RECENT PROGRESS FOR LIPASE-CATALYSED SYNTHESIS OF SUGAR FATTY ACID ESTERS

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ABSTRACT

Sugar fatty acid esters, important biobased value-added emulsifiers in foods, cosmetics, pharmaceuticals, and personal care products, are typically prepared using high temperatures (>100°C) and in the presence of organic solvents. A more environmental sustainable approach is to employ biocatalysis, particularly lipases, for their preparation, leading to reduced solvent usage, lower temperatures, and lower generation of waste products. In order for lipase-catalysed synthesis to be a robust alternative to chemical synthesis, several technical barriers need to be overcome, particularly the difficulty in co-solubilising acyl donor and acceptor, leading to a lower reaction rate. This article reviews two recent approaches to overcome the latter barrier via formation of suspensions of saccharide particles, in ionic liquids for one case and in solvent-free reaction media in another. The former provides 10-100-fold higher saccharide concentration, hence more rapid enzyme kinetics; but, both suspension types yield similar values for productivity (moles of product per mass of enzyme per time). The solvent-free approach offers improved enzyme activity retention, minimal requirements for downstream purification, and the absence of costs associated with solvent usage and recovery. Other recent trends for the enzymatic synthesis of sugar esters are also reviewed, particularly the utilisation of oligomeric acyl acceptors and new biocatalysts.

Keywords: biobased surfactant, lipase, sugar esters, ionic liquid, solvent-free bioconversions.

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INTRODUCTION

Globally, over 5500 t of saccharide fatty acid (FA) esters, are manufactured [*e.g.*, by Mitsubishi-Kagaku Foods Corp. (Tokyo, Japan) and Sisterna B.V. (Roosendaal, Netherlands)] at > 5500 t of production (Otomo, 2009). They are biodegradable, biocompatible nonionic biobased surfactants and emulsifiers derived from inexpensive renewable resources (therefore serving as 'value-added products'), widely employed in foods, pharmaceutical and cosmetics (Otomo, 2009). Applications for their use as food emulsifiers, outlined in *Figure 1*, are directly related to their inherent hydrophilic-lipophilic balance, or HLB. Surfactants with HLB ≥ 11 are more hydrophilic, hence water-soluble, and therefore commonly used to form oil-in-water (o/w)-emulsions (*e.g.*, in ice cream and cake batter, *Figure 1*). Alternatively, sugar esters possessing low HLB values (≤ 5) are oil-soluble and hence, used to form w/o-emulsions (*e.g.*, in chocolates, *Figure 1*), while those possessing intermediate HLB values (8-10) are equally balanced in hydrophilicity and lipophilicity (used in chewing gum, *Figure 1*) (Otamo, 2009).

The hydrophilicity of sugar esters increases with a decrease of the fatty acyl chain length and also a decrease degree of esterification; for instance, mixtures of mono and diesters, as typically formed

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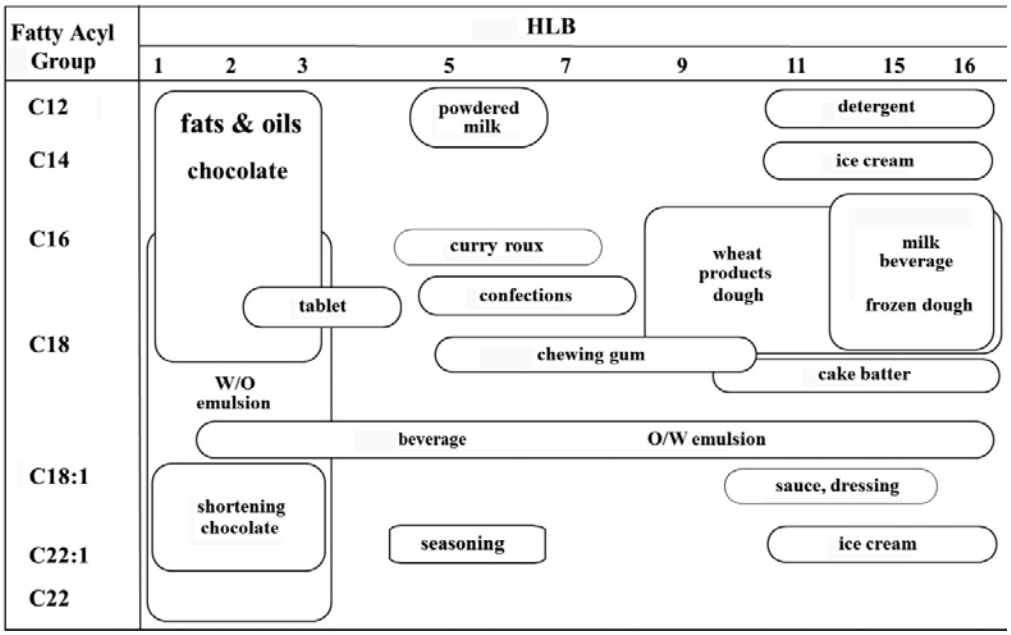

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Figure 1. Application map of sucrose fatty acid esters.

via enzymatic reactions, possess HLB values near 16, while surfactants enriched in sucrose penta- to –octa-esters have HLB values of ≤ 3 (*Figure 2*).

In addition, sugar esters exhibit anti-microbial, anti-tumour, and insecticidal activity (Zhao *et al*., 2014), are biocompatible with skin and as food components (*e.g*., 'generally regarded as safe', or GRAS, status by US Food and Drug Administration), among other important properties, thereby expanding their utility (*Table 1*).

Sucrose esters are typically prepared via transesterification of fatty acid methyl esters (FAME) at elevated temperatures (>100°C) and reduced pressure for several hours, often in the presence of toxic solvents such as dimethylformamide (DMF) or dimethylsulfoxide (DMSO) (Farone and Serfass, 1996; Polat and Linhardt, 2001; Feuge *et al*., 1970; Nelen and Cooper, 2004). Therefore, chemical synthesis method is not sustainable for the environment, and can also lead to formation of by-products (*e.g*., due to caramelisation of sugars) and sugar esters of poorer quality, *e.g*., possessing discolouration.

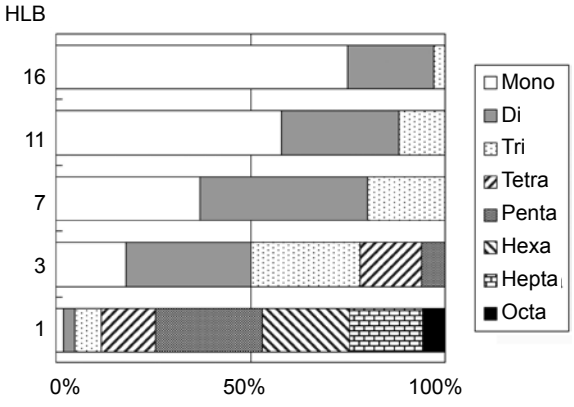

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Figure 2. Relationship between hydrophilic-lipophilic balance (HLB) and ester composition of sucrose stearates.

First reported in the 1980s, lipase-catalysed synthesis of saccharide-FA esters has been achieved utilising several different acyl acceptors: C_4-C_6 monosaccharides (*e.g*., fructose, glucose, xylose, and ribose), di- and tri-saccharides (*e.g*., sucrose, lactose, trehalose, and maltotriose), and sugar alcohols (*e.g*., sorbitol and xylitol, noting sorbitan esters are common commercial nonionic surfactants, Span®). The inherent region selectivity of biocatalysts greatly narrows the product distribution; for example, lipases typically can utilise only two of the eight hydroxyl groups present on sucrose (primarily the 6-OH moiety of the glucopyranose ring, with typically lower selectivity toward the 1'-OH group of the fructofuranose ring), *Figure 3*. However, reaction rates and yields are typically low due to the poor miscibility of the acyl donor and acceptor substrates, contributing toward mass transfer limitations and fouling of lipases preparations, and to the difficulty in controlling the water concentration of the reaction system, since the reaction co-product water induces the hydrolysis of ester product, thereby reducing the equilibrium conversion.

As reviewed (Ballesteros *et al*., 2007; Chang and Shaw, 2009; Gumel *et al*., 2011; Pyo and Hayes, 2009),

TABLE 1. APPLICATIONS OF SUGAR ESTERS AND THEIR DERIVATIVES

Application	Sugar esters type	Reference
Emulsifiers/Surfactants		
Food emulsifiers	Various	(Otomo, 2009; Nakamura, 1999)
Demulsification of heavy petroleum sludge	Ethoxylated glucose esters	(Abdul-Raheim *et al.*, 2013)
Skin and personal care products	Various	(Polat and Linhardt, 2001)
Gelators of organic solvents; oil spills	Diesters of trehalose, sugar alcohols	(John *et al*., 2006; Jadhav *et al*., 2010)
Span surfactants	Sorbitan esters	(Heuschkel *et al*., 2008)
Tween surfactants	Polyethoxylated sorbitan esters	(Bhattacharya and Palepu, 2004; Heuschkel *et al.*, 2008)
Drug Delivery Systems		
Transdermal	Sucrose esters	(Csoka *et al*., 2007, Okamoto *et al*., 2005)
Tableting	Sucrose esters	(Chansanroj and Betz, 2010)
Encapsulation of lidocaine in nanoparticles	Dextran decanoate esters	(Kaewprapan *et al*., 2012b)
Encapsulation of proteins in nanoparticles	Sucrose esters	(Abdel-Mageed *et al*., 2012; Youan Bi-Botti *et al*., 2003)
Anti-microbial Agents		
Bacillus sp., *Lactobacillus plantarum*	Lauric acid esters of sucrose and maltose	(Ferrer *et al*., 2005)
Heat-resistant bacteria	Sucrose monopalmitate	(Otomo, 2009)
Escherichia coli (on spinach)*	Sucrose monolaurate	(Xiao *et al*., 2011)
Food-borne pathogenic bacteria*	Sucrose esters	(Furukawa *et al*., 2010)
Insecticidal Agents		
Aphis glycines, Lymantria dispar	Diester of sucrose and octanoic acid	(Song *et al*., 2006)
Sweet potato whiteflies, aphids	Diesters of sucrose and heptanoic+nonanoic acid	(Chortyk *et al*., 1996)
Whiteflies	Sucrose esters	(Liu *et al*., 1996)
Tobacco aphid	Sucrose esters	(Xia *et al*., 1998)

Note: **Staphylococcus aureus*, *E. coli, Streptococcus mutans* and *Listeria monocytogenes.*

polar co-solvents (and/or solvent co-mixtures) are typically used in the bioconversions. In general, the reactions are operated near the solvent's boiling point under reflux using a Dean-Stark or molecular sieve trap to remove water, with the acyl acceptor adsorbed onto a macroscopic carrier such as silica gel and thereby suspended in solution via agitation. Finding a solvent (system) that possesses high solubility of saccharide (high polarity) and does not retain water (*e.g.*, does not form an azeotrope) nor promote enzyme inactivation (prominent in polar solvents such as DMSO), is a considerable challenge. The most common solvents for this application, *e.g.*, *tert*-butanol (t-BuOH) and dimethylformamide (DMF) are rather expensive; and the latter is not readily removed by evaporation. The employment of neat supercritical (SC)-CO_2 and its mixtures with acetone and ionic liquids (IL) have also been attempted [as well as CO_2-expanded acetone (Tai and Brunner, 2009)], an approach worthy of investigation (due to the low diffusivity of substrates and low solvent costs associated with SC-CO_2), but yet also suffers from low solubility of acyl acceptor [reviewed in (Shi *et al*., 2011)]. Many reports for enzyme-catalysed sugar esterification employed commercially available immobilised lipases, particularly *Candida antarctica* lipase B (CALB) immobilised onto macroporous acrylate, and to a lesser extent *Rhizomucor miehei* (RML) and *Thermomyces lanuginosa* lipase (TLL) adsorbed macro-porous polyacrylate, ion exchange resin, or silica gel. Of interest, the lipases possess different degrees of regioselectivity, with RML and TLL showing preference for the synthesis of monoesters and CALB-catalysed reactions yielding diester/monoester mixtures. For example, in the laboratory, when RML was replaced by CALB during the latter stage of the reaction for fructose-oleate synthesis, the

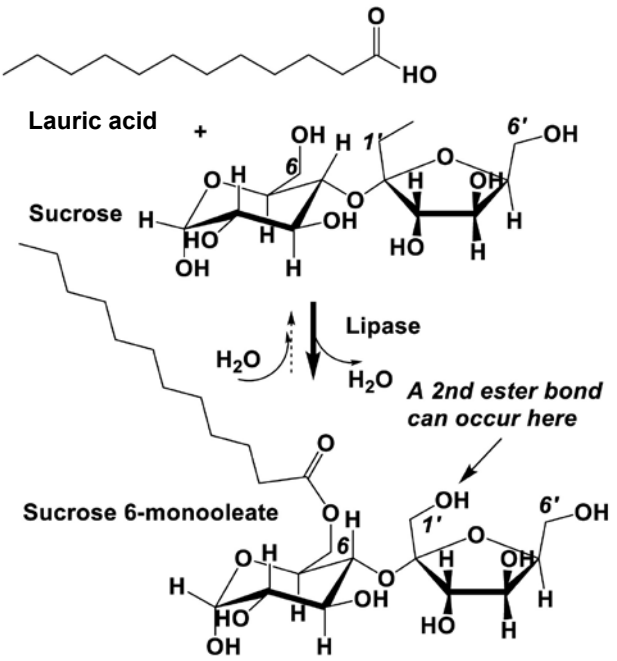
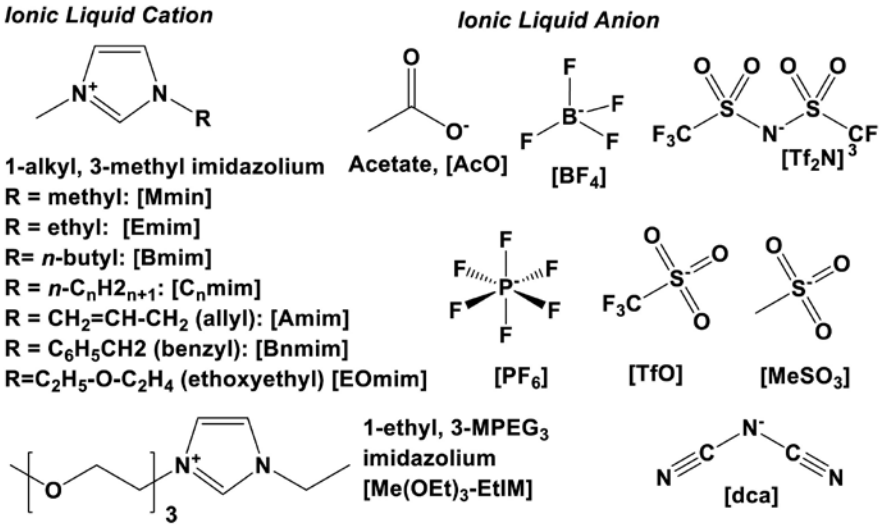


Figure 3. Reaction scheme for the lipase-catalysed synthesis of sucrose oleate.

fraction of monoester among the esters decreased from 90% to 65% (unpublished).

Several creative attempts have been employed to enhance miscibility, including the derivatisation of saccharides with protective groups (*e.g.*, isopropylidene) or complexation agents (*e.g.*, phenylboronic acid). However, derivitisation is not robust due to costs associated with reagents' and with protection + deprotection steps. A more promising approach is to carry out the reaction in a mainly solid-phase system consisting of saccharide, free fatty acid (FFA), and ester product in the presence of a small amount of solvent (*e.g.*, t-BuOH or acetone) at relatively low, often near ambient, temperature. The solvent serves as adjuvant to maintain a catalytic phase for the action of the enzyme (Cao *et al.*, 1999; 1997). Saccharides are poorly soluble in the system's liquid phase, which would typically lead to a low rate of reaction. However, reasonably high rates and extents of reaction were reported, due in part to the rapid crystallisation of the monoester from the liquid phase, driving the reaction into the forward direction via thermodynamic equilibrium. However, for the latter reaction, enzyme stability was subpar. CALB when reused in six subsequent batch reactions experienced a decrease in conversion of 25%. Other disadvantages of this approach include the use of organic solvent and the difficulty of separating the precipitated monoester from unreacted saccharide particles and immobilised biocatalyst.

The most promising approach according to the present authors' assessment of the recent literature on the lipase-catalysed synthesis of sugar esters is the use of suspensions of acyl donor in either IL or in liquid-phase solvent-free reaction media, the latter approach of which has been conducted by the authors. The employment of suspension-based media will be the focus of this review article. In addition, in recent years this biochemical reaction approach has been extended to additional acyl donors, acceptors (particularly to oligopolysaccharides), and biocatalysts. This topic will also be explored herein. These 'new directions' will also be described herein.



Note: 'mim' refers to imidazolium cation.

Figure 4. Molecular structure of ionic liquids used for conducting lipase-catalysed synthesis of sugar esters.

SACCHARIDE SUSPENSIONS IN IONIC LIQUIDS (IL) AS REACTION MEDIUM

Before a discussion based on IL-based suspensions for lipase-catalysed synthesis of sugar esters will be given, the employment of IL for this reaction will be briefly reviewed. Several different IL (*Figure 4*) may be potentially valuable solvents for lipase-catalysed esterification of saccharides, due to the high rates and extents of reaction that have been reported [reviewed in (Yang and Huang, 2012)]. Moreover, the solubilisation of glucose in IL is approximately 10 times higher than in commonly employed organic solvents; for example, the solubility of glucose at 60°C is 2.6 g litre^{-1} and 27.8 g litre^{-1} in 2-methyl 2-butanol and [Emim][TfO], respectively (Lee *et al*., 2008a). Generally, an increase of IL polarity increases saccharide stability but reduces enzyme stability. According to a recent review, the solubility of saccharides is more affected by the IL's anion than its cation, with effectiveness of anions towards glucose solubilisation occurring as follows (cf. *Figure 4*): [dca] > [TfO] > [BF_4] > [PF_6] > [TF_2N] (Yang and Huang, 2012). The solubilisation in [dca]-based IL is approximately one order of magnitude larger than IL based on the other anions listed above (Yang and Huang, 2012). The effect of the cation was also significant, with ethoxylated cations (*e.g*, [EOmim], cf. *Figure 4*) being the most effective (Yang and Huang, 2012).

Koo *et al*. have achieved large rates and extents of reactions through the formation of IL-based metastable suspensions of saccharide, formed by mixing aqueous saccharide solutions and IL, and then removing water *in vacuo*. This produced a high concentration of saccharide: > 500 g litre^{-1}, 113 g litre^{-1}, 46.3 g litre^{-1}, and 11.3 g litre^{-1} in [Emim][$MeSO_3$], [Emim][TfO], [Bmim][TfO], and [Emim][BF_4], respectively (Lee *et al*., 2008a). For CALB-catalysed esterification of lauric acid and glucose using a supersaturated solution of the latter in [Emim][TfO] at 50°C (with molecular sieves added to remove water), the same group achieved 91% conversion in 100 hr (Lee *et al*., 2008a). The product was recovered via precipitation, using water as an anti-solvent (Lee *et al*., 2008a). However, after use for five successive reactions conducted in [Bmim][TfO], CALB lost 64% of its original activity (Lee *et al*., 2008b). In subsequent research, to improve the stability, [Bmim][TFO] was mixed with the more hydrophobic IL, [Bmim][TF_2N], at 1:1 v/v, leading to a lower rate and extent of reaction, but to a significant improvement of stability: only 14% loss during five successive runs (Lee *et al*., 2008b). Similar trends were reported by the same group for [Bmim][TfO]/[C_8mim][Tf_2N] mixtures, with the optimal volume ratio of the IL for activity and stability being 9:1 and 1:1, respectively (Ha *et al*., 2010).

Other groups have also recently investigated IL for enzymatic sugar esterification. Wei *et al*. conducted sugar ester synthesis using FA esters as acyl donor and two- to three-fold molar excesses of donor relative to acceptor, glucose (Liang *et al*., 2012; Yao *et al*., 2012). Their trends in terms of optimal IL and biocatalyst reflect those described above. Two other recent reports employed IL/solvent mixtures as reaction medium, an approach developed previously (Ganske and Bornscheuer, 2005). In one report, galactose oleate was formed in a [Bmim][BF_4]/DMSO cosolvent mixture (20:1 v/v) using RML, achieving 87% conversion in 2 hr for a 3:1 mole ratio feed of oleic acid to galactose (Abdulmalek *et al*., 2012). In the second report, several IL, solvents, and their mixtures were examined for hosting the lipase-catalysed synthesis of maltose oleate mono- and di-esters. The highest conversions, 65%-75%, occurred for 1:1 v/v mixtures of acetone/DMF and [Emim][$MeSO_3$] / tetrahydrofuran, and CALB and *Pseudomonas cepacia* lipases as biocatalysts (Fischer *et al*., 2013). Optimal conversion did not correlate with the polarity of the solvent system (Fischer *et al*., 2013).

When one evaluated reactions based on productivity, moles of product per time per mass of enzyme, the advantage of employing IL (and polar organic solvents) is diminished (Ye *et al*., 2010). Equally as important, IL frequently promote enzyme inactivation [due to hydrogen bonding (Zhao *et al*., 2008)], are expensive, and many possess toxicity (Arning and Matzke, 2011; Pham *et al*., 2010; Yan *et al*., 2012). However, novel ethoxylated IL (cf. *Figure 4*) promote lower inactivation of enzymes, possess the high acyl acceptor solubility of other IL and have been successfully employed for the saccharide solubilisation and for lipase-catalysed esterification of D-glucose and cellulose with lauric acid vinyl ester and methyl methacrylate (Zhao *et al*., 2008; 2009). A promising alternative to IL for enzymatic transformations are deep eutectic solvents (DES), solvent-free IL-inspired reaction media formed by mixing solid organic salt (such as choline or other quaternary ammonium salts) and a complexing agent (*e.g*., hydrogen bond donors such as urea or glycerol), leading to a liquid at temperatures below 100°C when mixed in the proper proportions [reviewed in (Durand *et al*., 2013; Zhang *et al*., 2012; Zhao and Baker, 2013)]. DES systems have been used for several enzymatic transformations, and can be tuned to possess different polarities via choice of components, similar to IL. The major advantage of DES systems relative to IL is their lower cost, lower toxicity, higher biodegradability, and their lower propensity to denature enzymes. There are no reports for the use of DES systems to host enzymatic sugar esterification to date.

SACCHARIDE SUSPENSIONS IN SOLVENT-FREE REACTION MEDIUM

The authors have employed metastable suspensions of 10-200 μm-sized saccharide particles in solvent-free reaction medium for the lipase-catalysed synthesis of sugar esters. Here, sugar-FA esters, the desired product, serve as a cosolvent with acyl donor to improve the miscibility of donor and acceptor. To form the suspensions for the initial reaction medium, saccharide crystals are combined with a mixture of acyl donor (typically oleic acid) and ester, with the latter present at 5%-25%, and then vigorously stirred via magnetic stirring at ~800 rpm and 80°C for 6 hr, followed by centrifugation at 800 rpm for 0.5 min to remove the larger suspended particles, to lessen fouling in bioreactor system. The resultant supersaturated solution contained 1%-2% w/w saccharide. The suspensions were stable: <20% decrease in saccharide concentration occurred during a 12-hr period when the medium was stagnant (Ye *et al*., 2010). Subsequently, the suspension-based medium (~30 g) was introduced to a closed-loop bioreactor system operated under continuous recirculation that consisted of the following components: a reservoir open to atmosphere and maintained at 65°C (to enable free evaporation of the co-product, water); a peristaltic pump, and a packed-bed bioreactor (PBBR) operated at 53°C that contained immobilised lipase, typically, RML (Ye *et al*., 2010; Ye and Hayes, 2011). Periodically, recirculation of the reaction medium was paused, so that the consumed saccharide could be replenished, requiring off-line re-formation of the suspensions using the procedure described above.

During the initial reaction period, from 0% to ~60% conversion, free evaporation of water sufficiently removed water formed by the reaction. However, during the latter stages of the reactions, an additional means of water removal was required (either a combination of N_2 bubbling and vacuum pressure applied to the reservoir, or introduction of a molecular sieves packed column into the bioreactor system) to achieve a significantly high extent of reaction via lowering of the water content from ~0.8% to 0.4 % w/w. When the timing of suspension retreatment and introduction of more stringent water removal were optimised, for fructose oleate synthesis, 92.6% ester in the reaction medium was achieved within 132 hr (*Figure 5*) (Ye and Hayes, 2012a). This represents a productivity of 0.30 mmol hr^{-1} g^{-1}, which was among the highest reported values in the literature (Ye and Hayes, 2012a). The reaction medium, readily recovered, may serve as a technical grade product not requiring further purification. For the reaction depicted in *Figure 5*, in a 3.0 week period, four successive reactions were conducted without any loss of enzyme activity,

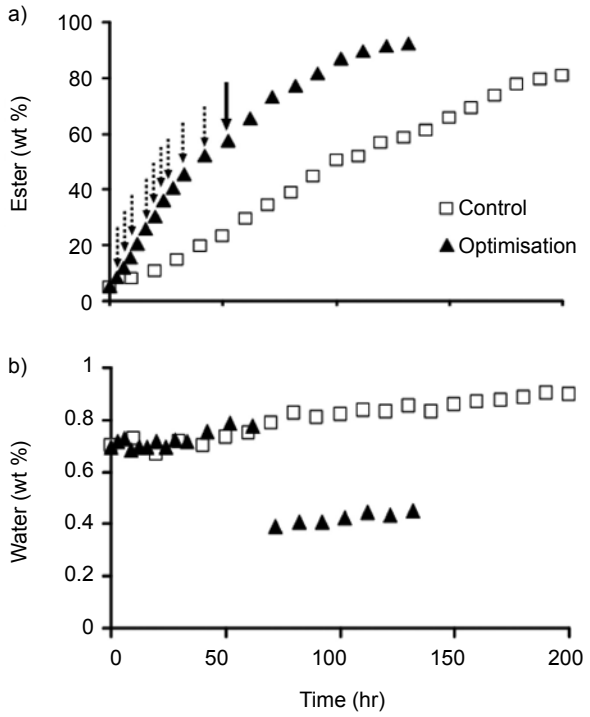


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Figure 5. Optimisation of interval time for between saccharide replenishment treatments and removal of water for the solvent-free Rhizomucor miehei (RML)-catalysed synthesis of fructose oleate using a packed-bed bioreactor system at 53°C. (a) Production of ester, (b) water control of liquid phase.'Optimisation': water removal via 67.7 kPa vacuum and 2.56 litre min^{-1} N_2 (g) was introduced to the reservoir upon reaching approximately 60% ester in the liquid phase, indicated by downward pointing arrows; timing of saccharide replenishment treatments indicated by dashed arrows. 'Control' experiment: vacuum plus N_2 bubbling were not incorporated; interval time between saccharide replenishment treatments was 10.0 hr.

indicating outstanding enzyme activity retention (Ye and Hayes, 2012a).

In more recent work, the solvent-free reactions were operated at the 20 g scale using the PBBR-based system described above, but using *in situ* addition of saccharide to the well-stirred reservoir, to replace off-line replenishment of acyl acceptor via formation of suspensions (Ye and Hayes, 2012b). This approach required employment of an in-line nylon filter of 180 micro metre nominal pore size placed at the inlet of the tubing that delivered the reaction medium from the reservoir to the pump, to prevent clogging. This system produced a similar conversion as achieved previously (85%-90% esters), but at a 30% lower productivity (0.20 mmol hr^{-1} g^{-1}) (Ye and Hayes, 2012b).

The solvent-free approach, using both the PBBR-based bioreactor system and stirred tank bioreactors (STBR), was applied to several different acyl donors and acceptors. A universal linear relationship occurred between the initial saccharide concentration and initial rate of reaction and final ester concentration, for all experimental conditions,

Figure 6 (Ye and Hayes, 2012a). Saccharide starting materials possessing the smallest average size produced suspension-based media with the highest saccharide concentration; moreover, saccharides with large particle size sedimented from the solution during the centrifugation step of the suspension formation procedure described above (Ye and Hayes, 2012a). Recently, the authors have been employing size reduction methods for the saccharide crystals to enhance the concentration and stability of the suspensions (unpublished data).

NEW DIRECTIONS FOR ENZYMATIC SYNTHESIS OF SUGAR ESTERS

Lipase-catalysed sugar esterification has recently been expanded to include a wider variety of acyl acceptors and donors. Regarding the former, the use of lipases and other hydrolases for the esterification of polysaccharides, such as dextrans, starches, and low molecular weight cellulose, has received particular interest, evidenced by the recent release of two review articles (Alissandratos and Halling, 2012; van den Broek and Boeriu, 2013). Recently, Durand *et al.* transesterified dextran (weight-average molecular weight of 40 kDa) and vinyl decanoate in DMSO using *Candida rugosa* lipase co-lyophilised with crown ether, achieving degrees of substitution (moles of ester bonds per mole of glucose units x 100%) up to 150% (Kaewprapan *et al*., 2012a). These materials may be useful amphiphiles (Kaewprapan *et al*., 2012b). Zhao *et al*. employed ethoxylated IL for the transesterification of Advicel® PH-100, a microcrystalline cellulose, with methyl methacryalate, yielding a multifunctional monomer (Zhao *et al*., 2008). Another example of a new acyl acceptor is monosaccharide modified with a dihydric alcohol. In a recent study, xylose dimers and oligomers first underwent transxylosylation with several different α,ω-diols, producing monoxylose attached to the alcohol via an ether linkage at the former's reducing end, shown in *Figure 7* (Kurakake *et al*., 2011). Subsequently, the remaining free primary hydroxyl of the xylose-conjugated dialcohol is readily acylated using a lipase, yielding a xylose ester possessing a dialcohol linker between the saccharide and fatty acid units (*Figure 7*). Regarding the extension of sugar esterification to new acyl donors, a recent study employed 4-hydroxyphenyl propionic acid methyl ester for the formation of sugar alcohol esters in DMSO/t-BuOH mixtures (Croitoru *et al*., 2011). Yields of 50% were obtained for xylitol and sorbitol as acceptors and CAL as biocatalyst, with the product potentially useful as an oil-soluble antioxidant.

Enzyme technology will also continue to play an important role in the improvement of this reaction's performance. In a recent study, whole cells were used as biocatalyst, specifically, *Pichia pastoris* cells that displayed CAL on their cell surface. A DMSO/2-methyl 2-butanol cosolvent mixture was used for hosting fructose laurate esterification using the whole cell biocatalysts (Jin *et al*., 2013). Yields of 60%-70% were obtained using a stoichiometric excess of donor. Importantly, the operational stability of *P. pastoris* was very good, with little or no loss of activity in 15 successive reactions, despite the use of the denaturing solvent DMSO.

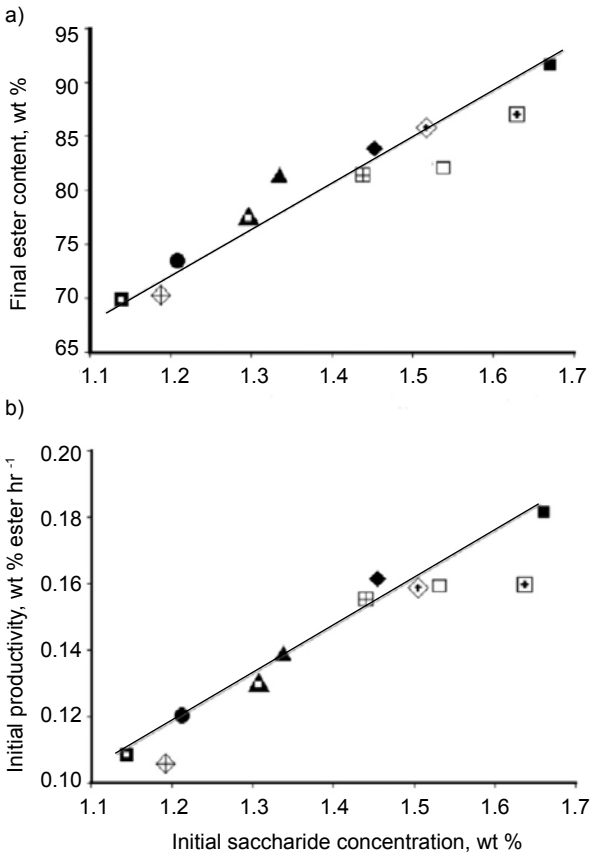

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Figure 6. Relationship between initial saccharide concentration, final ester concentration (a), and initial rate of reaction (b) for the solvent-free lipase-catalysed synthesis of saccharide-fatty acid esters in either a packed-bed bioreactor (PBBR) system at 53°C or a stirred-tank bioreactor (STBR) at 65°C. Legend: fructose (square), glucose (triangle), xylose (circle), sucrose (diamond); oleic acid (no embedded symbol), caprylic acid (embedded square), lauric acid (embedded medium-sized plus symbol) and myristic acid (embedded large-sized plus symbol); PBBR (filled), STBR (unfilled).

CONCLUSION

Biocatalytic synthesis of sugar esters has undergone several improvements during the past few years, leading to enhanced rates and extents of reactions, productivity, improved control of the product distribution, and an expanded range of possible carboxylic acyl and saccharide starting materials. As reviewed in this article, a major development

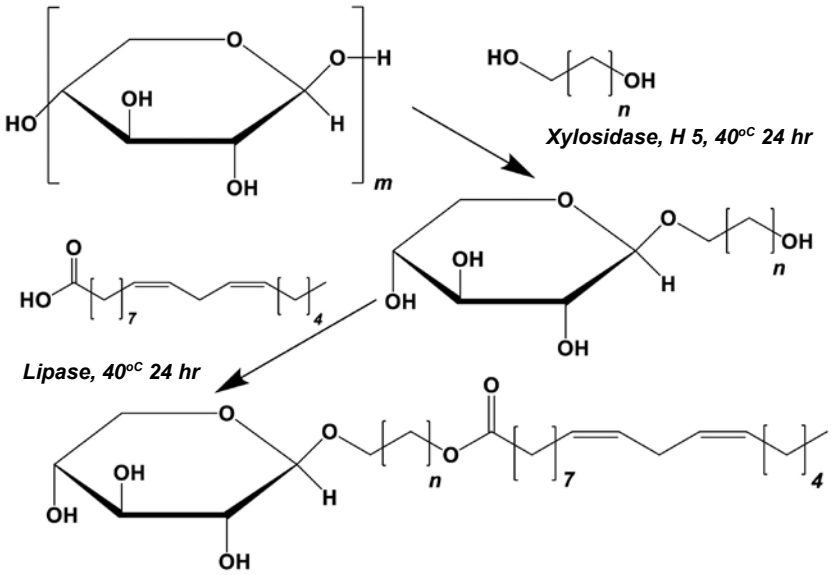

Figure 7. Formation of a xylose-linoleic acid ester possessing an α,ω,-diol (n=1-5) as a linker using xylosidase and lipase, as employed in (Kurakake et al.*, 2011), using xylobiose (m=2) or a xylooligosaccharide mixture (m=1-3) as a xylose donor for the first, transxylosylation, step of the reaction.*

to improve this reaction is the use of micron-sized (or smaller) suspensions of saccharide particles as reaction medium, either in ionic liquids or their cosolvent mixtures, or in solvent-free reaction medium. Further improvements in the reaction medium design, controlling of water content, and development of more active and stable biocatalysts will ultimately lead to a cost-effective bioprocessing approach for the sugar ester manufacturing that will be viable 'green' alternative to the high-temperature catalytic approaches currently used by industry, particularly for starting materials that are susceptible to degradation, such as polyunsaturated fatty acids.

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