CO_2 AS NH_2-BLOCKING GROUP IN ENZYMATIC INTERESTERIFICATION REACTIONS OF ALCOHOLS CONTAINING AMINO GROUPS

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Enzymatic catalysis in supercritical CO_2 is still in development. Although some enzymes lose their activity, CALB (CLEC Antarctica Lipase B) is one of the enzymes that remain active. The activity loss of enzymes in CO_2 can partly be explained by reversible formation of carbamates in CO_2. It is likely that also substrates containing amino groups will form carbamates with CO_2 and are therefore blocked for enzymatic reaction. In a high-pressure set-up the selectivity of CALB towards amino and alcohol groups is tested at different pressures. Although these reactions do not show complete inhibition of the amidification reaction at higher pressures, a significant decrease is achieved. As the enzyme remains its activity under the tested conditions, the decrease in formation of product can be ascribed to blocking of the amino group by CO_2.

INTRODUCTION

Enzymatic reactions in supercritical CO_2 are a relatively new area. Conventionally, enzymatic reactions are performed in aqueous solutions, but for some decades it is known that enzymes are also active in organic solvents and supercritical CO_2 [1,2]. As a result of the current awareness for the environmental aspects, supercritical CO_2 offers an interesting alternative for organic solvents. Furthermore, $scCO_2$ has tunable physical and chemical properties by changing temperature and/or pressure, which makes it possible to tune to the proper reaction environment.

The fact that many enzymes stay active in $scCO_2$ expands the scope of enzymatic reactions tremendously. A possible reason for some enzymes to lose their activity in $scCO_2$ is the formation of carbamate of the CO_2 with a terminal amine of the enzyme (Figure 1).

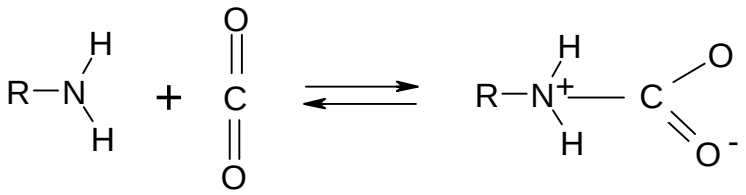

Figure 1 Carbamate formation of amine and carbon dioxide

This formation of carbamate occurs at higher pressure and is known to be reversible [3], which could make CO_2 an ideal NH_2-blocking group in reactions. Presently, most problems to

protect terminal amino groups are within the binding strength. The binding can either be not strong enough to protect the terminal amino group, or the blocking effect is too good but is not reversible. Using CO_2 at enhanced pressures for a reversible formation of carbamates could be a good solution for these problems.
In this work, the esterification of 4-aminophenol with vinyl acetate has been studied from atmospheric pressure up to 200 bars at 50 °C. The enzyme used is crystallized Candida Antarctica lipase B. This enzyme remains active in CO_2 and is able to catalyze reactions with both alcohol and amines.

EXPERIMENTAL
CLEC-Candida Antarctica lipase B was purchased from Altus Biologics. Before use, the CLEC was washed with tert-amyl alcohol and 1,4-dioxane for at least three times. The reactions at atmospheric pressure were performed in dioxane at 50°C in a stirred 10 ml reaction vessel. The reactions under pressure (100-200 bar) were performed in stirred autoclaves. The volume of these autoclaves was 100 ml. In the reactors 10 ml dioxane was added to act as a CO_2-expanded solvent. The concentration of 4-aminophenol and vinyl acetate used in these experiments was 100mM and 200mM, respectively. The CLEC concentration used was 0.5 mg/ml. The reaction time of the experiments varied between 3 hours to 23 hours. The experimental set-up is shown in figure 2. After performing the reaction, pressure was released and samples were taken and analysed with GC-MS.

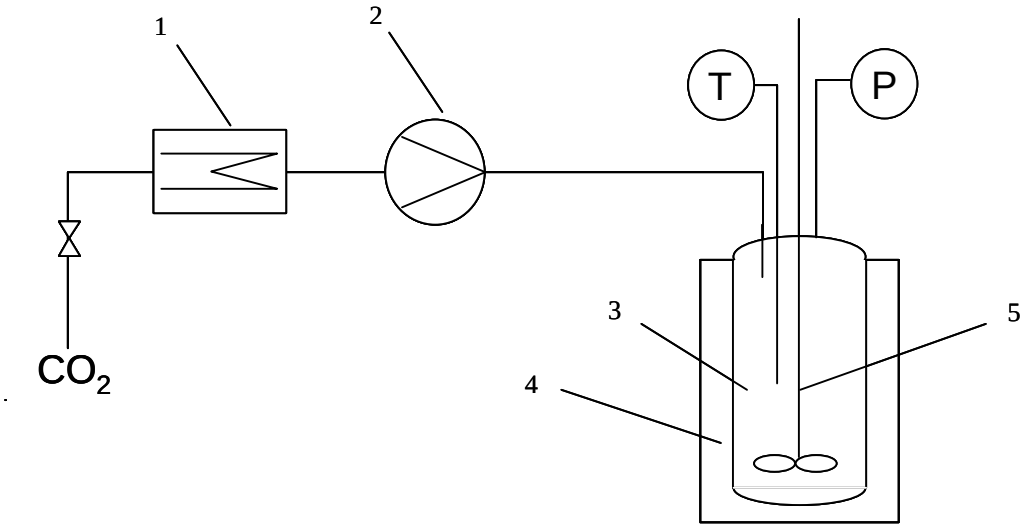

Figure 2. Schematic drawing of the high-pressure vessel. (1) cooler (2) pump (3) high-pressure vessel (4) cooling/heating jacket (5) magnetically coupled stirrer

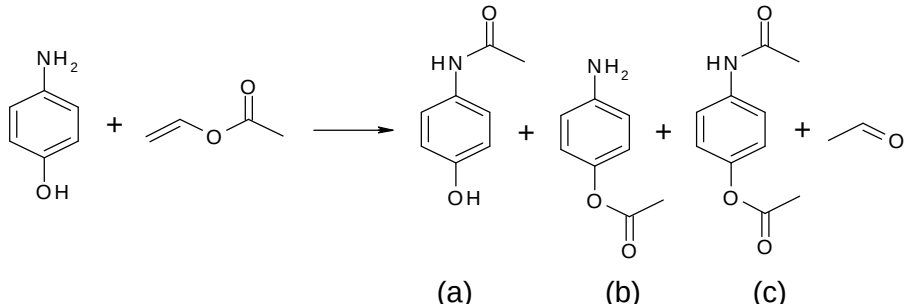

Figure 3 Model reaction of the esterification / amidification of 4-aminophenol with vinyl acetate

RESULTS

The reaction to be performed is the esterification / amidification of 4-aminophenol with vinyl acetate (Figure 3). The expected products are respectively acetaminophen (a), acetic acid 4-amino-phenyl ester (b) and acetic acid 4-acetylamino-phenyl ester (c). The last product mentioned does not appear in GC-MS analyses. Instead of this product, a compound with a mass of 163 is formed (Figure 4A). There are some possibilities which product this could be (Figure 4B), but further analysis is needed to determine the real nature of the product. In this report, this product is called MS163.

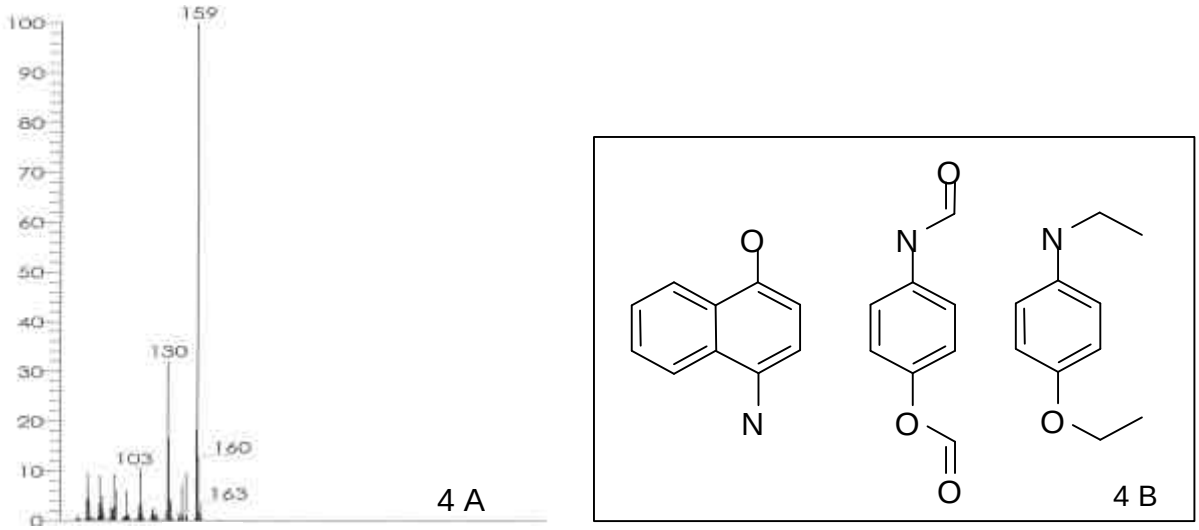

Figure 4A Mass spectrum of compound MS163
Figure 4B Possible molecular structures of compound MS163

The conversion of this reaction is very low. Products b and c are present in such a low concentrations or not present at all that they are not given in the results. The resulting concentrations of acetaminophen and MS163 are given in Figure 5.

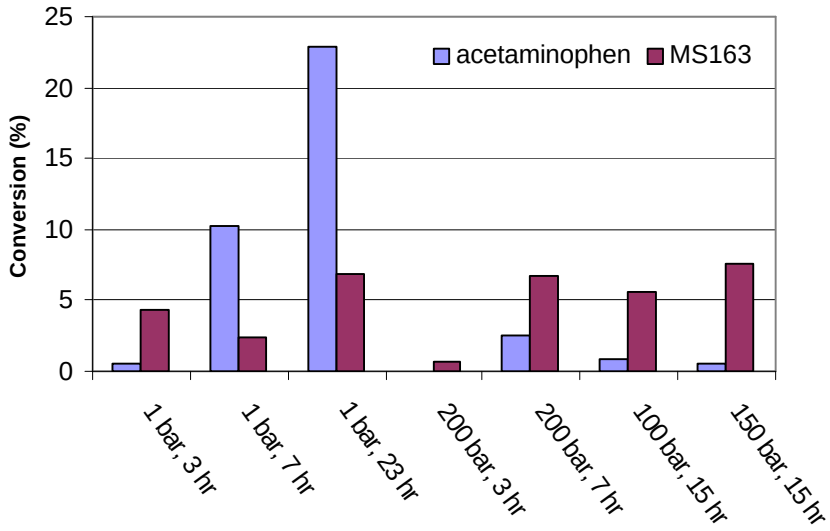

Figure 5 Reaction products at different pressure and the reaction times of the experiments

The main product formed at low pressure is acetaminophen. After 7 hours a conversion of about 10 % is reached and after 23 hours the conversion is increased to 23 %. At higher pressure, the conversion to acetaminophen is clearly suppressed, as the highest conversion reached is about 3 %. On the other hand, MS163 is formed at higher reaction rates at higher pressures. At 150 bar and 15 hours about the same conversion is reached as at 1 bar and after 23 hours. The figure clearly shows that it is possible to suppress the main reaction of the enzyme and increase the production of another by-product almost exclusively.

CONCLUSION

This enzyme in CLEC form is a suitable enzyme to perform esterification/amidification reactions in high-pressure CO_2, as it remains active. With increasing pressure the production of acetaminophen is suppressed, although the blocking effect is not 100%, a clear decrease in amide formation is shown. Also, the formation of the by-product is increased and formed almost exclusively. This shows a change in selectivity of the enzyme in CO_2 from the main product to a by-product, which is formed to a substantially lesser extent at atmospheric conditions.

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