1.10. References

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2. Aim of the work

Lignin is an amorphous polymer characterized by a wide range of molecular mass components, a disordered and branched three-dimensional structure, insoluble in water and in most common solvents. In order to perform lignin degradation, enzymatic treatment could represent an environmentally friendly alternative to chemical methods.

The purpose of this PhD project is to develop an "enzymatic tool-box" for an efficient oxidation and degradation of lignin into aromatic monomers. Laccases, Mn-peroxidases and lignin-peroxidases are attracting great scientific interest because of their very essential requirements and huge catalytic capabilities. These enzymes are commercially available or can be produced as recombinant and/or engineered proteins.

An evaluation of the biochemical properties of available commercial and recombinant ligninolytic enzymes under identical experimental conditions is necessary, with the final goal to identify interesting biocatalysts for lignin degradation. In order to assess the potential use of these enzymes in industrial processes, the effect of pH, temperature, NaCl, DMSO and Tween-80 on the enzymatic activity has to be investigated. Unfortunately, classical analytical methods for lignin analysis are extremely lengthy and complicated processes preventing the investigation of suitable large number of different condition: for this reason, an high-throughput colorimetric screening method to assay the oxidation of lignin groups by different enzymes represents a promising option to solve this bottleneck. A colorimetric screening method could facilitate the identification of optimal conditions for setup of a lignin treatment based on the combined use of various laccases and peroxidases. On this side, coupling the simple colorimetric assay with size exclusion chromatography and GC-MS analyses could be useful to identify changes in lignin molecular mass distribution and in production of small aromatic compounds.

Unfortunately, the known enzymatic activities alone are not able to depolymerize lignin. For this reason, novel enzymes must be taken into consideration. As an example, the membrane-bound polyphenol oxidase from the marine bacterium *Marinomonas mediterranea* (MmPPOA) represents an interesting enzyme for its biochemical properties: its recombinant expression and purification could make it a

suitable biocatalyst for lignin treatment. Furthermore, the exploration of new bacterial diversity may allow to discover and exploit enzymes from alternative sources involved in lignin metabolism: a novel and valuable peroxidase produced by the recently classified actinomycete *Nonomuraea gerenzanensis* could be taken in consideration.

Furthermore, based on recent literature, a chemo-enzymatic process to depolymerise lignin could represent an innovative and feasible way for valorisation of lignin under mild conditions.

3. Results

Comparison of different microbial laccases as tools for industrial uses

Fabio Tonin, Roberta Melis, Arno Cordes, Antonio Sanchez-Amat, Loredano Pollegioni, Elena Rosini (2016) *New biotechnology* 33(3): 387-398

Different recombinant forms of polyphenol oxidase A, a laccase from *Marinomonas mediterranea*

Fabio Tonin, Elena Rosini, Luciano Piubelli, Antonio Sanchez-Amat, Loredano Pollegioni (2016) *Protein expression and purification* 123: 60-69

Lignin degradation by actinomycetes: a valuable bacterial peroxidase activity from the novel species *Nonomuraea gerenzanensis*

Carmine Casciello; **Fabio Tonin**; Francesca Berini; Elisa Fasoli; Flavia Marinelli; Loredano Pollegioni; Elena Rosini (2016) (*Submitted*)

A novel, simple screening method for investigating the properties of lignin oxidative activities

Fabio Tonin; Elisa Vignali; Loredano Pollegioni; Paola D'Arrigo; Elena Rosini (2016) *Enzyme and Microbial Technology* (In Press)

Enzymatic and chemo-enzymatic depolymerisation of lignin

Fabio Tonin, et al. (*In preparation*)