Enzymatic process development for the extraction of ferulic from wheat bran

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The agro-industries generate thousands of tons of by-products, such as cereal bran or sugar beet pulps, each year. For instance, in the Walloon Region, wheat transformation industry produces about 200,000 t of bran annually. Most of those by-products are, at best, used for cattle feeding. Through biocracking, this biomass may however constitute a renewable source for various value-added molecules of interest. These include dietary fiber, proteins, antioxidants, etc. The Feruzyme project focuses on ferulic acid, a major example of the hydroxycinnamic acids. These phenolic compounds show excellent antioxidant ability, and are found in relative abundance in cereal bran (about 6.6 $mg \cdot g^{-1}$, dry basis, in wheat bran). Ferulic acid (along with other hydroxycinnamic acids) is in majority (usually about 80%) ester-linked to other constitutive elements of the cell wall, namely arabinoxylans. Its enzymatic release depends mainly on the breaking of its ester linkage by Ferulic Acid Esterases (FAE, EC 3.1.1.73), which works in synergy with arabinoxylan-degrading enzymes (hemicellulase, including xylanase). Cellulase and even protease may also help by "unweaving" further the complex, cross-linked structure of bran cell-wall. The aim of our project is to design a process, starting from raw wheat bran to obtain purified ferulic acid, either crystallized or in concentrated solution. Furthermore, this process should be feasible at pilot scale, as it is meant to commercial application. Bran pre-treatment may impact the efficiency of the enzymatic action, by facilitating the access of the enzymes to their substrate (grinding, micronisation), or by modifying cell-wall structure (extrusion, steam-explosion, etc. processes involving non-enzymatic hydrolysis). The composition of the bran may also be altered, for instance by destarching, but also by pearling, this process being able to separate richer layers within the bran. Simpler process, like fine sieving of ground bran, is sufficient to eliminate part of the starch (wheat bran starch granules are usually about 20 μm). Purified FAE are still not commercially available. However, several enzyme complexes do present FAE activity: Depol™ 740 l (Biocatalysts), Pentopan™ 500 BG and Novo™ 188 (Novozymes), Pectinase™ PE (Catalysts), Grindamyl™ S100 (Danisco), etc. These complexes are used as filtration aid or for bread making. FAE activity is usually a side activity, the major being hemicellulase and cellulase, except for Depol™ 740 l in which FAE activity is standardized (36 $U \cdot g^{-1}$). Even though it should be preferably integrated in a wider industrial scheme, a high potential of wheat bran valorization lies in the field of natural antioxidants extraction.
Keywords. Ferulic acid, wheat bran, biocracking, arabinoxylan.

Development of a biotransformation process of hydroperoxides into green leaf volatiles using sugar beet leaves

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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

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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*.