Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 340 (2005) 2097–2103
Carbohydrate RESEARCH

Ultrasound-accelerated enzymatic synthesis of sugar esters in nonaqueous solvents

Yong-mei Xiao,[a,b] Qi Wu,[a] Ying Cai[a] and Xian-fu Lin[a,*]

[a]*Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China*
[b]*Department of Chemistry, Zhengzhou Institute of Technology, Zhengzhou 450052, People's Republic of China*

Received 13 October 2004; accepted 13 June 2005

Abstract—Comparative studies of enzymatic synthesis of glucose esters under ultrasound and shaking were carried out in nonaqueous media. The influence of solvents, enzymes, chain length of the acyl donors, the power of the ultrasound bath, and intermittent ultrasound on the enzymatic synthesis was investigated. Among the eight solvents selected, pyridine was the most appropriate with alkaline protease from *Bacillus subtilis* whether under ultrasound or shaking. The acceleration effect of ultrasound with Novozym 435 and the alkaline protease from *B. subtilis*-catalyzed transesterification increased with the chain length of acyl donors, decreasing from C_{10} to C_4. We also investigated the influence of the power (50, 100, and 120 W) of the ultrasound irradiation and the manner of operation (continuous ultrasound, 10 min ultrasound/20 min shaking without ultrasound) on the transesterification. The results showed that higher power and continual operational gave the better acceleration. Ultrasound did not change the character and selectivity of the enzyme in the transesterification.

Keywords: Ultrasound; Enzymatic synthesis; Sugar ester; Nonaqueous solvent

1. Introduction

Sugar esters are biodegradable and nontoxic, and can be synthesized from renewable sources. The selective acylation of sugars is difficult because of the similar reactivity of the hydroxyl groups, and multi-step synthesis based on protection–deprotection reactions is necessary. As compared with the conventional chemical catalysts, enzyme catalysis can be performed under milder and simpler process conditions, and with higher selectivity. Such hydrolases as protease and lipase are widely used to catalyze transesterification of carbohydrates.[1–4] However, many enzymatic acylation reactions of sugars in nonaqueous media need long reaction times or give low yields.[5–9] Various methods, such as solvent engineering, immobilization of the enzymes, lyophilization of the enzyme, application of mechanical waves, and so on, have been done in attempts to improve the efficiency of enzymatic synthesis. For example, Yan et al. used a low-boiling-point azeotrope to obtain glucose octadecanoates in 93% yields after 48 h.[10] Cao et al. reported the synthesis of 6-*O*-hexadecanoyl-D-glucose in a mainly solid-phase system with an immobilized enzyme, with highest conversion of 84% in 24 h.[11] GeloPujic et al. investigated the esterification by lipase of some α-D-glucopyranosides in dry media under focused microwave irradiation. The advantages of performing the reactions in a microwave reactor are evident in all cases, either in terms of yields and (or) purities of products.[12] Roy and Gupta studied the effects of microwaves on protease-catalyzed esterification and transesterification, and in all cases, microwave irradiation was found to increase the initial reaction rates by 2.1–4.7 times at all hydration levels.[13] The stability of Novozym 435 in an organic medium under microwave radiation was superior as compared to conventional heating.[14]

Ultrasound is a mechanical rather than an electromagnetic wave. The chemical and physical effects of

* Corresponding author. Tel.: +86 571 87953001; fax: +86 571 87952618; e-mail: llc123@css.zju.edu.cn

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.06.027

ultrasound arise from cavitational collapse, which produces extreme conditions locally and thus induces the formation of chemical species not readily attained under conventional conditions, and driving a particular reactivity mode.[15] Ultrasound as an environmentally benign method has found many interesting applications in organic chemistry.[16,17] This technique derives its success from simple rate enhancement of specific reactions.[18] Because the mechanical effect of the acoustic waves enhances heterogeneous reactions and readily forms transient reactive species, ultrasound is also a useful tool in enzymatic reactions of carbohydrates, and displays a beneficial role in term of better rates, yields, and chemo-, regio-, and stereoselectivities.[19] Examples include ultrasound-accelerated enzymatic resolution of ethyl 3-hydroxy-3-phenylpropanoate[20] and enzymatic acylation of azido alcohols.[21] However, its application to enzymatic reactions is still little explored,[22] and no research on the use of ultrasound to accelerate the enzymatic synthesis of sugar esters has been reported.

In this work, continuing our research on the regioselective acylation of sugars,[23,24] we evaluated the influence of ultrasound on the transesterification of glucose and divinyl dicarboxylates in organic solvents. The influence of other factors on the acceleration effect of ultrasound, including solvent, chain length of acyl donors, the power (50, 100, and 120 W) of the ultrasound irradiation, and the operational manner, and enzyme catalysts were investigated.

2. Results and discussion

Based on the cavitation phenomenon, ultrasound can accelerate chemical transformations, affect the yield of product, and the catalytic activity.[22] Here, we evaluated the influence of ultrasound on the enzymatic synthesis of glucose esters.

2.1. Influence of solvent

First, we investigated the transesterification of glucose with divinylbutanedioate in different solvents by alkaline protease from *Bacillus subtilis*. Figure 1 shows that pyridine is most appropriate for the protease, and the acceleration effect of ultrasound was the best. However, no products were formed when *tert*-butanol and toluene were used as solvents. It is obvious that the activity and specificity of the enzymes depends on the solvents used. Degn et al. reported the efficient synthesis of glucose fatty acid esters catalyzed by *Candida antarctica* lipase in *tert*-butanol, a solvent was unfavorable for the alkaline protease from *B. subtilis* in our study.[25] Other researchers have reported that the enzyme activity in organic solvents was often correlated with solvent hydrophobicity, with the highest activities at high log *P* values. If the solubility of substrate is considered, hydrophobic solvents are not always good because of substrates such as glucose are hydrophilic. Furthermore, the solvent is a critical parameter in terms of cavitation. Figure 1 demonstrates the effects of ultrasound in pyridine, DMF, and *n*-hexane are more obvious than those in 1-4-dixoane, butanone, and acetonitrile. After the optimization of the reactive solvent, pyridine was used in the subsequent reactions.

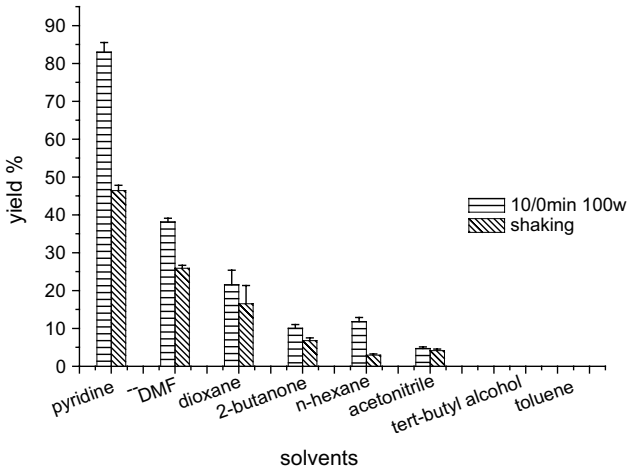

Figure 1. The influence of solvents on the enzymatic transesterification of glucose with divinylbutanedioate. Conditions: 0.2 M glucose, 0.8 M divinylbutanedioate, 10 mg/mL alkaline protease from *B. subtilis*, 50 °C, 3 h, 10/0 min 100 W continual ultrasound and shaking, yields determined by GC.

2.2. Influence of chain length of the acyl donors

We selected glucose as the sugar substrate, three divinyl dicarboxylates with different carbon chain lengths (C_4, C_6, C_{10}) as the acyl donors, and studied the influence of continual ultrasound on the synthesis of sugar esters using these acyl donors under catalysis by the alkaline protease from *B. subtilis*. The results under ultrasonication and shaking are presented in Figures 2–4. Figures 2 and 3 show that continual ultrasound increases the reaction rate and yields, especially with divinylbutanedioate as the acyl donor, where the yield under ultrasonication in the first 2 h was double that under shaking. However, no effect of ultrasound on the enzymatic reaction of divinyl decanedioate (Fig. 4) was observed, and the yields were lower relative to those with divinyl butanedioate and divinyl hexanedioate.

Figures 2–4 also showed that the chain length affected the reaction, whether under ultrasonication or shaking. The reaction rate increased with decreasing chain length of the acyl donor, the yield being the highest for divinylbutanedioate. It might be easier to access the active pocket of the enzyme with decreasing chain length. Pedersen et al. reported that the initial reaction rate increased with decreasing chain length of the acyl donor

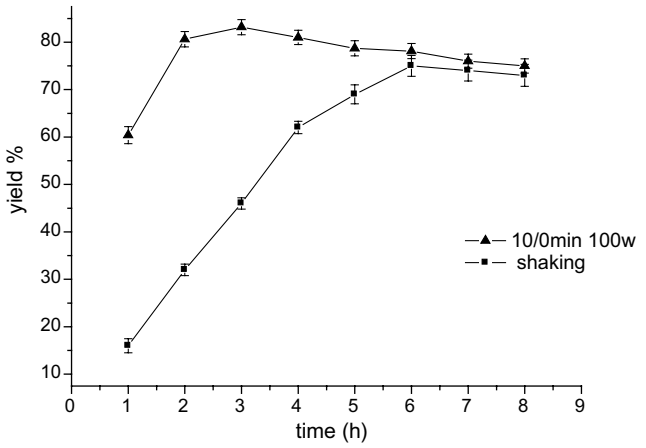

Figure 2. Ultrasound acceleration of the enzymatic acylation of glucose with divinylbutanedioate in pyridine. (▲) 10/0 min, 100 W continual ultrasound; (■) shaking. Conditions: 0.2 M glucose, 0.8 M divinylbutanedioate, 10 mg/mL alkaline protease from *B. subtilis*, 50 °C, yields determined by GC.

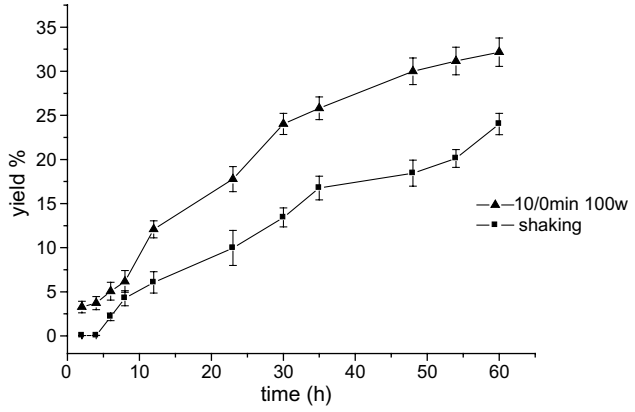

Figure 3. Ultrasound acceleration of the enzymatic acylation of glucose with divinylhexanedioate in pyridine. (▲) 10/0 min, 100 W continual ultrasound; (■) shaking. Conditions: 0.2 M glucose, 0.8 M divinylhexanedioate, 10 mg/mL alkaline protease from *B. subtilis*, 50 °C, yields determined by GC.

for disaccharides when fatty acids (chain length C_4–C_{12}) were used as donors. The highest initial reaction rate and yield were obtained with the shortest chain-length acyl donor.[26] Our results showed that application of ultrasound did not change the trend that the time of reaction is shortened with decreasing chain length of the acyl donor. The enhanced effect of ultrasound is possibly due to acceleration of the collision probability of the enzyme and substrate. Meanwhile, cavitation also accelerated mass transport, so that product diffused faster from the enzymatic site.[27]

The protease from *B. subtilis* was more active with shorter chains. We researched Novozym 435-catalyzed transesterification to compare a lipase with the protease. We chose *tert*-butanol as solvent because a positive correlation between the solubility of glucose and the product yield was observed, and *tert*-butanol was favorable for Novozym 435.[28] Figure 5 shows that ultrasound accelerates the reaction catalyzed by Novozym 435 and the influence of chain length under ultrasound was the same as that by the protease. The reaction of divinylhexanedioate under shaking was faster than that of divinyldecanedioate. However, research reported by Ghoul and co-workers[29] demonstrated that the enzymatic synthesis of fatty acid fructose esters, catalyzed by Novozym 435, gave lower yields of sugar ester when the chain length of the fatty acid decreased. The difference of the influence of chain length could be attributed to the fact that the acyl donors used in these studies were not similar.

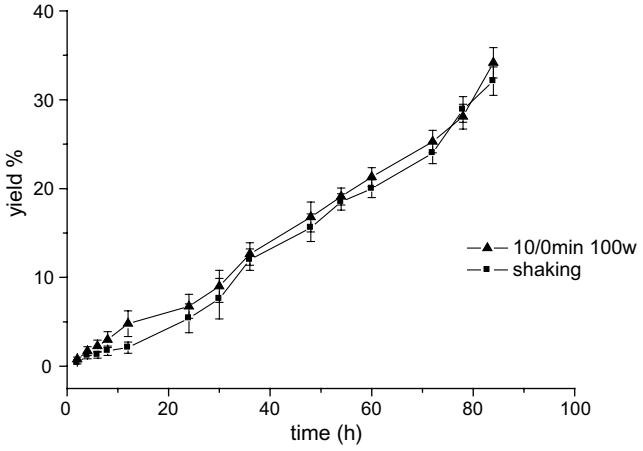

Figure 4. Ultrasound acceleration of the enzymatic acylation of glucose with divinyldecanedioate in pyridine. (▲) 10/0 min, 100 w continual ultrasound; (■) shaking. Conditions: 0.2 M glucose, 0.8 M divinyldecanedioate, 10 mg/mL alkaline protease from *B. subtilis*, 50 °C, yields determined by GC.

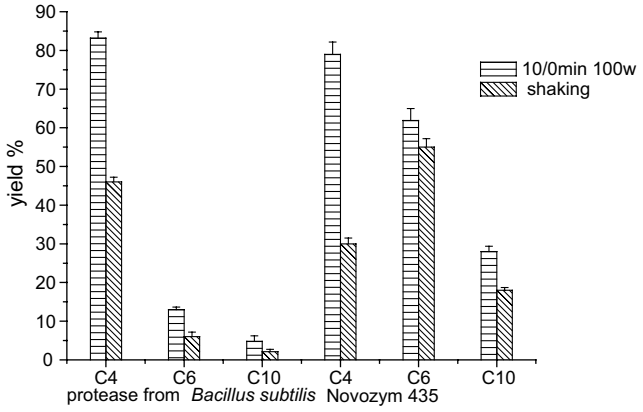

Figure 5. Influence of three different chain-length acyl donors on the synthesis of glucose esters. Conditions: 0.2 M glucose, 0.8 M acyl donors, 10 mg/mL alkaline protease from *B. subtilis*/pyridine, 3 h for C_4, 12 h for C_6, and C_{10}; 10 mg/mL Novozym 435/*tert*-butanol, 12 h for C_4, C_6, and C_{10}.

2.3. Influence of power and intermittence of ultrasound

For reactions under ultrasound irradiation, the operational procedure and ultrasound power were important influencing factors. In this study, we selected three different powers of 20 kHz ultrasound, namely 50, 100, and 120 W. Figures 6 and 7 showed the influence of ultrasound power on the yields of vinyl glucose butanedioate under continual and intermittent ultrasound, respectively. The yields increased with increasing ultrasound power. The intensity of the ultrasound irradiation affected the activity of enzyme. Ishimori et al. reported that the activity of free and immobilized α-chymotrypsin in aqueous media was accelerated with decreasing ultrasound power.[30] Sakakibara et al. reported that inactivation of the enzyme occurred at high ultrasonic intensities, and considered that the significant rate enhancement obtained at lower ultrasound intensities was probably due to a decrease in substrate inhibition and aggregation based on hydrogen bonding of molecules.[31] Brenelli and Fernandes reported that rates of acyl transfer increased 3.5 and 10 times using ultrasonication as compared to magnetic stirring, with lipase Amano PS- catalyzed acylations of 1,2-azido alcohols with vinyl acetate.[21] Other kinetic studies show that sonication increases the activity of the enzyme invertase by 130–150%.[31,32] At low acoustic power, some enzymes, such as those supported on porous silica were not deactivated.[33] Our research showed no inactivation of the enzyme with 20 kHz ultrasound, and the results showed that a suitable ultrasound power was necessary.

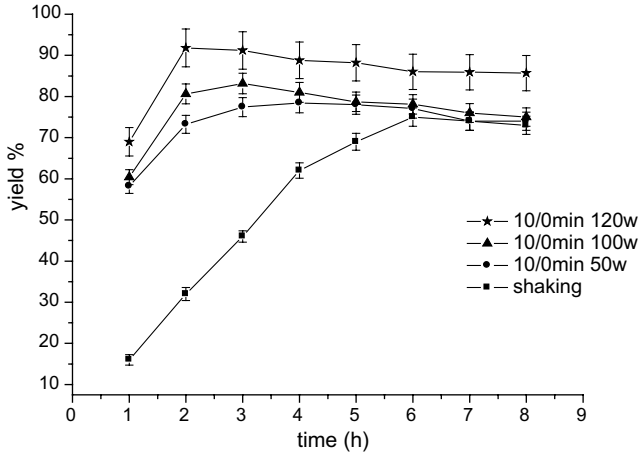

Figure 6. Influence of continual ultrasound on the enzymatic acylation of glucose with divinylbutanedioate in pyridine. (□) 10/0 min, 120 W continual ultrasound; (▲) 10/0 min, 100 W continual ultrasound; (●) 10/0 min, 50 W continual ultrasound. Conditions: 0.2 M glucose, 0.8 M divinylbutanedioate, 10 mg/mL alkaline protease from *B. subtilis*, 50 °C, yields determined by GC.

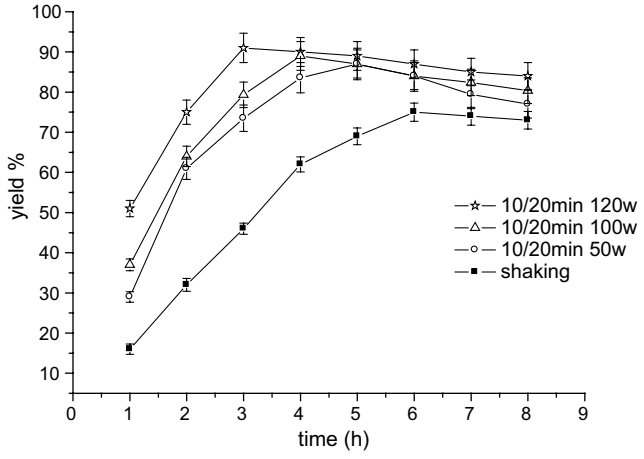

Figure 7. Influence of intermittent ultrasound on the enzymatic acylation of glucose with divinylbutanedioate in pyridine: (□) 10/20 min, 120 W intermittent ultrasound; (□) 10/20 min, 100 W intermittent ultrasound; (○) 10/20 min, 50 W intermittent ultrasound. Conditions: 0.2 M glucose, 0.8 M divinylbutanedioate, 10 mg/mL alkaline protease from *B. subtilis*, 50 °C, yields determined by GC.

The effect of continual ultrasound (Fig. 6) and interval ultrasound (10 min ultrasound/20 min shaking) (Fig. 7) was also investigated. Both Figures 6 and 7 show that ultrasound causes a remarkable acceleration of the transesterification, and the yields under continual or interval ultrasound were higher than that of shaking over the same reaction time. Furthermore, Figures 6 and 7 show that continual ultrasound causes greater rate acceleration than interval ultrasound. For example, the yields under continual ultrasound of 120 W were 70% after 1 h and 94% after 2 h, respectively, whereas the yields were only 50% and 75% under interval ultrasound (10 min ultrasound/20 min shaking). However, the equilibrium yields under continuous and interval ultrasound were nearly equivalent. The results showed that neither continual ultrasound nor interval ultrasound improved the equilibrium conversion.

The same influence of ultrasound power and operational procedure on the conversion of glucose was observed (not shown in this paper). Conversions of glucose under three continual or interval ultrasound powers (120, 100, and 50 W) were higher than that under shaking. A 98% conversion of glucose could be obtained after 2 h in 120 W in an ultrasound bath, whereas only 48% was observed in shaking under the same conditions.

2.4. Influence of the enzyme

The effect of ultrasound on different enzyme-catalyzed reactions is shown in Figure 8. Novozym 435 is an enzyme with high catalytic activity,[28,34,35] but the reaction of glucose with divinyl butanedioate its catalytic activity was lower than that of the alkaline protease, the yield being only 8.5% after 2 h, whereas it was 78% when catalyzed by the alkaline protease under the same ultrasound conditions (100 W, 20 Hz). Neither interval

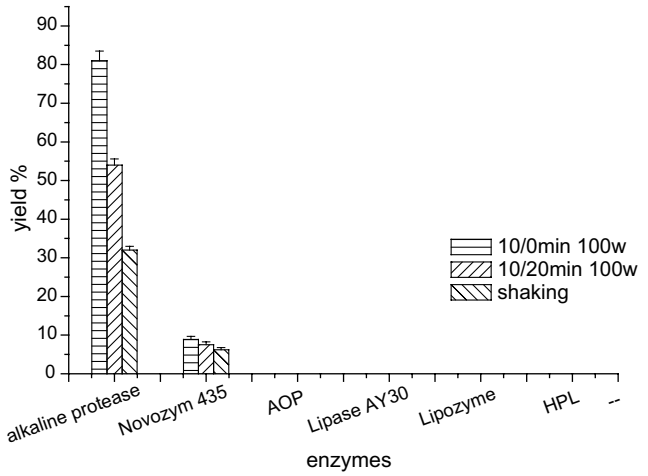

Figure 8. Influence of enzymes on the reaction of glucose and divinylbutanedioate in pyridine under ultrasound (10/0 min 100 W, 10/20 min 100 W) and shaking. Conditions: 2 h for protease and Novozym 435, 8 h for other enzymes.

ultrasound nor continual ultrasound increased the yield and the reaction rate of acylation glucose catalyzed by Novozym 435 in pyridine. It is known that the activity of enzymes in organic solvents is highly dependent on the nature of the solvents. Figure 5 shows that the yield of glucose esters in *tert*-butanol as catalyzed by Novozym 435 was higher than that in pyridine. Degn and Zimmerma reported that the activity of Novozym 435 was 0 μmol min^{-1} g^{-1} in pyridine, inactivation of the enzyme in pyridine being caused by interaction of pyridine with the solid support,[28] thus resembling our results.

We also investigated the catalytic activity of lipozyme, Lipase AY30, HPL, and AOP in with both ultrasound and shaking. GC and TLC showed that there were no products after 8 h with these enzymes. Immobilization of enzymes did not influence the effect of ultrasound. This implies that ultrasound only accelerates the reaction, but does not change the character of the reaction. Control experiments under ultrasound and shaking showed that reaction did not occur without enzymes, and using the enzymes pretreated in boiling water for 2 h for inactivation gave no products after 8 h by GC and TLC. NMR spectra of the products of reaction under shaking and ultrasound indicated that the use of ultrasound in enzymatic synthesis did not change the regioselectivity of acylation reaction.

The influence of ultrasound irradiation on the transesterification of glucose and divinyl dicarboxylates thus showed remarkable acceleration in the acylation of glucose in pyridine catalyzed by alkaline protease from *B. subtilis*. The acceleration effect increased as the chain length of acyl donors decreased from C_{10} to C_4 under catalysis by the alkaline protease from *B. subtilis* in pyridine and Novozym 435 in *tert*-butanol, and it was also influenced by the power of the ultrasound irradiation and the operational procedure. Higher power and continuous operation gave the better effect. The acceleration effects of ultrasound on different enzyme-catalyzed reactions were different, and the ultrasound did not change the regioselectivity of the enzymatic acylation.

3. Experimental

3.1. Materials

Alkaline protease from *B. subtilis* (Wuxi Enzyme Co. Ltd, Wuxi, PR China); Lipozyme, immobilized from *Mucor miehei* (Fluka); Lipase AY30 (Acros); Lipase acrylic resin from *C. antarctica* (Novozym 435, Sigma); Lipase from hog pancreas (Fluka); Protease from *Aspergillus oryzae* (Fluka). Pyridine and other solvents were dried over 3 Å molecular sieves for 24 h prior to use. Divinylbutanedioate, divinylhexanedioate, and divinyldecanedioate were produced and purified as described by the patent literature.[36] Butanedioic acid, hexanedioic acid, decanedioic acid, and vinyl acetate and all other chemicals were of the highest purity commercially available.

3.2. Analytical methods

The samples were analyzed using a Hewlett–Packard GC series 6890, fitted with a split injector (split ratio 1:50, N_2 as carrier gas), a fused silica capillary column (HP-5MS, crosslinked 5% PH ME siloxane, 30 m × 0.25 mm, 0.25 μm film thickness), and a flame-ionization detector. Prior to GC analysis, the glucose derivatives were subjected to precolumn derivatization with hexamethyldisilazane and Me_3SiCl according to a general methodology.[37] Both the injector and the detector were held at 280 °C. For **1** and **2**, the initial oven temperature was 200 °C for 2 min and the temperature was then increased to 240 °C at a rate of 40 °C min^{-1}, and was held at 240 °C for 10 min. For **3**, the temperature increased from 200 to 260 °C at a rate of 40 °C min^{-1}, and was held at 260 °C for 20 min. The internal standard for **1** and **2** was phentriazophos and for **3** it was dicyclohexyl phthalate. The following retention times were observed: 3.3 min (α anomer of glucose), 3.6 min (β anomer of glucose); 7.92 min (phentriazophos); 8.3 min (dicyclohexyl phthalate), 9.7 min (α anomer of **1**), 10.1 min (β anomer of **1**), 10.5 min (α anomer of **2**), 11.2 min (β anomer of **2**); 18.2 min (α anomer of **3**), 18.8 min (β anomer of **3**).

3.3. Transesterification reactions

Glucose (3.6 g, 0.02 M) was dissolved in 100 mL solvent containing 0.08 M divinyl dicarboxylate (C_4, C_6, C_{10}). After adding 1 g alkaline protease from *B. subtilis*

(10 mg mL^{-1}) or other enzymes (10 mg mL^{-1}), the reaction was carried out with shaking at 250 rev min^{-1} and in an ultrasound bath at 50 °C, respectively. Aliquots (0.2 mL) were transferred at timed intervals, and derivatized by adding 0.2 mL hexamethyldisilazane and 0.1 mL Me_3SiCl immediately. The samples were stored at −20 °C for later GC analysis. The products **1**, **2**, and **3** were 6-*O*-vinylbutanedioyl-D-glucose, 6-*O*-vinylhexanedioyl-D-glucose, and 6-*O*-vinyldecanedioyl-D-glucose, respectively. The position of substitution of glucose was proved by ^1H NMR and ^{13}C NMR.[23]

3.3.1. 6-*O*-Vinylbutanedioyl-D-glucose (1). Tan oil; 96% purity by GC; ^1H NMR (Me_2SO-d_6): δ 7.21 (dd, 1H, *J* 6.2, 13.9 Hz, –CH═), 6.7 (br s, 0.58H, β1-OH of D-glucose), 6.37 (br s, 0.42H, α1-OH of D-glucose), 5.25–4.50 (br m, other OH of D-glucose), 4.92 (d, 1H, *J* 13.7 Hz, CH_2═), 4.66 (d, 1H, *J* 5.9 Hz, CH_2═), 4.31 (m, 1.5H, H-6 (1H) and βH-1 (0.5H) of D-glucose), 4 (m, 1H, H-6′ of D-glucose), 3.75 (m, 0.5H, αH-5 of D-glucose), 3.5–3.2, 3.14, 3 (br m, other α or βH of D-glucose), 2.89 (m, 0.5H, βH-2 of D-glucose), 2.7–2.42 (m, 4H, $–CH_2–CH_2–$ of butanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.16, 169.82 (C═O), 141.41 (–OCH═CH_2), 98.51 (–OCH═CH_2), 97.12 (C-1β), 92.52 (C-1α), 76.62 (C-3β), 74.9 (C-2β), 73.68 (C-5β), 73.1(C-3α), 72.37 (C-2α), 70.35, 70.75 (C-4α, β), 69.31 (C-5α), 64.67 (C-6α, β), 28.57, 28.45 (–$(CH_2)_2$–).

3.3.2. 6-*O*-Vinylhexanedioyl-D-glucose (2). Colorless crystals; 100% purity by GC; Mp: 110–111 °C; ^1H NMR (Me_2SO-d_6): δ 7.21 (dd, 1H, *J* 6.3, 14 Hz, –CH═), 6.33 (d, 1H, *J* 4.6 Hz, 1-OH of α-D-glucose), 5.03 (1H, d, *J* 5.6 Hz, H-1 of α-D-glucose), 4.89 (m, 2H, one H of CH_2═ and 4-OH of α-D-glucose), 4.73 (d, 1H, *J* 4 Hz, 3-OH of α-D-glucose), 4.65 (dd, 1H, *J* 1.1, 6.3 Hz, CH_2═), 4.51 (d, 1H, *J* 6.3 Hz, 2-OH of α-D-glucose), 4.27 (d, 1H, *J* 11 Hz, H-6 of α-D-glucose), 3.99 (dd, 1H, *J* 6.3, 11.6 Hz, H-6′ of α-D-glucose), 3.77 (m, 1H, H-5 of α-D-glucose), 3.41 (m, 1H, H-3 of α-D-glucose), 3.13 (m, 1H, H-2 of α-D-glucose), 3 (m, 1H, H-4 of α-D-glucose), 2.44 (t, 2H, *J* 6.6 Hz, $–CH_2–$(COO–CH═CH_2)), 2.31 (t, 2H, *J* 6.9 Hz, $–CH_2–$(COO–glucose)), 1.56 (m, 4 H, other 2 $–CH_2–$ of hexanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.87, 170.43 (C═O), 141.41 (–OCH═CH_2), 98.23 (–OCH═CH_2), 92.44 (C-1α), 73.02 (C-3α), 72.33 (C-2α), 70.72 (C-4α, β), 69.27 (C-5α), 64.12 (C-6α, β), 32.84, 23.92, 23.59 (–$(CH_2)_4$–).

3.3.3. 6-*O*-Vinyldecanedioyl-D-glucose (3). Colorless sheets of crystals; 100% purity by GC; Mp: 101–102 °C; ^1H NMR (Me_2SO-d_6): δ 7.21 (dd, 1 H, *J* 6.3, 14 Hz, –CH═), 6.32 (d, 1H, *J* 4.5 Hz, 1-OH of α-D-glucose), 5.01 (d, 1H, *J* 5.6 Hz, H-1 of α-D-glucose), 4.88 (m, 2H, one H of CH_2═ and 4-OH of α-D-glucose), 4.72 (d, 1H, *J* 4.6 Hz, 3-OH of α-D-glucose), 4.64 (d, 1H, *J* 6.1 Hz, CH_2═), 4.5 (d, 1H, *J* 6.6 Hz, 2-OH of α-D-glucose), 4.26 (d, 1H, *J* 11.2 Hz, H-6 of α-D-glucose), 3.98 (dd, 1H, *J* 6.2, 11.6 Hz, H-6′ of α-D-glucose), 3.75 (m, 1H, H-5 of α-D-glucose), 3.42 (m, 1H, H-3 of α-D-glucose), 3.12 (m, 1H, H-2 of α-D-glucose), 3 (m, 1H, H-4 of α-D-glucose), 2.41 (t, 2H, *J* 7.3 Hz, $–CH_2–$(COO–CH═CH_2)), 2.26 (t, 2H, *J* 6.9 Hz, $–CH_2–$(COO–glucose)), 1.52, 1.25 (m, 12H, other CH_2 of decanedioyl part); ^{13}C NMR (Me_2SO-d_6): δ 172.93, 170.46 (C═O), 141.32 (–OCH═CH_2), 98.03 (–OCH═CH_2), 92.35 (C-1α), 72.96 (C-3α), 72.27 (C-2α), 70.65 (C-4α, β), 69.22 (C-5α), 63.9 (C-6α, β), 33.51, 33.09, 28.54, 28.5, 28.39, 28.32, 24.48, 24.07 (–$(CH_2)_8$–).

References

1. Potier, P.; Bouchu, A.; Gagnaire, J.; Queneau, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 2409–2419.
2. Kim, J.; Haam, S.; Park, D. W.; Ahn, I. S.; Lee, T. G.; Kim, H. S.; Kim, W. S. *Chem. Eng. J.* **2004**, *99*, 15–22.
3. Ferreira, L.; Gil, M. H.; Dordick, J. S. *Biomaterials* **2002**, *23*, 3957–3967.
4. Coulon, D.; Girardin, M.; Ghoul, M. *Process Biochem.* **1999**, *34*, 913–918.
5. Kitagawa, M.; Tokiwa, Y. *Biotechnol. Lett.* **1998**, *20*, 627–630.
6. Kitagawa, M.; Fan, H.; Raku, T.; Raku, T.; Shibatani, S.; Maekawa, Y.; Hiraguri, Y.; Kurane, R.; Tokiwa, Y. *Biotechnol. Lett.* **1999**, *21*, 355–359.
7. Raku, T.; Tokiwa, Y. *Macromol. Biosci.* **2003**, *3*, 151–156.
8. Castillo, E.; Pezzotti, F.; Navarro, A.; López-Munguía, A. *J. Biotechnol.* **2003**, *102*, 251–259.
9. Zhang, X.; Kobayashi, T.; Adachi, S.; Matsuno, R. *Biotechnol. Lett.* **2002**, *24*, 1097–1100.
10. Yan, Y. C.; Bornsheuer, U. T.; Schmid, R. D. *Biotechnol. Bioeng.* **2001**, *78*, 31–34.
11. Cao, L. Q.; Bornscheuer, U. T.; Schmid, R. D. *J. Mol. Catal. B: Enzym.* **1999**, *6*, 279–285.
12. GeloPujic, M.; GuibeJampel, E.; Loupy, A.; Galema, S. A.; Mathe, D. *J. Chem. Soc., Perkin Trans. 1* **1996**, *23*, 2777–2780.
13. Roy, I.; Gupta, M. N. *Tetrahedron* **2003**, *59*, 5431–5436.
14. Réjasse, B.; Lamare, S.; Legoy, M. D.; Besson, T. *Org. Biomol. Chem.* **2004**, *2*, 1086–1089.
15. Cintas, P.; Luche, J. L. *Green Chem.* **1999**, *1*, 115–125.
16. Nuchter, M.; Ondruschka, B.; Jungnickel, A.; Müller, U. *J. Phys. Org. Chem.* **2000**, *13*, 579–586.
17. De Groot, A. H.; Dommisse, R. A.; Lemière, G. L. *Tetrahedron* **2000**, *56*, 1541–1549.
18. Yachmenev, V. G.; Blanchard, E. J.; Lambert, A. H. *Ultrasonics* **2004**, *42*, 87–91.
19. Kardos, N.; Luche, J. *Carbohydr. Res.* **2001**, *332*, 115–131.
20. Ribeiro, C. M. R.; Passaroto, E. N.; Brenelli, E. *Tetrahedron Lett.* **2001**, *42*, 6477–6479.
21. Brenelli, E.; Fernandes, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1255–1259.
22. Wood, B. E.; Aldrich, H. C.; Ingram, L. O. *Biotechnol. Prog.* **1997**, *13*, 232–237.
23. Wu, Q.; Lu, D. S.; Cai, Y.; Xue, X. T.; Chen, Z. C.; Lin, X. F. *Biotechnol. Lett.* **2001**, *23*, 1981–1985.

24. Xiao, Y. M.; Wu, Q.; Wang, N.; Lin, X. F. *Carbohydr. Res.* **2004**, *339*, 1279–1283.
25. Degn, P.; Pedersen, L. H.; Duus, J. O.; Zimmermann, W. *Biotechnol. Lett.* **1999**, *21*, 275–280.
26. Pedersen, N. R.; Wimmer, R.; Emmersen, J.; Degn, P.; Pedersen, L. H. *Carbohydr. Res.* **2002**, *337*, 1178–1183.
27. Hagenson, L. C.; Doraiswamy, L. K. *Chem. Eng. Sci.* **1998**, *53*, 131–148.
28. Degn, P.; Zimmerma, W. *Biotechnol. Bioeng.* **2001**, *74*, 483–491.
29. Soultani, S.; Engasser, J. M.; Ghoul, M. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 725–731.
30. Ishimori, Y.; Karube, I.; Suzuki, S. *J. Mol. Catal.* **1981**, *12*, 253–259.
31. Sakakibara, M.; Wang, D.; Takahashi, K.; Mori, S. *Enzyme Microb. Technol.* **1996**, *18*, 444–448.
32. Sinisterra, J. V. *Ultrasonics* **1992**, *30*, 180–185.
33. Gao, D.; Chen, M.; Liang, H.; Min, Y.; Li, G. *Huanan Ligong Daxue Xuebao, Ziran Kexueban* **1994**, *22*, 70–74; *Chem. Abstr.* **1994**, *121*, 203454r.
34. Li, Y. Z.; Rethwisch, D. G. *Biotechnol. Bioeng.* **2002**, *79*, 15–22.
35. Potier, P.; Bouchu, A.; Descotes, G.; Queneau, Y. *Synthesis-Stuttgart* **2001**, *3*, 458–462.
36. John, E. D. M.; Henry, W. S.; Robert, J. P. Brit. Pat. 1960; pp 718–727.
37. Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497–2507.