Abstract

The use of circular economy processes to convert waste products to high added value molecules can be a good strategy towards an increasingly sustainable society. Therefore, in this thesis enzymatic processes were developed, which allow to work at low temperature and pressure, therefore without using much energy, to valorize the wastewater of anaerobic digestion and CO_2.

A problem with using free enzymes is their low stability. To overcome this limitation, different processes of immobilization on porous supports were studied in this thesis.

The experimental part, the materials and the biomolecules used are explained in detail in Chapter 2.

In Chapter 3 the CO_2 reduction process was studied, using formate dehydrogenase (FDH) to produce formic acid.

In the first section of Chapter 3, the influence of the pore size and morphology of the mesoporous silica as support was studied for *Candida boidinii* FDH, which catalyzes the reduction of carbon dioxide to formic acid. Specifically, a set of mesoporous silicas was modified with glyoxyl groups to covalently immobilize FDH. Three types of mesoporous silicas with different textural properties were synthesized and used as supports: i) SBA-15 (D_P = 4 nm); ii) MCF with 0.5 wt% mesitylene/pluronic ratio (D_P = 20 nm) and iii) MCF with 0.75 wt% mesitylene/pluronic ratio (D_P = 25 nm).

In the second section of Chapter 3, a strategy of immobilization of *Candida boidinii* FDH on natural zeolite (mordenite) was studied.

The support functionalization was carried out with glyoxyl (Z_G) or amino (Z_A) groups, to covalently bind the enzyme to the support. To carry out a covalent immobilization with Z_A, glutaraldehyde was added after the ionic bond formation between the enzyme and the support. The samples were evaluated in terms of

specific activity, immobilization yields and thermal stability. Finally, FDH immobilized on Z_G and Z_A supports were tested for the production of formic acid in a CO_2 saturated medium.

In the last section of Chapter 3, *Candida boidinii* FDH was used co-immobilized with the *Geobacillus stearothermophilus* glycerol dehydrogenase (GlyDH). In particular, the GlyDH enzyme was required for the NADH cofactor regeneration reaction.

A second type of natural zeolite, clinoptilolite, hetero functionalized with glyoxyl and amino groups was used as support to carry out a co-immobilization. The zeolite was also mesostructured to evaluate the variation of the pore size on the enzymatic activity, noting a great increase in activity for FDH.

Using GlyDH, it was possible to carry out the CO_2 reduction starting from the nicotinamide cofactor in oxidized form (NAD^+), as it is converted back to NADH in the first reaction phase (using glycerol as a 'sacrificial' molecule).

In Chapter 4, it was studied the immobilization of two enzymes, the alcohol dehydrogenase (ADH) and the aldehyde dehydrogenase (AldDH) both from *Saccharomyces cerevisiae*, which can be used to produce high value-added molecules from carboxylic acids embedded in anaerobic digestate.

In particular, three mesoporous siliceous materials, with different surface area and pore size, (MSU-H, MSU-F and $MCF_{0.75}$) were used as support for covalent immobilization. The support materials were characterized by complementary techniques. Then, after a functionalization, a covalent bond between the enzyme and the support was created. The specific activity and immobilization yield of the biocatalysts were then evaluated.

These biocatalysts were then characterized in terms of optimal pH and temperature and the stability factor was evaluated. Finally, the biocatalysts AldDH/MSU-H and ADH/MSU-H were used to perform the reduction reaction.

The ADH and AldDH enzymes have been successfully immobilized on mesoporous siliceous supports, considerably increasing their thermal stability and allowing to reuse them for several reaction cycles. The use of this immobilization procedure and these supports is adaptable to a wide variety of enzymes.