COMMUNICATION

# Enzymatic glycosidation in dry media under microwave irradiation

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**Glycosidase catalysed reversed hydrolysis and transglycosidations in dry media under focused microwave irradiation and classical heating conditions are described.**

## Introduction

Since the last decade enzymatic methods have become commonly used in carbohydrate synthesis, among them glycohydrolase [E.C. 3.2.1.] mediated reactions allow (predictable) stereo- and regio-selective synthesis of glycosides by transglycosidation[1] or reversed hydrolysis.[2] The glycosidases responsible for *in vivo* degradation of oligo- and poly-saccharides are able to catalyse glycoside synthesis under conditions of low water/high alcohol concentration, in the presence of an appropriate glycoside donor (phenyl-, nitrophenyl-glycoside *etc*.). The major drawback of this reaction is the hydrolysis of the donor and/or the product. As a consequence, yields do not exceed 40%. Likewise, reversed hydrolysis, which is an equilibrium controlled reaction, also gives poor yields. Addition of a water miscible organic co-solvent,[2b,f] two-phase reaction mixtures[3] and recent use of an alcohol/acceptor as solvent (with 10% water)[2c] can increase the yield of glycosidation.

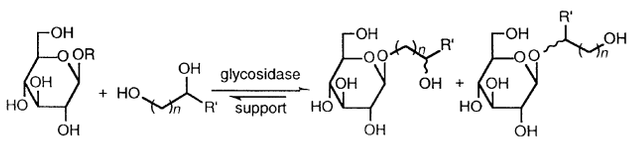
## Results and discussion

The aim of this work is to show that it is possible to shift the equilibrium by decreasing the amount of water and by working under dry conditions, *i.e.* with solid supports instead of organic solvents, and using a limited excess of acceptor. Indeed, the temperature effect allows acceleration of these often very slow reactions, while new thermophile and thermostable glycosidases allow the reactions to be performed at 75–80 °C.[4]

According to our previous work,[5] microwave activation used instead of classical heating in many cases improved the kinetics, the selectivity and the yields of enzyme mediated reactions. The use of a microwave monomode reactor allows a more homogenous heating of dry reaction media with high precision in the control of temperature, better energy usage and efficient elimination of secondary reaction products.

Some preliminary (trans)glycosidations (Scheme 1) were performed using almond-β-glucosidase, β-glycosidase present in crude homogenates of *Sulfolobus solfataricus* and *Pyrococcus furiosus* glucosidase. The synthesis by reversed hydrolysis was performed with glucose **1** and hexane-1,6-diol **4**, while transglycosidation was studied with phenyl-β-D-glucoside **2** and cellobiose **3** as donors and propane-1,2-diol **5** as acceptor. A dry reaction medium (water activity $a_w$ 0.75–0.95) was obtained by co-immobilisation of enzyme, donor and acceptor from water or acetonitrile–water solution. The reactions were performed in an open system, except entry 10 where a closed system was used. The results are given in Table 1.

Reversed hydrolysis catalysed by almond-β-glucosidase was studied using type C aluminium oxide as the support whose pH is close to that of the enzyme's optimal activity (pH 5). The maximum conversion was 16% after 1 h under microwave irradiation or after 6 h under classical heating (entries 1 and 2), while it was 60% after 5 days at 50 °C using 4 equiv. of acceptor in acetonitrile–water (9:1) mixture.[2c] In our experiments at 80 °C, however, the reaction time could not be prolonged due to the instability of this glucosidase. Transglycosidation with almond-β-glucosidase was not successful due to hydrolysis of the phenyl-β-D-glucoside, donor **2**, during the impregnation step.




R = H **1** R' = H *n* = 5 **4**
R = Ph **2** R' = $CH_3$ *n* = 1 **5**
R = 4-OGlc **3**
R' = H *n* = 6 **6**
R' = $CH_3$ *n* = 1 **7**
**8**

**Scheme 1**

However, thermostable enzymes like the crude homogenate of *Sulfolobus solfataricus* and recombinant β-glucosidase from *Pyrococcus furiosus* were successfully applied in transglycosilation reactions. The yield in the *Sulfolobus solfataricus* catalysed transesterification of phenyl-β-D-glucoside **2** was quantitative within 2 h at 110 °C with 10 equiv. of acceptor (entry 3) while it was 60% after 40 h under classical conditions (75 °C and 100 fold molar excess of acceptor in acetonitrile–water mixture).[6] However, the selectivity for primary *versus* secondary OH (products **7** and **8**) was found to be unchanged (4:1) and no enantioselectivity for the acceptor was observed. When lyophilised cells were used as support (entry 3) this system could not be reused, but when transglycosidation was performed in the presence of mineral support, here neutral aluminium oxide (entries 4–13), co-impregnated from water suspension of cells and donor, reusing the biocatalyst was found to be possible under microwave or classical heating conditions as well (entries 8, 9 and 12). Almost complete conversion (81% of conversion with 8% of hydrolysis) was obtained under microwave irradiation (entry 7) after 1 h, whereas in the experiment with the reused cells adsorbed on aluminium oxide, the conversion was slightly lower (55%, entry 8).

The commonly used temperature of 80 °C is insufficient for water elimination which leads to donor hydrolysis. The initial rates were also higher under microwave conditions when compared with classical heating (entries 11 and 13). In the closed system (entry 10) hydrolysis was the main reaction.

*Pyrococcus furiosus*-β-glucosidase catalysed transglycosilation of cellobiose **3** was complete within 3 h (95% of donor conversion) with 18% of hydrolysis.

## Conclusion

All the glycosidations performed in dry media were found to be faster than those described in the literature. The advantage of

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**Table 1** Glycosidase catalysed (trans)glycosidations of donors **1**, **2** and **3** under microwave irradiation (MW) and classical heating conditions (Δ)

| Entry | Donor | Acceptor (mmol) | *t*/h | *T*/°C | Support | Method | Conversion (%) | Yield of products (%) | | | Hydrolysis (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **6** | **7** | **8** | |
| 1 | **1** | **4** (2) | 1 | 80 | Type C $Al_2O_3$ | MW | 16 | 16 | | | |
| 2 | **1** | **4** (2) | 6 | 80 | Type C $Al_2O_3$ | Δ | 17 | 17 | | | |
| 3 | **2** | **5** (10) | 2 | 110 | No support | MW | 97 | | 77 | 20 | 0 |
| 4 | **2** | **5** (2) | 2 | 110 | Neutral $Al_2O_3$ | MW | 77 | | 62 | 15 | 0 |
| 5 | **2** | **5** (2) | 0.5 | 110 | Neutral $Al_2O_3$ | MW | 67 | | 49 | 11 | 7 |
| 6 | **2** | **5** (2) | 0.5 | 110 | Neutral $Al_2O_3$ | Δ | 47 | | 31 | 18 | 8 |
| 7 | **2** | **5** (2) | 1 | 95 | Neutral $Al_2O_3$ | MW | 81 | | 57 | 16 | 8 |
| 8[a] | **2** | **5** (2) | 1 | 95 | Neutral $Al_2O_3$ | MW | 55 | | 38 | 9 | 8 |
| 9[a] | **2** | **5** (2) | 2 | 95 | Neutral $Al_2O_3$ | Δ | 62 | | 40 | 9 | 13 |
| 10[b] | **2** | **5** (2) | 1 | 95 | Neutral $Al_2O_3$ | Δ | 100 | | 19 | 5 | 76 |
| 11 | **2** | **5** (2) | 2 | 80 | Neutral $Al_2O_3$ | MW | 52 | | 36 | 6 | 10 |
| 12[a] | **2** | **5** (2) | 2 | 80 | Neutral $Al_2O_3$ | MW | 40 | | 28 | 8 | 4 |
| 13 | **2** | **5** (2) | 2 | 80 | Neutral $Al_2O_3$ | Δ | 17 | | 12 | 3 | 2 |
| 14 | **3** | **5** (2) | 3 | 100 | Eupergit | MW | 95 | | 63 | 15 | 17 |

[a] Biocatalyst (cells/support) reused twice. [b] Reaction performed in a closed system.

microwave irradiation when compared to classical heating is evident. In particular, the transglycosidations result in complete conversion within 2–3 h, while hydrolysis is lowered to 10% and the excess of acceptor to only 2 equiv. The optimal conditions for enzymatic reactivity and stability under microwaves are 95 °C with water activity $a_w$ 0.80 and using an open system.

## Experimental

### Materials and methods

Almond-β-glucosidase (activity 7 U $mg^{-1}$ solid) was purchased from Sigma. *Sulfolobus solfataricus* crude homogenates were prepared by A. Trincone, Naples, Italy, and *Pyrococcus furiosus*-β-glucosidase immobilized on Eupergit (activity 400 U $mg^{-1}$ immobilizate) was a gift from L. Lischer, Braunschweig, Germany. Glucose, cellobiose and phenyl-β-D-glucoside were supplied by Fluka, neutral $Al_2O_3$ (pH 7.5) by Prolabo and type C $Al_2O_3$ (pH 4.6) by Degussa. All chemicals were commercially available. Solvents were used without any further purification.

### Microwave equipment

Reactions were performed in a monomode microwave reactor with focused electromagnetic field (Synthewave 402, Prolabo), fitted with a stirring system and an IR pyrometer.

### Analytical methods

NMR Spectra were recorded on Bruker instruments at 400 MHz in [$^2H_4$]methanol with $SiMe_4$ as an internal standard. Reactions were followed by GC after silylation on a 6000 Vega Series with FID detector, Spectra-Physics SP 4290 integrator and a OV1 column (12 m); detector 300 °C, injector 290 °C and column temperature in the range from 150 to 280 °C (10 °C $min^{-1}$). Water activity measurements were performed on Novasina Humidat IC-3 instrument (Suisse).

### Silylation procedure[7]

A sample (1 mg) was dissolved in dry pyridine (0.5 ml) and Hydrox-Sil reagent (0.15 ml) was added. The mixture was heated at 80 °C during 30 min, cooled to room temp., pyridine was removed by evaporation and the crude products were analysed by GC.

### Enzymatic glycosidation

Glucose (1 mmol) and almond-β-glucosidase (20 mg) were dissolved in water (1 ml) and impregnated on type C $Al_2O_3$ (pH 4.6, 1 g). The mixture was then dried under vacuum ($p \leq 5$ mmHg), then hexane-1,6-diol (2 mmol) was added and the samples were heated for the times indicated (Table 1). After cooling, the mixture was washed with methanol and the product **6** was analysed by GC after silylation.

### Enzymatic transglycosidation

To a suspension of *Sulfolobus solfataricus* cells (1 g) in water (1 ml) was added a solution of phenyl-β-D-glucoside (1 mmol) in a $CH_3CN$–water mixture (3:1, 1 ml). The final suspension was impregnated on neutral $Al_2O_3$ (pH 7.5, 1 g) and lyophilized. In the case of *Pyrococcus furiosus*-β-glucosidase, it was immobilized on Eupergit, cellobiose (1 mmol) was impregnated on 250 mg of immobilizate. Then propane-1,3-diol (2–10 mmol) was added and the mixture was irradiated in the microwave reactor as described in Table 1, or heated in a thermostatted oil bath. After cooling, the mixture was extracted with methanol and the products **7** and **8** were analysed by GC after silylation. The ratio and diastereomeric composition of products **7** and **8** were determined from $^1$H NMR shifts of anomeric signals ($\delta$ 4.27 and 4.30 for **7** and $\delta$ 4.41 and 4.43 for **8**).


## Acknowledgements

We are grateful to Dr Gabin Vic for useful discussions on almond-β-glucosidase catalysed reactions. We also thank Dr Lutz Fischer for a sample of *Pyrococcus furiosus*-β-glucosidase.


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*Paper* 7/00144D
*Received* 7*th January* 1997
*Accepted* 13*th February* 1997