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ORIGINAL PAPER

The impact of Ivan Málek's continuous culture concept on bioprocessing

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Abstract This paper summarizes research results and their industrial applications obtained by continuous culture in the former Czechoslovakia. Past achievements as well as recent trends and developments worldwide are presented. The term "Prague School of continuous culture" is put forward and its international activity is outlined. The impact of this school was pervasive across the entire field of applied microbiology and biotechnology in Czechoslovakia and, perhaps, even beyond the country's boarders. Continuous culture is a very mature field, and since its establishment it has become a powerful research tool. The present activity in this field amounts to a renaissance of continuous culture, emphasizing new dimensions in bioinformatics and systems biology.

Keywords Continuous culture · Fed-batch · Physiological state · ASCR · Ivan Málek · Prague School · Applied research

Introduction

Originally, continuous culture (CC) served as an experimental tool for the research into microbial physiology but later it was recognized as being vital to large-scale biotechnology: the technique became an industrial tool for production of microbial products, selection of the robust production organisms, and directed evolution of microorganisms with improved traits.

Continuous culture is an 'open'-culture system for the cultivation of microorganisms or cells in which fresh sterilized medium is introduced at a steady flow rate and from which the culture fluid emerges at the same rate [43]. So far, many types of a continuous culture technique have been developed, each being designed for a special purpose. As examples, we could name the chemostat [71], turbidostat [10], pH-auxostat [63], accelerostat [73], and adaptastat [102]. Among them, the most common type of continuous cultivation is a chemostat culture: this technique allows for steady-state concentrations of growth-limiting substrates to be maintained at a fixed level in the culture fluid, which results in highly reproducible 'steady-state' growth conditions. Reaching this state, changes in cell density, physiological state, and medium composition of the culture are no longer detectable and the kinetic parameters of the culture growth, formation of products, and mass balance in a system can be calculated. A mathematical theory of the continuous culture was elaborated in the 1950s and has been described elsewhere [e.g., 10, 29, 30, 55, 66].

Prague′s School of CC and international cooperation

The term "continuous flow environment" had already been coined and used by Ivan Málek in the 1940s, however, it took more than 20 years before a school of CC was established. In 1952, Málek took part in the formation of the Institute of Biology, ASCR, Prague, from which 12 years later the Institute of Microbiology of the ASCR (IM ASCR) and other ASCR institutions were founded. Research groups dealing with some aspects of CC were concentrated in the

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Department of Technical Microbiology (DTM) lead by Ivan Málek and Zdeněk Fencl. In 1966, I. Málek and Z. Fencl (as editors) published a multi-authored textbook entitled "Theoretical and Methodological Basis of Continuous Culture of Microorganisms" [61] under the auspices of the Academia Publishing House. The textbook could be considered as a "bible" of CC and a research group formed around Málek called the "Prague School of Continuous Culture".

Among the first activities of DTM, organization of the 2nd Symposium on Continuous Culture in 1962 should be mentioned. Later on, continuous culture symposia were held alternatively in Czechoslovakia and the UK, but a stop was put on this activity in 1987. As a result, nine symposia proceedings were published [13–15, 45, 56, 59, 60, 80, 94] from events held at Prague (the years 1958, 1962, 1968, and 1978), Porton Down (1967 and 1984), Oxford (1971 and 1975), and Hradec Králové (1987). The vigorous exchange of information between scientists of different countries and backgrounds was only possible on the grounds of symposia organized by the IM ASCR and their British colleagues: based on these meetings, long-term ties with researchers of many countries were established in the 1960s and 1970s.

The international activity of DTM was important for the fate of the whole IM ASCR as it set the stage for more vigorous pursuit of the research activities and opened the possibilities for better implementation of CC applications abroad.

Physiological state of microbial culture

To express the sum of properties of a culture, the term "physiological state of a culture" (PS) was proposed [57]. There is no unique definition of the term PS. Moreover, a view on the subject has been developing together with growing understanding of microbial physiology especially in connection with continuously grown microbial cultures. In the 1950s, PS was considered as a physical condition or status of a microbial culture at a particular time. Málek's concept was that the physiological state was defined by the growth rate and nutritional status of the environment [5, 57, 61]. In other words, the term PS was used as an auxiliary, operational expression serving to emphasize the fact that microbial populations grown under different conditions differ not only in properties expressed quantitatively (population kinetics, growth rates, etc.) but also in the sum of their physiological properties. In summary, the term PS was taken as a genetically defined set of metabolic activities of cultures, their integrated physiological unity of metabolic and genetic processes, with a clear dependence on the history of individual cells and populations. Later, the revised term was introduced: PS is the result of external conditions and the starting point for potential changes under the influence of new conditions within the framework of the genetic content. Thus, PS does not represent the actual, momentary state, but it is a time vector [58].

The principal application outcomes of CC in the former Czechoslovakia

Regarding applied microbiology, it has always been argued that to develop an economically viable production process based on the CC concept, this concept should be introduced right from the beginning, at the stage of process design. The introduction of the concept of CC by Málek has lead to a paradigm shift in the microbiologist's thinking and application of CC in many different industrial activities in Czechoslovakia, although a direct link cannot be always proven**.** The examples of research activities oriented towards applications are listed below.

Fodder yeast

A technological process has been developed for the production of fodder yeast (animal feed, *Candida*) from petroleum distillates and paraffins [78, 86, 108]. Likewise, a process for production of fodder yeast on synthetic ethanol was successfully developed and licensed abroad [54, 83, 87, 90, 93].

A continuous process was also applied at a cellulose production plant in Paskov, South Bohemia: the sulfite liquor was used as a medium nutrient for aerobic growth of liquor-adapted fodder yeast [3, 40, 111].

Beer production

For continuous beer production, many different technology designs have been suggested and tested at pilot and production scales, multistage, tower, immobilized cells, and some are quite recent [7–9, 69, 98]. The question remains on the organoleptic beer properties as compared to classical production modes.

Ethanol fermentation

The anaerobic process of ethanol production has been studied with free and immobilized microorganisms under conditions of multistage CC [2, 65, 75, 92]. The process was scaled-up and the product was used in food industry and for technical applications as a solvent.

Waste water treatment

CC is a standard processing mode in the waste-treatment industry. No suitable Czech reference exists as the government was not much in favor of disclosing details on such

activity. However, this field should be considered as a product of rather an empirical approach, not directly connected with the rational CC concept of Málek (e.g., 28).

Algae production

Based upon Málek's idea, an algae-production technology was developed at the division of IM ASCR located in Třebon, using fed-batch, semi-continuous, and continuous processing modes. It is essentially a thin layer sloped area unit. The technology has been tested in pilot and production systems in several countries (Cuba, Spain, Bulgaria, Kuwait, etc.) [6, 18, 19, 25, 53, 70, 82, 85] and is discussed in the article by Masojídek and Prášil [64] this volume.

Bioreactor design and control strategies

The design of laboratory and pilot-scale bioreactors has been pursued since the 1950s in the laboratory of Řičica of IM ASCR under leadership of Málek. He employed a concept of a Waldhof agitator to generate a deep vortex on the surface of a fluid with the help of a draught tube inserted inside the fermenter [55, 88]. The design was both revolutionary and unique. For many reasons, licensing abroad was never realized. Unfortunately, visiting foreign scientists reported on this design and as a result, the bioreactor design and manufacturing of fermenters became a lucrative business (e.g., in Switzerland). Later, the bioreactor design and scale-up was initiated in a research institute in Brno, Chepos, under the leadership of Kvasnička [44]. The result of these activities was a design of a 1,000-m^3 fermenter, agitated by three impellers on a common shaft, permitting regulation of the agitation regimen by impeller exchanges and eliminating the need for surfactants for foam control at the expense of increased power input into agitation. A draft tube insert allowed increased homogenization (necessary for hydrocarbon dispergation) and high oxygen transfer. The testing was carried in batch, semi-continuous, and continuous modes. Other designs were also proposed, e.g., a pilot multistage tower fermenter [74, 84]. A design for venting biofilters has also been proposed [76, 77].

Hospodka [33] has developed a substrate feeding strategy based on oxygen uptake rate and balance measured by dissolved oxygen probe. It was quickly adopted in the USA and became a part of control strategy for a computer-controlled bioreactor designed by Humphrey (Lehigh University) and Wilson (ABEC Inc.) and exhibited for the first time at the Conference on Microbial Engineering, held in Marienbad in 1972 [97]. Although the hardware was displayed in 1972, the very first system description was published in 1971 [34, 72]. The computer control became a standard in manufacturing of bioreactors/fermenters. A variation of a chemostat has been developed (pH–stat) to couple the pH control with substrate feed and applied for yeast growth on ethanol [1]. Other control strategies were also proposed [16, 17, 68].

Enrichment of microorganisms overproducing endoenzymes under selection pressure in CC

Experimental evolution of enzymes is another application that has been brought to attention in the context of continuous culture. Terms like "specific", "non-specific", or "periodic" selection were introduced in the 1950s when large populations of a single microorganism were grown at constant growth rate in a chemostat to study the physiological state of microorganisms after reaching culture steady-state. The terms reflect a repeated appearance of novel populations that takes over original populations due to a selective growth advantage [42]. The replacements of population occur much faster if an unnatural, slowly utilizable carbon source is used to limit the growth in chemostat and the evolved mutants frequently acquire new metabolic capabilities. The occurrence of mutations, such as chromosomal amplification of a structural gene encoding a catabolic function, promoter-up mutation increasing gene transcription, fusion of two operons under the control of a single regulatory region, and mutational activation of phenotypically "silent" genes, were identified as the reasons for accumulation of overproduction strains for endoenzymes in a chemostat. In these mutants, certain enzymes have simply been overproduced or exhibited changed substrate specificities and kinetic parameters [27, 46, 48, 49, 95, 96].

Improvement of the recombinant production strains in CC

It is generally accepted that the maintenance of a self-replicating, high-copy-number plasmid may impose a considerable metabolic burden upon a bacterial host [22]. Therefore, plasmid-less cells can out-compete their plasmid-bearing competitors under non-selective growth conditions. In the case of production microorganisms for endoenzymes based on the recombinant plasmids, the segregational instability of the plasmid is even more obvious and may result in marked reduction of the enzyme production at industrial fermentation stage. The techniques of continuous cultures were successfully applied to understand the process of competition in question and experimental data related to this subject were mostly obtained from the experiments carried out in chemostat cultures [47, 50]. It was found that the carbon-limited, chemostat culture of the plasmid-bearing production strain becomes rapidly heterogeneous and a selective disadvantage in growth rate associated with the plasmid carriage can be estimated.

Chemostat cultures of recombinant microorganisms have been used to study adaptation of the host *Escherichia*

coli to the dual metabolic burden resulting from overexpression of the product and maintenance of the recombinant plasmid. After 130 generations of slow growth in a carbon-limited chemostat, and in the presence of selection pressure for the maintenance of the recombinant plasmid [62], plasmid-bearing cells with a frame-shift mutation in the plasmid-borne gene encoding penicillin G acylase accumulated in the chemostat culture. The host also adapted to the selection pressure by increasing the specific growth rate by 30%. Re-transforming of the evolved host with original recombinant plasmid yielded a faster-growing overproduction strain for penicillin G acylase.

High-cell-density cultures

A unique and proprietary development was launched in the former Research Institute of Antibiotics and Biotransformations (RIAB), Roztoky near Prague, in cooperation with other institutions, primarily the Institute of Macromolecular Chemistry ASCR (IMC ASCR), Prague. A perfusion (continuous flow of nutrients), high-cell-density culture with full biomass retainment for production of amino acids was scaled up to an economically viable industrial process.

Industrial processes were also developed in the field of biotransformation of β-lactam antibiotics by RIAB, IMC ASCR, and IM ASCR. Initially, the research and development activities dealt with the preparation of robust biocatalysts for production of 6-aminopenicillanic acid (6-APA) and 7-aminocephalosporanic acid (7-ACA), precursors of semi-synthetic, β-lactam antibiotics, based on enzymes [4, 21, 79, 100, 104–107] or immobilized cells [106, 107, 113]. Recently, a new generation of catalysts has been developed for the enzymatic synthesis of β-lactam antibiotics: these catalysts are based on encapsulation of cross-linked enzyme aggregates [12, 51, 52]. The catalysts for biotransformation of β-lactam antibiotics are used industrially in a mode of repeated conversions in a stirred batch reactor.

The use of immobilized enzymes and cells on macroporous polymer carriers for amino acid production as well as the catalysts for biotransformation of β-lactam antibiotics has lead to the sale of licenses abroad.

Although the gel immobilization of enzymes was initiated earlier [38], one should contrast this with the very first gel cell immobilization patent and publication in the USA and Japan [11, 26]. The Czechoslovak approach was different: this approach could be denoted as "immobilization onto macroporous polymer carriers via covalent bond" in contrast to "immobilization using entrapment in gel" (Drobník, pers. comm.).

The concepts of a high-cell-density culture, fed-batch culture, and a full retainment of biomass for production goals, vigorously pursued by the above Czechoslovak scientists might have had an impact on other activities in this field. For example, a company in St. Louis, MO, USA, Invitron, developed immobilized perfusion mammalian cell culture for recombinant protein production and introduced a maintenance concept (e.g., maximizing protein production, minimizing biomass production). This is an extreme case of packaging cells into a tissue-like density. What followed were other approaches leading to a large-scale production of recombinant proteins via mammalian cells and high-cell-density cultures of microorganisms [23, 31, 36, 39, 41, 101].

Renaissance of continuous culture and current trends

Continuous culture is a very mature field, and since its establishment it has become a powerful research tool. On the practical side, it could be a method of choice for development of new technologies and as a production processing mode for applications in some areas of bioprocessing.

As a research tool, CC has recently been used to optimize a widely used expression system based on a high-cell-density fed-batch culture of *Pichia pastoris* utilizing methanol. Transient nutrient gradient applied in mixed substrate CC was used to optimize the ratio of glycerol to methanol in the feed medium so that technical improvement for the performance of high-cell-density culture connected with culture cooling and lowering oxygen consumption was reached [37].

The CC technique is a convenient tool to define and solve the problems arising from anthropogenic activities. The experimental approach based on chemostat culture (e.g., 109) is used for the research into biofilm formation on solid surfaces when the surface of solids is a growth-limiting substrate and planktonic microorganisms are present in technological fluids. Currently, chemostat studies of this type are extensively used to mitigate the problems of biocorrosion, a process of deterioration of surfaces of technological equipment by geochemical activities of microorganisms [20, 24, 89, 112].

Multistage CC may be important for stem cell improvement (and differentiation) in the near term. The successful transfer of human embryonic stem cell technology and cellular products into clinical and industrial applications needs to address issues of automation, standardization, and the generation of relevant cell numbers of high quality [91]. The microcarrier technology was combined with controlled stirred-tank bioreactors to develop an efficient and scalable system for expansion of stem cells. By controlling pO_2 conditions, a 12-fold improvement in the final cell yield was obtained when compared to static 2D cultures. The use of continuous perfusion systems further enhances metabolic performance of stem cells, ultimately facilitating bioprocess optimization including culture adaptation to growth conditions and production of cell-based products. In this

regard, it should be emphasized that Málek provided the concept of differentiation, which impacted many kinds of the research and development activity carried out at the Institute of Microbiology ASCR that continues to influence the current research work even today.

New horizons for CC emerge as CC provides reproducible, reliable, and homogenous data under defined conditions for functional genomics and post-genomics studies or fitness evaluation of different production strains. The precise control of a microenvironment is the most important asset that the CC provides.

A novel insight into the "state of overproduction" of industrial microorganisms has recently been obtained by combining CC with transcriptome profiling. Specific growth rate-dependent changes in expression of genes were studied in carbon-limited chemostat and accelerostat cultures of *E. coli* [67, 103] to understand the regulation of acetic acid synthesis, an overflow metabolism reducing growth rate and heterologous protein production by recombinant bacteria. Accelerostat is a cultivation method that enables real-time monitoring of culture parameters, e.g., culture optical density, oxygen consumption, and by-product formation during continuous change of specific growth rate. Studying the effect of specific growth rate on acetate metabolism, microarray data revealed a metabolic switch point, a range of specific growth rates, at which up- and down-regulation of expression of gene-encoding enzymes involved in overflow metabolism result in a loss of co-utilization of glucose and acetate.

Quantitative fitness assessment of the genetically modified and reference strain of industrially relevant filamentous fungi has been realized in mixed chemostat cultures by means of real-time PCR. The data on fitness together with transcriptome profiling and fermentation performance (e.g., maximum specific growth rate, substrate consumption, and product yields) have to be considered before the genetically modified strain is used for industrial application [99].

The latest effort in CC is supplemented with a global systems biology approach, where the whole organism and environment in CC studies is coupled in a brand new direction. At the same line, microfabricated nanoscale cultivation devices, operated in the CC and fed-batch/perfusion mode, particularly with mammalian cells, will provide a well-controlled and inexpensive way to produce multiplexed post-genomics data [110]. For proper cell culturing, continuous medium supply from a microfluidic channel and appropriate modification of the channel surface to accommodate cell attachment is required.

Conclusions

The impact of the Prague School of continuous culture was pervasive across the whole field of applied microbiology and biotechnology in Czechoslovakia and, perhaps, even beyond the country's boarders. It can be concluded that the above applications and licensing deals would have only been possible because of the excellent position of the Prague School of CC (pioneered and lead by Ivan Málek) in terms of international reputation and recognition. As a consequence, Czechoslovak research gained access to the international arena at that time and could exchange information at international meetings organized by the ASCR. Czech scientists, working now in a member country of the European Community, will undoubtedly contribute to the future development in the area of bioinformatics and systems biology, providing fundamental and applied results for further advancement of biomedical, environmental, small biotech, and pharmaceutical applications. Málek's CC concept will continue to provide a standardized basis for collecting uniform data towards such goals [32, 35, 81].

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References

1. Adámek L, Štros F, Švojgr M, Hauser K, Prokop A (1974) Mode of aerobic cultivation of yeasts on synthetic media. Patent CZ 158 954 (also British Patent 1,348,074, GFR Patent 2,217,909, USSR Patent 426,373)
2. Baleš V, Bukovska A, Pach L, Herain J, Langfelder I (1989) Influence of alginate gel composition on the productivity of an immobilized yeast bioreactor. Chem Papers (Chem Zvesti) 43:733–742
3. Bárta J (1969) Utilization of sulfite liquors and stillage by an isolate of *Cryptococcus diffluens*. Antonie Van Leeuwenhoek 35: (Suppl):7–8
4. Bečka S, Plháčková K, Kyslík P (1995) The way of stabilization of immobilized penicillin amidase from microorganism *Escherichia coli*. Czech Patent CZ 281678
5. Beran K (1962) Some remarks to the study of physiological state of microorganisms. In: Málek I, Beran K, Hospodka J (eds) Continuous culture of microorganisms: the 2nd symposium. Publishing House ASCR, Prague, pp 95–98
6. Bínová J, Tichý V, Lívanský K, Zahradník J (1998) Bacterial contamination of microalgal biomass during outdoor production and downstream processing. Archiv Hydrobiol Suppl 124:151–158
7. Brányik T, Silva DP, Vicente AA, Lehnert R, Silva JB, Dostálek P, Teixeira JA (2006) Continuous immobilized yeast reactor system for complete beer fermentation using spent grains and corncobs as carrier materials. J Ind Microbiol Biotechnol 33:1010–1018
8. Brányik T, Vicente A, Dostálek P, Teixeira JA (2005) Continuous beer fermentation using immobilized yeast cell bioreactor systems. Biotechnol Prog 21:653–663
9. Brányik T, Vicente A, Dostálek P, Teixeira JA (2008) A review of flavour formation in continuous beer fermentations. J Inst Brew 114:3–13
10. Bryson V, Szybalski W (1952) Microbial selection. Science 115:45–51

11. Chibata I, Tosa T, Sato T (1974) Immobilized aspartase-containing microbial cells: preparation and enzymatic properties. Appl Microbiol 27:878–885
12. Datla A, Rajasekar VW, Kyslík P, Bečka S, Krishnakant A, Yogesh ZS, Nikunj K (2009) Process for the preparation of immobilized recombinant penicillin acylase catalyst from *Achromobacter* sp. CCM 4824 expressed in *E. coli* BL21 CCM 7394 and its use for the synthesis of beta-lactam antibiotics. Patent application WO 2009/016642
13. Dean ACR, Ellwood DC, Evans CGT (1984) Continuous culture 8: biotechnology, medicine and the environment. Ellis Horwood Ltd., Chichester
14. Dean ACR, Ellwood DC, Evans CGT, Melling J (eds) (1976) Continuous culture 6: applications and new fields. Ellis Horwood Ltd., Chichester
15. Dean ACR, Pirt SJ, Tempest DW (eds) (1972) Environmental control of cell synthesis and function. In: Proceedings of the 5th international symposium, ISBN 0-12-208050-5, the UK
16. Dohnal M (1985) Fuzzy bioengineering models. Biotechnol Bioeng 27:1146–1151
17. Dohnal M (1991) Large quantitative models of complex chemical and bioengineering processes. Coll Czechoslovak Chem Commun 56:2107–2141
18. Doucha J, Lívanský K (2006) Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a middle and southern European climate. J Appl Phycol 18:811–826
19. Doucha J, Lívanský K (2009) Outdoor open thin-layer microalgal photobioreactor: potential productivity. J Appl Phycol 21:111–117
20. Doyle (ed) (1999) Methods in enzymology. Biofilms Academic Press, San Diego
21. Drobník J, Saudek V, Švec F, Kálal J, Vojtíšek V, Bárta M (1979) Enzyme immobilization techniques on poly(glycidyl methacrylate-co-ethylene dimethacrylate) carrier with penicillin amidase as model. Biotechnol Bioeng 21:1317–1332
22. Dykhuizen DE, Hartl DL (1983) Selection in chemostat. Microbiol Rev 47:150–168
23. Feder J, Tolbert WR (eds) (1985) Large-scale mammalian cell culture. Academic Press Inc, USA
24. Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18:1049–1056
25. Grobbelaar Ju, Nedbal L, Šetlík I (1995) Variation of some photosynthetic characteristics of microalgae cultured in outdoor thin-layered sloping reactors. J Appl Phycol 7:175–184
26. Guttag A (1973) Process of subjecting a microorganism susceptible material to a microorganism (methacrylate entrapment). US Patent 3,860,490
27. Hall BG, Yokoyama S, Calhoun DH (1983) Role of cryptic genes in microbial evolution. Mol Biol Evol 1:109–124
28. Hamer G (1984) Continuous culture kinetics and activated sludge processes. In: Dean ACR, Ellwood DC, Evans CGT (eds) Proc symp on cont culture 8. Ellis Horwood Ltd, Chichester, pp 169–184
29. Herbert D (1976) Stoichiometric aspects of microbial growth. In: Dean ACR, Ellwood DC, Evans CGT, Melling J (eds) Continuous culture 6: applications and new fields. Ellis Horwood LTD., Chichester, pp 1–30
30. Herbert D, Elsworth R, Telling RC (1956) The continuous culture of bacteria: a theoretical and experimental study. J Gen Microbiol 14:601–622
31. Himmelfarb P, Thayer PS, Martin HE (1969) Spin filter culture: the propagation of mammalian cells in suspension. Science 164:555–557
32. Hoskisson PA, Hobbs G (2005) Continuous culture—making a comeback? Microbiology 151:3153–3159
33. Hospodka J (1966) Oxygen-absorption rate-controlled feeding of substrate into aerobic microbial cultures. Biotechnol Bioeng 8:117–134
34. Humphrey AE (1971) Present limitations to the control and understanding of a fermentation process. Proc Labex Symp, Earls Court, London
35. Hung PJ, Lee PJ, Sabounchi P, Aghdam N, Lin R, Lee LP (2005) A novel high aspect ratio microfluidic design to provide a stable and uniform microenvironment for cell growth in a high throughput mammalian cell culture array. Lab Chip 5:44–48
36. Inloes DS, Smith WJ, Taylor DP, Cohen SN, Michaels AS, Robertson CR (1983) Hollow-fiber membrane bioreactors using immobilized *E. coli* for protein synthesis. Biotechnol Bioeng 25:2653–2681
37. Jungo C, Marison I, von Stockar U (2007) Mixed feed of glycerol and methanol can improve the performance of *Pichia pastoris* cultures: a quantitative study based on concentration gradients in transient continuous cultures. J Biotechnol 128:824–837
38. Katchalski E, Silman I, Goldman R (1971) Effect of the microenvironment on the mode of action of immobilized enzymes. Adv Enzymol Relat Areas Mol Biol 34:445–536
39. Knazek RA, Gullino PM, Kohler PO, Dedrick RL (1972) Cell culture on artificial capillaries: an approach to tissue growth in vitro. Science 178:65–66
40. Křen V, Kochtová M, Burianec Z, Rychtera M (1985) Continuous cultivation of *Candida utilis* yeasts growing on calcium bisulphate waste liquors: parameter estimation. Appl Miocrobiol Biotechnol 22:329–335
41. Kruse PF Jr, Keen LN, Whittle WL (1970) Some distinctive characteristics of high density perfusion cultures of diverse cell types. In Vitro 6:75–88
42. Kubitschek HE (1974) Operation on selection pressure on microbial populations. In: Carlile MJ, Skehel JJ (eds) Evolution in the microbial world. Proceedings of 24th Symposium SGM, Cambridge Univ Press, London, pp 105–130
43. Kuenen JG, Johnson OJ (2009) Continuous cultures (chemostats). In: Schaechter M (ed) Encyclopedia of microbiology. Academic Press, London, pp 130–14743
44. Kvasnička J, Sláma V, Pešl L (1981) High-productivity fermentation apparatus for the production of feed proteins from synthetic ethanol. Ropa Uhlie (Czechoslov) 23:9
45. Kyslík P, Dawes EA, Krumphanzl V, Novák M (eds) (1988) FEMS symposium No. 41: continuous culture in biotechnology and environment conservation. Academic Press, London
46. Kyslik P, Dobišová M (1986) Selection of ribitol dehydrogenase hyperproducing strains in a chemostat culture of *Escherichia coli* 1EA at different dilution rates. Biotechnol Lett 8:235–240
47. Kyslík P, Dobišová M, Marešová H, Sobotková L (1993) Plasmid burden in chemostat culture of *Escherichia coli*: its effect on the selection for overproducers of host enzymes. Biotechnol Bioeng 41:325–329
48. Kyslík P, Dobišová M, Sobotková L (1988) Selection of endoenzyme hyperproducing strains of *Escherichia coli* 1EA in pentitol-limited chemostat culture of the parent and evolvant strains. In: Kyslik P, Dawes EA, Krumphanzl V, Novák M (eds) FEMS symp on continuous culture. Academic Press, London, pp 17–28
49. Kyslík P, Sikyta B (1985) Changes of *Escherichia coli* populations in continuous culture limited by pentitols. Microbiol Sciences 2:25–27
50. Kyslík P, Sobotková L, Večerek B, Dobišová M, Chrudimský T (1989) Ribitol dehydrogenase overproduction and maintenance of plasmids pBR322 and pACYC184 in chemostat cultures of *Escherichia coli*. Biotechnol Lett 11:149–154
51. Kyslik P, Štěpánek V, Hollerová L, Bečka S, Vyasarayani RW, Datla A, Plháčková K, Maršálek J (2007) DNA sequence encoding penicillin acylase, novel recombinant DNA constructs and

recombinant microorganisms carrying this sequence. Czech Patent CZ 300 467
52. Kyslik P, Štěpánek V, Hollerová L, Bečka S, Vyasarayani RW, Datla A, Plháčková K, Maršálek J (2008) DNA sequence encoding penicillin acylase, novel recombinant DNA constructs and recombinant microorganisms carrying this sequence. Patent application WO 2008/093351
53. Lívanský K (1981) Effect of dilution rate, pH and temperature on the growth of *Chlorella kessleri* in a continuous autotrophic culture. Folia Microbiol 26:422–425
54. Machek F, Štros F, Prokop A, Adámek L (1976) Production and isolation of protein from synthetic ethanol. In: Dean ACR, Ellwood DC, Evans CGT, Melling JT (eds) Continuous culture 6: applications and new Fields. Ellis Horwood, Chichester, pp 135–145
55. Málek I (1955) About multiplication and cultivation of microorganisms, especially of bacteria (in Czech). Publishing House of the ASCR, Prague
56. Málek I (ed) (1958) Continuous culture of microorganisms. A symposium. Publishing House of the ASCR, Prague
57. Málek I (1958) The physiological state of microorganisms during continuous culture. In: Continuous culture of microorganisms. A symposium. Publishing House ASCR, Prague, p 21
58. Málek I (1976) Physiological state of continuously grown microbial cultures. In: Dean ACR, Ellwood DC, Ecans CGT, Melling J (eds) Continuous culture 6: applications and new fields. Ellis Horwood LTD. Chichester, pp. 31–39 717
59. Málek I, Beran K, Fencl Z, Munk V, Řičica J, Smrčková H (eds) (1969) Continuous cultivation of microorganisms. In: proceedings of the 4th symposium. Publishing House of the ASCR, Prague
60. Málek I, Beran K, Hospodka J (eds) (1964) Continuous cultivation of microorganisms: proceedings of the 2nd symposium. Publishing House of the ASCR, Prague
61. Málek I, Fencl Z (eds)(1966) Theoretical and methodological basis of continuous culture. Publishing House of the ASCR, Prague, Czech Republic, Academic Press, London, pp 95–104
62. Marešová H, Štěpánek V, Kyslík P (2001) A chemostat culture as a tool for the improvement of a recombinant *E. coli* strain overproducing penicillin G acylase. Biotechnol Bioengin 75:46–52
63. Martin GA, Hempfling WP (1976) A method for the regulation of microbial population density during continuous culture at high growth rates. Arch Microbiol 107:41–47
64. Masojídek J, Prášil O (2010) (this volume)
65. Melzoch K, Rychtera M, Markvichov NS, Pospíchalová V, Basařová G, Manakov MN (1991) Application of a membrane recycle bioreactor for continuous ethanol-production. Appl Microbiol Biotechnol 34:469–472
66. Monod J (1950) La technique de culture continue: theorie et applications. Ann Inst Pasteur 79:390–410
67. Nahku R, Valgepea K, Lahtvee P-J, Erm S, Abner K, Adamberg K, Vilu R (2010) Specific rate growth dependent transcriptome profiling of *Escherichia coli* k12 MG1655 in accelerostat cultures. J Biotechnol 145:60–65
68. Náhlík J, Burianec Z (1988) Online parameter and state estimation of continuous cultivation by extended Kalman filter. Appl Microbiol Biotechnol 28:128–134
69. Navrátil M, Dömény Z, Sturdík E, Smogrovicová D, Gemeiner P (2002) Production of non-alcoholic beer using free and immobilized cells of *Saccharomyces cerevisiae* deficient in the tricarboxylic acid cycle. Biotechnol Appl Biochem 35:133–140
70. Nečas J, Sulek J, Tetík K (1981) A multiple device for the cultivation device for the cultivation of algae in a liquid medium. Arch Hydrobiol Suppl 60:202–208
71. Novick A, Szilard L (1950) Experiments with the chemostat on spontaneous mutations of bacteria. Proc Natl Acad Sci USA 36:708–718
72. Nyiri LK (1971) A philosophy of data acquisition, analysis and computer control of fermentation processes. Proc Labex Symp, Earls Court, London
73. Paalme T, Kahru A, Elken R, Vanatalu K, Tiisma K, Vilu R (1995) The computer-controlled continuous culture of *Escherichia coli* with smooth change of dilution rate (A-stat). J Microbiol Methods 24:145–153
74. Páca J (1981) Effect of multistream ethanol feeding on growth and physiological-characteristics of *Candida utilis* in a multistage tower fermentor. Enzyme Microb Technol 3:123–128
75. Páca J, Grégr V (1977) Growth characteristics of *Candida utilis* on volatile substrate in a multistage tower fermentor. Biotechnol Bioeng 19:539–554
76. Páca J, Halecký M, Fitch M (2009) Steady-state performance of an activated carbon biofilter degrading styrene: effects of residence time and inlet concentration. J Air Waste Manag Assoc 59:45–51
77. Páca J, Halecký M, Maryška M, Jones K (2007) Gasoline vapor biofiltration. Eng Life Sci 7:469–474
78. Pilát P, Prokop A, Fencl Z (1973) Multi-stage continuous cultivation of *Candida lipolytica* on mineral oil. J Ferment Technol 51:249–253
79. Plháčková K, Bečka S, Kyslík P (1993) The way of preparation of stable form of 7β-(4-carboxybutanamido)-cephalosporanic acid hydrolase. Czech Patent CZ 280343
80. Powell EO, Evans CGT, Strange RE, Tempest DW (eds) (1967) Microbial physiology and continuous culture In: Proceedings of the 3rd international symposium, Her Majesty′s Stationary Office, Porton Down, the UK
81. Prokop A (2009) Systems biology in biotech and pharma. A new paradigm for innovation. BioPharm Knowledge Publishing, Lower Mill Office, Newnham Lane, Old Basing, Hants RG24 7AT, the UK, pp 1–220 (an industrial report)
82. Prokop A, Fekri M (1984) Potential of mass algae production in Kuwait. Biotechnol Bioeng 26:1282–1287
83. Prokop A, Fencl Z, Sikyta B, Machek F (1983) Survey on research and development of fodder and food protein production (SCP) in Czechoslovakia. In: Senez J-C (ed) International symposium on single cell proteins. A.P.R.I.A, Paris, pp 218–231
84. Prokop A, Janík P, Sobotka M, Krumphanzl V (1983) Hydrodynamics, mass transfer and yeast culture performance of a column bioreactor with ejector. Biotechnol Bioeng 25:1147–1160
85. Prokop A, Řičica J, Málek I, Thomas J (1967) Growth and physiological characteristics of a high temperature strain of *Chlorella pyrenoidosa* in continuous culture. Nature 214:1234–1235
86. Prokop A, Volfová O (1972) SCP from hydrocarbons. Process Biochem 7:31–32
87. Prokop A, Votruba J, Sobotka M, Panoš J (1978) Yeast SCP from ethanol: measurements, modeling and parameter estimation in a batch system. Biotechnol Bioeng 20:1523–1540
88. Řičica J (1977) New laboratory fermentor. Folia Microbiol 22:482
89. Rosche B, Li XZ, Hauer B, Schmid A, Buehler K (2009) Microbial biofilms: a concept for industrial catalysis? Trends Biotechnol 27:636–643
90. Rut M, Štros F (1975) Mass balance during synthesis of yeast biomass from ethanol. Folia Microbiol 20:67
91. Serra M, Brito C, Sousa MFQ, Jensen J, Tostoes R, Clemente J, Strehl R, Hyllner J, Carrondo MJT, Alves PM (2010) Improving expansion of pluripotent human embryonic stem cells in perfused bioreactors through oxygen control. J Biotechnol 148:208–215
92. Šestáková M (1976) Growth of *Candida utilis* on a mixture of monosaccharides, acetic acid and ethanol as a model of waste sulphite liquor. Folia Microbiol 24:318–327
93. Šestáková M, Adámek L, Štros F (1976) Effect of crotonaldehyde on the metabolism of *Candida utilis* during the production of single cell protein from ethanol. Folia Microbiol 21:444–454

94. Sikyta B, Fencl Z, Poláček V (eds) (1980) Continuous cultivation of microorganisms In: Proceedings of the 7th symposium, Institute of Microbiology, the ASCR, Prague
95. Sikyta B, Kyslík P, Voleský B, Pavlasová E, Stejskalová E (1981) Overproduction of endoenzymes in *Escherichia coli*—selection of hyperproducing strains in a chemostat. In: Krumphanzl V, Sikyta B, Vaněk Z (eds) FEMS symposium on overproduction of microbial products. Academic Press, London, pp 593–600
96. Sikyta B, Pavlasová E, Kyslík P, Stejskalová E (1984) Population changes of *Escherichia coli* strain hyperproducing endoenzymes in continuous cultures. In: Dean ACR, Ellwood DC, Evans CGT (eds) Proc Symp on Continuous Culture 8. Ellis Horwood Ltd, Chichester, pp 205–212
97. Sikyta B, Prokop A, Novák M (eds) (1973) Advances in microbial engineering. Proc Symp Biotechnology and Engineering Nos. 4 and 5. Wiley, New York
98. Smogrovicová D, Dömmny Z, Svitel J (2001) Modeling of saccharide utilization in primary beer fermentation with yeasts immobilized in calcium alginate. Appl Biochem Biotechnol 94:147–158
99. Snoek ISI, van der Krogt ZA, Touw H, Kerkman R, Pronk JT, Bovenberg RAL, van den Berg MA, Daran J (2009) Construction of an hdfA *P. chrysogenum* strain impaired in non-homologous end-joining and analysis of its potential for functional analysis studies. Fungal Genet Biol 46:418–426
100. Švec F, Kálal J, Menyailova II, Nakhapetyan LA (1978) Immobilization of amyloglucosidase on poly(glycidyl methacrylate-co-ethylene dimethacrylate) carrier and its derivatives. Biotechnol Bioeng 20:1319–1328
101. Tolbert WR, Feder J, Kimes RC (1981) Large-scale rotating filter perfusion system for high-density growth of mammalian suspension cultures. In Vitro 17:885–890
102. Tomson K, Barber J, Vanatalu K (2006) Adaptastat—a new method for optimizing of bacterial growth conditions in continuous culture: interactive substrate limitation based on dissolved oxygen measurements. J Microbiol Methods 64:380–390
103. Vemuri GN, Altman E, Sangurdekar DP, Khodursky AB, Eiteman MA (2006) Overflow metabolism in *E. coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. Appl Environ Microbiol 72:3653–3661
104. Vojtíšek V, Bárta M, Zeman R, Čulík K, Kálal J, Drobník J, Švec F (1978) Imobilizovaná penicilinacyláza k přeměně penicilinu na kyselinu 6-aminopenicilánovou a způsob její přípravy (immobilized penicillinacylase for conversion of penicillin into 6-aminopenicillinic acid and mode of its preparation). Czech patent application PV, pp 376–378
105. Vojtíšek V, Bárta M, Zeman R, Čulík K, Kálal J, Drobník J, Švec, F (1978) Immobilisierte penicillinacylase and verfahren zu ihrer herstellung. DE 2849764
106. Vojtíšek V, Vlasák J, Bárta M, Čulík K (1972) Způsob obohacování a čištění penicilinacylázy (Mode of enrichment and purification of penicillinacylase). Czech patent application PV 6355-1975
107. Vojtíšek V, Žůrková E, Zeman R, Drobník J, Čulík K, Slavíček M, Švec F (1977) Pevný biokatalyzátor k enzymové přeměně benzylpencilinu na kyselinu 6-aminopenicilanovou a způsob jeho přípravy (Solid biocatalysts for enzymatic conversion of benzylpenicillin to 6-aminopenicillinic acid and mode of preparation). Czech patent application PV 2317-1977
108. Volfová O, Pilát P, Prokop A (1975) Growth of yeasts on solid wax. Microbiologiya (USSR) 44:1046–1050 (in Russian)
109. Wardel JN, Brown CM (1984) Bacterial growth on inert surfaces. In: Dean ACR, Ellwood DC, Evans CGT (eds) Proc Symp on Continuous Culture 8. Ellis Horwood Ltd, Chichester, pp 159–168
110. Wikswo JP, Prokop A, Baudenbacher F, Cliffel D, Csukas B, Velkovsky M (2006) The engineering challenges of BioMEMS: the integration of microfluidics, micro- and nano-devices, models, and external control for systems biology. IEE Proc Nanobiotechnol 153:81–101
111. Zahradník J, Rychtera M, Kratochvíl J (1983) Production of biomass from sulfite liquors in tower fermenter with forced circulation. Coll Czechoslovak Chem Commun 48:1984–1995
112. Zuo R (2007) Biofilms: strategies for metal corrosion inhibition employing microorganisms. Appl Microbiol Biotechnol 76:1245–1253
113. Žůrková E, Drobník J, Kálal J, Švec F, Tyráčková V, Vojtíšek V, Zeman R (1983) Immobilization of *Escherichia coli* cells with penicillin-amidohydrolase activity on solid polymeric carriers. Biotechnol Bioeng 25:2231–2242