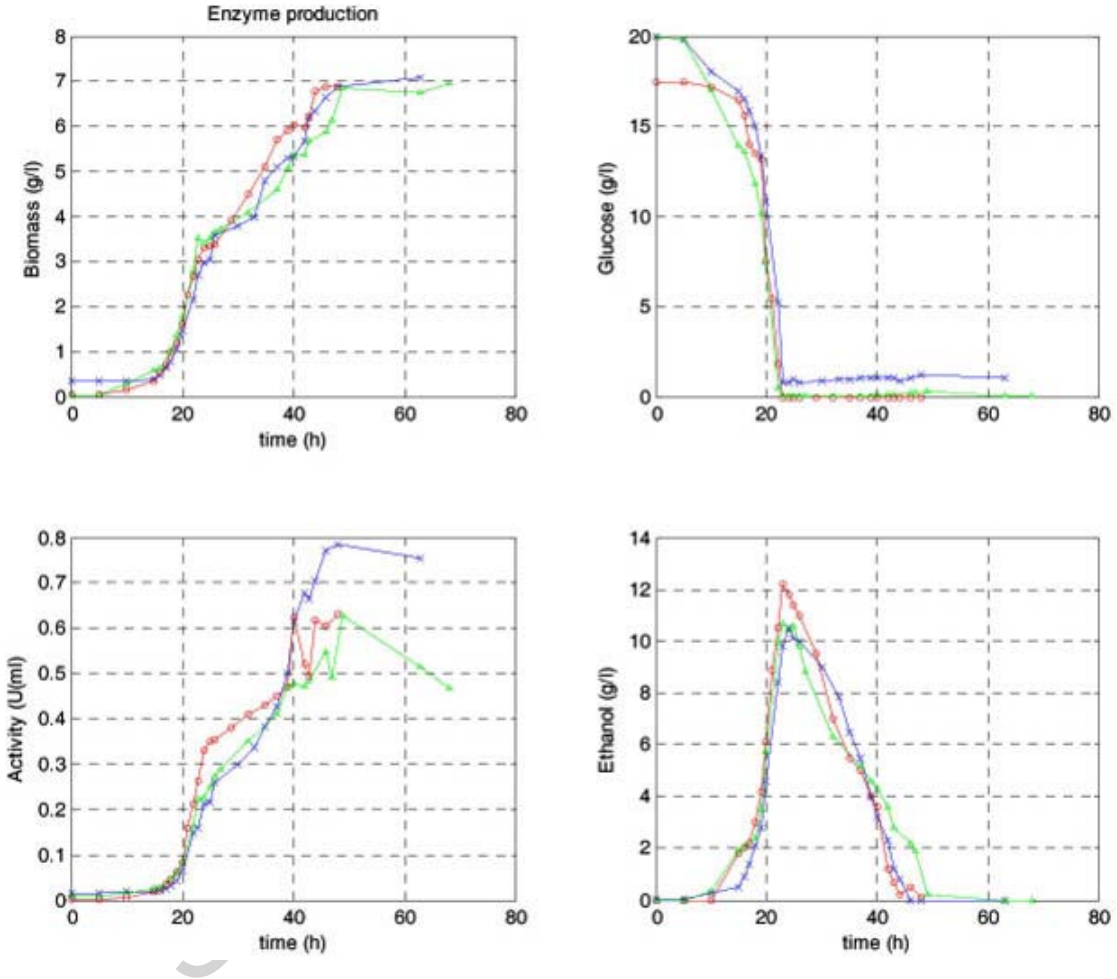

Figure 2: Batch fermentation

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Biographical Sketches

José M. Bruno-Bárcena graduated as a Biologist at University of Oviedo, Spain, in 1991. From 1994 to 1999, he worked at the Pilot Plant for Microbial Processes (CONICET-PROIMI) and successfully completed his Ph.D. in 1997 at the University of Tucumán, Argentina. From 1999 to 2002, he was a researcher at the Institute of Agrochemical and Food Technology (IATA) in Valencia, Spain. There, he assembled the fermentation and process development capabilities of the center, being also responsible for managing the technical operations of the laboratory, including resource and personnel management. He developed processes for pharmaceutical companies and actively promoted IATA´s capabilities to produce custom fermentations for third parties in Europe. From 2002 to 2005, at North Carolina State University, he served as a researcher studying the molecular responses of microorganisms to reactive oxygen species. In 2005, NC State University appointed him as Research Assistant Professor to serve in the Biomanufacturing Training and Education Center. Since then, he has developed bioprocessing related courses and provided the expertise that led to establishment of the process development BTEC laboratory. Presently, he manages daily operations while actively serving in the Microbiology Department, his primary appointment. His research interests are microbial physiology, including process development and optimization of cell culture. He has written over 15 publications in peer-reviewed journals and books.

Enric Picó-Marco graduated at the Technical Univ. of Valencia (UPV) in 2000 after getting his Master Thesis in Lund University (LTH, Sweden) as an Erasmus student. He received his European PhD. in 2004 at the UPV with a thesis on the control of bioreactors. During the doctoral studies period he stayed one year in the Institute for Agrochemistry and Food Technology within the Spanish Research Council (IATA-CSIC, Valencia, Spain) and 3 months at Supelec (Paris, France). Now he is a lecturer in the Dept. of Control Eng. & Automation in the UPV. His research interests include geometric methods for control and applications to biological systems. He has participated as author or coauthor in 4 publications in international journals and in one international bookchapter.

Jose Luis Navarro graduated as an Electronic Engineer at Polytechnic University of Valencia, Spain, in 1987 and received his Ph.D. in 1994 at the same University. He held several positions at the Polytechnic University of Valencia where now is an Assistant Professor at the Department of System Engineering and Automatic Control. His research interests include fuzzy control, system identification, dynamic simulation, dynamic observers, nonlinear control and sensors for biotechnological processes. He has participated in numerous research and development projects applying control and system engineering techniques to very different fields, as cement kiln and mill, ship cruise control, knowledge based systems, container terminal management and, lately, biotechnological processes. He has participated as author or coauthor in 12 publications in peer-reviewed journals, and more than 30 full paper conferences.

Jesús Picó graduated at the Technical University of Valencia (UPV) in 1989 and received his Ph.D. in 1996 at the same University. From 1991 he has held several positions as lecturer at the Department of Systems Engineering and Control of the UPV, where currently he is Associated Professor. From 2004 he is also member of the Institute for Industrial Computing and Control Systems (AI2) at UPV. His main research interests are in applications of nonlinear control to biomedical and biotechnological systems, including: modeling, analysis and control of reaction and metabolic networks, robust nonlinear control of bioreactors, uncertainty management in physiological systems, robust drug dosage. He has participated as author or co-author in more than 20 scientific publications in international journals and book chapters, and over 35 articles in peer reviewed international congresses.