Poster GC11

Enzymatic Acylation of 6-amino-1-hexanol by *Candida Antarctica* Lipase B (CALB) in Supercritical Carbon Dioxide: Use of DOE for Evaluating the optimal Pressure-Temperature conditions

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Candida Antarctica lipase B (CAL B) is a well-known enzyme that can be used for O- or N- acylation processes of biomolecules using supercritical carbon dioxide as non-aqueous solvent. While numerous studies investigated CAL B performances for (trans)esterification or N- acylation in given fixed conditions of pressure and temperature, unfortunately, few attempts were described to identify optimal CO_2 pressure-temperature conditions for CAL B activity based on Design of Experiment approaches (DOE). In the present study, a central composite design was chosen using STAVEX 5.1 software (AICOS Technologies AG, Basel, Switzerland). Eleven pressure-temperature conditions were investigated from 45°C to 115°C and from 100 to 300 bars. Enzymatic acylation reactions were carried out during 3 hours under batch conditions in a 10 mL magnetically stirred reactor (figure 1). 100 mg of CAL B immobilized on macroporous acrylic beads (a_w=0.12) were mixed together with 6-amino-1-hexanol (0.02 M) and oleic acid (0.04 M). Target P-T conditions were achieved in 30 seconds. Gentle depressurization was performed by bubbling into 5 mL methanol during 15 min. Reactor and basket contents were recovered in 15 mL methanol for appropriate rinsing. The consumption of oleic acid was quantified by measurement of the residual oleic acid using LC-MS. As depicted in figure 2, an appropriate surface response model could be generated (R^2=0.94) with coresponding isoresponse curves. Residuals were normally distributed (Shapiro-Wilk test). After 3 hours of reaction, the maximum oleic consumption found by the model was **64.8%** at **250 bars and 75°C** which was quite similar to the 67.8% experimentally observed in similar conditions. At low (45°C) and high temperatures (115°C) the 6-amino-1-hexanol acylation reaction by CALB appeared to be favored (+10%) at high pressure (300 bars) compared to low pressure (100 bars). N oleyl and N,O dioleyl 6-amino-1-hexanol were quantified in each pressure-temperature condition.

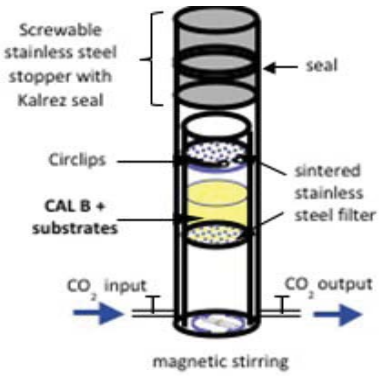

Figure 1: 10 mL batch reactor with its 2.33mL volume reaction basket

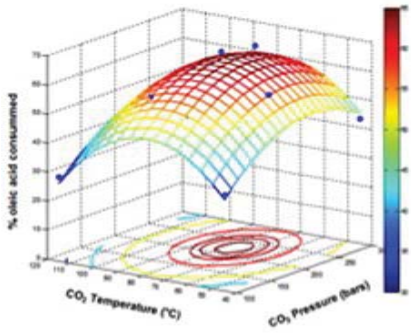

Figure 2: Surface response and iso-response curves of 6-amino-1-hexqnol acylation by CALB based on oleic acid consumption after 3 hours of reaction in supercritical CO_2. Experimental points (.) have been superimposed