Lipase assisted synthesis of potential bio-based surfactants starting from lignocellulosic carbohydrates

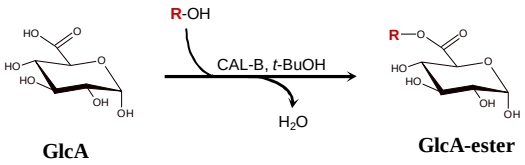
G. Richard [(a),(b)], **K. Nott**[(a),(b)], **M. Paquot**[(a)], **J.-P. Wathelet**[(b)]

[(a)] *Unit of Biological Chemistry, Gembloux Agro-Bio Tech, Liège University, Passage des Déportés, 2, B – 5030 Gembloux, Belgium*
[(b)] *Unit of General and Organic Chemistry, Gembloux Agro-Bio Tech, Liège University, Passage des Déportés, 2, B – 5030 Gembloux, Belgium*

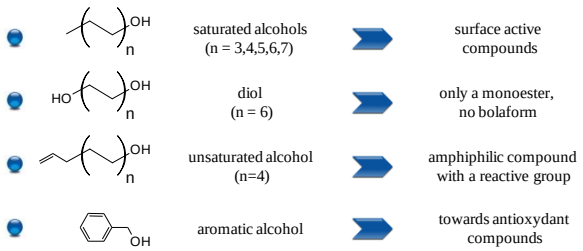
Surfactants represent an important class of compounds with many applications, especially in the food and beverage industries (emulsion formation and stabilization, antiadhesive and antimicrobial activities)[1]. White biotechnology offers efficient tools for synthesising new active compounds. Indeed, the use of enzymes as biocatalysts provides an interesting alternative to the chemical route that often requires high reaction temperatures and suffers from a lack of specificity, resulting in complex mixtures. Among all the biocatalysts available, lipases are one of the most interesting enzymes for industry[2,3]. In parallel, raw material is also an important parameter. Due to the ongoing depletion of petroleum reserves, its increasing price and various environmental aspects, using renewable or biomass resources is unavoidable.
Within the frame of a biorefinery project, we focused on the lipase-assisted esterification of glucuronic acid (GlcA). This carbohydrate obtained from hemicellulose degradation can react with fatty alcohols in the presence of the *Candida antarctica* lipase B (CAL-B), leading to potential surface active compounds.

Synthesis of various GlcA esters

The figure below illustrates the general procedure followed to synthesize GlcA esters in the presence of CAL-B. This lipase and the *t*-BuOH solvent were demonstrated to be the most suitable. Noticeably, no reaction occurs with galacturonic acid, which differs from GlcA only by its hydroxyl orientation on C4.

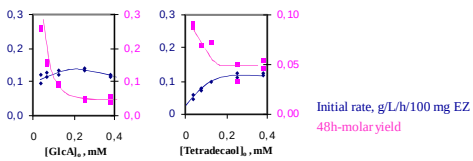


Depending on the acyl donor used, the structure of the synthesised esters can be modulated resulting in different potential properties. The alcohols successfully tested, and their potential applications are listed below.



Influence of initial concentrations

The graphs below show the influence of the initial GlcA and tetradecanol concentrations on the yield and initial rate of the GlcA-O-C14 synthesis.



Increase of the initial tetradecanol concentration leads to higher initial rate and is maximum at the saturated C14OH concentration determined to be 0.25 M. However, 48h-yield decreases.

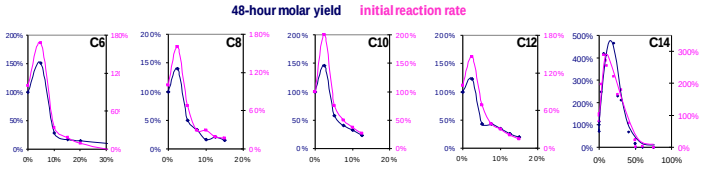
With GlcA, initial rates are hardly influenced, since the concentrations tested are higher than the GlcA solubility (4mM). As for the yields, they are higher at low $[GlcA]_0$.

For the synthesis of GlcA-O-C14, performing the reaction at 0.04M GlcA and 0.25M tetradecanol results in a 48h-molar yield of 30% with an initial rate of 1.2 g/L/h/g of enzyme. The steady state is reached after 9 days, with a yield of 50%.

Influence of co-solvent addition

The main drawback of carbohydrate modification is their low solubility in organic media. The use of the polar solvent *t*-BuOH enables a limited solubilisation of GlcA.

To further increase the GlcA solubility, and thus the esterification rate, several co-solvents were tested. Pyridine was shown to be the best. With alcohols from C6 to C14, the addition of 5 % (V/V) of pyridine leads to an improvement of 150-300% and of 120-500% for the initial rate and the 48-yield respectively.



Several other co-solvents were tested (DMSO, dioxane, triethylamine, DMF, DMI), but none lead to a better or similar improvement as when pyridine was used.

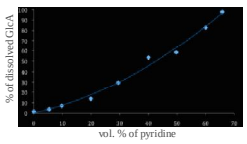
In the case of tetradecanol, performing the reaction at 5-10% (V/V) of pyridine results in a 48h-molar-yield of 40% and an initial rate of 5 g/L/h/g of enzyme, which is the best result we have obtained.

Towards an explanation of the role of pyridine

Surprisingly, further addition of pyridine did not lead to a linear increase of the initial rate. A bell-shape curve was observed instead. To try to understand this result, the GlcA solubility and the lipase activity were evaluated as a function of the volumetric percentage of pyridine added.

Determination of the GlcA solubility as a function of the percentage of pyridine added

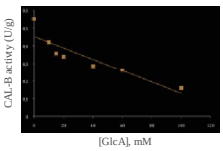
The solubility was measured from *t*-BuOH solutions saturated in GlcA and differing by the volumetric percentage in pyridine. After stirring over-night, the solubilized GlcA in the supernatant was quantified by RP-HPLC.



As expected, it is observed that the amount of GlcA dissolved in *t*-BuOH increases with the percentage of pyridine added. As a consequence, the initial rate increases with the amount of pyridine added.

Measurement of the activity of CAL-B

Pyridine could be expected to inhibit CAL-B. However, we have already reported[4] the use of pyridine up to 20% V/V without the negative effect as observed in this study.



The graph on the left shows the pNPB hydrolytic activity of CAL-B as a function of the GlcA amount introduced. The results show that CAL-B is inhibited by GlcA. Consequently, initial rate will decrease with increasing percentage of pyridine. Lipase inhibition by small polar acidic compounds has already been observed with acetic acid or lactic acid [5].

CAL-B is an efficient tool for the esterification of GlcA in *t*-BuOH. Different kind of surface active compounds are obtained when various alcohol structures are used. Reactive groups can be introduced to enable subsequent modifications. Yields and initial rates are increased by adjusting the initial concentrations in GlcA and in acyl acceptor. The addition of pyridine as co-solvent was shown to be very valuable for further improvement. However, the amount of pyridine introduced must be carefully optimised. Indeed, the addition of pyridine increases the GlcA solubility but also indirectly inhibits the lipase due to too high GlcA dissolved concentrations.

This work was carried out in the framework of the « excellence program » TECHNOSE supported by the Walloon Region (BELGIUM).
The Walloon Region is gratefully acknowledged for its financial support.

[1] Kralova I. et al. *J. Journal of Dispersion Science and Technology* **2009** 30 : 1363-1383
[2] Hasan F. *Enzyme and Microbial Technology* **2006** 39: 235-251
[3] Houde A. *Applied Biochemistry and Biotechnology* **2004** 118: 155-170
[4] Brognaux A. *Journal of molecular catalysis B: Enzymatic* **2010** submitted
[5] Bousquet M.P. *Journal of Biotechnology* **1999** 68 : 61-69