



International Union of Microbiological Societies  
IUMS Congresses '94

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7th International Congress  
of Bacteriology and Applied  
Microbiology Division  
7th International Congress  
of Mycology Division

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Prague, Czech Republic  
July 3rd — 8th, 1994

Czechoslovak Society for Microbiology

**ABSTRACT  
BOOK**

## Physiology and metabolism in continuous culture

BC-3/29

CONSUMPTION OF GLYCEROL AS CO-SUBSTRATE OF GLUCOSE IN CONTINUOUS CULTURES OF *CLOSTRIDIUM ACETOBUTYLICUM* ATCC 824. I. C. Andrade, I. Vasconcelos, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida 4200 Porto, Portugal

To maximise the synthesis of solvents in the acetone-butanol fermentation it is essential to control the carbon and energy flow patterns. This can be achieved by a choice of suitable feedstock mixtures. Using mixtures of different proportions of glucose and a more reduced chemical, like glycerol, it is possible to manipulate the overall degree of reduction of the carbon source and, therefore, to change quantitatively the metabolic flow of available reduced nucleotides.

Co-fermentation of glucose and glycerol in continuous cultures of *Clostridium acetobutylicum* ATCC 824, on a phosphate-limited synthetic medium, with a dilution rate of  $0.05 \text{ h}^{-1}$  and maintained at pH 6.5, results in the synthesis of butanol and ethanol as the major fermentation products (1). Optimal solvent yields are obtained when the molar ratio glycerol/glucose in the feed medium is 1.96. In these conditions cultures are glucose-limited, but glycerol is not entirely consumed.

To increase glycerol degradation higher amounts of glucose (the most oxidised substrate) must be co-metabolized, which allows the synthesis of reduced products, consuming the excess of NADH formed in glycerol degradation.

Experiments were performed in the same operating conditions, but without phosphate limitation and with initial molar ratios glycerol/glucose lower than 1.96. Results showed that culture stability and solvent production could be maintained in these cultures. Higher glucose levels were fermented, leading to an increase in glycerol consumption and to higher alcohol productivities.

(1) I. Vasconcelos, L. Girbal and P. Soucaille. Regulation of carbon and electron flow in *Clostridium acetobutylicum* grown in chemostat culture at neutral pH on mixtures of glucose and glycerol, *J. Bacteriol.* (in press).

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PEPTIDOGLYCAN BIOSYNTHESIS DURING REPEATED FED BATCH AND CONTINUOUS CULTIVATIONS OF *BREVIBACTERIUM DIVARICATUM*. V. Carić Duić<sup>1</sup>, I. Friganović<sup>1</sup>, M. Wrischer<sup>2</sup>, M. Bošnjak<sup>1</sup>, <sup>1</sup>PLIVA, Research Institute, Baruna Filipovića 89, 41000 Zagreb, Croatia, <sup>2</sup>Ruder Bošković<sup>\*</sup> Institute, Bijenička c. 54, 41000 Zagreb, Croatia

Cells of *Brevibacterium divaricatum* NRRL 2311 after penicillin treatment release uncross-linked peptidoglycans into culture media. In this work, behaviour of *Brevibacterium divaricatum* in batch (BC), repeated fed batch (RFBC) and continuous culture (CC) was studied in order to evaluate their convenience with respect to peptidoglycan (PG) production. Experiments were performed on a laboratory scale applying glass bioreactors of 14 L, different cultivation media as well as an aeration (10 L/min) and agitation (500 r.p.m.) during cultivation. Inocula were prepared applying the rich complex medium (bacto peptone 2g/L, yeast extract 6g/L, bacto triptone 4g/L). The poor mineral medium showed to be quite convenient for BC, but not for RFBC and CC. For RFBC and CC it appeared necessary to enrich the mineral medium with bacto peptone (0.1-1g/L), yeast extract (0.3-3g/L) and bacto triptone (0.2-2g/L). In BC the mean specific growth rate during exponential phase was observed to be 0.278/h, maximal PG yield 0.6g/L was obtained by treating the harvested cells after 10 hours of cultivation and an average PG production rate of 0.043g/L/h was realised. In CC the specific growth rate varied (0.202-0.489/h) according to applied dilution rate (0.2-0.5/h). PG productivity varied similarly (0.089-0.188g/L/h). RFBC experiments were performed by varying the dilution (feed) rate range and frequency of periodical withdrawals. When dilution rate range of 0.15-0.214/h and withdrawals of every two hours were applied the observed specific growth rates ranged from 0.19 to 0.32/h whereas PG productivity varied from 0.016 to 0.029g/L/h. An increasing of periodical withdrawals frequency to every half an hour with a simultaneous dilution rate increase to 0.4-0.5/h caused an increased PG productivity (0.055g/L/h). Cultivation conditions affected cell morphology, size and structure, as established by an electron microscope. To conclude, RFBC and CC could be applied for PG production, both being superior to BC.

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GROWTH OF *Candida utilis* ON GLUCOSE AND MALTOSE MIXTURES IN A CHEMOSTAT: REGULATION OF THE SIMULTANEOUS CONSUMPTION OF BOTH SUGARS UNDER GLUCOSE DEREPRESSION. A. E. Espineli and J.M. Peinado, Dpt. Microbiology, Faculty of Biology, U.C.M., E-28040 Madrid, Spain.

*Candida utilis* can not consume simultaneously glucose and maltose in batch culture because glucose represses maltose permease and maltase and also inactivates the permease. However the level of repression and inactivation depends on the glucose concentration in the growth medium and in a continuous culture at low dilution rates (D) it is possible to get cells growing on steady state glucose concentrations below the level that triggers repression. At these low D values the cells are able to consume simultaneously glucose and other sugars. We have used a chemostat to study the regulation of the uptake of glucose and maltose under non-repressing glucose concentrations. Under those conditions, the total catabolic flux is proportional to the dilution rate (i.e. to the specific growth rate), at any of the sugar mixtures assayed. However the individual consumption rate for each sugar depends on its proportion in the mixture and is not related, as the Monod equation predicts, with the steady-state concentration of the sugars in the fermenter. These results have been confirmed, in batch cultures, with derepressed mutants of these strain. Three spontaneous mutants able to consume maltose in the presence of repressing concentrations of glucose were obtained and assayed. Also in these cases, the relation between the two specific consumption rates for glucose and maltose, was the same that their proportion in the growth medium composition. We submit that in a mixed sugar culture, under non-repressing conditions or with catabolic repression resistant cells, the steady-state concentration of each sugar does not determine its specific consumption rate; instead, it seems to be the proportion of sugars in the inflowing medium what determines the relative catabolic flux for each sugar and its steady-state concentration.

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MAINTENANCE OF A RECOMBINANT PLASMID IN CONTINUOUS CULTURE IN *PSEUDOMONAS PUTIDA*. J.D. Brinck<sup>1</sup>, T. Williamson<sup>2</sup>, B. Abbishaw<sup>2</sup>, J. Cole<sup>1</sup>, <sup>1</sup>School of Biochemistry, University of Birmingham, University Road West, Birmingham B15 2TT, UK, <sup>2</sup>Zeneca Bio Products, PO Box 2, Belasis Avenue, Billingham, Cleveland TS23 1YN, UK.

Zeneca Bio Products use a recombinant plasmid transformed into a *Pseudomonas putida* strain to produce an enantiomer-specific dehalogenase protein in an industrial, continuous culture process. The physiology of plasmid retention and enzyme production has been investigated. The plasmid, which encodes an enantiomer specific dehalogenase, was typically lost after 200-400h of continuous growth at a dilution rate of  $0.1 \text{ h}^{-1}$ . Although 25% of the total cell protein was produced as the dehalogenase, a large proportion of this was inactive. The effect of various parameters on these phenomena was studied to see whether the overall productivity of the culture could be improved. The dilution rate (D) or the growth-limiting nutrient were varied. Significant changes in specific dehalogenase activity were not seen when either parameter was changed. However, when the dilution rate was increased from 0.1 to  $0.2 \text{ h}^{-1}$  an improvement in plasmid maintenance was seen. The plasmid had still been retained by all of the bacteria after 122 generations whereas in cultures grown at  $D=0.1 \text{ h}^{-1}$  the plasmid was typically lost after 28-58 generations. Analysis of samples taken from continuous cultures has shown that the dehalogenase protein does not appear to be prematurely inactivated by proteolysis or to form inclusion bodies. The low activity of the protein produced could be due to a limiting amount of a cellular factor such as a chaperone required for correct post-translational protein folding.