



Enzymatic Modifications of Sugars in Supercritical CO₂

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Beyond a specific temperature and pressure (the critical point), CO₂ becomes a supercritical fluid, a state that is neither a gas nor a liquid, but has properties of both. Supercritical CO₂ (Sc-CO₂) has many advantages (environmentally friendlier and safer, non-flammable...) and constitutes an interesting alternative to the organic solvents.¹ Since there are many examples in literature of carbohydrates esterification catalyzed by lipases in organic medium,²⁻⁴ few reports describe the enzymatic synthesis of sugars in Sc-CO₂.⁵⁻⁹ So the **aim of this work** is to develop the synthesis of sugars esters catalyzed by lipases in Sc-CO₂.

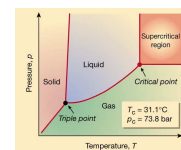


Fig 1. Pressure-temperature phase diagram for CO₂

Esterification of D-Mannose catalyzed by lipase

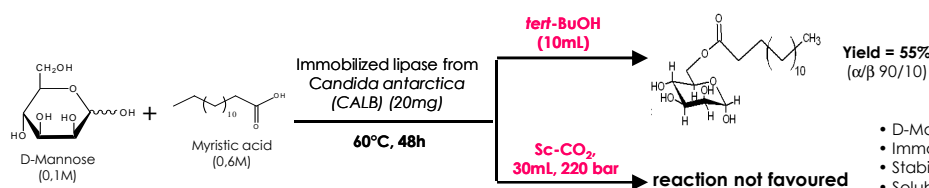
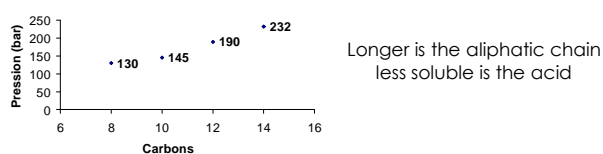


Fig 2. High pressure vessel

Solubility of aliphatic acids in Sc-CO₂ at 60°C



Effect of Sc-CO₂ on D-Mannose

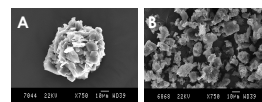


Fig 3. SEM pictures of D-Mannose particles before (A) and after (B) exposure to Sc-CO₂ (220 bar).

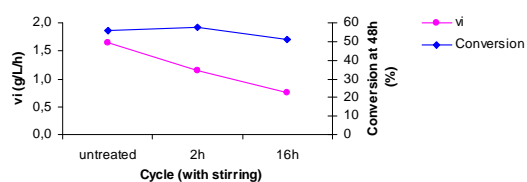
A: particles size 50-107 μm
B: particles size 17-35 μm

Pressurization/depressurization has strong effect on D-Mannose and leads to the decrease of particles size

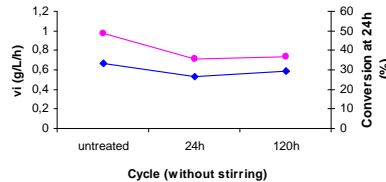
Stability of immobilized lipase from *Candida antarctica* in Sc-CO₂

Before performing reactions, the immobilized lipase from *Candida antarctica* (CALB) was incubated in Sc-CO₂ at 200 bar and 60°C for different times and several cycles. The same lipase preparations, recovered from the reactor after depressurization, were used as catalysts for esterification reactions of D-Mannose with myristic acid in *tert*-BuOH at 60°C and atmospheric pressure. For comparison the same esterification reaction was also catalyzed by non-incubated lipase (untreated). (vi = initial reaction rate)

Effect of Sc-CO₂ on lipase activity



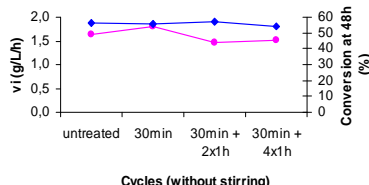
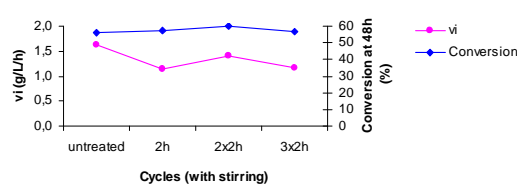
Activity is preserved, low effect on conversion but **decrease** of initial reaction rate. Destruction of the enzyme support: effect of stirring or depressurization?



Activity is preserved, low effect on conversion and initial reaction rate. No effect of depressurization

No effect of Sc-CO₂ on lipase activity
Strong effect of stirring leading to destruction of the enzyme support

Effect of cycles of pressurization/depressurization



Activity is preserved after several cycles of pressurization/depressurization
Low effect on initial reaction rate and conversion

Conclusions

This preliminary study allowed us to assess the influence of various parameters such as solubility of acids in Sc-CO₂ and effect of Sc-CO₂ on D-Mannose and CALB. The enzymatic synthesis of sugar esters in Sc-CO₂ seems to be a promising approach but other factors still need to be evaluated (influence of water in medium, pressure, temperature...) in order to favour esterification reaction in such media.

References

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