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Lipase catalyzed synthesis of benzyl acetate in solvent-free medium using vinyl acetate as acyl donor

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Abstract—Use of vinyl acetate as acyl donor in transesterification of benzyl alcohol catalyzed by a commercially available lipase (Lipozyme® RM IM) gave 100% conversion in 10 min. The excess acyl donor and the enzyme could be recovered and reused. Unlike the chemical catalytic processes, it produced no undesirable side product.

The chemical synthesis of benzyl acetate is carried out by acetoxylation of toluene by using inorganic catalysts.[1,2] This chemical synthesis produces unwanted side product[3,4] and it also has an associated problem of catalyst deactivation.[5] There has been some work on the formation of benzyl esters using enzymes.[6,7] However, no work seems to have been carried out on the lipase catalyzed synthesis of benzyl acetate.

Benzyl acetate finds extensive uses in perfumery, food, and chemical industries.[3] The present work shows that a commercially available lipase (Lipozyme® RM IM), under optimized conditions, in solvent-free medium, can be a very efficient biocatalyst for conversion of benzyl alcohol to benzyl acetate.

For enzyme catalyzed esterification and transesterification reactions in non-aqueous media vinyl acetate is considered a very good choice for acyl donor.[8] Isomerization of the unstable vinyl alcohol to acetaldehyde as the product drives the reaction in this forward direction. Lipases have proven to be versatile catalysts for such reactions. Lipozyme® RM IM, a commercially available lipase, is among the more economical industrial level catalysts which has been extensively employed in several esterification and transesterification reactions.[9,10] Hence, Lipozyme® RM IM was used in the present work.[11] The solvent-free medium (i.e., using reactants as such as medium) was employed. Some advantages of employing solvent-free medium have been discussed previously.[12] It was seen that for efficient and fast conversion, it was necessary to operate with high amount of enzyme (10% of the mass of the two reactants) and excess acyl donor (6× benzyl alcohol on a molar basis) (Table 1 and Fig. 1). It may be added that similar high level of enzyme load has been generally reported to be required in such reactions.[9,13,14] 100% Conversion in enzyme catalyzed reactions has been reported earlier,[9,15–17] however, the 100% conversion obtained in 10 min is among the fastest reported for enzyme catalyzed transesterification reactions.

Figure 2a shows further optimization to reduce the required amount of enzyme and the acyl donor. With vinyl acetate just at 1:1 molar ratio (with respect to the benzyl alcohol), 10% enzyme (w/w, reactants)[18] was necessary to obtain 100% conversion (as monitored by GC)[19]; although with the reduced concentration of acyl donor, it required 180 min (96% conversion though could be obtained in 60 min) (Fig. 2a). With vinyl acetate at 1:6 molar excess, even 5% enzyme (w/w, reactants) could also produce 100% conversion (Fig. 2b). Again the reaction time was greater than 60 min and 180 min was adequate. Thus, a combination of high level of enzyme and excess acyl donor was required to obtain 100% conversion in a short period of 10 min. There is obviously a trade off behavior of enzyme

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Table 1. Benzyl alcohol and vinyl acetate were taken in varied molar ratios with 10% (w/w, reactants) lyophilized enzyme

BA–VA (mol:mol)	Conversion (%) (in 10 min)	Initial rate ($\times 10^3$ μmoles/mg/h)
1:1	75	108.9
1:2	82	114.2
1:4	95	146.4
1:6	99.5	160.0
1:8	100	166.2
1:10	100	169.7

The incubation was done at 45 °C under a constant shaking at 200 rpm. BA stands for benzyl alcohol, VA stands for vinyl acetate.

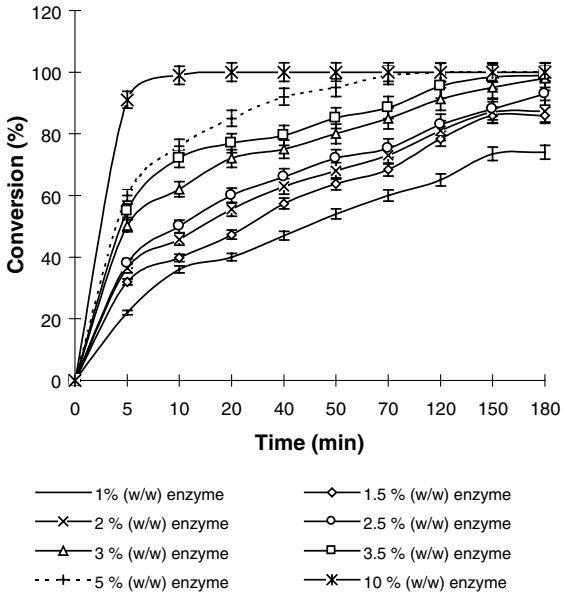

Figure 1. Effect on conversion values by enzyme amount. The enzyme load was varied from 1% to 10% (w/w, reactants). The reactants were taken at a fixed molar ratio (BA–VA = 1:6) and incubation was done at 45 °C at 200 rpm. Aliquots were taken at various time intervals and the product was analyzed by GC.

concentration and vinyl acetate concentration (Fig. 2a and b). 5% enzyme gave similar conversion ~78% in 10 min with excess (1:6 molar) vinyl acetate as 10% enzyme with 1:1 molar ratio of the acyl donor in the same time period. In fact it was found that with 6 times molar excess of vinyl acetate, 100% conversion could be obtained even with 2.5% enzyme but it required 240 min (data not shown). These results define the possible flexibility in this process design with respect to the enzyme concentration, acyl donor excess, and the reaction time. From product isolation[20] point of view, 100% conversion is very convenient as both vinyl acetate and acetaldehyde could be separated from the product by distillation. The vinyl acetate could be recovered (79% of the starting amount) by distillation and could be reused.[21]

Lipozyme® RM IM is an immobilized enzyme preparation and this offers an opportunity of reusability of this biocatalyst. Any biocatalyst reusability obviously

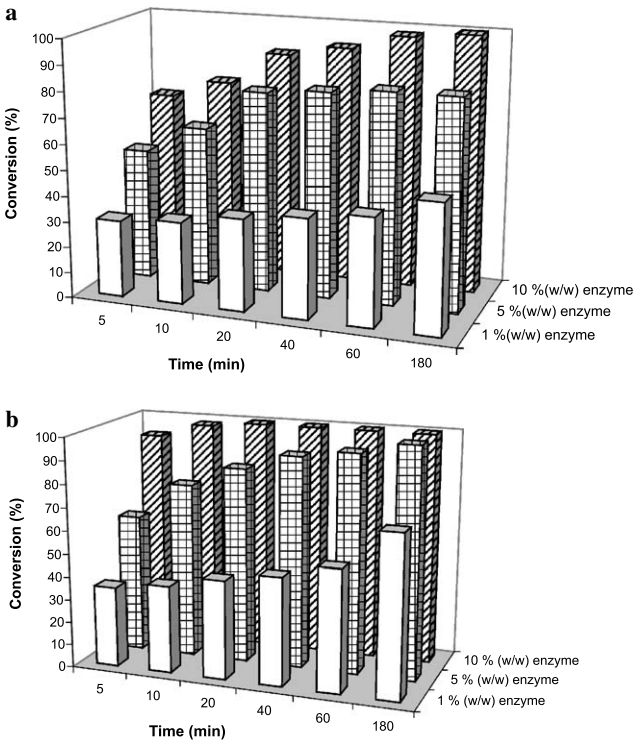

Figure 2. Variation of enzyme amounts with substrate concentration. Three enzyme loads viz. 1%, 5%, and 10% (w/w, reactants) were taken with two different molar concentrations of vinyl acetate viz. 1:1 (a) and 1:6 (BA–VA) (b). The media were incubated at 45 °C at 200 rpm. The values correspond to the mean of the experiments done in pair; the individual values were in ±2% (not shown).

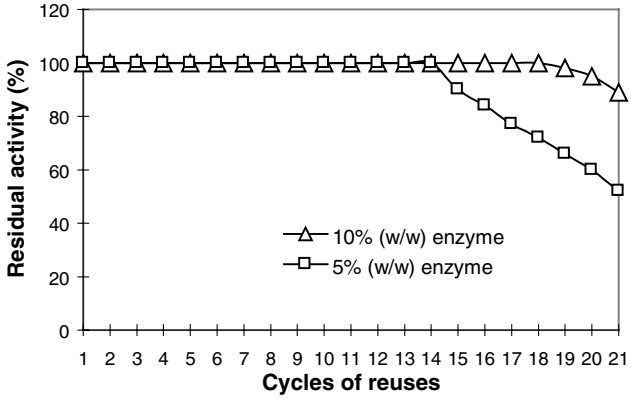

Figure 3. Reusability of the lipase used. Reusability was checked with two different enzyme loads viz. 5% and 10% (w/w, reactants). In this case, the residual activity for 10% enzyme load corresponds to that of its 100% conversion and for 5%, it actually corresponds to the 78% conversion. All the other parameters were kept constant.

impacts upon the process economics. Figure 3 shows that 10% biocatalyst (w/w, reactants) could be reused up to 18 times without any loss of conversion efficiency. One way of looking at this point of view of process conomics, it amounts to using enzyme at 10/18 (=0.55) % (w/w, reactants). The synthesized product benzyl acetate was characterized by IR and ^{1}H NMR[22] by comparing with the spectral database (Figs. 4–6).[23]

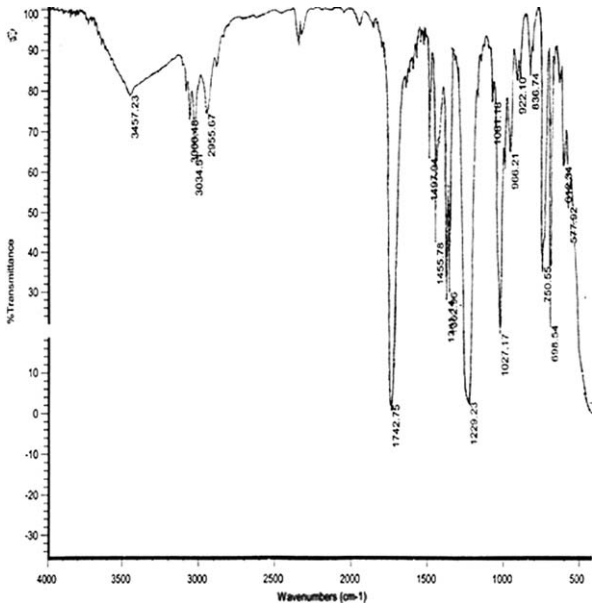

Figure 4. IR spectrum of the product.

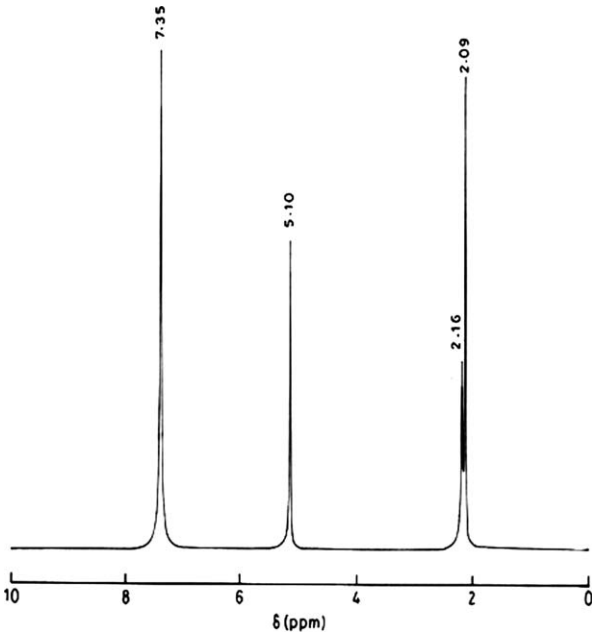

Figure 5. 1-D H NMR spectrum before the completion of the reaction. The reaction mixture was distilled to remove the excess vinyl acetate and then was analyzed by 1-D ^{1}H NMR spectrum which shows the formation of the acetyl peak of benzyl acetate.

Enzyme catalyzed reactions constitute an important component of the move toward green chemistry. One major consideration is the comparison with the existing industrial route. The present work shows, that in fortuitous cases like this, the enzyme based route can be employed with advantage.

Acknowledgments

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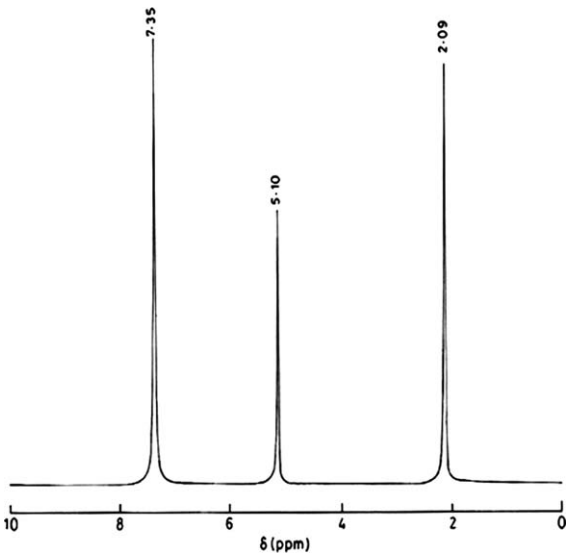

Figure 6. 1-D ^{1}H NMR spectrum after the completion of the reaction. It shows the complete disappearance of alcoholic ^{1}H-peak.

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References and notes

1. Komatsu, T.; Inaba, K.; Uezno, T.; Onda, A.; Yashima, T. *Appl. Catal. A* **2003**, *251*, 315.
2. Benhmid, A.; Narayana, K. V.; Martin, A.; Lucke, B. *Chem. Commun.* **2004**, 2118.
3. Augustine, R. L.; Tanielyan, S. K. *J. Mol. Catal.* **1994**, *87*, 311.
4. Benhmid, A.; Narayana, K. V.; Martin, A.; Lucke, B.; Bischoff, S.; Pohl, M.; Radnik, J.; Schneider, M. *J. Catal.* **2005**, *230*, 420.
5. Radnik, J.; Benhmid, A.; Kalevaru, V. N.; Pohl, M.; Martin, A.; Lucke, B.; Dingerdissen, U. *Angew. Chem. Int. Ed.* **2005**, *44*, 6771.
6. Tewari, Y. B.; Ihara, T.; Phinney, K. W.; Mayhew, M. P. *J. Mol. Catal. B: Enzym.* **2004**, *30*, 131.
7. Tewari, Y. B.; Vanderah, D. J.; Rozzell, J. D. *J. Mol. Catal. B: Enzym.* **2003**, *21*, 123.
8. Bornscheuer, U. T. In *Methods in Non-Aqueous Enzymology*; Gupta, M. N., Ed.; Birkhäuser Verlag, 2000; pp 90–109, Chapter 6.
9. Akoh, C. C.; Yee, L. N. *J. Mol.Catal. B: Enzym.* **1998**, *4*, 149.
10. Chatterjee, T.; Bhattacharyya, D. K. *J. Am. Oil Chem. Soc.* **1998**, *75*, 651.
11. *Rhizomucor miehei* lipase immobilized on macro porous anion exchange resin (Lipozyme® RM IM) was a kind gift from Dr. J.S. Rao, Novozymes, Bangalore, India. Benzyl alcohol (99%, GC grade) was purchased from Merck, Mumbai, India. Vinyl acetate (>99%, GC grade) was purchased from Merck, Honenbrunn, Germany. *Preparation of freeze-dried (Lyophilized) lipase*: the Lipozyme® RM IM was suspended in 0.5 ml of 10 mM phosphate buffer, pH 7, at 25 °C for 1 h. The mixture was frozen at −20 °C and lyophilized for 36 h.

12. Dossat, V.; Combes, D.; Marty, A. *Enzyme Microb. Technol.* **2002**, *30*, 90.
13. Bornscheuer, U. T.; Herar, A.; Wendel, V.; Capewell, A.; Meyer, H. H.; Sheper, T.; Kolisis, F. N. *Tetrahedron: Asymmetry* **1993**, *4*, 1007.
14. Carnell, A. J.; Swain, S. A.; Bickley, J. F. *Tetrahedron Lett.* **1999**, *40*, 8633.
15. Lee, K. K. B.; Poppenborg, L. H.; Stuckey, C. *Enzyme Microb. Technol.* **1998**, *23*, 253.
16. Masja, N. N. G.; de Bont, J. A. M. *Appl. Environ. Microbiol.* **1998**, *64*, 3009.
17. Macedo, G. A.; Lozano, M. M. S.; Pastore, G. M. *Electron. J. Biotechnol.* **2003**, *6*, 72.
18. *Transesterification of benzyl alcohol.* Benzyl alcohol and vinyl acetate were taken in the different (mol/mol) ratios in screw-capped vials in duplicates, followed by the transfer of lyophilized lipase Lipozyme® RM IM (% w/w, reaction medium) in cold. The reaction mixture was incubated at 45 °C with a constant shaking at 200 rpm. Aliquots were taken at various time intervals and the benzyl acetate formed was analyzed by gas chromatography (GC) in % conversion (mol/ mol).
19. *Gas chromatographic analysis.* Benzyl acetate formed was analyzed by Nucon 5700 gas chromatograph with a flame ionization detector . The capillary column (length 30 m, internal diameter 0.25 mm) was packed with 70% phenylpolysilphenylenesiloxane. Nitrogen was used as the carrier gas at a constant pressure of 4 kg/cm^2. The initial oven temperature was at 160 °C and the final oven temperature was fixed at 250 °C with a rate of 10 °C/ min. The injector and the detector temperatures were at 240 and 250 °C, respectively. From each duplicated set the mean of the data was taken and represented with an error bar that falls within ±3%. The corresponding retention times of benzyl acetate and benzyl alcohol were, respectively, 5.39 and 6.31.
20. *Product recovery*. The product mixture benzyl acetate (bp 206 °C) and excess vinyl acetate (bp 72–73 °C) was separated by distillation at 75–86 °C under atmospheric pressure for 20 min. The other side product, acetaldehyde being very low boiling (bp 20–21 °C), could not be recovered. The recovered excess vinyl acetate was reused.
21. *Reusability of the enzyme.* The reaction medium was decanted carefully so that there was no enzyme loss. Then the lipase was rinsed with dry hexane (water = 0.015%) three times taking 2 ml each time, dried in air for 10 min at 25 °C until it became free flow and used.
22. *Product characterization.* The product was characterized by IR (Nicolet 460 Spectrometer) and ^{1}H NMR (Bruker-S-300) spectroscopies. The IR spectrum shows the characteristic ester peaks at: 1742.75, 1229.23, 1027.17 (cm^{-1}). The ^{1}H NMR shows: $\delta = 2.09$ [3H, s], 2.16 [1H, s], 4.7 [2H, s], 7.2–7.5 [5H, aromatic, pseudo s] in the middle of the reaction. At the end of the reaction, the singlet at 2.16 [hydroxylic proton of benzyl alcohol] disappears showing the 100% conversion to benzyl acetate.
23. Spectral Database for Organic Compounds, SDBS. [www.aist.go.jp/R1ODB/SDBS].