

# **CONTINUOUS CULTIVATION OF MICROORGANISMS**

**Proceedings of the Second Symposium  
held in Prague  
June 18-23, 1962**

**Edited by I. Málek, K. Beran and J. Hospodka**



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## Preface

This volume includes the full proceedings of sessions of the IInd International Symposium on continuous cultivation of microorganisms, which took place in Prague, June 18th to 23rd, 1962. The First International Symposium, held in Prague in June 1958, has requested our laboratory to help in coordinating scientific research concerned with the development of this method, which is so important at a time when microbiology has become an important field of biological and biochemical fundamental research, and when it has broadened its applications in industrial production. One of the means by which we are attempting to fulfil this task has been the organisation of this second symposium and the publication of its materials. We wish to bring this material and the progressive development of continuous processes reflected in them to the attention of the broadest public of scientists.

We thank all those who have by their participation at the symposium, and by cooperation in the publication of this volume helped us to fulfil our task.

*Academician Ivan Málek*

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## SITUATION AND MAIN TRENDS IN FURTHER DEVELOPMENTS OF CONTINUOUS CULTIVATION

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In my initial reflections, I wish to start out from the first Symposium on Continuous Cultivation of Microorganisms, which took place in Prague in 1958. I shall attempt to show what use has been made in the past four years, in order better to bring out those questions which ought to be discussed and judged at the present meeting. The first Prague Symposium has not only presented a review of the work which had already been done in the field of continuous cultivation of microorganisms, what problems were being worked on and where this method has been applied in fermentation practice, but it was, at the same time, the first occasion on a world-wide scale for those scientists who are working on the development of this method to exchange opinions and to help discern the future trends of the development of this method.

The Prague Symposium has, beside practical application, concentrated the attention on two important questions. The first was the physiological state of microorganisms under conditions of continuous cultivation, the second question involved multi-stage cultivation and conditions under which it becomes advantageous.

With respect to the first question, it was then shown in the initial speech (Málek, 1958) as well as in the detailed discussion (Beran, 1959), that continuous cultivation presents quite new possibilities of forming intentionally such conditions as are necessary for maintaining a certain state of the physiological properties, in the cultured microorganisms required for our work, e. g. their propagation activity connected with a certain optimum amount and mutual ratio of RNA and DNA, a certain enzymic equipment, kinetics of variable processes, etc. It has been stressed, however, that results obtained in conventional static cultures can be applied to continuous cultures only with considerable reserve: it has also been concluded that the present-day mathematical theory of continuous cultures cannot express fully this qualitative aspect of continuous cultivation and therefore the practical results also correspond to the assumptions made on the basis of this mathematical theory only up to a certain degree. It has been especially noted that the influence of the growth rate on the physiological state under various forms of limitations in a chemostat has been elucidated only incompletely: thus, e. g., it was stated that the present results of experiments do not present a clear picture of the difference between cultures propagated under a very low flow-rate and those cultivated at a flow rate that is optimal for rapid propagation. It has been accepted as a conclusion to all these reflections that

for successful application of continuous cultivation, either for experimental biochemical or genetic research, or for practical application in fermentation, it is necessary to devote the greatest attention precisely to this field of study of the physiological state of cultures, to the analysis of its individual components and their kinetics. Especially in those cases where continuous cultivation is used to obtain a biomass with certain required properties (as, for instance, in biochemical research, or in the production of baker's yeast or in the preparation of vaccines) such detailed investigations are imperative. The need of these studies is even more distinct in the case of more complicated metabolic products. A number of special examples for all these conclusions have been presented at the Symposium in the experimental papers as well as in the discussion (Málek, 1958; Beran, 1959).

The second question—multi-stage continuous cultivation—has followed quite logically from the first distribution of the culture into several stages and thus the formation of several steady states makes it possible to divide the kinetics of the physiological state as a whole into a number of stages and to study the conditions of their origin separately, as was shown for the first time in 1952 (Málek, 1952). This makes it possible to find the most suitable conditions for the formation of products, originating in the course of several metabolic intermediate stages. All possible situations were analyzed in the discussion, under which multi-stage processes may be applied to the greatest advantage. The conditions of multi-stage processes were only seldom used in the experimental material presented at the Symposium.

The Prague Symposium has stressed for the first time the method of continuous cultivation as an important method, which makes our equipment more precise and versatile when dealing with biochemical or genetic research where microorganisms are used as models. The method opens a new epoch for the fermentation industry.

These main aspects have been confirmed at the panel organized on the occasion of the VIth International Congress for Microbiology in Stockholm in the same year (1959), as well as at various other international symposia and conferences (New York 1959, Moscow 1958, London 1960, Rome 1960). When studying the original papers that have appeared during the same period, one may see the increasing number of papers dealing in more detail with the various manifestations of the physiological state under various conditions of continuous cultivation, as well as with possible applications of this process. Both questions are only seldom associated for purposes of application of multi-stage equipment to a deeper study of the physiological state. Among the numerous papers a few should be noted: Herbert (1959), Maaløe, Kjeldgaard, Schaechter (1958), Ierusalimsky (1960, 1961), Ierusalimsky and Rukina (1959), Fencel et al. (1961), Hospodka and Beran (1960), Řičica (1960), Pirt and Callow (1959a,b,c), Konovalov and Grebeshova (1959), as we have already mentioned and evaluated in our annual reviews of the literature.

Among the newer work, that of Sikyta, Slezák and Herold (1961) ought to be mentioned. These authors have studied the production of chlorotetracycline and pigments by *Streptomyces aureofaciens* from the point of view of dependence on the type and concentration of the limiting nutrient and on the rate of dilution: I am mentioning this work in the first place because it shows that the laws, valid for nonfibrous microorganisms, are analogously valid for fibrous microorganisms, among other reasons since they clearly show the necessity of a detailed study of the kinetics of antibiotic formation in dependence on the factors mentioned, and of the questions of physiological activities of mycelia connected with this, when it is desired to obtain optimum results in the production of the