Lipase-Catalyzed Synthesis of Butyl Butyrate by Alcoholysis in an Integrated Liquid−Vapor System

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This paper reports experimental work pertaining to alcoholysis between butanol and ethyl butanoate, catalyzed by an immobilized lipase in a liquid−vapor system where chemical reaction and physical separation are simultaneously carried out. The processing setup was tested for various compositions of the starting feedstock and operated under reduced pressure. Samples were withdrawn both from the boiler and the condenser, and they were chromatographically assayed for butyl butyrate. The integrated configuration tested is quite effective toward improvement of the final yield of the desired product.

Introduction

Biochemical reaction processes are designed to transform reactant(s) into product(s) that satisfy a number of preset specifications, within constrained time frames. However, due either to thermodynamic and/or kinetic limitations, the final yield and purity are often poor. To overcome such shortcomings, reaction units have traditionally been connected in series with separation units, which bring about separation of components via selective mass transfer between phases (e.g. filtration, distillation, adsorption, desorption, and membrane-mediated processes) (*1*). However, postreaction separation processes in the bioengineering field are expensive because they require considerable amounts of energy to drive separation. A better rationale, aimed at improving the performance of said systems, relies on process intensification via cascading reactors and separators. Such a configuration permits increase in efficiency since product is removed incrementally at each reactor/separator set; the stepwise product recovery may already lead to acceptable degrees of purity, while the reaction equilibrium is shifted favorably.

An alternative approach, which may be seen as the limit of cascading when the number of units increases without limit, has been scrutinized in recent years by several researchers worldwide; it consists of integration of reaction and separation in a single unit. Such processing configuration allows product(s) formed by reaction to be continuously (and immediately) removed from the reaction medium; in this fashion, effectiveness of separation is improved because bulk concentrations are not allowed to build up. A review on uses of integrated configurations when lipase is used as biocatalyst has been published elsewhere (*2*). Studies pertaining to comparison of the performance of sequential, cascaded, and integrated configurations, in either thermodynamic, kinetic, or economic terms, are also available in the literature (*3 8*).

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One possibility of integrating reaction and separation in the same unit is reactive distillation. Operating a distillation column as a chemical reactor offers distinctive advantages over the classical process of a chemical reactor followed by a distillation column. In such an integrated unit, two processes take place simultaneously in a single piece of equipment; as a result, lower capital and operating costs are expected, mainly because several pumping, piping, and instrumentation devices can be eliminated. Furthermore, continuous removal of reaction products while reaction is still in progress increases the overall conversion, because a true chemical equilibrium is never attained. Finally, the heat generated by reaction may not affect the operating temperature (and hence the equilibrium constant)—in the case of an exothermic reaction, part of the (or the whole) heat of vaporization may in fact be directly supplied as heat of reaction (*9*). Therefore, chemical reactions that are characterized by unfavorable reaction equilibria are particularly good candidates for reactive distillation (*10−12*).

The original concept of reactive distillation is not recent; this technique was applied early in the last century for the continuous production of ethyl acetate using sulfuric acid as homogeneous catalyst (*13, 14*) and was later comprehensively reviewed by Keyes (*15*).

Use of reactive distillation to effect reactions that are brought about by solid heterogeneous catalysts is a more recent development, first described by Sennewald et al. (*16*). Use of the catalyst in a solid form, rather than dissolved in the reaction system, permits its confinement to the distillation column by physical methods for eventual reuse and concomitantly will prevent it from showing up in the product. Implementation of such a device with enzymes is somewhat difficult because these (proteinaceous) catalysts are thermolabile and become soluble above a certain threshold of water in the system; hence, uniform and sustained dispersion of the activity of a lipase through the various stages will be hard owing to the high boiling temperatures of the mixture of interest under atmospheric pressure (with consequently high deactivation rates for the enzyme), coupled with the tendency for the lipase to solubilize in the lower trays (which are richer in water).

To avoid both of these drawbacks, the more feasible configuration for lipase-catalyzed reactions would be to use the lipase immobilized onto an external solid carrier. In the ideal integrated process, the product(s) should be completely and instantaneously taken away from the catalytic site, so as to reduce the chance for the reverse reaction to occur. The alcoholysis reaction of interest here yields two products (1-butyl-1-butanoate and ethanol); due to the difference in volatilities, ethanol is preferentially removed in the top of the experimental setup, whereas butyl butanoate accumulates in the bottom. No previous reports exist—as far as our knowledge goes—on the use of this type of integrated configuration for lipase-catalyzed BuBu synthesis, so it can be taken as fully innovative. To minimize the number of operating devices (viz., pumps), a batch configuration was selected for our experiments.

Materials and Methods

Selection of System Configuration and Chemical Reaction. When integrating chemical reaction with physical separation via reactive distillation, the first decision is to choose the configuration of the integrated setup. Three basic configurations may be potentially considered: (i) the catalyst is placed in the boiler (*boiler reactor*), which is appropriate for reactions where the boiling points of both the reactants are higher than those of the products, and which therefore promotes reaction in the boiler and allows products to be separated in the distillate; (ii) the catalyst is located at the outlet stream of the condenser (*condenser reactor*), which is appropriate for reactions where the reactants are more volatile than the products, thus allowing products to be recovered in the boiler; and (iii) the catalyst is located in the distillation column itself (*column reactor*), in which the reactants coexist inside the column, and which will allow product separation only if (at least) one of the reaction products has a boiling point sufficiently different from those of the other product and reactants.

Taking these three possibilities into account, a preliminary (theoretical) screening was conducted in order to select a compatible lipase-catalyzed reaction, as well as the most appropriate configuration of the integrated setup. Two factors had to be taken into account so as to attain this goal: (i) the chain length of the molecules at stake and (ii) the relative boiling points of reactants and products. Since all components involved in the chemical reaction must be rather volatile so as to allow distillation to be effective in separating them, a set of esterification and alcoholysis reactions were screened for the production of esters of up to five carbon atoms, in both the acyl and alcohol moieties (not shown).

Of the 122 reactions considered, and assuming ideal behavior (i.e., ignoring the possibility of formation of azeotropes), none was in principle appropriate for use in the condenser-reactor setup, and only one was appropriate for use in the boiler-reactor setup; for the remaining 121 reactions, the reactants/products possessed intermediate volatilities and were thus adequate for use in the column-reactor setup. The column-reactor configuration, coupled with the alcoholysis reaction between 1-butanol (bp = 117 °C) and ethyl-1-butanoate (bp = 124 °C) to yield ethanol (bp = 79 °C) and 1-butyl-1-butanoate (bp = 166 °C), was eventually selected, because the boiling points of the reactants are similar but the alcohol product is much more volatile, therefore leading to selective removal thereof by distillation at the condenser, whereas the ester product accumulates in the boiler.

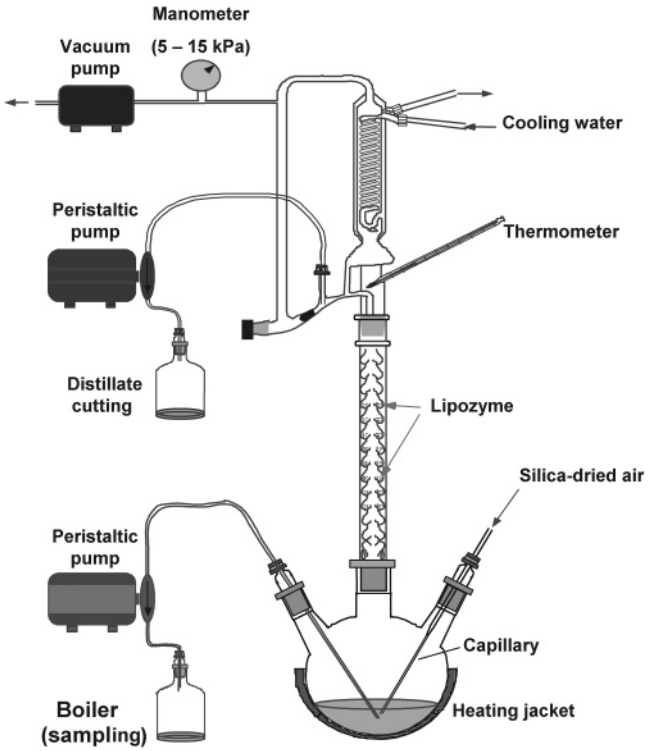

Figure 1. Schematic representation of the column-reactor experimental setup.

Enzyme. A lipase from *Mucor miehei*, immobilized on a macroporous anion-exchange resin (Lipozyme, 5 BAUN g^{-1}; BAUN = batch acidolysis units novo), was kindly provided by NOVO Nordisk (Bagsvaerd, Denmark) and was used without further purification. The water content of the enzyme, calculated by comparison of its weight before and after heating at 110 °C for 24 h, was ca. 4.5% (w/w).

Chemicals. Ethyl butanoate (EtBu) was purchased from Merck (Darmstadt, Germany), 1-butanol (BuOH) from Romil Chemicals (Cambridge, U.K.), 1-butyl-1-butanoate (BuBu) from Fluka (Buchs, Switzerland), and ethanol (EtOH) from AGA (Sacavém, Portugal). All chemicals were proanalysis grade.

Experimental Setup. The experimental setup was constituted by a 500 mL, three-necked, round-bottom flask (boiler), which was heated by an electrical heater; this boiler was connected to a distillation column and a reflux cooler (Figure 1).

A Pasteur pipet was inserted in the boiler to avoid superheating of the system (as a pressure safety valve). A 23 cm long distillation column, with an internal diameter of 3 cm, was fitted to the top of the boiler. This column contained 13 inverted pear-bulbs 1.8 cm in length, each containing immobilized enzyme (ca. 200 mg/pear-bulb). An extra pear-bulb was placed upright in the bottom of the column, so as to fit the narrowing neck of the column and mechanically support the others.

A liquid-dividing fractional distillation head (reflux), refrigerated to −20 °C in order to reduce evaporation of distillate, was mounted on the top of the column, with a thermometer inserted just below the cooler. The temperature of the system was maintained low by applying vacuum (ca. 15 kPa) to the outlet on the top of the reflux head. A suction flask was placed between the pump and the reflux head; a needle valve was also placed between the pump and the suction flask to bleed air in order to set the desired vacuum level; and a manometer was further placed in the suction flask.

The distillate was collected in a weir; via manipulation of a stopcock, it was then either directed backward to the column top (in the *reflux mode*, with all distillate refluxing into the column) or directed outward (in the *cutting mode*, with all distillate being removed for sampling). Peristaltic pumps were connected so as to allow periodic sampling of both the distillate and the boiler contents.

All connections were made of ground glass greased with silicone or of Teflon (tubing and stopcocks); only the connection from the pump to the boiler was made of Masterflex tubing.

Reaction Conditions. Mixtures prepared with different concentrations of the four active components of the reaction of interest were initially inserted in the boiler, and the progress of reaction was accompanied from the very moment of boiling of the mixture. Samples were periodically withdrawn both from the boiler and the distillate.

Corrections were duly performed for all progress reaction curves, to allow for sample removal and evaporation, based on the assumptions that the evaporate composition was equal to that of the distillate, and that the composition of the column hold-up (ca. 10% of the total system volume) was the average of the distillate and boiler compositions (in either case, the resulting corrections were always minor in magnitude).

Analytical Methods. The concentration of butyl butyrate was determined by gas chromatography (Perkin-Elmer Autosystem XL) using flame ionization as detection mode. A capillary DB-1 column (J&W), 60 m long and 0.32 mm of internal diameter, with a stationary, 0.25 μm thick layer of dimethylpolysiloxane (with a polarity of 5) was used as resolution medium. After injection of samples (1.0 μL), the temperature of the column was held constant at 130 °C; the temperatures of the injector and detector were both set equal to 250 °C. The flow rate of the carrier gas (He at 85 kPa, split-open at 32 mL min^{-1} during the first minute) was 1 mL min^{-1}. Quantitative data were collected and processed with the TurboChrom software (Perkin-Elmer). Calibration curves were prepared for the components assayed for.

Results and Discussion

Distillation of Quaternary Mixture. To anticipate the formation of azeotropes in the system selected for our study, the experimental setup was operated for 3.5 h in the absence of enzyme, with an approximately equimolar quaternary mixture consisting of 140 mL of ethyl-1-butanoate (25.45% mol/mol), 1-butanol (27.48% mol/mol), 1-butyl-1-butanoate (22.12% mol/mol), and ethanol (24.95% mol/mol). The distillation column was run under total reflux, with sample withdrawal from the distillate and the liquid in the boiler throughout time, for later chromatographic assay for chemical composition (Figure 2).

Inspection of Figure 2 indicates that there was an azeotrope, which consists of ca. 50% (mol/mol) 1-butanol and ca. 35% (mol/mol) ethyl-1-butanoate, with the remainder 15% (mol/mol) consisting of EtOH and BuBu. In addition, EtOH, the most volatile component in the system (bp = 79 °C), accumulates almost completely in the distillate (as expected), while BuBu, the least volatile component in the system (bp = 166 °C), remains essentially in the boiler. When ethanol is depleted in the boiler, its concentration in the distillate obviously decreases, and the 1-butanol concentration becomes eventually ca. 1.5-fold that of EtBu.

The situation which prevails during reactive distillation (see below) corresponds presumably to the stage during which there is no ethanol in the boiler and less than 20% (mol/mol) ethanol in the distillate; during that stage, it is feasible to effectively remove ethanol (which is a product of reaction) in the distillate, whereas 1-butyl-1-butanoate (which is the desired ester product) is for its major part retained in the boiler; the azeotrope is then distilled off.

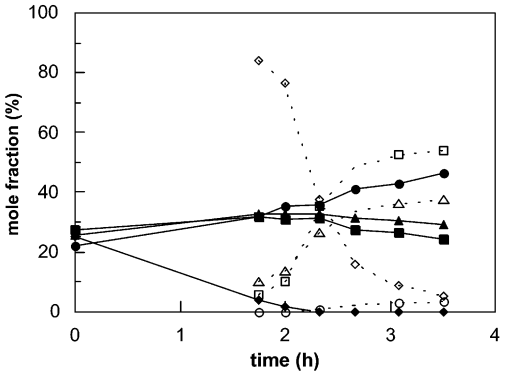

Figure 2. Evolution of the mole fractions of BuBu (●, ○), BuOH (■, □), EtBu (▲, △), and EtOH (◆, ◇) in the distillate (--, open symbols) and in the boiler (—, solid symbols), during the distillation process of an approximately equimolar mixture of reactants and products in the absence of enzyme.

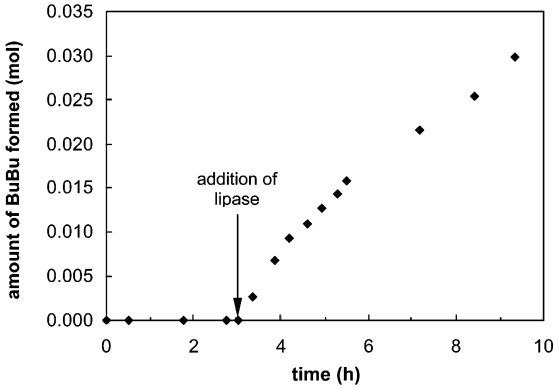

Figure 3. Effect of addition of lipase on 1-butyl-1-butanoate production, when departing from an approximately equimolar mixture of reactants.

Effect of Enzyme Concentration. To assess the chemical effectiveness of the experimental setup designed, experiments were performed with and without Lipozyme. The results depicted in Figure 3, representing the evolution of the total number of moles of BuBu produced in the system (either present in the boiler, in the distillate, in the column, or in the evaporate), indicated that no product is formed in the absence of enzyme.

The same process, when carried out in a batch system at 60 °C (*17*), did not as well provide evidence of chemical reaction taking place in the absence of Lipozyme.

Effect of Reactant Concentration. The effect of the initial concentrations of BuOH and EtBu on product formation rates was assessed for three initial BuBu mole fractions (0, 24, and 50% (mol/mol)), using the proposed integrated experimental setup. Parts a−c of Figure 4 depict the evolution of the number of moles of BuBu as a function of reaction time.

Inspection of Figure 4 indicates that, in the initial binary mixture of BuOH and EtBu (Figure 4a) or in a ternary mixture of BuOH, EtBu, and BuBu (Figure 4b,c), at every initial level of BuBu considered (i.e., 0, 24, and 50% (mol/mol)), decreasing BuOH concentrations enhance product formation. Such realization is in agree-

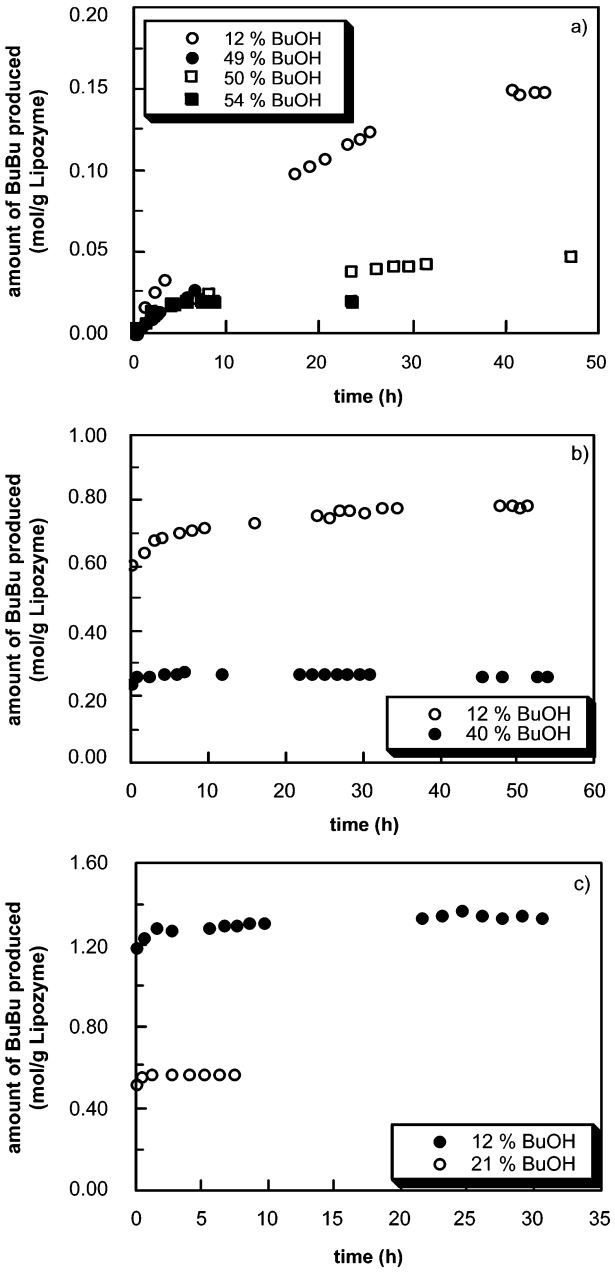

Figure 4. Evolution of the mole amount of BuBu produced as a function of reaction time, for various initial mole fractions of BuOH and EtBu, and for the initial mole fraction of BuBu of (a) 0, (b) 24, and (c) 50% (mol/mol).

ment with previous works (*17*), where it has been demonstrated that BuOH acts as a competitive inhibitor of Lipozyme in this type of reaction; it has also been shown (*17*) that no enzyme deactivation took place under these processing conditions. Moreover, unfavorable reactant concentrations, i.e., high BuOH and low EtBu concentrations, apparently cause a rapid halting of the reaction; this effect, which is most clearly observed at higher BuBu concentrations, may be explained by a faster enzyme deactivation under such conditions.

Effect of Product Removal. The aim of integrating reaction with separation is to remove reaction products in situ, so as to decrease the rate of the reverse reaction. To assess the effect of removing a product (EtOH) in the distillate upon the production of BuBu accumulated in the boiler, a few experiments were performed in which, for an initial BuBu mole fraction of ca. 24% (mol/mol) and several initial concentrations of EtBu and BuOH,

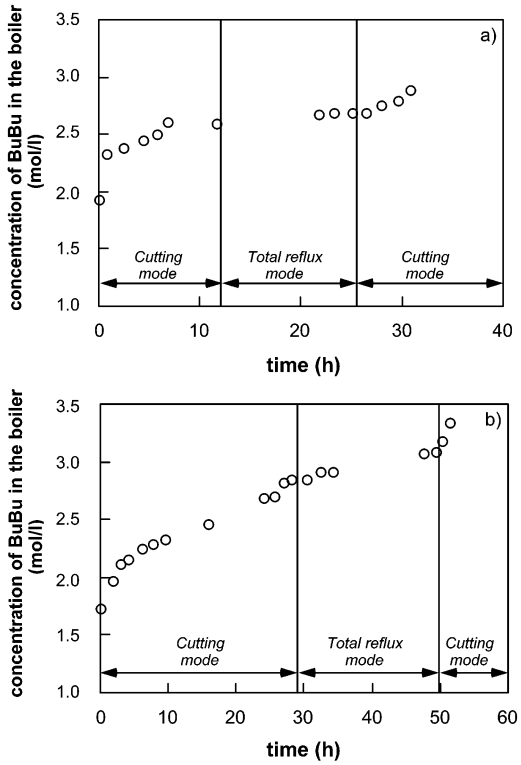

Figure 5. Evolution of the mole concentration of BuBu in the boiler as a function of reaction time, for an initial mole fraction of BuBu of ca. 24% (mol/mol), and initial mole fractions of BuOH and EtBu of (a) 40 and 36% (mol/mol) and (b) 12 and 64% (mol/mol), respectively.

removal of EtOH-rich distillate (i.e., cutting mode) was alternated with total reflux of the distillate into the distillation column (i.e., total-reflux mode), as represented in Figure 5.

Inspection of Figure 5a,b suggests that (i) the concentration of BuBu in the boiler increases when EtOH is removed in the distillate (cutting mode), (ii) the formation of BuBu clearly slows down and eventually stops when ethanol is not removed (total-reflux mode), and (iii) BuBu accumulation resumes when EtOH is again removed in the distillate (cutting mode).

Upon setting the system in the total-reflux mode, it was also observed that product formation continues until the ethanol load increases to such an extent that the concentrations at the reactive zone of the column (i.e., the lower half) apparently attain thermodynamic equilibrium conditions, thus leading to (net) zero production. When the system is bleached again of distillate (to remove the ethanol generated), production of BuBu resumes, hence confirming that this experimental setup effectively operates (and behaves) as a fully integrated system.

Effect of Product Concentration. The effect of various initial levels of BuBu on the reaction progress was studied for a constant initial fraction of BuOH (ca. 12% (mol/mol)) and varying initial molar fractions of BuBu; the results are compiled in Figure 6.

Observation of Figure 6 indicates that the amount of BuBu in the system increases when the initial BuBu fractions take the values of 0, 24, and 50% (mol/mol) and decreases when it takes the value of 69% (mol/mol). Apparently, there is a promotion of the activity of catalyst with increasing concentrations of BuBu at low levels of this product, which overrides any effect of thermodynamic inhibition arising from occurrence of the reverse

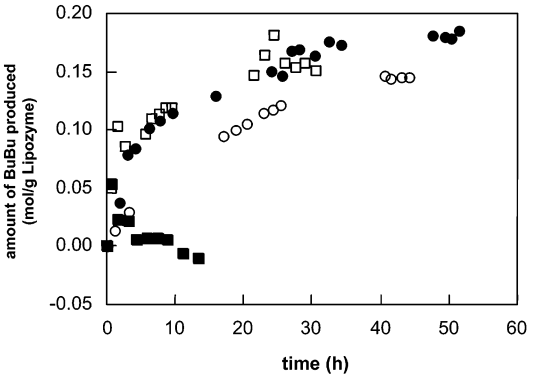

Figure 6. Evolution of the mole amount of BuBu in the boiler as a function of reaction time, for an initial mole fraction of BuOH of ca. 12% (mol/mol) and initial mole fractions of BuBu of ca. 0 (○), 24 (●), 50 (□), and 69% (mol/mol) (■). (The number of moles represented is the difference between the number of moles at the time indicated and at time zero.)

reaction; the former putative effect eventually vanishes at high levels of BuBu, where kinetic inhibition may also be claimed to play a role. It can also be observed that, at initial BuBu fractions of 24 and 50% (mol/mol), the production rates of BuBu are very similar to each other (164 and 158 mmol g^{-1} Lipozyme, respectively) at 30 h. Such results provide strong evidence that continuous removal of ethanol allows the forward reaction to proceed more or less independently of the BuBu concentration—at least at intermediate concentration ranges, thus unfolding the effectiveness of the integrated configuration. On the other hand, when the BuBu initial molar fraction is ca. 69% (mol/mol), the BuBu rate of production decreases, which indicates that, above a certain threshold of BuBu, the reverse reaction is actually favored, as already discussed.

Conclusions

This paper reports experimental work, aimed at testing the effectiveness of an integrated reactive-distillation setup in the lipase-mediated production of butyl butyrate via alcoholysis. The results produced indicate that (i) no reaction takes place in the absence of enzyme, (ii) BuOH acts as inhibitor of the enzyme in the molar range tested, and (iii) the aforementioned integrated configuration is quite effective toward improving the final yield of (purified) butyl butyrate, especially when one of the products of reaction (i.e., ethanol) is removed continuously. One can, therefore, conclude that such a reactive distillation setup behaves as an integrated system, and that it can effectively be used to promote lipase-catalyzed alcoholysis reactions, at least between butanol and ethyl butanoate.

Acknowledgment

The authors received financial support provided by JNICT and FCT (Portugal), through programs CIENCIA (Grant BD/2081/92-IF, doctoral fellowship) and PRAXIS XXI (Grant BD/5568/95, doctoral fellowship; and Grant 2/2.1/BIO/34/94—Extractive Biocatalysis, research project), and by EU, through the program Human Capital and Mobility (Grant ERBCHBGCT-940591, postdoctoral fellowship).

Notation

bp	boiling point (°C)
BuBu	butyl butanoate
BuOH	butanol
EtBu	ethyl butanoate
EtOH	ethanol

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