**Enzymatic synthesis of tyrosol galactoside: Screening of immobilization resins.**

Veronika Hollá1, Monika Antošová1, Milan Polakovič1

*1 Department of Chemical and Biochemical Engineering, Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia*

*\*Corresponding author: milan.polakovic@stuba.sk*

**Highlights**

* Screening of six various carriers for immobilization of *β*-galactosidase.
* Tyrosol adsorption on carriers and calculation of Langmuir parameters.
* Synthesis of tyrosol β-galactoside by immobilized biocatalyst.
* Study of operational stability of immobilized biocatalyst on one selected resin.

**1. Introduction**

Nature produces a vast variety of glycosides with interesting biological properties which could be used in pharmaceutical and cosmetics industry. However, demand for naturally occurring glycosides in reasonable quantities and purity requires their synthesis, either chemical or enzymatic. Enzymatic synthesis represents an effective and direct alternative to the synthesis of glycosides thanks to the stereo- and regioselectivity of enzyme. Enzymatic synthesis of glycosides is carried out using hydrolytic enzymes such as glucosidases, fructosidases and galactosidases. These enzymes possess both hydrolytic and transferase activity, but the ratio of these activities depends on the source of enzyme. Glycosides containing tyrosol or hydroxytyrosol group possess bioactive properties. Tyrosol *β*-galactoside owns many advantageous properties for humans, such as antioxidant, anti-fatigue and anti-hypoxia effects. This study is focused on enzymatic synthesis of tyrosol *β*-galactoside by immobilized *β*-galactosidase from *A. oryzae*. Immobilization was conducted on various commercial resins. All studied resins were used for the enzymatic synthesis of tyrosol *β*-galactoside. Furthermore, adsorption of tyrosol on the resins were measured at two different temperatures and subsequently Langmuir parameters were calculated. Based on the results, one immobilization resin was chosen for study of operation stability in repeated batch experiments.

**2. Methods**

The investigated resins were Dowex Marathon MSA (Dowex), LifetechTM ECR1508 (1508), LifetechTM ECR8309M (Amino C2), LifetechTM ECR8409M (Amino C6), LifetechTM ECR8209M (Epoxy) and LifetechTM ECR8285M (Epoxy Butyl). Before immobilization and adsorption measurement was each resin prepared according to immobilization protocol from supplier. Enzymatic synthesis of tyrosol *β*-galactoside and adsorption of tyrosol on resins were carried out as batch experiments. Samples from adsorption measurements were analyzed by the diode array spectrophotometer HP 8452A (Hewlett Packard, USA) at a wavelength of 275 nm. The HPLC system Agilent 1200 (Agilent Technologies, USA) with diode array detector and a Zorbax Eclipse XDB C-18 column with a guard column was used to determine the concentrations of tyrosol and tyrosol *β*-galactoside in diluted samples from enzymatic synthesis.

**3. Results and discussion**

The primary aim of this study was to prepare an immobilized biocatalyst suitable for the synthesis of tyrosol *β*-galactoside. At first, tyrosol adsorption was measured for each studied resin and isotherms at 37°C are illustrated in Figure 1. Carriers 1508 and Dowex are based on non-polar, hydrophobic matrices containing benzene rings, however Amino and Epoxy, Epoxy Butyl carriers are made from more hydrophilic and polar methacrylate resins. Since tyrosol contains a non-polar benzene ring it has a greater binding capacity on non-polar particles. It is clear from the results that Amino C2 and C6 carriers are the most polar particles and therefore they adsorb the least amount of tyrosol which results in the greatest amount of tyrosol available in the reaction solution for synthesis of tyrosol *β*-galactoside.

**Figure 1.** Adsorption isotherms for studied carriers at 37°C. Points represent experimental data, lines represent calculated Langmuir isotherms.

Subsequently, free *β*-galactosidase was immobilized on all studied carriers and was used for synthesis of tyrosol *β*-galactoside from lactose and tyrosol. Experiments were carried out with two different amounts of enzyme. Evident hydrolysis of product was observed for higher amounts of enzyme which is typical for transglucosylation. [1, 2] The highest volumetric productivities and yields were achieved with Amino and Epoxy resins. Epoxy resins were afterwards used for the study of operational stability in fourteen repeated cycles. The formation of tyrosol *β*-galactoside was kept nearly constant during all cycles. The differences among individual runs were within the experimental error. Epoxy resins exhibit covalent binding with enzyme which resulted in high operational stability and no leakage of the enzyme out of the matrix.

**4. Conclusions**

This work provides the screening of immobilization carriers suitable for enzymatic synthesis of tyrosol *β*-galactoside. Hydrophilic, polar resins and resins with covalent bonding results as the best immobilization carriers for tyrosol galactoside synthesis.

**5. Acknowledgements**

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**References [Calibri 10]**

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