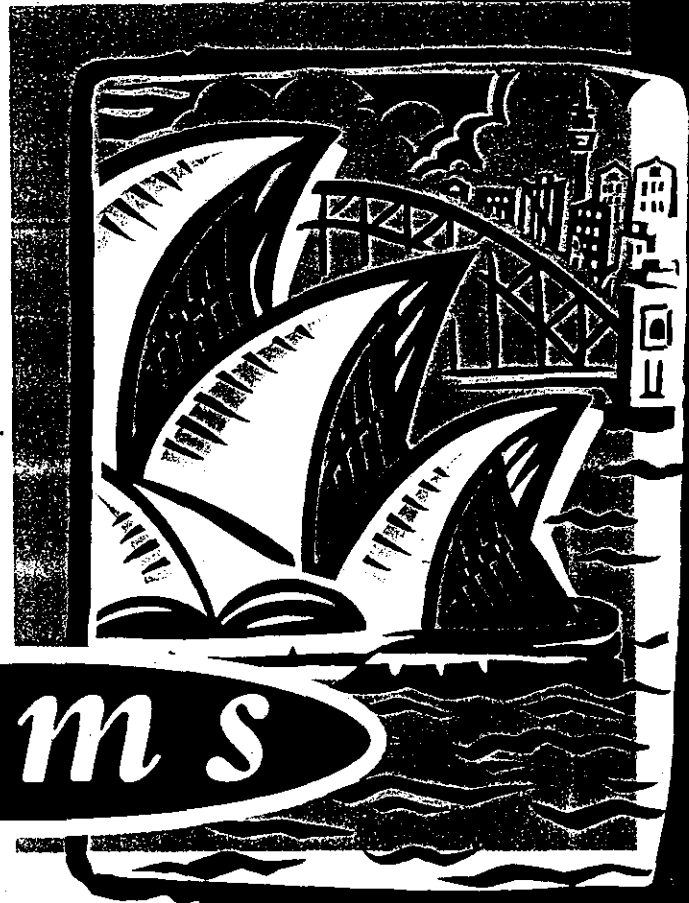


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BPO15.12**Xylitol production using recombinant *Saccharomyces cerevisiae* containing multiple xylose reductase genes at chromosomal Ψ -sequences**Yong Sung Kim^{*1}, Jung Hoe Kim¹, Sun Chang Kim¹¹Department of Biological Sciences, Korea Advanced Institute of Science and Technology

Xylitol production from xylose was studied using recombinant *Saccharomyces cerevisiae* 2805 containing xylose reductase genes (XYL1) of *Pichia stipitis* at chromosomal Ψ sequences. *S. cerevisiae* 2805-39-40, which contains about 40 copies of the XYL1 gene on the chromosome, was obtained by a sequential transformation using a dominant selection marker *neor* and an auxotrophic marker URA3. The multiple XYL1 genes were stably maintained on the chromosome even after 21 days and 10 days in the non-selective sequential batch and chemostat cultures, respectively, whereas *S. cerevisiae* 2805 :pVTXR, which harbors the episomal plasmid pVTXR having the XYL1 gene, showed mitotic plasmid instability and more than 95 % of the cells lost the plasmid under the same culture conditions. In the first batch (3 days) of the sequential batch culture, volumetric xylitol productivity was 0.18 g/l/h for *S. cerevisiae* 2805-39-40, as compared to 0.21 g/l/h for *S. cerevisiae* 2805 :pVTXR. However, the xylitol productivity of the latter started to decrease rapidly in the third batch and dropped to 0.04 g/l/h in the seventh batch, whereas the former maintained the stable xylitol productivity at 0.18 g/l/h through the entire sequential batch culture. The xylitol production level in the chemostat culture was about 8 g/l for *S. cerevisiae* 2805-39-40, as compared to 2.0 g/l for *S. cerevisiae* 2805:pVTXR after 10 days of cultures even though the xylitol production level of the latter was higher than that of the former for the first 5 days. The results of this experiment indicate that *S. cerevisiae* containing the multiple XYL1 genes on the chromosome is much more efficient for the xylitol production in the long-term non selective culture than *S. cerevisiae* harboring the episomal plasmid containing the XYL1 gene.

BPO15.13**Fermentative ability of *Zymomonas mobilis* under various stirring intensity conditions in continuous culture**Malda Maija Toma^{*1}, Andrejs Berzins¹, Maris Rikmanis¹, Uldis Viesturs¹,¹Institute of Microbiology and Biotechnology, University of Latvia

The performance of *Z. mobilis* 113 S under various stirring intensity regimes in continuous culture was quantitatively investigated. Specially developed devices-counterflow impeller and stirring intensity measurer (SIMD) were used for fermentation performance. It was very important for keeping even stirring intensity in the media volume during the cultivation. Growth of *Z. mobilis* 113 S in continuous culture revealed that the productivity of biomass and ethanol increased with increasing stirring intensity within the range of 300 to 1100 rpm. Specific glucose uptake rate also increased and had generally been calculated to be 10 g/g.h. Changes in the stirring intensity affected not only metabolic rate of glucose conversion and yield characteristics, but also appearance and content of by-products. The distribution of by-products differed significantly according to the applied stirring intensity and affected carbon balance. Stirring intensity also affected the morphology of the cells and an increase in stirring intensity resulted in the elongation of the cells. An increase in the stirring intensity within the range of 300 to 1100 rpm. resulted in the change in

morphology from normally dividing cells to filaments of increasing lengths.

BPO15.14**1,3-Propanediol continuous production by *Clostridium butyricum* VPI 1718: effect of dilution rate and substrate concentration**M Gonzalez-Pajuelo^{*1}, A P Ribeiro-Cruz¹, C M Sousa-Monagas¹, J C Andrade¹, I Vasconcelos¹¹Escola Superior de Biotecnologia

1,3-propanediol is a very versatile degradable intermediate compound for the synthesis of heterocycles and a monomer for the production of polymers such as polyesters and polyurethanes. As an alternative to its chemical synthesis, it has been shown that glycerol can be converted to 1,3-propanediol by *Klebsiella*, *Citrobacter* and *Clostridia* strains. Due to its safety record *Clostridium butyricum* is the preferred strain for this process. In this work the effects of dilution rate and substrate feed concentration on continuous glycerol fermentation by *Clostridium butyricum* VPI 1718 were studied. Different values of dilution rate ($D=0.05-0.5\text{ h}^{-1}$) were tested at a fixed substrate concentration (30 g/l). An improve in the volumetric productivity was achieved increasing the dilution rate up to 0.3 h^{-1} . At D values between 0.05 and 0.3 h^{-1} glycerol was exhausted from the culture medium. Significant concentrations of residual glycerol were observed at D values of 0.4 h^{-1} and 0.5 h^{-1} . Concentrations of glycerol in the feed medium of 60-70 g/l were tested. A higher 1,3-propanediol final level and a higher productivity (10.3 g/l/h) were achieved at $D=0.3\text{ h}^{-1}$; a switch in the acetate/butyrate ratio was also observed. Further improvement of the 1,3-propanediol productivity is being tested with a membrane bioreactor.

BPO15.15***Methylobacterium extorquens* P14 as a source of poly(β -hydroxybutyrate)**Marek Ostafin^{*1}, Jerzy Haber¹, Aleksander P. Sokolov², Yuriy A. Trotsenko²,¹Institute of catalysis and surface chemistry Polish Academy of Sciences, ²Institute of physiology and biochemistry Russian Academy of Sciences

As known, the accumulation of polyhydroxybutyrate (PHB) by methylotrophic bacteria begins only after consuming of nitrogen, phosphorus or some other medium components (1,2,3). We studied the influence of these components on PHB production and activity of the appropriate enzymes of PHB metabolism under batch, fed-batch and continuous cultivation of new strain *Methylobacterium extorquens*. As found *Methylobacterium extorquens* could synthesize PHB in batch, fed-batch and continuous culture. During continuous fermentation in less quantities than during batch or fed-batch cultivations. Intracellular PHB content increased when nitrogen and/or phosphorus shifted to the lower level. The influence of nitrogen was more expressed in the range of concentrations used. Enzymological analysis revealed a negative effect of nitrogen on the activity of β -ketothiolase and in a less extent on hydroxybutyrate dehydrogenase. NADPH-dependent acetoacetyl-CoA reductase was derepressed at higher level of phosphate. Neither nitrogen nor phosphorus controlled the activity of acetoacetate- succinate CoA-transferase in the range of concentrations used (4,5). 1. Suzuki T., Yamane T., Shimizu S. (1986) Appl. Microbiol. Biotechnol., 23, 322-329. 2. Trotsenko Y.A., Doronina N.V., Sokolov A.P., Ostafin M.