On-Line Estimation of Growth-Linked Extracellular Protein Synthesis by pH Control Analysis

I. Ratão[1], J.I. Castrillo[2], J.A. Teixeira[1], U. Ugalde[2] and A.A. Vicente[1]

[1] Centro de Engenharia Biológica – IBQF, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Phone: +351.253.604400; fax: +351.253.678986.
e-mail: avicente@deb.uminho.pt

[2] Grupo de Bioquímica II, Departamento de Química Aplicada, Facultad de Ciencias Químicas, Universidad del País Vasco, P.O. Box 1072, 20080 San Sebastián, Spain. Phone: +34.943.015452

Key words: On-line control, growth monitoring, type 1 product synthesis, proton titration

Abstract

The study of on-line control strategies involving pH as the reference variable constitutes a field of particular interest, since it may be applied to a wide range of biotechnological systems for the production of biomass, proteins and growth-linked products (1-4). Experimental evidence of the existence of a direct relationship between growth and proton production in fermentation processes has been reported in the past (5-7) but the metabolic basis of the underlying relationships remained unclear. A formal physiological study conducted under aerobic controlled conditions has shown that the nitrogen assimilation pathway is the main net proton-contributing pathway and that there is a direct relationship between the specific nitrogen uptake rate (qN) and the specific proton production rate (qH^+) (8). From this knowledge specific relationships between growth (associated with nitrogen assimilation) and proton production could be derived (9).

Since the above obtained relations may be applied as formal rules it should be possible to extend these results to the monitoring of growth-linked products. Thus, for a given fermentation where biomass and a given primary metabolite are obtained a yield coefficient, $Y_{P/X}$, can be defined as the mass of product formed over the increase of cell mass. As a direct relationship between product synthesis and biomass formation occurs in growth-linked products, similar relations to the ones established for biomass dependence on qH^+ will also be expected.
This work presents the application of this concept to the on-line monitoring of extracellular protein synthesis in aerobic fermentations. The model system is endopolygalacturonase production by a strain of *Kluyveromyces marxianus* growing on glucose, with potential participation of additional acid/base contributions. Tests in batch and chemostat culture confirm the validity of qH^+ as a formal control parameter in aerobic fermentations.

1. Chen, W., Graham, C. and Cicarelli, R. B. (1997) *J. Ind. Microb. Biotechnol.* **18** (1), 43-48.
2. San, K-Y and Stephanopoulos, G. (1984) *Biotechnol. Bioeng.* **26**, 1209-1218.
3. Siano, S. A. (1995) *Biotechnol. Bioeng.* **47** (6), 651-665.
4. Suzuki, T., Yamane, T. and Shimizu, S. (1987) *Appl. Microb. Biotechnol.* **25**, 526-531.
5. Huth, J., Werner, S. and Mueller, H. G., *J. Basic Microbiol.* **30** (8), 561-567, 1990.
6. Kotyk, A., (1989) In: S. Fleischer, and B. Fleischer (eds.). Methods in Enzymology, vol. 174 (Biomembranes, Part U) Academic Press, New York, pp. 592-603.
7. Roos, W. and Luckner, M. (1984) *J. Gen. Microbiol.* **130**, 1007-1014.
8. Castrillo, J. I., de Miguel, I. and Ugalde, U. O. (1995) , *Yeast* **11**, 1353-1365.
9. Vicente, A., Castrillo, J. I., Teixeira, J. A. and Ugalde, U. O. (1998) *Biotechnol. Bioeng.* **58**, 445-450

International Conference on

Measurement, Analysis and Control in BIOPROCESS TECHNOLOGIES

Current Status and Future Prospects

24th - 26th September 2000
Robinson College
Cambridge
UK

Programme and Abstracts

108th Event of the European Federation of Biotechnology

Organised by the Biotechnology Group of the SCI and co-sponsored by IEE and IChemE