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ORIGINAL PAPER

Designs of Bioreactor Systems for Solvent-Free Lipase-Catalyzed Synthesis of Fructose–Oleic Acid Esters

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Abstract Fructose–oleic acid esters, biodegradable, biocompatible and biobased surfactants and value-added products were synthesized under solvent-free conditions at 65 °C in stirred-batch mode and using several different bioreactor systems. For a stirred-tank bioreactor (STBR) using fed-batch fructose addition and 5.0 wt.% immobilized *Rhizomucor miehei* lipase (Lipozyme® IM, Novozymes, Franklinton, NC), the conversion yield was over 80%, and the initial rate of the reaction was comparable to previously obtained results using *tert*-butanol during the initial phase. The bioreactor systems contained a packed "desorption" column (DC) containing fructose crystals and silica gel for delivery of saccharide, and either a STBR or packed-bed bioreactor (PBBR). The liquid stream, initially containing oleic acid and a mixture of fructose–oleic acid esters at a ratio of 75/25 w/w, was continuously recirculated throughout the system. The PBBR system yielded the highest conversion (84.4%) and rate of reaction subsequent to the addition of 10 wt.% molecular sieves during the latter stage of reaction; however, the reaction rate was several-fold lower than the batch mode reactions due to the lower fructose concentrations provided by the DC.

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Introduction

The foods, cosmetics, and pharmaceutical industries have recently employed saccharide–fatty acid esters, which are value-added products derived from natural products such as corn and plant oils, as "green," biodegradable, and biocompatible surfactants or emulsifiers [1–3]. Saccharide–fatty acid esters are typically produced by chemical methods that require costly and environmentally unsafe conditions such as high temperature, high pressure, alkaline pH, and use of organic solvents [4–6]. Additionally, chemical reactions can often yield a broad product distribution of partial esters, and the employment of unsaturated fatty acyl donor substrates might yield additional by-products via degradation of the double bonds, which may be harmful or toxic [7].

In contrast, employment of immobilized lipase provides milder and environmentally friendly operating conditions and leads to no chemodegradation of double bonds and a very narrow product distribution of typically 1–3 mono- and di-ester species due to the inherent regioselectivity of the enzyme [6, 8–10]. However, there are several problems to overcome for the biocatalytic approach, the main one being the poor miscibility of the polar saccharide and nonpolar fatty acid substrates [11]. Non-solubilized saccharide promotes fouling of the bioreactor, adsorbs water from the air headspace of the bioreactor, and increases its water content, thus lowering the conversion, and/or adsorbs to the immobilized lipase, leading to inactivation and a low reaction rate [12, 13]. Several different approaches have been employed

that have improved the miscibility with success, but most are not economically viable, environmentally safe, or readily scaled up, including the addition of polar organic solvents [9, 14] and ionic liquids [15] and the derivatization of the acyl acceptor [15–17] [reviewed in [11].

In a previous study by our group [14], it was demonstrated that the product, saccharide–fatty acid ester, significantly increased the miscibility of substrates, suggesting the possibility of the lipase-catalyzed solventless synthesis of saccharide–fatty acid ester; moreover, the presence of ester greatly increased the solubility to an extent that a co-solvent would not be required at 65°C. A solvent, *tert*-butanol, was employed only during the initial period to enhance fructose solubility and was allowed to evaporate away completely upon reaching ~25% conversion. Conversions of 80–90% were achieved for immobilized *Rhizomucor miehei* lipase- (RML-) catalyzed esterification of oleic acid and saccharide (fructose and sucrose) at 65 °C using near-stoichiometric amounts of substrates in batch mode. Immobilized lipase exhibited excellent activity retention, with no loss of activity during the three successive batches, equating to 24 days of operational time [14].

This paper describes the adaptation of our group's batch mode research into bioreactor systems operated under solvent-free conditions that contain a reservoir open to the atmosphere, a peristaltic pump, a packed column to deliver fructose [18], and a bioreactor. The liquid phase is continually recirculated between the listed units. Systems operating in continuous mode provide several advantages, such as reduced labor costs, improved productivity, and more robust process control. The use of a packed column for delivery of acyl acceptor in a bioreactor system was demonstrated recently for the lipase-catalyzed esterification of monosaccharides and sugar alcohols in the presence of acetone at ambient pressure or under subcritical conditions [19–21]. This investigation focuses upon the comparison of bioreactor systems with an emphasis on the relationship between the system design (particularly the use of a stirred tank versus a packed bed bioreactor), water content, and the reaction rate, conversion, and selectivity.

Materials and Methods

Materials

Technical grade oleic acid, 98% pure, as determined by HPLC [14], and Lipozyme® IM, lipase (EC 3.1.1.3) from *R. miehei* immobilized onto macroporous anionic beads, or "RML," the latter a product manufactured by Novozymes, Inc. (Franklinton, NC), were purchased from Sigma-Aldrich (St. Louis, MO). Fructose (>98% purity), acetonitrile (HPLC-grade), molecular sieves (Type 3A, 4–8 mesh, Grade 562) were purchased from Fisher Scientific (Pittsburgh, PA). Silica gel with particle size 32–63 μm was purchased from Selecto Scientific (Suwanee, GA). All materials were used without further purification. Technical grade fructose–oleic acid ester, FOE, the reaction product and a component of the initial charge to the bioreactor system, was synthesized by the RML-catalyzed fed-batch reaction protocol given in our modified from previous report [14], as described elsewhere [18]. The purity of fructose–oleic acid ester was 77.4%, with the mono and di-ester present at a ratio of 8.3:1.7 (g/g). This product was mixed with oleic acid to obtain the desired proportions of oleic acid and ester, and fed to the bioreactor system.

Solventless, Batch Mode Operation of Stirred-Tank Bioreactor (STBR)

Batch mode operation of a STBR was conducted in a 20 ml scintillation vial placed on a four-position hot-plate magnetic stirring device (Super-Nuova from Barnstead, Dubuque, IA) open to the atmosphere, consisting of 5.7 g (20 mmol) oleic acid and 0.9 g (5 mmol) fructose initially, and incubated at 65 °C for 1.0 h under magnetic stirring at a rate of 350 rpm. Esterification was initiated by adding 1–10 wt.% of RML. Additional fructose was added in small, 0.9 g (5 mmol) amounts on the days 1, 3, and 5 of the time course. Small aliquots were taken from the solution for compositional analyses. Replicate experiments were performed for all data given in the figures and table, with error bars reflecting the standard deviation.

Preparation of Packed Fructose-Silica Gel Column

The fructose desorption column ("DC") was prepared in an Omnifit® 100 × 10 mm ID chromatography column (Bio-Chem Fluidics, Boonton, NJ). The columns were packed with a mixture of ground crystalline fructose and silica gel at 70 and 30 wt.%, respectively, which was found to be the optimal composition according to previously published results [18]. The columns were first equilibrated by recirculating 6.0 g oleic acid at 0.1 ml min^{-1} for 4 h at 65 °C in a convection oven prior to its use in the bioreactor systems, using the peristaltic pump and tubing described below for the bioreactor systems. Substitution of oleic acid with an oleic acid/fructose oleate mixture in the equilibration process did not significantly affect the performance of the DC.

Operation of Bioreactor Systems Containing a Packed Bed Desorption Column Under Continuous Recirculation Mode

Several bench-scale bioreactor systems were configured and operated that consisted of a peristaltic pump, a packed

desorption column (described above), a packed bed or stirred tank bioreactor (PBBR and STBR, respectively, with the latter described above), and for the PBBR-based systems, a reservoir open to the atmosphere (Fig. 1). The units were connected in series using C-FLEX® 1.6 mm ID tubing made of a styrene-ethylene-butylene modified block copolymer from Cole-Parmer (Vernon Hills, IL), forming a closed-loop system that underwent continuous recirculation. A BioLogic LP® peristaltic pump from Bio-Rad (Hercules, CA), operated at 0.1 ml min^{-1}, provided the energy required for transporting the fluid. PharMed® BPT 1.6 mm ID tubing (Saint-Gobain Performance Plastics Corp., Akron, OH) was used within the peristaltic pump apparatus. The PBBR was prepared by packing a fixed amount of RML, either at 0.05 or 0.10 g of RML per gram of reaction mixture (oleic acid + FOE), into a 50 mm $L \times 10$ mm ID Omnifit® chromatography column. For one of the PBBR-based systems, a molecular sieve column (MSC) was employed, prepared in a 50-mm $L \times 10$ mm ID Omnifit® chromatography column containing 0.10 g of 3A molecular sieves per gram of reaction mixture. The reservoir consisted of an empty 20 ml scintillation vial opened to the atmosphere.

The bioreactor systems investigated, displayed in Fig. 1, consisted of a STBR-based system (STBR → pump → DC → STBR), a PBBR system (reservoir → pump → DC → PBBR → reservoir), and a PBBR system equipped with a molecular sieve column (reservoir → pump → DC → MSC → PBBR → reservoir). The initial charge to the bioreactor systems typically consisted of 2.5 g FOE and 7.5 g oleic acid, equivalent to a reaction mixture that has achieved 25% conversion. The amount of RML employed in the systems consisted of either 0.05 or 0.10 g per g of FOE + oleic acid. The packed columns (DC, PBBR, and MSC), and the tubing that interconnected them, were placed within a convection oven that retained a constant temperature of 65°C. The hot plate-stirrer described above for the STBR batch reactions was employed under the same operating conditions (65 °C and 350 rpm of stirring). To supplement the removal of water that occurred during free evaporation from the STBR, granules of molecular sieves (0.1 g per g of oleic acid + fructose oleate) were added after reaching 60–70% ester content. Small aliquots were taken from the bioreactor's effluent stream or from the reservoir or STBR for compositional analysis by HPLC and Karl-Fischer titration.

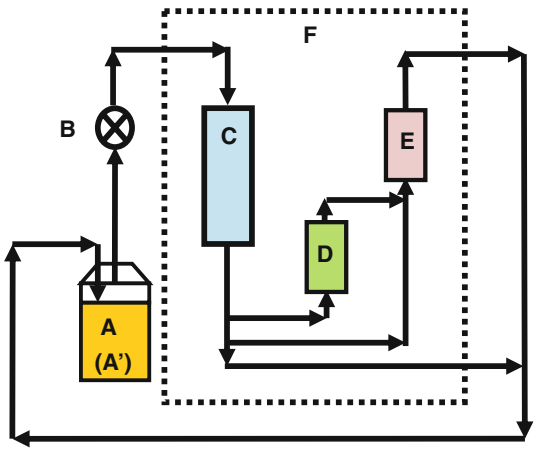

Fig. 1 Bioreactor systems for solventless synthesis of fructose–oleic acid ester, FOE, that undergo continuous recirculation *A*. Reservoir tank (no lipase), *A′*. Stirred tank bioreactor (STBR; with lipase) heated by hot plate to 65°C, *B*. Peristaltic pump (0.1 ml/min), *C*. Fructose desorption column (DC; 100 × 10 mm ID), *D*. Molecular sieves column (MSC; 1 g, 50 × 10 mm ID), *E*. Packed-Bed Bioreactor (PBBR; 50 × 10 mm ID) packed with RML, *F*; 65 °C oven. Systems given in this figure are referred to in Figs. 4 and 5

Monitoring of Water and Esters Content on the Time Course of Reation

The water content of the reaction mixture diluted using methanol was analyzed by Karl-Fischer titration using a Coulometric KF Titrator (Denver Instrument Company, Denver, CO). Quantitative analysis of oleic acid and its mono- and di-esters on a fructose-free basis was performed using a dual-pump system from Varian (Walnut Grove, CA) and a model Mark III evaporative light scattering detector from Alltech Associates, a division of WR Grace (Deerfield, IL) [18]. An analytical reversed phase C_{18} column (4.6 × 250 mm, pore diameter 5 μm) from Alltech was employed using separation conditions consisting of a column temperature of 25 °C and an isocratic solvent system, acetone/acetonitrile/acetic acid (45/45/10 v/v/v), at a flow rate of 1.0 ml min^{-1}. Response factors were measured and employed to convert peak areas into concentrations.

To analyze the fructose content, 40 mg-sized aliquots of column effluent were subjected to liquid–liquid extraction by the system of *n*-hexane and water (500 μl of each) [18]. The extraction was carried out three times at 35 °C for 2 h using a thermomixer (Eppendorf AG, Germany). The aliquots from the pooled aqueous extraction solutions were diluted with acetonitrile to match the composition of the HPLC mobile phase to prevent peak broadening in the HPLC analysis. An analytical Prevail Carbohydrate ES column (4.6 × 250 mm, pore diameter 5 μm) from Alltech was employed using a column temperature of 25 °C and an isocratic solvent system, acetonitrile/deionized water (80/20 v/v) at a flow rate of 1 ml min^{-1}. Standard curves for fructose concentration in an oleic acid/fructose oleate liquid phase versus peak area were obtained and found to be independent of the liquid phase composition.

Results and Discussion

Operation of a Stirred-Tank Bioreactor (STBR) with Fed-Batch Addition of Fructose

To compare the difference between solvent-containing versus solvent-free synthesis and to serve as a control for the bioreactor systems described herein, the experimental protocol employed previously in our group [14], namely, the RML-catalyzed synthesis of fructose oleate in stirred batch mode at 65 °C using stoichiometric proportions of the substrates but with fed-batch addition of the acyl acceptor, was performed, except in this report, the bioreactor medium was completely solvent-free. Previously, *tert*-butanol was present initially, but was allowed to completely evaporate away during the initial portion of the time course [14]. Fed-batch delivery of fructose in four equal amounts at a frequency of every 48 h was implemented to maintain a low level of undissolved fructose, since it is known that excess undissolved polyol acyl acceptor promotes fouling and aggregation of immobilized lipase particles, leading to inactivation and a low reaction rate [12, 13]. However, particularly for the initial period, not all of the fructose was solubilized, but the dissolution of fructose was greatly increased as the amount of fructose ester was increased [14].

The effect of RML concentration on the time course of the reaction is given in Fig. 2. Reactions employing higher RML concentrations (5.0 and 7.5%) underwent nearly identical time courses, with a linear increase of the percentage of fructose–oleic acid esters (FOE) up to 65% between 0 and 5 days, demonstrating the excellent repeatability of the results and that the further increase of RML beyond 5% did not increase the reaction rate, in agreement with the previously published work by our group for systems that utilized *tert*-butanol initially [14]. The time course was similar to that reported previously when *tert*-butanol was added initially, except in the previous report the linear time course region extended to nearly 70% conversion [14]. In fact, the rate of the linear increase depicted in Fig. 2, when normalized by the concentration of RML, was identical with the rate calculated for the cited paper [14]. The underlying cause of the slow rate of reaction for the increase of conversion from 65 to 80% in Fig. 2 was at least in part related to the high water concentration (discussed below). Moreover, when molecular sieves were added at 11 days, the conversion increased from 73.5 to 80.0% within 2 days. The final conversion of 80% was lower than the conversion achieved previously using *tert*-butanol during the initial stage of reaction, 90%, with the conversion increased to 93% when operated in a closed system that possessed low water activity [14].

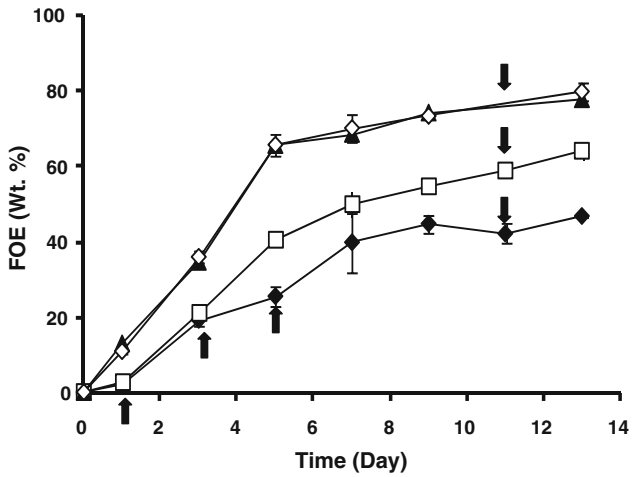

Fig. 2 Effect of RML concentration on the time course of fructose oleic acid ester (FOE) synthesis in a stirred-tank bioreactor (STBR) operated under solvent-free conditions in batch mode at 65 °C. Fructose was added in fed-batch mode: 0.25 moles per mole of oleic acid added initially and on days 1, 3, and 5 (indicated by *upward-pointing arrows*). RML amount (g per g oleic acid + FOE initially × 100%): 1.0 (*filled diamond*), 2.5 (*open square*), 5.0 (*filled triangle*), and 7.5 (*open diamond*). Molecular sieves addition (0.10 g per g of oleic acid + FOE) indicated by *downward-pointing arrow*

Regarding the product distribution, five hydroxyl groups in fructose are possible to serve as acyl acceptor. Among them, groups at the 1- and 6-position are the only ones utilized by lipases, yielding two species of fructose monooleate (FMO) and dioleate (FDO) [9, 22]. Initially, the product distribution for the reactions employing 5.0 and 7.5% RML contain a low proportion of the FMO (10–20 wt.%, with the remainder of products being FDO), but the FMO proportion increased with time, approaching 40–50% (Fig. 3a). The underlying cause for the increase of the FMO fraction is mainly due to the increase of fructose concentration relative to its competing acyl acceptor, FMO. A secondary factor is that for enzyme-catalyzed esterification of polyols, the product distribution favors formation of fewer ester bonds per polyol molecule as hydrophilicity increases [21, 22].

Initially, the solubility of fructose is low due to the very low fraction of FOE in the reaction mixture (Fig. 3a) [14]. Moreover, the majority of the fructose exists as suspended crystals or as aggregates adsorbed onto RML particles; hence, the liquid phase fructose concentration is very low and of the same or smaller value than FMO, resulting in the favored formation of FDO. As the reaction proceeds, the solubility of fructose increases because of the increased amount of FOE, leading to a gradual increase of the FMO proportion (Fig. 3a). This trend mirrors the results obtained when *tert*-butanol was used in the initial phase as a solvent, except that there was a higher fraction of FMO in the initial period because of the presence of solvent (which rapidly decreased as the solvent evaporated away) and that the

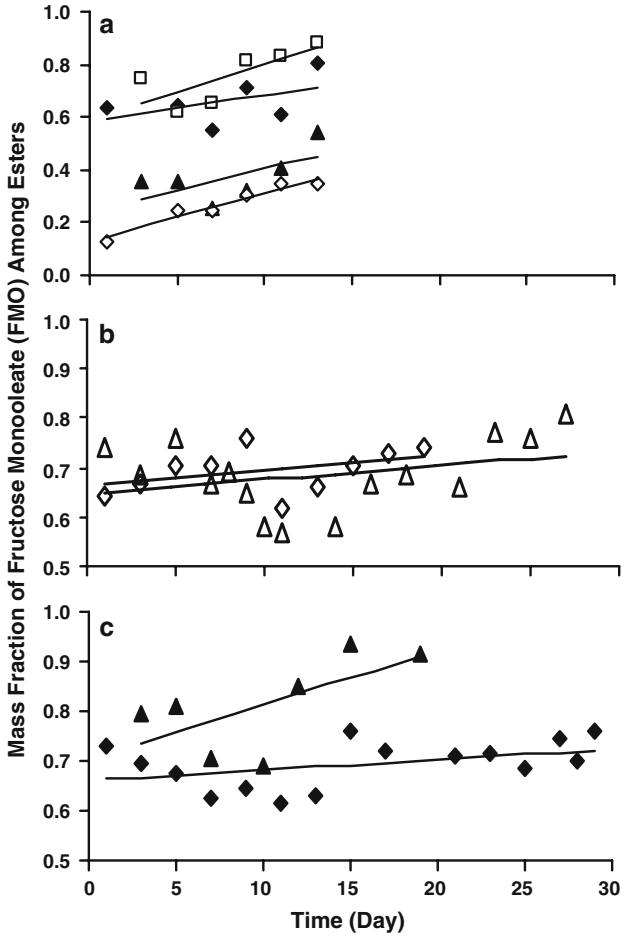

Fig. 3 Mass fraction of fructose monooleate (FMO) among the fructose oleate esters (FOE) for the reservoir's liquid phase during the time course of lipase-catalyzed solvent-free FOE synthesis at 65 °C for various bioreactor systems. (**a**) STBR operating in batch mode, with fed-batch addition of fructose; (**b**) DC/STBR-based system undergoing continuous recirculation with 5.0% RML; (**c**) DC/PBBR-based system undergoing continuous recirculation, with conditions and explanation of the symbols given in Figs. 2, 4, and 5, respectively

increase of the FMO fraction with time was higher, yielding a FMO:FDO ratio of approximately 9:1 w/w at the onset of 80% conversion [14].

When a lower concentration of RML was employed (1 and 2.5 wt.%), the time course underwent a lag phase during the initial day (Fig. 2), presumably because of a high ratio of adsorbed fructose mass per RML particle. After the lag period elapsed, the increase of conversion with time was similar in rate to that achieved using the higher concentrations of RML until ~5 days, after which the conversion slowly increased, similar in trend to 5.0 and 7.5% RML (Fig. 2). Regarding the product distribution, the 1.0 and 2.5% RML reactions yielded higher fractions of FMO than 5.0 and 7.5% RML, presumably because of the higher local concentration of fructose in RML's microenvironment, and they increased linearly to achieve the 9:1 FMO/FDO ratio previously reported [14].

Operation of Solvent-Free Bioreactor Systems in Continuous Recirculation Mode that Contained a Fructose/Silica Gel-Packed Desorption Column

With the goal being to develop a bioreactor system that can be operated under continuous mode for the solvent-free lipase-catalyzed synthesis of saccharide–fatty acid esters, several different bioreactor systems were assembled that operated under continuous recirculation at 65 °C (Fig. 1). For these systems, a packed column containing a physical mixture of fructose crystals and silica gel, the latter to provide mechanical support (desorption column, or DC), was used as a means to deliver fructose to the reaction medium. It was anticipated that the employment of a DC would produce a solvent-free liquid phase saturated with fructose and that during the time course of the reaction, the fructose saturation level would increase because of the increase of FOE concentration, as demonstrated previously [14]. The DC was characterized previously [18]. The cited report demonstrated the optimal composition of the DC was fructose:silica gel at a 7:3 mass ratio; hence, the DCs employed herein were of the same composition initially [18]. Under most conditions, the initial charge to the bioreactor system consisted of oleic acid/FOE (monoester: diester 9:1 g/g) at a 1:3 ratio, equivalent to a reaction medium that underwent 25% conversion, to achieve higher solubilization during the initial stage of reaction.

The first system to be investigated consisted of a DC in series with a STBR ($A' \rightarrow B \rightarrow C \rightarrow A'$ in Fig. 1). The time course of reaction for two different RML concentrations, 5 and 10 wt.%, given in Fig. 4a., were nearly identical, demonstrating that operation of this bioreactor system was highly repeatable and that an increase of RML beyond 5% did not increase the rate of reaction, similar to the results achieved using the STBR in batch mode (Fig. 2). The time versus conversion profile for the STBR-bioreactor system (Fig. 4a) was very similar to the STBR-based system operated under batch mode (Fig. 2) except that the rate of reaction was a factor of 2 lower. Similar to the STBR operated under batch mode, the addition of molecular sieves (0.10 g per g of reaction medium) increased the conversion at the latter stage of the reaction, from 71.5 to 74.5% within 2 days of addition, with the final conversion being slightly higher than the results achieved using batch mode (Fig. 2). However, unlike the batch mode reaction conducted in the STBR, the product distribution more greatly favored the production of FMO, at a mass fraction of 0.6–0.8 among the FOEs and a slight increase during the time course of reaction (Fig. 3b). Of note, the initial rate of reaction was independent of the initial fraction of FOE between 0 and 25 wt.% (Fig. 4a inset).

The underlying reason for the slower reaction rate is the reduced concentration of fructose. In our previous work

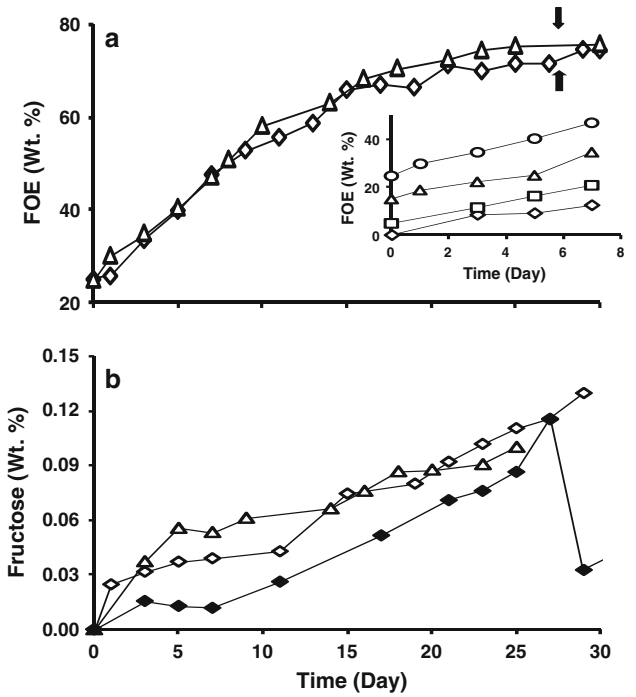

Fig. 4 Effect of RML concentration on the change of the (**a**) fructose oleic acid ester (FOE) and (**b**) fructose concentrations of the desorption column (DC) effluent (*outlined symbols*) and the STBR (darkened symbols) during the time course of reaction for a DC/stirred tank bioreactor (STBR) system undergoing continuous recirculation (A′ → B → C → A′ in Fig. 1) at 65°C. The addition of molecular sieves (0.10 g per g of oleic acid + FOE) is indicated by an *arrow*. RML amount (g per g oleic acid + FOE initially × 100%): 5.0% (*open diamond*, *filled diamond*) and 10.0% (*open triangle*). DC stationary phase consisted of 70 wt.% fructose and 30% silica gel. Inset: Effect of initial FOE content [(*open diamond*) 0 wt.%, (*open square*) 5%, (*open triangle*) 15%, (*open circle*) 25%] on the time course of reaction using 10.0% RML. (Other conditions same as those employed for main figure)

involving stirred batch reactions, fructose concentrations were typically on the order of 1–7 wt.% [10]. In contrast, the stream leaving the DC contained a concentration of 0.01–0.14 wt.% (0.1–1.4 mg g^{-1}), with higher concentrations achieved during the latter portion of the time course due to the increased concentration of FOE (Fig. 4b). As described previously, the difference in fructose concentration between the DC effluent and the stirred batch systems is that the latter yields small, ~100–300 μm-sized suspensions of fructose; moreover, the reported concentration for the latter is "apparent" [18]. In contrast, the DC column effluent is completely devoid of suspensions and is optically clear [18].

For the 5.0% RML reaction, the fructose concentration-time profile was obtained for both the DC effluent and in the STBR (Fig. 4b). The fact that the latter was consistently lower than the former is evidence of the acyl acceptor's consumption. The agreement of fructose concentration for both the DC effluent and STBR at 26 days suggests the stoppage of the reaction due to thermodynamic equilibrium; however, the addition of molecular sieves led to an increased conversion, indicated by the decrease of the STBR's fructose concentration (Fig. 4b).

The second system to be investigated consisted of a DC in series with a packed bed bioreactor, or PBBR (A → B → C → E → A in Fig. 1). The PBBR-based bioreactor system yielded a slightly higher initial rate of reaction (60% conversion in 7 days, Fig. 5a) compared to the STBR-based system (60% conversion in 10 days, Fig. 4a). These results reflect the improved efficiency frequently reported for PBBR systems [20]. PBBRs also possess the advantages of low capital cost and ease of operation, maintenance, and scale-up. The rate of reaction, however, remained low compared to the stirred batch experiments [14] because of the low fructose concentrations (Fig. 5b), which were comparable to those obtained for the STBR-based bioreactor system (Fig. 4b). Beyond 7 days in the time course, the reaction progressed slowly,

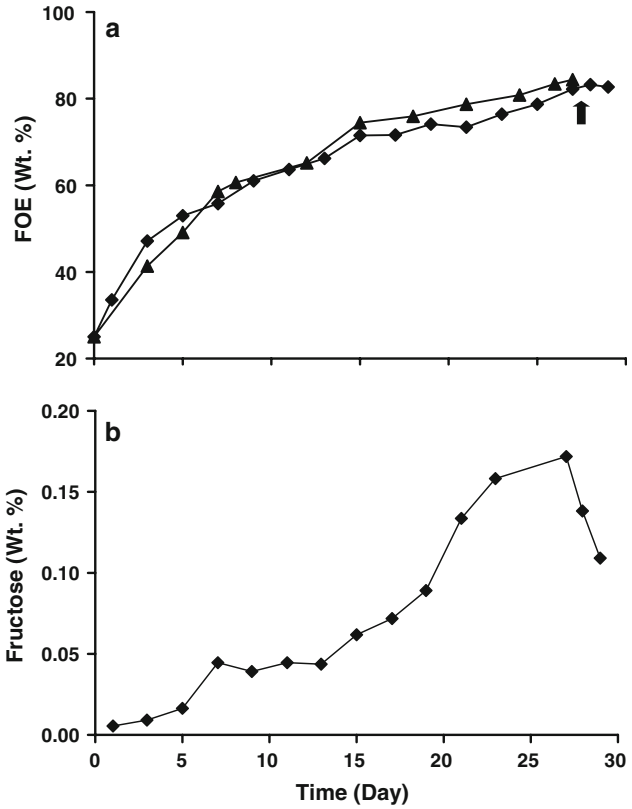

Fig. 5 Time course of (**a**) fructose oleic acid ester (FOE) and (**b**) fructose concentrations of the reservoir for a desorption column DC/packed bed bioreactor (PBBR) system undergoing continuous recirculation in the presence (*filled triangle*) versus absence (*filled diamond*) of a molecular sieve column (MSC; A → B → C → E → A and A → B → C → D → E → A in Fig. 1, respectively) at 65°C. PBBR- and PBBR/MSC-based systems: 5.0 and 10.0% RML (g per g oleic + FOE initially × 100%), respectively. Other conditions are given in Fig. 4

similar to the STBR-based system (Fig. 4a), but yielded 78.7% conversion, 83.2% conversion after the addition of molecular sieves (Fig. 5a). The latter conversion is comparable with the results obtained previously for operation using the stirred batch mode [14]. The product distribution favored FMO production, similar to the STBR-based system, with the FMO mass fraction increasing from 0.65 to 0.75 wt.% during the time course (Fig. 3c).

To determine if the reaction rate and yield can be increased by the removal of additional water, a molecular sieve column (MSC) was placed in series between the DC and the PBBR (Fig. 1, A → B → C → D → E → A), and the volume of the PBBR was doubled from 5 to 10% RML (per mass of reaction medium). Figure 5a demonstrates the time course of reaction for this system is not majorly different from that of the PBBR bioreactor system that employed only 5% RML and did not possess a MSC; in fact, the conversions achieved are nearly identical. The similarity of both time-conversion profiles supports the strong repeatability of the data obtained using this bioreactor system. The results agree with those obtained using the STBR-based systems showing that an increase of RML above 5% does not greatly enhance the rate or extent of reaction and that the time course of the reaction is not very sensitive to the water concentration during the initial phase of the time course for the reaction conditions employed [23]. The fraction of FMO among the FOE increased from 0.7 to 0.9 during the time course of the reaction (Fig. 3b).

A summary of the results obtained by the different bioreactor systems is given in Table 1. As demonstrated in the table, the highest conversion was achieved using a PBBR-based system in the presence of a MSC or after the addition of molecular sieve to the system's reservoir. The role of the water content on the conversion is discussed below. Of note, the STBR- and PBBR-based systems were significantly slower than the rate achieved in batch mode, reflecting the low saccharide concentration achieved using the DC.

Water Content in Each Bioreactor System and its Effects on Reaction Rate

As described previously, for the lipase-catalyzed synthesis of fructose oleate under solvent-free or nearly solvent-free conditions, the removal of water via free evaporation was sufficient for the reaction to proceed toward completion up to 60–80% conversion, with additional means of water removal (e.g., via molecular sieves) not being necessary; however, above 60% conversion, the employment of a low water activity environment (implemented through enclosure of the bioreactor in a closed container whose water activity was controlled by a saturated salt solution) or the addition of molecular sieves (Figs. 2, 4, 5) significantly increased the conversion [14, 23]. Figure 6 compares the water content of the STBR operated in batch mode versus the STBR and PBBR/MSC systems during the time course of reaction. As demonstrated in the figure, the latter system consistently yielded a lower water content throughout the time course. Perhaps the underlying reason for this result is that the reservoir open to the atmosphere, where removal of water by free evaporation occurred, had an absence of suspended RML particles and fructose crystals; in contrast, STBRs contained RML and, in the case of batch mode operation, fructose crystals. Both particles would adsorb water, particularly fructose crystals, which would result in an increased retention of water.

Figure 7a presents the relationship between the percent conversion and the water content achieved for the STBR operated in batch mode, and the STBR and PBBR-based bioreactor systems. It demonstrates clearly that the conversion increases with a decrease of water content, consistent with previous results [23, 24]. The lowest water content was achieved by employing molecular sieves during the latter part of the reaction (darkened symbols in Fig. 7a; or, employing a MSC) and by employing the PBBR rather than a STBR. Figure 7b demonstrates that the selectivity toward FMO increases as the water content increases, which reflects the influence of increased fructose

Table 1 Summary of final yield and product distribution for fructose oleic acid ester synthesis in bioreactor systems

System	System configuration (Fig. 1)	RML (wt.%)	Reaction time (day)		Water concentration (wt.%)		FOE content (wt.%)		Monoester/FOE, g/g
			Before MS addition	After MS addition	Before MS addition	After MS addition	Before MS addition	After MS addition	After MS addition
STBR	A′	7.5	11	13	0.75 ± 0.06	0.45 ± 0.07	73.5 ± 0.3	80.0 ± 2.6	0.35 ± 0.14
STBR/DC	A′ → B → C → A′	5	25	30	1.08 ± 0.18	0.78 ± 0.16	71.5 ± 0.9	74.5 ± 1.7	0.74 ± 0.04
PBBR/DC	A → B → C → E → A	5	25	29	0.64 ± 0.01	0.52 ± 0.01	78.7 ± 2.7	83.2 ± 0.5	0.76 ± 0.03
PBBR/DC/MSC	A → B → C → D → E →A	10		27		0.29 ± 0.01		84.4 ± 0.7	0.92 ± 0.05

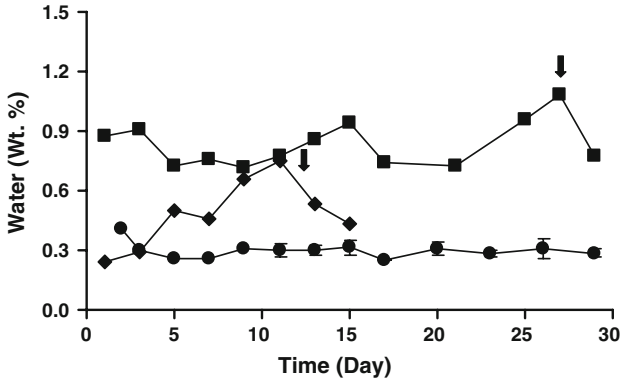

Fig. 6 Change of the reservoir's liquid phase water content during the time course of lipase-catalyzed solvent-free fructose oleic acid ester (FOE) synthesis at 65 °C for various bioreactor systems. (*Filled diamond*), STBR operating in batch mode, with fed-batch addition of fructose (Fig. 2, 7.5% RML); (*filled square*) DC/STBR-based system undergoing continuous recirculation (Fig. 4, 5.0% RML); (*filled circle*) DC/MSC/PBBR-based system undergoing continuous recirculation (Fig. 5). The addition of molecular sieves (0.10 g per g of oleic acid + FOE) indicated by *arrows*

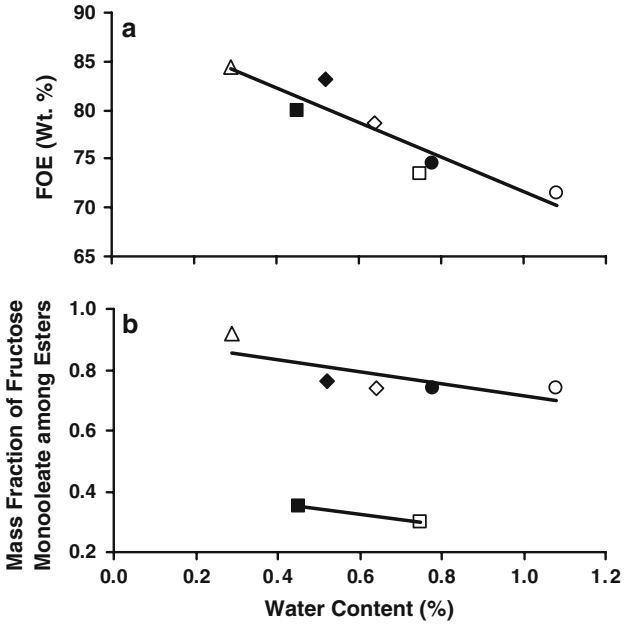

Fig. 7 Relationship of water content (wt.%) with (**a**) fructose oleic acid ester (FOE) concentration (wt.%) and (**b**) mass fraction of fructose monooleate (FMO) among FOEs. *Outlined* and *darkened symbols* represent results that occurred before and after the addition of 10 wt.% molecular sieves, respectively. Reactor systems: (*open square*, *filled square*) STBR operating in batch mode, with fed-batch addition of fructose (Fig. 2, 7.5% RML), (*open circle*, *filled circle*) DC/STBR-based system undergoing continuous recirculation (Fig. 4, 5% RML), (*filled diamond*, *open diamond*) DC/PBBR-based system undergoing continuous recirculation (Fig. 5), and (*filled triangle*) DC/PBBR/MSC-based system undergoing continuous recirculation (Fig. 5)

solubility and medium polarity with an increased production of FOE rather than a direct relationship with the water content, since it is known that a decrease of water content will promote a more hydrophobic environment for lipase, which would result in an increased selectivity toward FDO, as described above. The underlying reason for the lower selectivity toward FMO for the STBR reactions operated in batch mode displayed in Fig. 7b is due to the relatively low initial fructose concentration, as described above.

Conclusions

The solvent-free lipase-catalyzed synthesis of saccharide-fatty acid using a stirred-tank bioreactor operated in batch mode and using bioreactor systems containing a desorption column packed with saccharide crystals and silica gel operating under continuous recirculation is reported here. Batch-mode operation yielded a similar time course to that achieved previously using similar conditions except for the inclusion of solvent, *tert*-butanol, during the initial phase, except for a slower rate of reaction at the latter stage, which may have been a function of the programming used for the fed-batch addition of fructose, which was not optimized [14]. The employment of bioreactor systems provided a comparable yield to the batch mode reactions, but occurred more slowly because of the relatively low concentration of fructose achieved by the packed desorption column. Moreover, the stirred batch approach appears to produce suspensions of 100–300-μm-sized crystalline fructose particles, yielding a higher apparent saccharide concentration [18]; the suspension is readily utilized by immobilized lipase without loss of the inherent enzyme activity during a month of continuous operation [14]. The solvent-free approach, environmentally friendly due to its use of mild reaction conditions and the absence of solvents or other additives, yields a product containing 80–85% FOE and strong selectivity toward monoester production, a technical-grade biobased surfactant product that does not require further downstream purification. Therefore, there is potential value for the solvent-free approach.

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