



Review

Enzymatic synthesis of amphiphilic carbohydrate esters: Influence of physicochemical and biochemical parameters



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ABSTRACT

Glycolipids, carbohydrate fatty esters or sugar esters are amphiphilic molecules containing hydrophilic groups bonded to hydrophobic parent structures. Recently, glycolipids have shown their antimicrobial and antitumor capacities. Their surface activity properties have applications in the food, pharmaceutical and cosmetic industries. Sugar esters' building blocks can be obtained from natural resources and/or be transformed by biochemical pathways for uses as surfactants. Biosurfactants are non-ionic, nontoxic, biodegradable, tasteless, and odourless. The biocatalysis of these molecules involves sustainable, green, and safer methods. The advantages of producing biosurfactants from enzymatic catalysis are the energy economy, high selectivity, production of natural products, reduction of the use of fossil-based solvents and chloride compounds. This review presents the most recent studies concerning the evaluation of the impact of the main parameters and their levels influencing the enzymatic synthesis of glycolipids. Various enzyme catalysed synthetic methods were described. The parameters studied were temperature, reaction time, solvent system, type of biocatalyst, substrates molar ratio proportion and the nature of substrates. This review discusses the influence of different biocatalysts in the conversions of glycolipids; The reactivity from mono to polysaccharides and their interaction with fatty acids of different carbon chain lengths in the presence of specific enzymes; The effect of the solvent polarity, the use of multiple solvents, ionic liquids, supercritical CO₂, and solvent-free media in sugar ester conversions; And the optimization of temperature and reaction time in different enzymatic systems.

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1. Introduction

Surfactant (surface active agent) molecules are constituted by a hydrophobic long alkyl carbon chain linked to a hydrophilic polar head. They present the ability to form aggregates (micelles) in the interface between solvents with different polarities reducing the interface tension between them. Surfactants can also reduce the surface tension of aqueous media in systems of water-gas, for instance. Due to their affinity to the different phases in a heterogeneous medium, they are named as amphiphilic (from the Greek *amphi*, “on both sides” and *philos*, “friend”). Surfactants are currently used as emulsifiers [1–3], detergents [4,5], wetting agents, foaming agents [6–8], agents in drug delivery systems [9], and bioactive molecules [10–16]. Biosurfactants are naturally produced by various microorganisms such as yeasts, fungi, and especially by bacteria present in lipid environments [17–20]. These

substances can be isolated from their biological medium by extraction, distillation, or precipitation processes. The properties of biosurfactants are related to their origin and chemical structures. There are different types of biosurfactants. The five main groups are the lipopeptides, lipoproteins, glycolipids, phospholipids, and the polymeric ones [11]. Carbohydrate fatty acid esters, also called “Glycolipids”, are the most popular biosurfactants. They are also known as fatty acid sugar esters due to their structure formed by a sugar head (mono- or oligosaccharide) and a fatty acid tail. Carbohydrate fatty esters are non-ionic, non-toxic, tasteless, odourless and biodegradable biosurfactants, and can exhibit antimicrobial [10,16] and antitumor [12] properties. These molecules present several applications such as in food, pharmaceutical, cosmetic, and petrochemical industries [21,22]. Moreover, they can play an important environmental role in the biodegradation of spill oil as in the treatment of industrial effluents and soil [23]. Glycolipids can be produced by microbial fermentation [18,24], by chemical or enzymatic synthesis using renewable resources [25,26]. The advantages of biocatalysed reactions are the possibility of working in mild conditions. Enzyme-catalysed reactions are normally conducted under controlled temperatures from 30 °C to 70 °C and can use eco-

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friendly solvents. Enzymes are specific, selective, versatile, reusable, and biodegradable catalysts [27]. Enzymatic pathways can take place either in water or in organic solvents with low water activity with low water activity, they demand fewer steps of purification and generate safe products for food, cosmetic and pharmaceutical industries. Esterification reactions using enzymes as catalysis are safer for food process as they normally only need carboxylic acids, amino acids or vinyl esters derivatives as reagent instead of acyl chlorides as acyl donors [28]. This review presents the most recent works on the biocatalysis of carbohydrate fatty esters as a resume of the studies of the past ten years. For comparison purposes, the results of efficiency of the biocatalysed reactions on this review are presented in percentage of conversion of the acyl donor.

2. Chemical parameters and their levels influencing the enzyme-catalysed synthesis of glycolipids

2.1. Influence of the solvent

The degree of solubility of the saccharide in the reaction medium seems to be one of the most important factors in the glycolipid syntheses. Solvents as DMSO [12,29–31], DMF, THF [1], acetone [32], acetonitrile [33], tertiary alcohols [7,28,34–36], pyridine, and even ionic liquids were described to be efficient in the solubilisation of saccharides during their biocatalysed esterification. It is well known that lipases act in the hydrophobic/hydrophilic interface of heterogeneous media and can catalyse hydrolysis and esterification reactions, mainly depending on the amounts of water in the system. Even for the reverse reaction of hydrolysis, *i.e.* esterification, lipases need a minimum quantity of surrounding water to keep their active conformation. A good solvent system for the esterification of sugar by fatty acids should provide a good solubilisation of both reagents (saccharide and fatty acyl donor) taking into account distinct polarities and produced esters while keeping unchanged the enzyme activity and avoiding the hydrolysis of the products. Therefore, the degree of hydration of the reaction medium is a determinant factor in the conversion of substrates into glycolipids especially in enzymatic reactions. As water is formed as a by-product during esterification reactions, mostly works cited in this review used water absorbents such as molecular sieves or sodium sulphate in the reaction medium to avoid hydrolysis and improve conversion rates. Indeed, it has been demonstrated that initial water activity significantly affected sugar esters lipase-catalysed synthesis in organic media [37]. The use of molecular sieves to trap the water formed in the medium during esterification reaction was proved to be an efficient method to improve sugar ester conversions. For instance, it has been tested ten different organic solvents on the synthesis of 6-*O*-glucose palmitate catalysed by the lipase B of *Candida antartica* [34]. They also studied the influence of three solvent pre-treatments (A: no-treatment; B: drying; C: addition of 3 Å molecular sieves) on the monoester production. The best conversions of vinyl palmitate into 6-*O*-glucose palmitate after 72 h reaction at 45 °C were from 80 % to 100 %. These results were observed when using acetone, *t*-butanol, THF, dioxane, and acetonitrile under pre-treatments B and C, with slightly improvements from B to C. Authors concluded that drying the solvents could boost conversions of vinyl palmitate as described: 80 % in dioxane, 88 % in *t*-butanol and in THF, 93 % in acetone and full conversion when using acetonitrile. This behaviour confirms the influence of residual water contents on the saccharide conversion rates and corroborates the use of anhydrous solvents in biocatalysed esterification reactions.

In industry, as reactions occur in larger scale, organic solvents instead of aqueous ones are more suitable as they are easier to remove, can be recovered and reused and their use avoid microbial

contamination [21]. Some authors found that the use of co-solvents with distinct polarities could improve the biocatalysed synthesis of biosurfactants [32]. They investigated two different binary solvent systems for the lipase-catalysed synthesis of dilauroyl maltose. The highest lauric acid conversion (34 %) (or 69 % diester production) were obtained using acetone/*n*-hexane (60:40, v/v) as solvent system, at 50 °C for 72 h. The authors discussed the importance of the hydrophobicity of the reaction solvents on the selective yields of mono and diesters. According to the observed kinetic synthesis of maltose lauroyl esters, in an acetone/*n*-hexane binary solvent system, increasing hydrophobicity of the medium led to better conversions in diester while low hydrophobicity enhances the yields in monoester. These results also confirm that mixtures of solvents with different polarities can contribute to both solubilisation of the sugar (as acetone, in the present case) and good stability of the enzyme (*n*-hexane, in this case), which can afford optimal conversions and high selective synthesis of sugar esters. Despite the 34 % fatty acyl donor conversion, the system studied [32] should be a good choice for the synthesis of bioactive molecules as acetone and *n*-hexane are acceptable in food and pharmaceutical industrial processes. Similarly, another authors [33] presented a novel simultaneous reaction-extraction process based on the presence of two immiscible organic solvents. The aim of this alternative system was to improve selectivity and conversion of the catalysis of dilauryl mannose by the Novozym 435 lipase. The solvents tested were acetonitrile and *n*-hexane. It was determined that acetonitrile served as the reaction media and *n*-hexane as extraction solvent. The best ester conversions were obtained with ratio of 1:1 (*n*-hexane/acetone). Under the optimal conditions (50 °C, 72 h, 4:1 acyl donor/saccharide), 25 % of the initial lauric acid molar amounts were converted into diesters (51 % total yield). Reaction total yields in monoesters (76 %) were mostly produced in contrast to diesters (51 %). These results confirmed the selectivity of the two-phase system towards the monoesters. Recently, it has been studied the effect of using organic solvents with different polarities [1], *i.e.* *n*-hexane, THF, and *t*-butanol, in the synthesis of glucose esters catalysed by Novozym 435 (*Candida antartica* B). Enzymes tend to be more active in poor polar reaction media. However, it is important to consider that the solubility of the saccharide plays an important role in esterification reactions. This could explain the profile found [1] where THF and *t*-butanol showed higher conversion into glucose monoesters compared to those using *n*-hexane as reaction solvent. The authors compared the efficiency of the reaction in pure solvent (THF or *t*-butanol) with that obtained in different THF/*n*-hexane mixtures. They found that a solvent mixture of 1:1 (v/v) THF/*n*-hexane presented the best results (52 % conversion of acyl donor in 9 h or 78 % ester yield as from the original data). Strongly hydrophilic solvents can deactivate the enzyme by restricting the water layer that keeps the active native conformation of the enzyme structure. Thus, the high solubility of D-glucose in THF and the increase of the lipase activity in *n*-hexane justify the results obtained by [1] where the best solvent medium for the Novozym 435 catalysed synthesis of glucose esters was 1:1 (v/v) THF/*n*-hexane.

Due to saccharides polarity, high polar solvents showed the best conversions in the works presented in this review. The high reaction conversions obtained could confirm the importance of the good solubilization of the substrates. A recent study, [29] had the best conversion (64 % in 24 h) of caproic acid to form xylose caproate esters in a Novozym 435 catalysed reaction using a dual solvent system constituted by DMSO/acetone (1:10, v/v) at 60 °C. Similarly, another authors [31] studied the effect of different amounts of DMSO in the esterification reactions of L-(+)-arabinose with palmitic acid catalysed by the lipase B of *Candida antartica*. The best results (22 % palmitic acid conversion) were obtained in

24 h using 10 % (v/v) DMSO in *t*-butanol, at 60 °C and with equimolar ratio of substrates. In one hand, higher amounts of DMSO in the reaction medium led to both decrease of conversion rates and of biocatalyst activity. In the other hand, smaller amounts of DMSO promote the synthesis of diester instead of monoesters: also used DMSO [38] in a binary solvent system DMSO/*t*-butanol for the synthesis of different fatty acid esters of glucose catalysed by the immobilized lipase B of *Candida antarctica*. This work provided conversions above 70 % in 24 h and at 55 °C. As mentioned above, the solubility of the saccharide is one of the most important parameters in the biocatalysed synthesis of biosurfactants from sugar molecules. DMSO is a good solvent for the solubilisation of saccharides. However, this solvent can negatively affect biocatalysis as in high amounts it can inactivate the enzyme. In 2019, a study [39] described the dependence of sugar solubility on the biocatalytic esterification rates. Water, organic solvents, and mixture solutions were tested and compared to a solvent-mediated method using supersaturated sugar solutions in hydrophobic and mixture of hydrophilic/hydrophobic ionic liquids. DMSO, DMF, methanol, ethanol, 1-propanol, 2-propanol, acetonitrile, and acetone were tested as organic solvents. Among the pure organic solvents, DMSO showed the best solubility. It was observed, as expected, that glucose solubility increased with the polarity of the solvent. Indeed, sugar molecules showed high solubility in ionic liquid mixtures. Furthermore, the tested mixture of hydrophilic/hydrophobic ionic liquids provided conversions two times higher than those using only organic solvents. Besides the good solubility of sugars in ionic liquids, lipases tend to be more stable and active under amphiphilic media, which justifies the rising researches on the use of ionic liquids as main solvent or in mixtures with water or organic solvents on biocatalytic reactions.

A comparison of the effect of using organic solvents [30] (acetone, acetonitrile and 2-methyl-2-butanol) or ionic liquids with different polarities on the lipase-catalysed synthesis of galactose oleate monoester. In this study, preliminary tests using organic solvents gave a maximum conversion of 40 % compared to 25 % when using an imidazolium-based ionic liquid in the same reaction conditions (60 °C, 1:2 galactose/oleic acid molar ratio, 2% of lipase, w/w). They observed that D-Galactose was not completely dissolved in the ionic liquid and found that the most important criterion for higher conversion rates was the ability of the solvent to dissolve the saccharide. Due to their polarity, saccharides tend to be more soluble in solvents with high polarity. In this study, authors [30] investigated the use of different amounts of a high polar organic solvent (DMSO) as co-solvent for the ionic liquid system to improve dissolution and conversion of D-Galactose into its oleate ester. The best ratio of DMSO to the ionic liquid ratio was 1:20 (v/v) which converted 77 % of the oleic acid in 2 h reaction. Lower amounts of DMSO as co-solvent were not able to completely dissolve D-Galactose, and higher amounts would be responsible for denaturation of the enzyme. In 2013, a publication [40] also studied different solvent systems in the synthesis of glycolipids catalysed by the lipase Novozym 435. In this case, D-maltose was esterified into a mixture of mono-6- and -6'-*O*-linoleyl- α -D-maltose, mono-acylation occurred mainly on 6'-*O*-linoleyl-D-maltose (29 % linoleic acid conversion, or 58 % maltose conversion) and secondly on 6-*O*-linoleyl-D-maltose (21 % of linoleic acid conversion, or 42 % maltose conversion). Four pathways, according to reaction media: solvent-free, were tested, *i.e.* organic solvents; ionic liquids; and mixtures of both organic solvents and ionic liquids. The organic solvents tested were acetone, *n*-hexane, DMSO, *t*-butanol, toluene, dioxane, and THF. Eleven ionic liquids with different polarity were also tested. In this work, DMF provided the best conversions among the single solvent systems. The best conversions of linoleic acid were 20% (DMF), 19 % (acetone), 17 % (THF) and 15 % (acetonitrile). The other organic

solvents led to lower conversion rates in the following order: *t*-butanol > *n*-hexane > dioxane > DMSO > toluene. Binary solvents, such as acetone/DMF and acetone/ionic liquids, were able to double ester conversions compared to single solvent systems, using 1:1 binary solvents at 65 °C for 72 h. Contrary to the studies carried out [30], described acetone as the best solvent as it was able to provide good reaction conversion rates, is easily recovered, induces the inverse hydrolysis reaction with lipases, and it is considered as a green solvent that can be produced from renewable feedstock. In a molecular scale, a study [41] investigated the influence of solvent-to-protein interaction in the conformation of the lipase B of *Candida antarctica* (CALB) in terms of efficiency and selectivity of the lipase for the synthesis of lauric acid fructose esters. The solvents tested were 2-methyl-2-butanol and imidazolium-based ionic liquids in different proportions. The 12 h reactions were carried at 50 °C, using equimolar ratios of fructose and lauric acid. The use of a binary solvent system significantly improved conversions in fructose monoesters compared to the reactions without 2-methyl-2-butanol. In 60:40 (v/v, ionic liquid / 2-methyl-2-butanol) and 20:80 (v/v, ionic liquid / 2-methyl-2-butanol), mono-acylated fructose ester were obtained, respectively, with 85 % and 78 % conversion of lauric acid (calculated as per fructose transformation in an equimolar reaction). Only monoesters were produced at the beginning of the reaction [33]. The formation of diester would be associated with the consumption of substrates and the production of high amounts of mono-acylated esters. Changes in enzyme conformation were detected in the presence of ionic liquids as a matter of diesters production. Previous studies demonstrated that the flexibility of CALB can be limited by the co-solvent system [42]. Different from organic solvents, in the presence of ionic liquids, lipase conformation become less flexible. Authors suggested that these changes would contribute to alterations in the lipase activity and selectivity. As from their Fourier transform infrared spectra analysis, when the lipase binds to lauric acid and fructose, the second structure changed in co-solvent systems. This could be explained by changes in the affinity of the enzyme towards the substrates in the presence of imidazolium-based ionic liquids. With the advantages from the use of ionic liquids as low evaporation, recycling possibility, selectivity, etc, their concomitant use with organic solvents could furnish an efficient system in the enzymatic synthesis of biosurfactants.

Alternative pathways for the enzyme-catalysed esterification of fatty acids and carbohydrates considered the reduction of the amounts of organic solvents to the reaction system. Another authors [43] studied the lipase (Novozym 435) catalysed esterification of glucose with palmitic acid in a high-pressure acetone saturated with CO₂ system. Authors investigated the effect of acetone and water concentrations on the initial reaction rates and found the optimal conditions (conversions higher than 20 %) as 3% (v/v) and 0.5 % (v/v), respectively. The optimal temperature and CO₂ pressure were found as 50 °C and 65 bar, respectively. Another alternative system was described [44]. In this study, a solvent-free packed bed bioreactor continuous system was developed to synthesize fructose oleates using immobilized *Rhizomucor miehei* lipase. The optimum conditions were 1:4 (fructose/oleic acid molar ratio), 144 h, and 65 °C. The system was able to convert 19 % of oleic acid into fructose oleate monoester (88 % total yield, which 92 % of monoester) saccharide-fatty acid esters within 144 h at 65 °C. With the reduction of organic solvents amounts, these methods could be a sustainable alternative in the production of amphiphilic surfactants in industrial scale.

2.2. Effect of temperature level

Temperature has an important role in synthetic reactions, especially on enzymatic pathways. Even if high temperatures tend

to improve the mass transfer in the reaction medium, enzymes can be thermosensitive and usually work in a specific temperature range. Due to its versatility, stability, and low price, one of the most used lipases in biocatalysis is the lipase B of *Candida Antarctica* (commercially available as Novozym 435, immobilized). According to literature, this lipase has shown to be active in a range of temperature from 40 °C to 80 °C with good conversion rates. Several studies evaluated the influence of different temperature levels in the efficiency of the lipase Novozym 435 to synthesize glycolipids by esterification reactions. The parameter was investigated for the transformation of monosaccharides as glucose [1,34], fructose [45] and mannose [7,45], but also for more complex saccharides as starch [46] in the presence of the lipase B of *Candida antarctica*. For the esterification of D-Glucose with *N*-lauroyl acyl glycine, [1] obtained conversions from 22 % to 76 % when the reaction temperature was increased from 40 °C to 55 °C. Increments in temperature to 60 °C or more were prejudicial. The lipase can undergo thermal denaturation when submitted to higher temperatures. In accordance to that, a publication [34] showed the effect of different temperature levels (20 °C, 30 °C, 45 °C, 60 °C and 70 °C) in the conversions of vinyl palmitate into 6-*O*-glucose palmitate in acetonitrile for 40 h. The kinetic of the reaction showed that conversions in 6-*O*-glucose palmitate significantly increased from 20 °C to 60 °C. Moreover, conversion rates were higher at higher temperatures. At 60 °C, a 94 % acyl donor conversion was observed at 20 h reaction, while the same conversion was only achieved after 40 h at 45 °C. Only a maximum of 86 % of conversion was observed when heating the medium at 70 °C. After 20 h reaction, no improvement was observed in 6-*O*-glucose palmitate synthesis, probably due to lipase thermal deactivation. Two papers [45] studied the influence of different parameters on the synthesis of glycolipids in the presence of the lipase B of *Candida antarctica* and found that temperature was the most important factor in the conversion of monosaccharide to corresponding esters. First paper [45] studied the esterification of fructose with oleic acid and second paper [7] studied the reaction between mannose and hemifluorated acid derivatives. Both studies showed that the increase in temperature improved significantly conversion rates. In the case of mannose with hemifluorinated acid derivatives reaction, the conversions from was increased from 12 % to 19 % (25%–39% of ester production as per percentage of esterified OH at mannose C6 position) when temperature increased from 60 °C to 80 °C, respectively [7]. Only slight differences were observed when other parameters were changed. This behaviour demonstrates the thermostability of the lipase. Increases in reaction temperature could also facilitate the mass transfer in the reaction medium as the solubility of the reactants may be increased. The same work demonstrated that the synthesized hemifluorinated carbohydrates have better tensioactive properties than their aliphatic counterparts. In the enzymatic synthesis of fructose fatty acid ester, authors [45] studied the influence of temperature, agitation and reaction time on esterification percentage using a response surface methodology with central composite rotatable design. The tested temperature levels were 46.6 °C, 50 °C, 55 °C, 60 °C, and 63.4 °C. The optimum temperature level was 57.2 °C giving a maximum conversion of 88 % (± 0.3 %) of oleic acid in 37.8 h reaction, corresponding to an improvement of about 15 % comparing to previous reported studies. Novozym 435 has also proved to be efficient in the esterification of complex carbohydrates such as starch. The influence of different temperatures (from 50 °C to 70 °C) in the synthesis of starch palmitate in a solvent-free system was recently studied [46]. The conversion of palmitic acid increased from 50 °C to 60 °C and then decreased significantly until 70 °C. In 4 h, the higher conversion rate was obtained at 60 °C (around 70 %). It demonstrated the good thermostability of the lipase and suggested

that increases in temperature until approximately 60 °C could improve the synthesis of glycolipids.

Other enzymes and lipases from other microorganisms were also tested in the biocatalytic esterification of mono- and disaccharides. The immobilized lipase of *Candida rugosa* was tested in the biocatalytic synthesis of lactose caprate esters [47], fructose oleates [8] and glucose fatty acids esters from coconut oil [48]. In the first case, the fatty acid conversions were about 20 % greater when increasing temperature from 45 °C to 55 °C. This observation could be explained as a result of increasing the frequency of contact of the molecules in the reaction medium due to the application of greater energy levels to the system. However, increases in temperature to 60 °C did not improved the synthesis of lactose ester significantly. In contrast, losses in conversion rate were observed in the case of the free lipase catalysed reaction. These results demonstrate that immobilization can boost enzyme thermal stability. Higher energy levels applied to the reaction can improve conversion rates. Enzymes tend to be sensitive to temperature variation. Their immobilization on supports soluble in the reaction medium and which promote the accessibility of the enzyme active site are very important parameters to achieve good catalytic transformation rates. The same lipase catalysed the synthesis of fructose oleic esters in a fluidized bed reactor and provided a conversion of oleic acid of 80 % in 48 h at 60 °C [8]. For [48], the reaction conversions were very low (about 9%), even at the optimum temperature (40 °C). Slight increases in the temperature level (45 °C), drastically decreased the reaction conversions of about 25 %. Differently, an another study [6] evaluated the influence of reaction temperature (30 °C, 50 °C and 70 °C) in the synthesis of glucose vinyl ester using an alkaline protease of *Bacillus subtilis*. At 30 °C, the conversion rate of the acyl donor (divinyl adipate) was linearly increased with the time (from 40 % in 24 h to 80 % in 120 h). No significant differences were observed at 50 °C and 70 °C after 24 h, both presenting a plateau over 90 % conversion. It is important to observe that, even in mild temperature conditions, the enzyme was able to catalyse the reaction with great conversion rates. This energy saving profile is important especially for industrial and environmental concerns.

Lipases from different microorganisms and a protease demonstrated their ability to catalyse sugar esterification with good conversion rates. These works investigated the influence of various parameters as substrate concentration, saccharide to acyl donor molar ratio, lipase amounts, temperature and reaction time. Temperature seemed to be the most important reaction condition that controls the conversion of sugar esters. The other parameters influencing the biocatalytic esterification of saccharides will be discussed thereafter.

2.3. Effect of the substrate molar ratio

In order to improve the acylation of saccharides molecules in the presence of fatty acid, a molar excess of the acyl-donor is desirable. Some authors studied the optimization of the molar ratio between the saccharide and the acyl donor in biocatalysed synthesis of glycolipids. A study in which [29] tested different molar ratios sugar to fatty acid to produce xylose caproate in the presence of Novozym 435. They found that an equimolar ratio was less efficient (45 %) in acid conversion than the use of an excess of three molar equivalent of caproic acid (64 %). An excess of one or two molar equivalent of fatty acid in the reaction medium gave conversions 28 % lower than the one using 1:4 (sugar/fatty acid) molar equivalent. Similarly, the effect of carbohydrate to fatty acid molar ratio in the lipase (Novozym 435) catalysed esterification of pre-treated starch with palmitic acid was studied by [46]. In a first time, the investigated palmitic acid to starch molar ratios were 1:1.7, 1:2.5, 1:5, 1:10, 1:20. The initial rates of conversion of palmitic

acid were enhanced from 52 % to 68 % in 24 h by increasing palmitic acid to starch molar ratio from 1:1.7 to 1:5. Slight decreases in palmitic acid conversions were observed by increasing starch molar ratio: 51 % and 48 % in 24 h for 1:10 and 1:20 (palmitic acid/starch), respectively. In a second time, starch amounts were kept constant (10 mmol) with varying the amounts of the acyl donor as follows: 1:0.014; 1:0.017; 1:0.02; 1:0.025; 1:0.033; 1:0.05; 1:0.1; 1:0.2 (starch/palmitic acid molar ratio). Initial reaction rates increased from around 10 % to above 70 % in 24 h. Palmitic acid conversions increased by increasing palmitic acid amounts with an optimum at 1:0.02 (starch/palmitic acid molar ratio). Authors claim that the basic composition of starch has not been changed after the pre-treatment and that esterification was carried out at the hydroxyl groups of D-glucopyranosyl structural unit of the starch polymer. The specific evolution of the conversion rate according to palmitic acid molar equivalents could be attributed to the formation of complexes between enzyme and exceeding amounts of starch as justified by the authors. As explained before, mass transfer limitations and the saturation of the reaction medium could also explain the reduction in the reaction rates.

The study [48] of the effect of substrates molar ratio on the *Candida rugosa* lipase catalysed esterification of glucose by fatty esters and found optimal proportions of 1:60 (glucose/fatty acids). With a very high molar proportion of fatty acid to saccharide when compared to the other studies, the authors suggested that increasing the proportion of fatty acid could negatively affect the lipase conformation due to an acidification of the reaction medium. Similarly, [47] study of the effect of sugar to acyl donor molar ratio in the synthesis of lactose caprate esters catalysed by the lipase of *Candida rugosa*. Different molar ratios of lactose to capric acid (3:1, 2:1, 1:1, 1:2 and 1:3) were tested. It was observed that the conversion of capric acid increased from about 30 % to almost 70 % by decreasing the sugar concentration until 2:1. Increases in acyl donor molar ratio to 1:1, 1:2, and 1:3 slightly reduced conversion rates.

In the case of the protease-catalysed synthesis of glucose vinyl ester, author [6] studied the sugar to divinyladipate molar ratio (1:1, 1:2, 1:4). Reaction rates only slightly increased when using 1:1 and 1:2 (glucose/divinyladipate) along 120 h being 33 % and 50 % the maximum divinyladipate conversion, respectively. However, conversion rates increased from around 40 % to more than 80 % in 24 h and achieved a total conversion in 120 h when the molar ratio was of 1:4 (glucose/divinyladipate).

A small excess of sugar could favour the conversion of fatty acids. Sugars could act by reducing the water activity of the reaction medium, favouring the esterification way. However, higher amounts of sugar could inhibit the synthesis of ester as sugar could denature the enzyme by removing the enzyme water layer essential to keep its active conformation.

2.4. Effect of the reaction time

In terms of efficiency, reaction time is still a challenge for some enzyme-catalysed reactions when compared to chemical pathways. Indeed, enzymes can allow syntheses of products with good conversion rates and high purity, but these reactions can be slow. Though, recent studies proved that enzymatic pathways can be as efficient as some established chemical protocols or even better if sustainability point of view is taken into account. For example [30], the study the kinetics of the esterification of D-Galactose using 2% (w/w) Lipozyme RM IM (from *Rhizomucor miehei*) at 60 °C, 1:2 (galactose/oleic acid molar ratio), and 1:20 (v/v, DMSO/ionic liquid) for 72 h. The maximum oleic acid conversions obtained (77 %) was achieved in 2 h reaction with no significant increases in the conversion after that time. The authors justified the efficiency of the reaction due to complete solubilisation of the sugar in the

reaction medium. A recent study [29] showed that 24 h reaction gave the higher conversion rate (64 %) of caprylic acid when catalysed by 16 % (w/v) of Novozym 435 in 1:10 (DMSO/acetone) and at 60 °C. In this work, the reaction conversion rates increased in 40 % from 1 h to 24 h and then decreased significantly with an increment of reaction time. The decrease in fatty acid conversion rates along the time could be explained by the formation of water, by-product of the esterification reaction, which allows the lipase to hydrolyse sugar esters. In the lipase-catalysed synthesis of glucose esters with an alkyl chain from C10 to C16, esterification rates increased linearly from 1 h to 9 h with a maximum conversions of 76 % (C10), 78 % (C12), 65 % (C14), and 64 % (C16) with no significant improvements with time increasing from 9 h to 11 h [1]. Authors justified this behaviour as per the reach of kinetic equilibrium.

Other authors used factorial designs to optimize the reactions. In a response surface methodology with central composite rotatable design, study [45] of the optimization of reaction time on the esterification of fructose with oleic acid catalysed by the lipase B from *Candida antarctica*. The different reaction times evaluated were 3.8 h, 12 h, 24 h, 36 h, and 44.2 h. This study furnished an optimum conversion (88 %) at 37.8 h. However, as from the profile observed during the reaction kinetics, good conversions of oleic acid (78 %) were already obtained after 3.5 h. Therefore, as authors argued, from a practical point of view, the reaction could be stopped at 3.5 h to avoid losses in productivity (moles of ester per hour) keeping good conversion rates.

A publication [6] demonstrated that sugar to acyl donor molar ratio as the temperature level had stronger influence in the enzymatic synthesis of glucose vinyl ester than reaction time. The kinetic profile of the reaction significantly changed from 24 h to 48 h reaction while a plateau was observed after that time with no significant influence of the other parameters. It could be related to the enzyme thermal stability or its ability to avoid or reduce hydrolysis reactions in the presence of an excess of esterification substrates.

In a different system, biocatalysis conducted under fluidized-bed continuous systems could show different a behaviour towards reaction time as the reaction medium is in constant flow. An another [8] studied the *Candida rugosa* lipase catalysed synthesis of fructose oleic ester in a fluidized bed reactor. In this continuous system, the best conversion rate was of 80 % at 48 h. After that time, keeping the system running for more 24 h decreased conversion rates in about 25 % due to the reverse hydrolysis reaction. An important observation from this study was that increases in saccharide molar ratio tended to favour the monoester production, while increases in the acyl donor concentration tended to favour diester and tri-ester conversions.

3. Effect of biochemical parameters and their levels influencing the enzymatic catalysis of glycolipids

3.1. Effect of the type of saccharide and acyl donor

Some authors studied the effect of the esterification of different saccharides with different acyl donors. Besides the importance of knowing the right proportion between the two substrates in the reaction medium, the nature and polarity of the acyl donor as the number and reactivity of the hydroxyls groups of the saccharide play an important role in the type of interactions between these molecules, their disposition in the reaction medium and their affinity to the biocatalyst.

The lipase (Novozym 435) catalysed the synthesis of fructose, sucrose and lactose oleic esters were investigated [2]. The reactions were conducted at 40 °C, for 72 h by using ethanol as the organic solvent. All three saccharides were efficiently converted into their respective esters. The highest oleic acid

Table 1
Main parameters influencing the biocatalytic synthesis of amphiphilic carbohydrates in recent studies.

| Saccharide (carbohydrate) | Acyl donor | Enzyme | Solvents (v/v) | Time (h) | Saccharide/acyl donor (molar ratio) | T (°C) | Conversion of fatty acyl donor (%) | Refs |
|---------------------------|--|---|--|----------|-------------------------------------|--------|------------------------------------|------|
| Glucose | Vinyl palmitate (C16) | <i>Candida antarctica</i> immobilized lipase B | Acetonitrile | 72 | 1:1 | 45 | 100 % | [34] |
| Glucose | N-lauroyl glycine (C12) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | THF/n-hexane (1:1) | 9 | 1:1,5 | 55 | 52 % | [1] |
| Glucose | Palmitic acid (C16:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Acetone saturated with CO ₂ | 4 | 1:2 | 50 | > 20 % | [43] |
| Glucose | Vinyl laurate (C12:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | IL* solutions | 12 | 1:2 | 40 | 55 % | [39] |
| Glucose | Caproic acid (C6:0) | <i>Candida antarctica</i> immobilized lipase B | DMSO/tert-butanol (8:2) | 24 | 1:3 | 55 | 113 % | [38] |
| Glucose | Lauric acid (C12:0) | <i>Candida antarctica</i> immobilized lipase B | DMSO/tert-butanol (8:2) | 24 | 1:3 | 55 | 77 % | |
| Glucose | Palmitic acid (C16:0) | <i>Candida antarctica</i> immobilized lipase B | DMSO/tert-butanol (8:2) | 24 | 1:3 | 55 | 97 % | |
| Fructose | Lauric acid (C12:0) | <i>Candida antarctica</i> immobilized lipase B | IL*/2-methyl-2-butanol (6:4) | 12 | 1:1 | 50 | 85 % | [41] |
| Fructose | Oleic acid (C18:1) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Ethanol | 72 | 1:1 | 40 | 74 % | [2] |
| Fructose | Oleic acid (C18:1) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Ethanol | 38 | 1,2:1 | 57 | 88 % | [45] |
| Sucrose | Oleic acid (C18:1) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Ethanol | 72 | 1:1 | 40 | 56 % | |
| Lactose | Oleic acid (C18:1) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Ethanol | 72 | 1:1 | 40 | 84 % | |
| Xylose | Caproic acid (C6:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | DMSO/Acetone (1:10) | 24 | 1:4 | 60 | 64 % | [29] |
| D-Xylose | Vinyl laurate (C12:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | 2-methylbutan-2-ol | 4 | 1:3 | 50 | 25 % | [49] |
| Mannose | Lauric acid (C12:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | n-hexane/acetone (1:1) | 72 | 1:4 | 50 | 25 % | [33] |
| Mannose | Hemifluorinated acid derivatives | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | 2-methyl-2-butanol | 24 | 1:2 | 80 | 19 % | [7] |
| Maltose | Linoleic acid (C18:2) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Acetone | 72 | 1:2 | 65 | 19 % | [40] |
| Maltose | Lauric acid (C12:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Acetone/n-hexane (6:4) | 72 | 1:4 | 50 | 34 % | [32] |
| Arabinose | Palmitic acid (C16:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | DMSO/tert-butanol (1:9) | 24 | 1:1 | 60 | 22 % | [31] |
| Trehalose | Lipoic acid (C8) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | DMSO/2-methyl-2-butanol (4:1) | 96 | 1:4 | 40 | 19 % | [13] |
| Oligo fructose | Caprylic acid (C8:0); Lauric acid (C12:0); Palmitic acid (C16:0); Stearic acid (C18:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | DMSO/butanol (1:9) | 69 | 1:3 | 60 | C18 > C16 > C12 > C8 | [50] |
| Starch | Palmitic acid (C16:0) | Novozym 435 (<i>Candida antarctica</i> immobilized lipase B) | Solvent-free | 4 | 1:5 | 60 | 70 % | [46] |
| Trehalose | Palmitic acid (C16:0) | Fermase CALB™ 10000 lipase | Acetone | 4 | 1:5 | 60 | 14 % | [14] |
| Glucose | Fatty acids of coconut oil (C8:0; C10:0; C12:0; C14:0; C18:0; C18:1; C18:2; C18:3) | <i>Candida rugosa</i> immobilized lipase | n-hexane | 16 | 1:60 | 40 | 9 % | [48] |
| Fructose | Oleic acid (C18:1) | <i>Candida rugosa</i> immobilized lipase | Solvent-free | 48 | 1:4 | 60 | 80 % | [8] |
| Lactose | Capric acid (C10:0) | <i>Candida rugosa</i> immobilized lipase | Acetone | 48 | 1:1 | 55 | 67 % | [47] |
| Ribose | Capric acid (C10:0) | <i>Candida rugosa</i> immobilized lipase | Acetone | 48 | 1:1 | 55 | 63 % | |
| Mannose | Capric acid (C10:0) | <i>Candida rugosa</i> immobilized lipase | Acetone | 48 | 1:1 | 55 | 62 % | |
| Trehalose | Capric acid (C10:0) | <i>Candida rugosa</i> immobilized lipase | Acetone | 48 | 1:1 | 55 | 56 % | |

Table 1 (Continued)

| Saccharide (carbohydrate) | Acyl donor | Enzyme | Solvents (v/v) | Time (h) | Saccharide/acyl donor (molar ratio) | T (°C) | Conversion of fatty acyl donor (%) | Refs |
|-------------------------------|---|--|-------------------|----------|-------------------------------------|--------|------------------------------------|------|
| D-galactose | Oleic acid (C18:1) | <i>Candida rugosa</i> immobilized lipase | DMSO/IL* | 2 | 1:2 | 60 | 77 % | [30] |
| α - β -galactose | N-decanoylglycine (C10) | Lyozyme RM IM (<i>Rhizopus mucor</i> immobilized lipase) | (1:20) | 6 | 1:1,5 | 40 | 94 % | [56] |
| | | Lyozyme TL IM (<i>Thermomyces lanuginosus</i> immobilized lipase) | <i>t</i> -butanol | | | | | |
| Agave tequilana fructans | Vinyl laurate (C12:0) | Lipozyme 435 (L435) (lipase B from <i>Candida antarctica</i>) | n-hexane | 96 | nc** | 60 | 80 % | [4] |
| Fructose | Oleic acid (C18:1) | Lipozyme IM (<i>Rhizomucor miehei</i> immobilized lipase) | Solvent-free | 144 | 1:4 | 65 | 19 % | [44] |
| Maltodextrin | Capric acid (C10:0); Lauric acid (C12:0); Palmitic acid (C16:0) | <i>Thermomyces lanuginosus</i> lipase | DMSO | 4 | 1:0,5 | 60 | C10 > C12 > C16 | [3] |
| Glucose | Divinyl adipate | <i>Bacillus subtilis</i> protease | DMF | 120 | 1:4 | 50 | 80 % | [6] |
| Isomaltriose | Palmitic acid vinyl ester (C16:0) | <i>Bacillus thermoproteolyticus rokko</i> metalloprotease | DMSO | 24 | 1:1 | 45 | 90 % | [5] |

*IL: Ionic liquids**.

nc: Not cited.

conversions were detected for lactose (84 %), followed by fructose (74 %) and sucrose (56 %) transformations. Authors also tested the emulsifying ability of the synthesised biosurfactants and found that lactose ester showed the best surface tension activity and emulsification index. Concerning the synthesis of D-Xylose and L-arabinose laurate esters in 2-methylbutan-2-ol at 50 °C, using the lipase B of *Candida antarctica*, similar conversion rates were obtained [49]. The optimal conversions of D-xylose into xylose lauryl esters were achieved at 4 h (75 %, or 25 % vinyl laurate conversion), while the higher amounts of arabinose lauryl esters were obtained at 24 h (57 %, or 19 % vinyl laurate conversion). Both saccharides furnished different proportions of mono and diesters. In the case of D-xylose, two monoesters and two diesters were formed with a majority (38 %) of monoesters. From the 57 % global conversion into L-arabinose esters, one diester (30 %) and one monoester (19 %) were formed. This behaviour demonstrated the regioselectivity of the lipase for the primary hydroxyl group for both pentoses. Reaction rates were better when using D-xylose as substrate.

The effect of the fatty acid alkyl chain length in the synthesis of glucose esters catalysed by the lipase B of *Candida antarctica* was investigated [38]. In this work, the acyl donors investigated were palmitic, lauric and hexanoic acids. The best conversions for each fatty acid were obtained at 24 h: 77 % for glucose laurate (C12), 97 % for palmitate (C16), and 113 % for hexanoate (C6). However, the equation used in this work to quantify the conversion rates, only takes into account the fatty acid concentrations in the reaction medium. Authors observed the presence of non-dissolved glucose crystals along the esterification, which means that a total conversion was not achieved as one of the substrates was still present in the medium as its initial form. The biocatalyst used was the commercial lipase of *Candida antarctica* and the reactions were carried out at 55 °C. Authors justified the higher conversions of the fatty acid into hexanoate esters as, in one hand, hexanoic acid is the most polar fatty acid among the three fatty acids tested making it more soluble in the solvent mixture (80/20, v/v, DMSO/2-methyl-2-butanol). In the other hand, hexanoic acid has a shorter carbon chain length which could enable less steric hindrance than palmitic and lauric acid and could favour the access to enzyme active sites. This higher activity with hexanoic acid could also justify the higher conversion rates on its respective esters, as diesters might be produced along with monoesters.

In the case of more complex saccharides, as maltodextrin, decanoic acid (C10), lauric acid (C12) and palmitic acid (C16) were tested as acyl donors [3]. The biocatalyst used was the lipase from *Thermomyces lanuginosus* and the optimum conditions were 1:0.5 (maltodextrin/fatty acid molar ratio), DMSO as solvent, 60 °C, for 4 h reaction time. It was observed that shorter fatty acid chain lengths gave rise to higher rates of ester conversion. Authors justified this tendency by the easier mobility of the smaller acyl donors in the polar reaction medium. Similarly, the effect of carbon chain length of the acyl donors were tested [50] in the enzyme-catalysed esterification of oligofructose. The reactions were carried out in DMSO/butanol (10/90, v/v) at 60 °C for 69 h, using immobilised lipase B of *Candida antarctica* as catalyst. The tested acyl donors were caprylic (C8), lauric (C12), palmitic (C16), and stearic (C18) acids. In this case, longer fatty acid chain length favoured the production of oligofructose esters. As a consequence of lipase specificity, mainly monoesters were formed, and the higher conversions were observed with palmitic acid (C16). Authors related the preference of the lipase active site to interact with larger molecules due to the anatomy of its catalytic cleft (elliptical, steep funnel, and with dimensions of 9.5 × 4.5 Å) [51], which would allow a better interaction between lipase and substrate.

Different carbohydrates were studied in the production of lactose, trehalose, ribose and mannose esters catalysed by free and immobilised lipases of *Candida rugosa* [47]. The lipase-catalysed acylation of sugars was found to be regioselective. The lipases catalysed preferentially the esterification of lactose (67 % capric acid conversion) followed by 63 %, 62 %, and 56 % for ribose, mannose, and trehalose, respectively. Two possibilities were proposed to justify this behaviour, the sugar solubility degree, and the preference of the lipase to esterify primary hydroxyl groups of sugars substrate. Monosaccharides such as ribose and mannose tend to have higher solubility in acetone than disaccharides such as lactose and trehalose due to the lower molecular weights of monosaccharides. However, lactose showed the higher ester conversions, which could be explained by the presence of two primary OH at the C6 positions of both monosaccharides (glucose and galactose) in lactose, increasing its reactivity towards the acyl group [52]. The optimised conditions for sugar fatty acid ester syntheses are 48 h at 55 °C, using a 2:1 of molar ratio of lactose to capric acid.

Recently [14] a study of the influence of the trehalose esters, synthesized using the lipase Fermase CALB™ 10000 as catalyst and caprylic (C8), lauric (C12), palmitic (C16) acids as acyl donors, on its anti-microbial and anti-inflammatory activities. During the syntheses, the effect of substrates molar ratio, temperature, enzyme amount, and reaction time were evaluated for the conversions of trehalose palmitate mono- and diesters. In all cases, diesters were mainly obtained and in significant larger amounts. All parameters seemed to be significant. The profiles of the conversion rates were similar for the parameters: substrates molar ratio, temperature, and enzyme amount. Graphs presented showed bell curves with significant difference between each level. The investigated trehalose to palmitic acid molar ratios were 1:2, 1:3, 1:4, 1:5, 1:6, and 1:7. Temperature levels varied from 30 °C to 70 °C and enzyme load from 5 to 35 % (w/w of substrates). Reaction kinetic was followed for 8 h. Ester conversion increased in about 80 % from 1 h to 4 h reaction. However, no significant differences were observed from 4 h reaction for both mono- and diesters. The best conversions were obtained with 1:5 trehalose/palmitic acid molar ratio, 15 % (w/w to substrates) enzyme load, 60 °C, and 4 h reacting. At these optimum conditions, trehalose palmitate diesters were obtained in a conversion rate of 35 % (14 % conversion of the initial amounts of palmitic acid). This study also demonstrated the importance of the hydrophobic moiety in trehalose esters as functional and biological activities of the fatty acid esters were improved with increase in fatty acid chain length. This affirmation was corroborated as diesters presented higher bioactivity than their corresponding monoesters, demonstrating the importance of the hydrophobic moiety in the glycolipid's bioactivity. This phenomenon was explained as the addition of a long carbon chain to the trehalose structure could increase the affinity of this molecule to the lipoxygenase 15-LOX which could improve the inhibitor effect of trehalose towards oxidative effects.

3.2. Effect of the type of enzyme and enzyme amount

From the recent works analysed in this review concerning the biocatalytic synthesis of glycolipids for application as biosurfactants, most of them showed that lipases were the best enzymes to catalyse the reactions (Table 1). Lipases were tested for the esterification of monosaccharides, such as xylose, galactose, glucose, mannose, fructose, arabinose, and ribose, oligosaccharides (maltose, isomaltotriose, trehalose, sucrose, and lactose) to polysaccharides such as maltodextrin and starch. Lipases from different microorganisms were also tested and the lipase of *Candida antarctica* was often appointed as the best choice due to its high activity, its easy immobilization, a good availability, and low price. Recently, [1] a test of five different commercial immobilized lipases was realized for the synthesis of glucosyl esters (Novozym 435, Lipozyme 435, *Candida cylindracea* lipase, *Porcine pancreas* lipase and thermophilic fungal lipase). The reactions were carried out at 55 °C for 9 h using a molar ratio of 1:1.5 [D-glucose/*N*-fatty acyl glycine (C10, C12, C14, and C16)], and 5% of lipase (w/w, based on the weight of D-glucose). The reaction using Novozym 435 showed the highest acyl donor conversions for C10 (51 %), C12 (52 %), C14 (45 %), and C16 (41 %). Lipozyme 435 also provided almost good conversions of 41 %, 45 %, 41 % and 39 % for C10, C12, C14, and C16 *N*-fatty acyl glycine, respectively. The other lipases led to conversions from around 50%–80% lower than the reactions using Novozym 435 for all acyl donors, *Porcine pancreas* lipase being the less efficient. Novozym 435 has shown to be a versatile and highly efficient biocatalyst. Additionally, the lower price of Novozym 435 as its availability make it one of the most suitable biocatalysts for the biosynthesis of amphiphilic carbohydrates. Same authors [1] also studied the application of 1%–9% (w/w, to D-glucose weight) of Novozym 435 in the esterification of D-glucose. The increase in the

enzyme amounts from 1% to 7% (w/w) significantly increased the conversions in glucose ester while no significant difference was observed when loading the reaction medium with from 7% to 9% (w/w) of lipase. According to the authors, this phenomenon could be due to the saturation of the lipase active sites with the substrate. The efficiency of four commercial lipases on the synthesis of *A. tequilana* fructans vinyl laurate esters was investigated [4]. The reactions were carried out in *n*-hexane for 96 h, at 60 °C and using 10 % (w/v) lipase. The lipases tested were Lipozyme 435 (lipase B from *Candida antarctica*), Lipozyme RM IM (from *Rhizomucor miehei*), Lipozyme TL IM (from *Thermomyces lanuginosa*), and Lipase PS (from *Burkholderia cepacia*). The Lipozyme 435 was the most efficient lipase with a conversion of over 80 % of the initial lauric acid and was able to produce both mono and diacylated products with proved good emulsifying properties in water/oil emulsions. The efficiency of ten different lipases in the synthesis of linoleyl-maltose mono-esters were investigated [40]. The lipases that led to the highest maltose conversions were the lipase of *Pseudomonas cepacia* (58 %, or 29 % conversion of the initial linoleic acid) and the immobilized lipase of *Candida antarctica* (57 %, or 28 % of linoleic acid conversion) after 72 h reaction, in acetone and at 40 °C. Lipases from four different strains were evaluated [46] regarding to their effectiveness to synthesise starch palmitate in a solvent-free system. The commercial lipases Novozym 435 and the immobilized thermophilic fungal lipase (TLIM) were tested together with the lipases of *Candida cylindracea* and *Porcine pancreas*. The best palmitic acid conversions were obtained with Novozym 435 (57 %) and TLIM (23 %) after 24 h reaction. Both lipases *Candida cylindracea* and *Porcine pancreas* led to conversions of less than 7% in 24 h. These results could be explained by the preference of these lipase to follow the hydrolysis route instead of the esterification one. Authors claimed that TLIM lipases have shown high activity towards long chain length fatty acids in esterification reactions. In the present study, as in most of the presented works, Novozym 435 has shown to be the best catalyst for the esterification of carbohydrates from simple sugar molecules to more complex ones as trehalose and starch. In a recent work [13] the efficiency of different lipases in the production of trehalose mono-ester derivatives with lipoic acid was investigated. Novozym 435, Lipase AY, and Trypsin were tested as catalysts. After 72 h reaction, Novozym 435 gave conversions at least 34 times higher for trehalose ester synthesis than lipase AY and trypsin. Authors chose Novozym 435 as the best biocatalyst for the synthesis of trehalose ester in the study conditions. At optimum conditions [1:4 (trehalose/lipoic acid), 4:1 (DMSO/2-methyl-2-butanol), 40 °C, 96 h], the highest weight conversion of lipoic acid was of 19 % (76 % trehalose conversion). The novel synthesized molecule structure was confirmed by NMR as 6-*O*-trehalose lipoate and its radical scavenging ability was significantly higher than that of trehalose or lipoic acid. Authors claimed that this trehalose ester could be applied as a natural food emulsifier due to its green nature.

The optimum amounts of biocatalyst were also tested as the form of the enzyme to be applied to the reaction medium (free or immobilized). Enzyme immobilization can improve the biocatalyst efficiency. The active conformation is normally kept, and the active site is more available after immobilization. Besides that, immobilized enzymes tend to be more stable to temperature and solvent polarity variations as they can be reused. Physicochemical characteristics of the support are likewise important factors that can alter the efficacy of the enzyme and need to be chosen according to the desired reaction. A study [53] of the effect of ten different supports used for the immobilization of the lipase from *C. antarctica* B on the conversion of 6-*O*-glucose palmitate in *t*-butanol was described. They suggested that a decrease in the hydrophilicity of the support increased the rate of reaction conversions. They proposed that hydrophobic supports tend to

favour the partition of water between the support and the enzyme which minimizes the lack of hydration of the enzyme and prevents denaturation [53].

Five different commercial lipases were tested for the synthesis of galactose oleates in a binary solvent medium of DMSO/ionic liquid [30]. Three of them were immobilized lipases: Novozym 435 (from *Candida antarctica* B), Lipozyme RM IM (from *Rhizomucor miehei*) and Lipozyme TL IM (from *Thermomyces lanuginosus*) and two free lipases: from *Candida rugosa* and from *Geobacillus zalihae*. The commercial immobilized lipase RM IM from *Rhizomucor miehei* showed the best conversions of oleic acid, 77% in 2 h (2% of lipase, w/w). Novozym 435, the most current used commercial lipase, showed similar conversion rates (73%) at the same conditions, demonstrating to be an excellent choice to esterification reactions. Free lipases showed lower conversion rates. This could be explained by the higher stability of immobilized lipases to the polarity of the medium and due to the rigidity of enzyme conformation once immobilized [30,54]. A study in 2012 [30] also demonstrated that higher amounts of lipase were not advantageous for the conversion of the saccharide as we could expect. The authors justified decreases in the conversion of D-galactose when amounts of Lipozyme RM IM were increased from 2% to 3%, 4% and 5% (w/w) by a limitation of the substrate and a reduction of the mass transfer as they observed an increase in the viscosity of the reaction media. For the synthesis of xylose caproate, it was found [29] that by increasing the amounts of Novozym 435 from 1% to 16% (w/v) in the reaction medium the conversion of the caproic acid increased in 89%. Higher amounts of enzyme (24%, w/v) slightly decreased the reaction conversion rate in 5%. Likewise, this phenomenon could be explained by limitations in mass transfer due to the increase in the viscosity of the medium when the biocatalyst is in excess or even the occurrence of reversible reactions. Similarly, a recent study [34] compared the efficiency of the immobilised lipases from *Candida Antarctica* (A and B), *Rhizomucor miehei*, *Thermomyces lanuginosa*, *Pseudomonas cepacia*, and *Fusarium solani pisi* in the synthesis of 6-O-glucose palmitate. The reactions were carried out under argon atmosphere and the conditions were: 45 °C, 72 h, 5% (wt) lipase. Five solvents were tested: acetonitrile, acetone, *t*-butanol, dioxane and THF and independently of the solvent, the lipase B of *Candida antarctica* showed the best vinyl palmitate conversions, from 57% in dioxane to 100% in acetonitrile. The lipase of *Thermomyces lanuginosa* was the second most efficient of the tested catalysts in the synthesis of 6-O-glucose palmitate, with maximum conversions of 32% and 36% in *t*-butanol and acetonitrile, respectively. Only poor conversion rates were observed for *Rhizomucor miehei*, *Pseudomonas cepacia*, and *Fusarium solani pisi* (from 0% to 18%) with slight differences between the tested solvents. The lipase A of *Candida antarctica* did not show any conversion whatever the solvent used. This behaviour could be explained by the compatibility of the substrate to these enzymes. The authors justified it as matter of geometry of the enzyme active site. Authors also studied the influence of immobilization type of the lipase B of *Candida Antarctica* on the acrylic beads of support, whether if it was by adsorption or covalent linkage. According to conversion results, the covalently-linked lipase was significantly less efficient than the adsorbed one in all solvents studied, from 57% in *t*-butanol to 85% in dioxane. Depending on the type of lipase-support interaction, the active site of the enzyme could be more or less encumbered/exposed which could affect its accessibility to the substrate.

Concerning lipases from other microorganisms, it was evaluated the pre-treatment effect of the lipase of *Candida rugosa* on the conversion of vinyl caprate sucrose ester in non-aqueous biphasic medium [55]. The lipase was pre-treated by pH adjustments in the presence of crown ether in order to avoid losses in its catalytic activity in the presence of DMSO. Authors argued that interactions between the enzyme and crown ethers, as the presence of buffer

conditions during pre-treatment, could preserve lipase active conformation even in polar media. Also, using polar solvents, such as DMSO, in enzymatic esterification reactions is more suitable for the dissolution of saccharide which could allow greater conversion rates by increasing reactants availability. In this work, a total conversion on sucrose monoester was obtained in 1 h reaction and at 50 °C.

Alternatively, proteases were able to provide glycolipids by good conversion rates from mono and trisaccharides. So a test of two different proteases from *Bacillus subtilis* (neutral and alkaline) in the synthesis of a water-soluble glucose vinyl ester were realized [6]. Conversion rates were significantly higher when using alkaline protease. In 24 h reaction, divinyl adipate conversion rates (from 42% to 80%) were about two times higher with alkaline protease than those obtained for neutral protease (from 18% to 40%) at 30 °C. Over 120 h, almost complete conversions (>98%) were observed.

A synthesis of isomaltotriose palmitate using a thermostable metalloproteinase (thermolysin from *B. thermoproteolyticus rokko*) in DMSO was realized [5]. Reaction time, temperature, water content, and enzyme concentration were the parameters studied. At an optimum temperature of 45 °C, the monoester isomaltotriose palmitate was produced with 90% conversion rate (in an equimolar reaction) in 24 h reaction. Concerning the enzyme concentration, three different levels were tested: 0.03%, 0.05%, and 0.1% (w/w). At 45 °C, the conversions increased from 51% to 90% when increasing the enzyme concentration from 0.03 to 0.05% (w/w) in 24 h. The highest enzyme level [0.1% (w/w)] only gave a conversion of 49%. Decreases in conversion rates could be explained by overloaded catalyst amounts which limits medium fluidity. Also, overcrowding biocatalysts could prevent the access of the substrate to the enzyme active site.

4. Conclusions

All parameters presented in this review seemed to be relevant in the biocatalytic synthesis of glycolipids. From the studies involving factorial designs that compared the influence of each parameter, some authors described temperature and saccharide to acyl donor molar ratio as the most important parameters affecting the reaction. One of the advantages of using enzymes as catalysts is the possibility of working under mild temperatures. The enzymes showed to be highly efficient at low temperatures as from 40 °C. The choice of the solvent system was also described as a crucial factor as not only the polarity of the reaction medium would define the degree of solubility and availability of substrates and their interactions with the biocatalyst. According to literature, DMSO was shown as one of the most powerful solvents, due to the high solubility of saccharides in this solvent. Increases in biocatalyst amounts, time and the length of the carbon chain of acyl donors did not necessarily improve conversion rates. Factors as mass transfer in the medium, enzyme saturation and their specificity should be considered to optimize biocatalytic transformations. Considering substrates molar ratio, enzymes can present distinct affinity to the substrate according to its polarity, structure, and availability in the medium. For lipases, for instance, high concentrations of fatty acid acyl donors can reduce the enzyme activity. As fatty acids are the perfect substrates of lipases, the presence of high amounts of these molecules could saturate the active site of the enzyme and so reducing its activity. Strong interactions between the enzyme and the fatty acid due to the high affinity between both structures could avoid the catalytic role of the lipase in saturated reaction media.

On a sustainable point of view, the production of biosurfactants by enzymatic pathway can save energy, reduce the use of fossil-based solvents and the number of steps during product treatment. It is important to consider the enzymatic synthesis of biosurfactants for applications in food, cosmetic and pharmaceutical

industry as consumers tend to prefer natural processes and products. Enzymes, such as lipases, can be isolated from natural lipid medium and can be applied in the transformation of saccharides extracted from agricultural co-products. This type of system could be a good choice as an environmentally friendly process, which would consist of helping to solve actual environmental concerns.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publishing of the paper in Biotechnology Reports, that the paper has not been published elsewhere, and doesn't include any form of plagiarism. All the authors mentioned above have approved the manuscript and have agreed with the submission of the manuscript.

References

- [1] D. An, X. Zhang, F. Liang, M. Xian, D. Feng, Z. Ye, Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants, *Colloids Surf. A Physicochem. Eng. Asp.* 577 (2019) 257–264, doi:<http://dx.doi.org/10.1016/j.colsurfa.2019.05.079>.
- [2] Ndo A.S. Neta, J.C.S. dos Santos, S. de O. Sancho, S. Rodrigues, L.R.B. Gonçalves, L.R. Rodrigues, J.A. Teixeira, Enzymatic synthesis of sugar esters and their potential as surface-active stabilizers of coconut milk emulsions, *Food Hydrocolloids* 27 (2012) 324–331, doi:<http://dx.doi.org/10.1016/j.foodhyd.2011.10.009>.
- [3] S. Udomrati, S. Gohtani, Enzymatic esterification of tapioca maltodextrin fatty acid ester, *Carbohydr. Polym.* 99 (2014) 379–384, doi:<http://dx.doi.org/10.1016/j.carbpol.2013.07.081>.
- [4] L. Casas-Godoy, J. Arrizon, D. Arrieta-Baez, F.J. Plou, G. Sandoval, Synthesis and emulsifying properties of carbohydrate fatty acid esters produced from Agave tequilana fructans by enzymatic acylation, *Food Chem.* 204 (2016) 437–443, doi:<http://dx.doi.org/10.1016/j.foodchem.2016.02.153>.
- [5] K.J. Liu, Enzymatic synthesis of isomaltotriose palmitate and evaluation of its emulsifying property, *Enzyme Microb. Technol.* 101 (2017) 51–56, doi:<http://dx.doi.org/10.1016/j.enzmictec.2017.03.004>.
- [6] M.R. Borges, J.A. dos Santos, M. Vieira, R. Balaban, Polymerization of a water soluble glucose vinyl ester monomer with tensoactive properties synthesized by enzymatic catalyst, *Mater. Sci. Eng. C* 29 (2009) 519–523, doi:<http://dx.doi.org/10.1016/j.msec.2008.09.013>.
- [7] A. Favrelle, C. Boyère, P. Laurent, G. Broze, C. Blecker, M. Paquot, C. Jérôme, A. Debuigne, Enzymatic synthesis and surface active properties of novel hemifluorinated mannose esters, *Carbohydr. Res.* 346 (2011) 1161–1164, doi:<http://dx.doi.org/10.1016/j.carres.2011.04.004>.
- [8] C. Hidayat, K. Fitria, Supriyanto, P. Hastuti, Enzymatic synthesis of biosurfactant fructose oleic ester using immobilized lipase on modified hydrophobic matrix in fluidized bed reactor, *Agric. Agric. Sci. Procedia* 9 (2016) 353–362, doi:<http://dx.doi.org/10.1016/j.aaspro.2016.02.150>.
- [9] A. Szűts, M. Budai-Szűcs, I. Eros, N. Otomo, P. Szabó-Révész, Study of gel-forming properties of sucrose esters for thermosensitive drug delivery systems, *Int. J. Pharm.* 383 (2010) 132–137, doi:<http://dx.doi.org/10.1016/j.ijpharm.2009.09.013>.
- [10] M.N. AlFindee, Q. Zhang, Y.P. Subedi, J.P. Shrestha, Y. Kawasaki, M. Grilley, J.Y. Takemoto, C.W.T. Chang, One-step synthesis of carbohydrate esters as antibacterial and antifungal agents, *Bioorg. Med. Chem.* 26 (2018) 765–774, doi:<http://dx.doi.org/10.1016/j.bmc.2017.12.038>.
- [11] I.M. Banat, A. Franzetti, I. Gandolfi, G. Bestetti, M.G. Martinotti, L. Fracchia, T.J. Smyth, R. Marchant, Microbial biosurfactants production, applications and future potential, *Appl. Microbiol. Biotechnol.* 87 (2010) 427–444, doi:<http://dx.doi.org/10.1007/s00253-010-2589-0>.
- [12] M. Ferrer, G. Perez, F.J. Plou, J.V. Castell, A. Ballesteros, Antitumour activity of fatty acid maltotriose esters obtained by enzymatic synthesis, *Biotechnol. Appl. Biochem.* 42 (2005) 35, doi:<http://dx.doi.org/10.1042/ba20040122>.
- [13] S. Hsieh, M. Lee, C. Tsai, L. Lai, Food and Bioproducts Processing Enzymatic Synthesis, Purification and Identification of Bioactive Trehalose Ester 5, (2015) , pp. 163–172.
- [14] S.J. Marathe, N.N. Shah, R.S. Singhal, Enzymatic synthesis of fatty acid esters of trehalose: process optimization, characterization of the esters and evaluation of their bioactivities, *Bioorg. Chem.* 94 (2020), doi:<http://dx.doi.org/10.1016/j.bioorg.2019.103460>.
- [15] X. Yang, X. Shi, R. D'arcy, N. Tirelli, G. Zhai, Amphiphilic polysaccharides as building blocks for self-assembled nanosystems: molecular design and application in cancer and inflammatory diseases, *J. Control. Release* 272 (2018) 114–144, doi:<http://dx.doi.org/10.1016/j.jconrel.2017.12.033>.
- [16] L. Zhao, H. Zhang, T. Hao, S. Li, In vitro antibacterial activities and mechanism of sugar fatty acid esters against five food-related bacteria, *Food Chem.* 187 (2015) 370–377, doi:<http://dx.doi.org/10.1016/j.foodchem.2015.04.108>.
- [17] O.S. Amodu, T.V. Ojumu, S.K.O. Ntwampe, Kinetic modelling of cell growth, substrate utilization, and biosurfactant production from solid agrowaste (Beta vulgaris) by *Bacillus licheniformis* STK 01, *Can. J. Chem. Eng.* 94 (2016) 2268–2275, doi:<http://dx.doi.org/10.1002/cjce.22631>.
- [18] M.G. Healy, C.M. Devine, R. Murphy, Microbial production of biosurfactants, *Stud. Environ. Sci.* 66 (1997) 179–195, doi:[http://dx.doi.org/10.1016/S0166-1116\(97\)80043-X](http://dx.doi.org/10.1016/S0166-1116(97)80043-X).
- [19] M. Ohadi, G. Dehghannoudeh, M. Shakibaie, I.M. Banat, M. Pournamdari, H. Forootanfar, Isolation, characterization, and optimization of biosurfactant production by an oil-degrading *Acinetobacter junii* B6 isolated from an Iranian oil excavation site, *Biocatal. Agric. Biotechnol.* 12 (2017) 1–9, doi:<http://dx.doi.org/10.1016/j.bcab.2017.08.007>.
- [20] R.D. Rufino, J.M. de Luna, G.M. de Campos Takaki, L.A. Sarubbo, Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988, *Electron. J. Biotechnol.* 17 (2014) 34–38, doi:<http://dx.doi.org/10.1016/j.ejbt.2013.12.006>.
- [21] A. Kumar, K. Dhar, S.S. Kanwar, P.K. Arora, Lipase catalysis in organic solvents: advantages and applications, *Biol. Proced. Online* 18 (2016) 1–11, doi:<http://dx.doi.org/10.1186/s12575-016-0033-2>.
- [22] B. Pérez, S. Anankanbil, Z. Guo, Synthesis of sugar fatty acid esters and their industrial utilizations, *Fat. Acids* (2017) 329–354, doi:<http://dx.doi.org/10.1016/b978-0-12-809521-8.00010-6>.
- [23] A.P. Karlapudi, T.C. Venkateswarulu, J. Tammineedi, L. Kanumuri, B.K. Ravuru, V. ramu Dirisala, V.P. Kodali, Role of biosurfactants in bioremediation of oil pollution—a review, *Petroleum* 4 (2018) 241–249, doi:<http://dx.doi.org/10.1016/j.petlm.2018.03.007>.
- [24] F.M. Bento, C.C. Gaylarde, The production of interfacial emulsions by bacterial isolates from diesel fuels, *Int. Biodeterior. Biodegrad.* 38 (1996) 31–33, doi:[http://dx.doi.org/10.1016/S0964-8305\(96\)00021-2](http://dx.doi.org/10.1016/S0964-8305(96)00021-2).
- [25] J. Chopineau, F.D. McCafferty, M. Therisod, A.M. Klivanov, Production of biosurfactants from sugar alcohols and vegetable oils catalyzed by lipases in a nonaqueous medium, *Biotechnol. Bioeng.* 31 (1988) 208–214, doi:<http://dx.doi.org/10.1002/bit.260310305>.
- [26] J. Grüniger, A. Delavault, K. Ochsenreither, Enzymatic glycolipid surfactant synthesis from renewables, *Process Biochem.* 87 (2019) 45–54, doi:<http://dx.doi.org/10.1016/j.procbio.2019.09.023>.
- [27] P. Villeneuve, Lipases in lipophilization reactions, *Biotechnol. Adv.* 25 (2007) 515–536, doi:<http://dx.doi.org/10.1016/j.biotechadv.2007.06.001>.
- [28] T. Kobayashi, Lipase-catalyzed syntheses of sugar esters in non-aqueous media, *Biotechnol. Lett.* 33 (2011) 1911–1919, doi:<http://dx.doi.org/10.1007/s10529-011-0663-z>.
- [29] E. Abdulmalek, N.F. Hamidon, M.B. Abdul Rahman, Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents, *J. Mol. Catal. B Enzym.* 132 (2016) 1–4, doi:<http://dx.doi.org/10.1016/j.molcatb.2016.06.010>.
- [30] E. Abdulmalek, H.S. Mohd Saupi, B.A. Tejo, M. Basri, A.B. Salleh, R.N.Z. Raja Abd Rahman, M.B. Abdul Rahman, Improved enzymatic galactose oleate ester synthesis in ionic liquids, *J. Mol. Catal. B Enzym.* 76 (2012) 37–43, doi:<http://dx.doi.org/10.1016/j.molcatb.2011.12.004>.
- [31] V.M. Pappalardo, C.G. Boeriu, F. Zaccheria, N. Ravasio, Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification, *Mol. Catal.* 433 (2017) 383–390, doi:<http://dx.doi.org/10.1016/j.mcat.2017.02.029>.
- [32] C. Jia, J. Zhao, B. Feng, X. Zhang, W. Xia, A simple approach for the selective enzymatic synthesis of dilauryl maltose in organic media, *J. Mol. Catal. B Enzym.* 62 (2010) 265–269, doi:<http://dx.doi.org/10.1016/j.molcatb.2009.11.003>.
- [33] W. Zhang, Y. Wang, K. Hayat, X. Zhang, A. Shabbar, B. Feng, C. Jia, Efficient lipase-selective synthesis of dilauryl mannoses by simultaneous reaction-extraction system, *Biotechnol. Lett.* 31 (2009) 423–428, doi:<http://dx.doi.org/10.1007/s10529-008-9889-9>.
- [34] D. Arcens, E. Grau, S. Grelier, H. Cramail, F. Peruch, 6-O-glucose palmitate synthesis with lipase: investigation of some key parameters, *Mol. Catal.* 460 (2018) 63–68, doi:<http://dx.doi.org/10.1016/j.mcat.2018.09.013>.
- [35] M. Ferrer, J. Soliveri, F.J. Plou, N. López-Cortés, D. Reyes-Duarte, M. Christensen, J.L. Copa-Patiño, A. Ballesteros, Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties, *Enzyme Microb. Technol.* 36 (2005) 391–398, doi:<http://dx.doi.org/10.1016/j.enzmictec.2004.02.009>.
- [36] G. Olive, G.A. Pompeu, C. Blecker, Comptes Rendus Chimie `se enzymatique d' esters de fructose Synth Enzymatic synthesis of fructose esters 15, (2012) , pp. 1037–1047.
- [37] F. Chamouveau, D. Coulon, M. Girardin, M. Ghoul, Influence of water activity and water content on sugar esters lipase-catalyzed synthesis in organic media, *J. Mol. Catal. B Enzym.* 11 (2001) 949–954, doi:[http://dx.doi.org/10.1016/S1381-1177\(00\)00166-1](http://dx.doi.org/10.1016/S1381-1177(00)00166-1).
- [38] K. Ren, B.P. Lamsal, Synthesis of some glucose-fatty acid esters by lipase from *Candida antarctica* and their emulsion functions, *Food Chem.* 214 (2017) 556–563, doi:<http://dx.doi.org/10.1016/j.foodchem.2016.07.031>.
- [39] D.W. Shin, N.L. Mai, S.W. Bae, Y.M. Koo, Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids, *Enzyme Microb. Technol.* 126 (2019) 18–23, doi:<http://dx.doi.org/10.1016/j.enzmictec.2019.03.004>.
- [40] F. Fischer, M. Happe, J. Emery, A. Fornage, R. Schütz, Enzymatic synthesis of 6- and 6'-O-linoleyl- α -D-maltose: from solvent-free to binary ionic liquid

- reaction media, *J. Mol. Catal. B Enzym.* 90 (2013) 98–106, doi:<http://dx.doi.org/10.1016/j.molcatb.2013.01.019>.
- [41] L. Li, F. Ji, J. Wang, B. Jiang, Y. Li, Y. Bao, Efficient mono-acylation of fructose by lipase-catalyzed esterification in ionic liquid co-solvents, *Carbohydr. Res.* 416 (2015) 51–58, doi:<http://dx.doi.org/10.1016/j.carres.2015.08.009>.
- [42] P.R. Burney, J. Pfaendtner, Structural and dynamic features of *Candida rugosa* lipase 1 in water, octane, toluene, and ionic liquids BMIM-PF6 and BMIM-NO₃, *J. Phys. Chem. B* 117 (2013) 2662–2670, doi:<http://dx.doi.org/10.1021/jp312299d>.
- [43] H.P. Tai, G. Brunner, Sugar fatty acid ester synthesis in high-pressure acetone-CO₂ system, *J. Supercrit. Fluids* 48 (2009) 36–40, doi:<http://dx.doi.org/10.1016/j.supflu.2008.09.009>.
- [44] R. Ye, S.H. Pyo, D.G. Hayes, Lipase-catalyzed synthesis of saccharide-fatty acid esters using suspensions of saccharide crystals in solvent-free media, *JAOCS J. Am. Oil Chem. Soc.* 87 (2010) 281–293, doi:<http://dx.doi.org/10.1007/s11746-009-1504-2>.
- [45] N.S. Neta, A.M. Peres, J.A. Teixeira, L.R. Rodrigues, Maximization of fructose esters synthesis by response surface methodology, *N. Biotechnol.* 28 (2011) 349–355, doi:<http://dx.doi.org/10.1016/j.nbt.2011.02.007>.
- [46] Y. Wang, J. Xin, J. Shi, W. Wu, C. Xia, A kinetic study of starch palmitate synthesis by immobilized lipase-catalyzed esterification in solvent free system, *J. Mol. Catal. B Enzym.* 101 (2014) 73–79, doi:<http://dx.doi.org/10.1016/j.molcatb.2014.01.003>.
- [47] U.H. Zaidan, M.B. Abdul Rahman, S.S. Othman, M. Basri, E. Abdulmalek, R.N.Z.R. Abdul Rahman, A.B. Salleh, Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase, *Food Chem.* 131 (2012) 199–205, doi:<http://dx.doi.org/10.1016/j.foodchem.2011.08.060>.
- [48] S. Hudiyono, S. Handayani, B. Susilo, Esterification of glucose fatty acids of coconut oil catalyzed by *Candida rugosa* lipase EC 3.1.1.3 immobilized on an Indonesia's natural zeolite matrix, *World Appl. Sci. J.* 19 (2012) 1105–1111, doi:<http://dx.doi.org/10.5829/idosi.wasj.2012.19.08.2065>.
- [49] T. Méline, M. Muzard, M. Deleu, H. Rakotoarivonina, R. Plantier-Royon, C. Rémond, d-Xylose and l-arabinose laurate esters: enzymatic synthesis, characterization and physico-chemical properties, *Enzyme Microb. Technol.* 112 (2018) 14–21, doi:<http://dx.doi.org/10.1016/j.enzmictec.2018.01.008>.
- [50] S.E.H.J. Van Kempen, C.G. Boeriu, H.A. Schols, P. De Waard, E. Van Der Linden, L. M.C. Sagis, Novel surface-active oligofructose fatty acid mono-esters by enzymatic esterification, *Food Chem.* 138 (2013) 1884–1891, doi:<http://dx.doi.org/10.1016/j.foodchem.2012.09.133>.
- [51] J. Pleiss, M. Fischer, R.D. Schmid, Anatomy of lipase binding sites: the scissile fatty acid binding site, *Chem. Phys. Lipids* 93 (1998) 67–80, doi:[http://dx.doi.org/10.1016/S0009-3084\(98\)00030-9](http://dx.doi.org/10.1016/S0009-3084(98)00030-9).
- [52] L.A.W. Thelwall, Recent aspects of the chemistry of lactose, *J. Dairy Res.* 49 (1982) 713–724, doi:<http://dx.doi.org/10.1017/S0022029900022846>.
- [53] L. Cao, U.T. Bornscheuer, R.D. Schmid, Lipase-catalyzed solid-phase synthesis of sugar esters. Influence of immobilization on productivity and stability of the enzyme, *J. Mol. Catal. B Enzym.* 6 (1999) 279–285, doi:[http://dx.doi.org/10.1016/S1381-1177\(98\)00083-6](http://dx.doi.org/10.1016/S1381-1177(98)00083-6).
- [54] J. Ge, D. Lu, J. Wang, Z. Liu, Lipase nanogel catalyzed transesterification in anhydrous dimethyl sulfoxide, *Biomacromolecules* 10 (2009) 1612–1618, doi:<http://dx.doi.org/10.1021/bm900205r>.
- [55] P. Inprakhon, N. Wongthongdee, T. Amornsakchai, T. Pongtharankul, P. Sunintaboon, L.O. Wiemann, A. Durand, V. Sieber, Lipase-catalyzed synthesis of sucrose monoester: increased productivity by combining enzyme pretreatment and non-aqueous biphasic medium, *J. Biotechnol.* 259 (2017) 182–190, doi:<http://dx.doi.org/10.1016/j.jbiotec.2017.07.021>.
- [56] D. An, X. Zhao, Z. Ye, Enzymatic synthesis and characterization of galactosyl monoesters, *Carbohydr. Res.* 414 (2015) 32–38, doi:<http://dx.doi.org/10.1016/j.carres.2015.05.011>.