Natural green leaf volatiles (GLVs) are commonly sole AS aldehydic and alcoholic flavors; their synthesis is a great challenge for industry. Especially, the bioconversion step of fatty acid hydroperoxides into aldehydes by the hydroperoxide lyase (HL). This widely studied enzyme is present in cell membranes of green organs from superior plants. Extracted from its natural condition, HL is subject to a suicidal behavior, being irreversibly inhibited by its own substrate. Furthermore, GLVs produced are highly volatile and quickly degraded by other plant enzymes. Thence, high GLVs levels in industrial production are very difficult to obtain, but several biotechnological tools could be developed to enhance this natural synthesis level more than hundred times. This paper will describe a new method for GLVs production in bioreactor using sugar beet leaves as source of HL. One step reaction, including hydroperoxide metabolisation and GLVs extraction, is performed during a short time process. Downstream processing to dispose of natural and pure GLVs molecule will also be discussed.

Keywords. Hydroperoxide lyase, green leaf volatiles, natural flavor, suicidal behavior.

Dynamic analysis of microbial behavior face to environmental heterogeneities encountered in large-scale bioreactors

Sirichai Sunya [1], Carine Bideaux [1], Jean-Louis Uribelarrea [1], Frank Delvigne [2], Tina Van Dyk [3], Carole Molina-Jouve [1], Nathalie Gorret [1]

[1] INSA Toulouse. CNRS UMR 5504. INRA UMR 792. Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés. Avenue de Rangueil, 135. F-31077 Toulouse Cedex 04 (France). E-mail: sunya@insa-toulouse.fr

[2] Univ. Liege - Gembloux Agro-Bio Tech. Walloon Center of Industrial Biology (WCIB). Bio-Industries Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium).

[3] DuPont Company. Central Research and Development Department. Rt. 141 and Powdermill Road. P.O. Box 80173. Wilmington, DE 19880-0173 (USA).

Heterogeneities caused by deficient mixing in large-scale bioreactors have been identified and described in literature. These heterogeneities affect physiological changes of microorganisms through its passage in different zones of concentrations. Consequently the differences in terms of productivities, qualities and/or yields of products of interest have been observed during scaling-up from laboratory to larger scales. For this reason, large-scale process improvement depends on the understanding of dynamic interactions between microbial responses and physical phenomena inside bioreactors. The dynamic responses of microorganisms are used as a tool for gaining insight into the fundamentals of microbial changes under a mixing-well controlled environment. Our research group has not only applied scale-down methodology to study the microbial responses at molecular, microscopic and macroscopic levels of observation, but also has used innovative strains and process engineering tools to evaluate fast dynamic responses of microorganism at time scales from seconds to minutes. This presentation focuses on the application of rational strategies in order to characterize distributed relaxation times of microorganisms which are considered to be constant at all fermentation scales. Experiments were conducted with reporter bioluminescent strains of *Escherichia coli* in which the luxCDABE operon was fused to promoters responding to different selected environmental stresses (dissolved oxygen, pH, temperature and substrate, etc.). Such strains allow real-time recording of the expression of genes involved in stress responses. Kinetic analyses of biomass, extracellular metabolites, inlet/exhaust gas, were carried out in order to determine mass balances and biological kinetic parameters. We will present our approach and the results concerning continuous culture of *E. coli* DPD2417 (nirB::luxCDABE) to monitor the microbial responses to oxygen limitation.

Keywords. Dynamic responses, oxygen limitation, reporter bioluminescent strains, *Escherichia coli*.