kinetics (very close to plug flow). For these reasons, FBBR was chosen for experimental investigations and theoretical analysis of performance.

The experimental apparatus was constituted by two reservoir tanks, a pump and the reactor. The reactor was equipped with a ball distributor at the bottom to guarantee uniform flow conditions at the reactor inlet and a diverging section at the top to prevent fine solids from elutriating.

Tracing experiments were run in order to determine fluid-dynamic conditions of non-ideal flow within the bed and a dispersion model was applied. The fluid-dynamic model testes in a vast set of experimental conditions, at different flow rates and bed void fractions. Once fluid-dynamics were known, they were implemented into a reaction model taking into account three main issues: reaction kinetics of the Michaelis-Menten type, mass transport resistances and biocatalyst efficiency as a function of Damköhler number and saturation parameter, non-ideal flow behaviour by means of dispersion coefficient. Experimental tests were run and the model was validated.

The work covers those aspects of reactor engineering that are hardly dealt with in biocatalysis studies where a crossing of biochemical and engineering competences is more and more requested.

doi:10.1016/j.jbiotec.2010.09.487

[P-I.122]

Determination of yeast cell viability: viable count *vs* ATP-based bioluminescence assay

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Keywords: cell viability; bioluminescence; viable count; ATP determination

During fed-batch cultivation to produce heterologous protein with yeast cells, cell viability should be determined quickly. Indeed, the early arrest of cell proliferation is a phenomenon often observed in auxotrophic yeast strains such as *Saccharomyces cerevisiae* BY4741 and ascribable to stressful environmental conditions arising in fed-batch systems.

Choosing a cell viability assay can be a challenging task because the growth arrest is not easily attributable to one of the many mechanisms of cell death. In this work, two techniques used to assay cell viability have been compared: the viable cell count on agar plate and the bioluminescence assay based on the luciferase reaction to measure the amount of ATP from viable cells. To compare the methods, either the maximum specific velocity of growth or its specific death rate (k_d) at 50 and 53 °C of BY4741 strain, were determined. In the first case, both the viable count and the bioluminescence assay gave the same results, showing that the amount of ATP in exponentially growing cells correlates with cell viability. On the contrary, during thermal inactivation the k_d value obtained via bioluminescence, resulted always smaller than that obtained by viable cell count even though it was always possible to correlate the k_d value obtained by bioluminescence with that obtained by viable count, through a factor. Apparently, in the operative condition examined, cell death either did not lead to the loss of membrane integrity, nor allow the endogenous ATPases to destroy any remaining ATP; thus the ATP levels did not fall precipitously.

In the light of the results obtained, due to the ease of use, high sensitivity and action in real-time, ATP-based bioluminescence assay is the natural candidate to replace the cell viable count method which contrarily requires many replicates and extended incubation periods, provided that a correlation factor is considered.

doi:10.1016/j.jbiotec.2010.09.488

[P-I.123]

Calcium signalling and heterologous protein production in *Kluyveromyces lactis*

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Keywords: *K.lactis*; heterologous protein secretion; calcium signalling

Among various host–vector systems, yeast is a very useful cell factory for the extracellular production of heterologous gene products, since it is a safe eukaryotic microorganism with well established recombinant and fermentation technologies. In the last years *Kluyveromyces lactis* has been successfully used as an alternative host to *Saccharomyces cerevisiae* in the food and pharmacology industries. In fact, stable multicopy vectors, tightly regulated promoters and reliable procedures are available for the genetic manipulations of this yeast.

In eukaryotic cells cytosolic free calcium is a major signal transducing element employed to induce a variety of physiological responses, such as muscle contraction, neurotransmitter release, and cell proliferation. In addition, calcium is involved in the transport of secretory proteins from the endoplasmic reticulum. It is well established that heterologous overexpression of proteins is connected with different stress reactions. A prominent player is the endoplasmic reticulum (ER) where the protein quality control system is often rate limiting. Heterologous expression of secreted proteins can saturate the cell capacity to properly fold proteins, triggering the unfolded protein response and resulting in overall reduction of protein expression. A regulatory pathway connecting the accumulation of misfolded proteins in ER and Ca^{2+} influx at plasma membrane has been reported.

We found that increased calcium concentration in the growth medium resulted in enhanced protein production whereas the addition of EGTA, a calcium chelator, reduced the amount of secreted proteins. The expression of genes encoding for relevant players in calcium homeostasis was analyzed and found to correlate with ER homeostasis. The impact of modulating calcium/calmodulin based signalling on the secretion of heterologous proteins in *K.lactis* was studied.

doi:10.1016/j.jbiotec.2010.09.489

[P-I.124]

Elicitors as Stress Factors in Cultures of *Bacillus licheniformis*

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Keywords: Elicitation; *Bacillus licheniformis*; Phosphorylation

Elicitation is a phenomenon where the addition to a living system of a substance (elicitor) in trace amounts activates certain metabolic, physiological, and/or morphological responses in the system. Oligosaccharides with a degree of polymerisation (DP) of 7-10 derived from galactomannans and alginates have been reported to exert strong biological activity in a number of fungal and bacterial cultures. However, little has been reported about the effects