model was then extrapolated to simulate different fed-batch operating conditions. The potential use of the model to identify right nutrient feeding strategies for fed-batch cultivation to improve the productivity of 1,3-propanediol will be demonstrated.

doi:10.1016/j.jbiotec.2010.09.559

**[P-I.194]**

## Development of a culture strategy to produce a bacteriocin type substance utilizing a strain of *Enterococcus mundtii*

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*Keywords:* Bacteriocins; *Enterococcus mundtii*; Culture strategy

Lactic acid bacteria produce a variety of bacteriocins, peptide molecules that act inhibiting growth of Gram-positive bacteria. The strain of *Enterococcus mundtti* used in this work was isolated at a salmon processing factory located southern Chile. Growing in MRS medium it exhibits a high production capacity of a bacteriocin type substance (BTS) very active against *Listeria monocytogenes*.

Previous studies using MRS medium shown that BTS is produced growth associated obtaining a maximum volumetric productivity of $3.74*10^8$ (AU/L*h) at pH 6.2 and 30 °C.

The objective of this work was to develop a fermentation strategy to maximize the volumetric productivity of BTS using MRS medium.

Experiments were conducted batch wise in a 2 L bioreactor using MRS medium, at 30 °C and 1 vvm. The agitator was a Rushton type turbine operated at 300 rpm.

Initially four fermentations were run intending to evaluate the effect of aeration and pH control policy on BTS productivity. First and second runs were aerated and pH controlled automatically adding KOH in one case and using phosphate buffer in the other. Third and fourth runs were non-aerated and the same pH policy as before respectively. The condition using aeration and phosphate buffer gave the best results of BTS volumetric productivity equal to $1.8710^9$ (AU/Lh) and a product yield of $6.810^9$ (UA/gcel). It was observed that high BTS production was stimulated by pH fall from 6.2 to 4.8 during growth phase.

These results allowed designing a fermentation strategy consisting of a two stage batch operation. The first stage was aerated and pH controlled automatically and the second also was aerated but without pH control. BTS volumetric productivity was $2.1710^{10}$ (AU/Lh) and product yield was $5.210^{10}$ (UA/gcel).

By using this strategy volumetric productivity was increased by a factor of 11.6, using in both cases the same MRS culture medium.

### Acknowledgments

PUCV Proyect BIOACTIVES, FONDEF PROYECT D04I1153.

doi:10.1016/j.jbiotec.2010.09.560

**[P-I.195]**

## Condition of Mild Hypothermia does not Promote an Increase in Specific Productivity of Recombinant Protein in Continuous Culture of CHO Cells

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*Keywords:* CHO Cells; Continuous Culture; Sub-physiological temperature; Proteomics

Mammalian cells for recombinant protein production are usually grown at 37 °C. However, mild hypothermia condition (28–34 °C) often leads the production improvement. Grow at low temperatures can improve the maintenance of cell viability, decrease the consumption of carbon and energy sources and also decrease the protease and sialidase activity. Nevertheless, the mechanisms regulating these phenotypes are yet poorly understood.

Chinese Hamster Ovary (CHO) cells, producing human recombinant tissue plasminogen activator (tPA) were grown in HyClone CDM4CHO medium supplemented with 10 mM of glucose and 6 mM of glutamate using spinner flask adapted to continuous cultures (140 ml) at 37 °C and 33 °C under controlled atmosphere by the same dilution rate ($D = 0{,}0167 \pm 0{,}0007\ h^{-1}$). Number of viable cells was determined using a haemocytometer and trypan blue exclusion method. After 3 time of residence we considered stationary state and we take samples of cells and supernatant.

The main metabolites were measure: glucose, lactate and glutamate concentration using biochemical analyzer (YSI2700), ammonium concentration by the Berthelot reaction. Secreted and intracellular tPA concentrations were quantify via ELISA kit (Imulyse t-PA, Biopool) and identified through westernblot. The mRNA-tPA was quantified by means of RT-PCR. The differential protein expression was investigated using 2D-DIGE and mass spectrometry.

Our results demonstrate that the $Y_{lac/glc}$ was not affected by the decrease of temperature, while the consumption of glutamate remarks decreased to 33 °C, but not the production of ammonium. Specific production of tPA in cells growing at mild hypothermia condition but at the same specific growth rate is 67% lower than specific production at 37 °C; this is consistent with our results of quantification of mRNA-tPA.

Then, we conclude that a decrease in temperature is not responsible for the increase in specific productivity of recombinant protein, would rather a decrease in specific growth rate that occurs in batch culture.

doi:10.1016/j.jbiotec.2010.09.561

**[P-I.196]**

## Optimization of Exo-Polygalacturonase Production from Orange Peel by *Aspergillus sojae*

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*Keywords:* Polygalacturonase; *Aspergillus sojae*; optimization; submerged fermentation

Pectinases catalyze the degradation of pectic substances, thus they are used extensively in fruit juice and wine industry to facil-

itate extraction and clarification. *Aspergillus* species, in particular *Aspergillus niger*, have long been utilized for production of pectinases. Previous studies of our group showed that *A. sojae* has a potential to produce enhanced amount of polygalacturonase, which is one of the pectic enzymes, in both submerged and solid-state cultures (Gogus et al., 2006; Tari et al., 2007). In this study, several agricultural products were screened in an effort to find a cheap and abundant substrate for submerged polygalacturonase production using a UV-mutated *A. sojae* strain. Medium composition was optimized to further enhance the enzyme level. Experiments were designed and analyzed statistically using the trial version of the statistical software, Design Expert.

Shake flask cultures were used in screening and optimization studies. In the screening part, highest activities were obtained using orange peel as inducer and potassium phosphate as the phosphate source. As the additional carbon source, sugar beet syrup was the best, followed by maltrin and glucose. Concentrations of orange peel, sugar beet syrup and potassium phosphate as well as ammonium sulphate, which was used as the nitrogen source, were optimized using response surface methodology. The optimum concentrations for high polygalacturonase production were found as (g/l); orange peel: 10, sugar beet syrup: 61.87, and ammonium sulphate: 8.43. Potassium phosphate had no effect on the enzyme activity. Under these conditions enzyme activity level around 150 U/ml were obtained. This level was verified by validation experiments.

Under these conditions an activity of 120 U/ml was obtained in the fermenter with a four liter working volume. pH dropped from initial level of 4.2 to 2.0 in four days. In an experiment performed at constant pH of 4, enzyme activity was only 40 U/ml.

This study showed that, high amount of polygalacturonase could be produced by *A. sojae* in submerged cultures using available and economical substrates at uncontrolled pH conditions.

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doi:10.1016/j.jbiotec.2010.09.562

**[P-I.197]**

### Incidence of Mild Hypothermia on Metabolic Behavior and Synthesis of tPA in CHO Cells Inhibited ERAD Degradation Pathways

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*Keywords:* CHO Cells; ERAD degradation pathway; endoplasmic reticulum; degradation of glycoproteins

Mammalian cells have become the main system for the production of recombinant proteins because of their capacity for proper protein folding, assembly and post-translational modification. However, these systems have inherent difficulties, so it is of great interest to the study and development of more efficient processes. In particular, manipulation of operational parameters, such as low temperature, was shown a significant improvement in specific productivity of several recombinant proteins. However, this fact is not well explained from the physiological point of view.

Our group has recently observed that the stage of protein synthesis developed in the endoplasmic reticulum appears to be critical in this phenomenon observed. Initially we intend to determine the rate of synthesis of t-PA protein enters the endoplasmic reticulum at two temperatures, observing changes in protein production and metabolic behavior.

To evaluate this, two sets of cultures at 37 °C and 33 °C were carried out, using a control culture and blocked ERAD degradation pathways culture by inhibitors MG132 (ERAD-I), pepstatin-A, leupeptin, E64d (ERAD-II). Metabolites: glucose, glutamate and lactate were measured using biochemical analyzer (YSI2700) and ammonium by Berthelot reaction. t-PA was measured by ELISA (Imulyse t-PA, Biopool).

The results indicate that cells inhibited at 37 °C and 33 °C increased the specific productivity of t-PA by 50% and 44% regarding the control of each temperature. In addition, cells inhibited at 37 °C showed an increase in glucose uptake and production of ammonium and lactate by 15%, 23% and 31% respectively and decrease in glutamate uptake by 70%. Inhibited cultures at 33 °C showed a glucose and lactate metabolism similar to the control at 37 °C and a decrease in glutamate uptake and ammonium production by 35% and 20% respectively.

We conclude that the inhibition of ERAD degradation pathways does not alter the positive effect of low temperatures on specific productivity.

doi:10.1016/j.jbiotec.2010.09.563

**[P-I.198]**

### Raw starch digesting amylase (RSDA) immobilization and stabilization by multipoint attachment on polyglutaraldehyde activated chitosan beads

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Benefits of utilization of raw starch digesting enzymes (RSDA) are reduced time and energy, greater process efficiency and increased net yield. As a result of instability issues, product inhibition and problems of contamination due to application at low temperature use of RSDAs are limited. RSDA from *Aspergillus carbonarius* was stabilized through immobilization on chitosan beads by conjugation, spontaneous crosslinking, and adsorption-crosslinking using glutaraldehyde or polyglutaraldehyde (PG). Influence of immobilization on enzyme kinetics, catalytic, operational and storage stability of immobilized enzyme were evaluated. Maximum amount of glutaraldehyde for activation of chitosan or crosslinking of enzyme adsorbed chitosan was 2.5% for 100 min at pH 7. PG activation for 30 min at pH 6 gave the best condition for production of PG activated derivative. PG derivative gave the highest immobilization yield (100%), though expressed activity (75%) was lower than that of the glutaraldehyde derivative (83%). Glutaraldehyde derivative was most pH stable and retained over 95% activity at pH 3.5 and 90% at pH 9. PG derivative had 75% activity compared to 70% of the soluble enzyme at pH 3.5, however it maintained over 80% activity at pH 9. Optimal enzyme activity for the soluble RSDA and all its chitosan derivatives was at 30 °C. At 80 °C soluble enzyme retained 42% activity, glutaraldehyde 70%, adsorbed-crosslinked 97% and PG derivative 95%. From thermoinactivation studies of free and immobilized RSDA at 60 °C, 98% activity, 94% activity, 78% and 76% activity was retained by PG,