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β-Glycosidases: An alternative enzyme based method for synthesis of alkyl-glycosides

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Abstract

Alkyl-glycosides and -polyglycosides are environment friendly, non-ionic surfactants with favourable properties like biodegradability and chemical stability. These are extensively used in personal care products, pharmaceutical preparations and membrane protein research.

Commercial production of these surfactants is carried out in multiple steps through Fischer glycosylation reactions under extreme conditions in the presence of toxic catalysts.

β-glycosidases provide an alternative enzymatic method for their synthesis as these are easily available from microbial systems and exhibit broad substrate specificity and high stereo-selectivity. This review highlights the recent progress in glycosidase catalyzed synthesis of alkyl-glycosides. Several reaction parameters that affect the overall reaction kinetics, such as, water activity, nature of the glycosyl donor, pH of the reaction medium, reaction time, temperature and source of enzyme are discussed. Strategies available to enhance the yield, including two-phase solvent systems and immobilization are described. Current challenges and future prospects in the biological routes of synthesis are also reviewed.

Keywords: Eco-friendly surfactants, Transglycosylation, Alkyl glycosides, Alkyl polyglycosides, Two-phase system

Alkyl -glycosides and -polyglycosides

Growing ecological concern and commercial demand has generated considerable interest in eco-friendly, biodegradable and non-ionic surfactants such as alkyl-glycosides (AGs) and alkyl-polyglycosides (APGs) [1]. These are better surfactants than Triton X-100, carbohydrate esters because of their superior protein extraction capability [2], chemical stability in water and alkali and non-reactivity towards oxygen [3]. AGs contain a carbohydrate head group (which could be glucose, galactose, maltose, xylose, α-cyclodextrin) and a hydrocarbon tail (usually a primary alcohol) of different chain length of saturated or unsaturated nature [3-6]. These find diverse and attractive applications in personal care products, cosmetics, extraction of organic dyes and membrane protein research [1,7]. APGs contain more than one sugar group at the hydrophilic end and can be composed of a complex mixture of alkyl homologues, oligomers, anomers and isomers. These also find extensive use in preparation of micro emulsions, detergents, cosmetics and pesticide formulations because of their excellent behaviour at interfaces [8,9]. Commercial grade APGs contain short carbohydrate head groups, primarily monoglycosides and diglycosides, and their properties depend on the structure and bonding between the oligomeric head groups [8]. Selective elongation of AGs, such as extension of dodecyl-β-D-maltoside to dodecyl-β-D-maltooctaside by addition of α-cyclodextrin to the head group has resulted in improving their water solubility and reducing cytotoxicity [3].

The commercial production of AGs is carried out by Fischer glycosylation through direct synthesis or transacetalization. In direct synthesis, which is a one step approach, a fatty alcohol is reacted with glucose producing a complex mixture of AGs due to condensation at equally reactive hydroxyl groups. In the transacetalization synthesis, a carbohydrate is first coupled to a short alcohol like methanol producing methyl glycoside which subsequently acts as a substrate in the transacetalization with a longer alcohol [10]. The chemical route involves extremes of temperature, pressure, use of toxic catalysts, and multiple steps of protection, deprotection and activation [11]. AGs can also be prepared enzymatically by Glycosidases (E.C.3.2.1.–). These are co-factor independent hydrolytic

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enzymes and cleave glycosidic linkages *in vivo* to form mono- or oligosaccharides from poly-sugars. They can be used to synthesize glycosides *in-vitro* with judicious selection of reaction conditions. Most glycosidases used for synthetic purposes are either retaining or inverting enzymes, classified on the basis of whether the stereo character of the donor's anomeric bond is retained or inverted. They may exhibit broad specificity with respect to their natural substrates or engineered substrates which broaden their applications [12]. Availability of glycosidases from natural (microorganisms, plant) and commercial source (almond seeds), robust nature and easy handling make them more attractive for synthesis. Another advantage of using glycosidases is that they operate at near neutral pH, ambient temperature, atmospheric pressure and are highly enantio- and regio-specific [13,14].

Several reaction parameters affect the yield of AGs using glycosidases. These are water activity, temperature, time of incubation, pH of the reaction system, nature of the substrate and source of enzyme [15,16]. These operating conditions can be manipulated to give high yield of the end products. Some other strategies such as immobilization of glycosidases, presence of organic solvents and use of reverse micelle system [13,17-19] have been adopted to make the enzymatic route more economical than the chemical route by allowing reuse of enzymes and suppressing parasitic hydrolysis of the synthesized products to give high yield per unit of enzyme. Medium engineering has also been successfully used for improving yield and employs micro-aqueous, two-phase and ionic liquid systems [20-22].

In recent years, synthesis has also been attempted, with good success, with whole cell systems. The microbial cells can be cultivated on inexpensive media, stored and often the associated enzyme activities are stable. These systems are also being investigated extensively [23-26]. Our laboratory has successfully shown the use of whole yeast cells for synthesis of medium and long chain AGs, APGs and aryl-oligosaccharides [20,24,25]. The reaction specificity is decided by the cell bound glycosidases resulting in formation of regio- and stereo-specific products. Since side reactions are absent, purification of the products is relatively straight forward. This makes it a commercially viable option. A variation of this approach has been to display glycosidases on the cell surface by fusion with the cell wall anchored proteins. This, in a way, mimics the concept of immobilized enzyme reactions [27-29]. A significant progress has been made in the past few years to improve the transglycosylation yields by suppressing the secondary hydrolysis of the synthesized products by using engineered enzymes. These are a novel class of glycosidases, called as glycosynthases, and are produced by replacement of the carboxylate nucleophile by non-nucleophilic amino acid residue [30]. Glycosynthases promote glycosidic bond formation by providing a suitable activated glycosyl donor, such as a sugar fluoride [31]. The glycosynthase technology is now well established and substantial improvements have been made in this area to make enzymatic synthesis a more attractive option.

In this review, we focus on the use of glycosidases as alternative tools for alkyl glycoside synthesis with emphasis on process parameters that affect the yield. Novel strategies employed for overproduction of AGs and current challenges are also discussed.

Routes of alkyl glycoside synthesis

Chemical routes

In the chemical route, preparation of an anomerically pure AG requires numerous protection, deprotection and activation steps. Typically, all hydroxyl groups of sugars are first protected by acetylation using acetic anhydride/sodium acetate, aldehydes, acetals or benzoyl halides at 140°C in organic solvents or by microwave irradiation under mild reaction conditions (i.e., without solvent or with reduced catalyst loading) followed by deprotection by sodium methoxide in methanol (Figure 1). Activation step involves exchange of anomeric hydroxyl group with a bromine or chlorine atom. Large amounts of catalyst, mostly $FeCl_3$, $SnCl_4$, phosphotungstic acid, phosphomolybdic acid (50-100% by weight compared to the amount of sugars) are used at high temperature (80°C) to promote synthesis of AGs with moderate yield. Under the chemical method, two routes are described, namely, the Fischer glycosylation method and the Koenigs-Knorr method. Fischer glycosylation is simpler and less expensive than the Koenigs-Knorr method but the main drawback here is the use of bulk alcohol and strong mineral acids, which complicate the purification of the final products [10,32,33]. Furthermore, an equilibrium mixture of α- and β- glycosidic products is formed in a ratio that is dependent on thermodynamic stability of the isomers [34]. Koenigs-Knorr method is also a widely used method for AG synthesis but the limiting factor for large scale synthesis is the requirement of heavy metal salts like cadmium carbonate which are used as promoters [35]. Microwave irradiation has been recently used by synthetic chemists and is simple to execute, decreases reaction times and increases the product yield and purity [34,36]. However, this is not suitable for large scale preparation as most polar alcohols undergo quick degradation under microwave irradiation [33].

Biological routes

In contrast to multi-step and catalyst dependent chemical routes, glycosidases do the same job using either of the two pathways, namely, the thermodynamically controlled reverse hydrolysis path or the kinetically driven transglycosylation route. In the former, alkyl glycoside is synthesized by reacting a monosaccharide with an

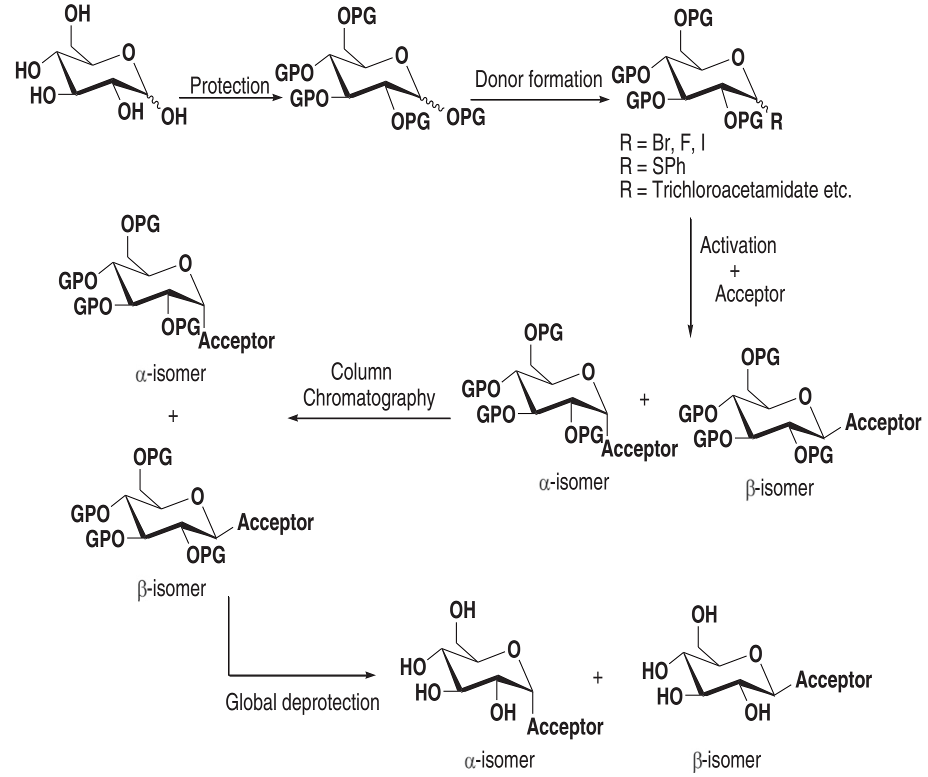

Figure 1 General strategy of glycosylation for α/β-alkyl-glucoside synthesis via chemical routes. Global protection (GP) of all hydroxyl groups using acetic anhydride/sodium acetate, aldehydes, acetals or benzoyl halides at 140°C. Activation step involves exchange of anomeric group by bromine (Br), fluorine (F), Iodine (I), Thiophenol (SPh) or tricholoroacetamide. Deprotection of final product by sodium methoxide in methanol.

alcohol in which the equilibrium of the reaction is shifted towards synthesis by reducing water activity. This is done by addition of co-solvents or by increasing the substrate concentration [21,37]. In the second approach, activated glycoside donors (mostly aryl-glycosides) and an alcohol (as nucleophilic acceptor) are used to generate a new glycosidic bond [15,21,38].

Reaction mechanism

Glycosidases fall in to two most commonly employed mechanistic classes; one with net retention and the second with inversion of configuration after hydrolyzing the glycosidic bond at the anomeric centre. Essential features have been recently described [39,40] for these two types of reaction mechanisms. The active site of both classes of enzymes contains two carboxylic acid residues (Glu/Asp), designated as R_2 and R_3 in Figure 2, which play a key role in the catalytic process. In case of inverting glycosidases, these residues are positioned approximately 10Å apart: one residue acts as an acid catalyst and the other as a base (Figure 2) and catalyzes the reaction by single displacement mechanism. The positioning of the two residues at 10 Å distance presumably allows the water and the other substrate to bind simultaneously [41]. Inverting glycosidases do not catalyze transglycosylation reaction; they just work in reverse hydrolysis mode. Unlike inverting glycosidases, the reaction mechanism of the retaining glycosidases involves a nucleophile group and an acid/base catalyst located approximately 5.5 Å apart. The reaction proceeds by double displacement mechanism in which a glycosyl enzyme intermediate is formed (glycosylation step) that has an anomeric configuration opposite to that of the starting substrate (Figure 2). Aglucone departure is facilitated by the protonation of the glucosidic oxygen by the acid catalyst. The second step of this reaction (deglucosylation step) involves the hydrolytic breakdown of the glycosyl-enzyme intermediate with net retention of anomeric configuration (Figure 2) [41]. In this, a water molecule is activated by the catalytic base to serve as a nucleophile for the hydrolysis of the glucosidic bond [42]. Under suitable reaction conditions, covalently bond glucose of the glucosyl-enzyme intermediate is transferred to other nucleophile (NuH) designated as R_4 in Figure 2, which can be an alcohol or a second sugar. Both inverting as well as the retaining glycosidases involve oxocarbenium-ion-like transition state.

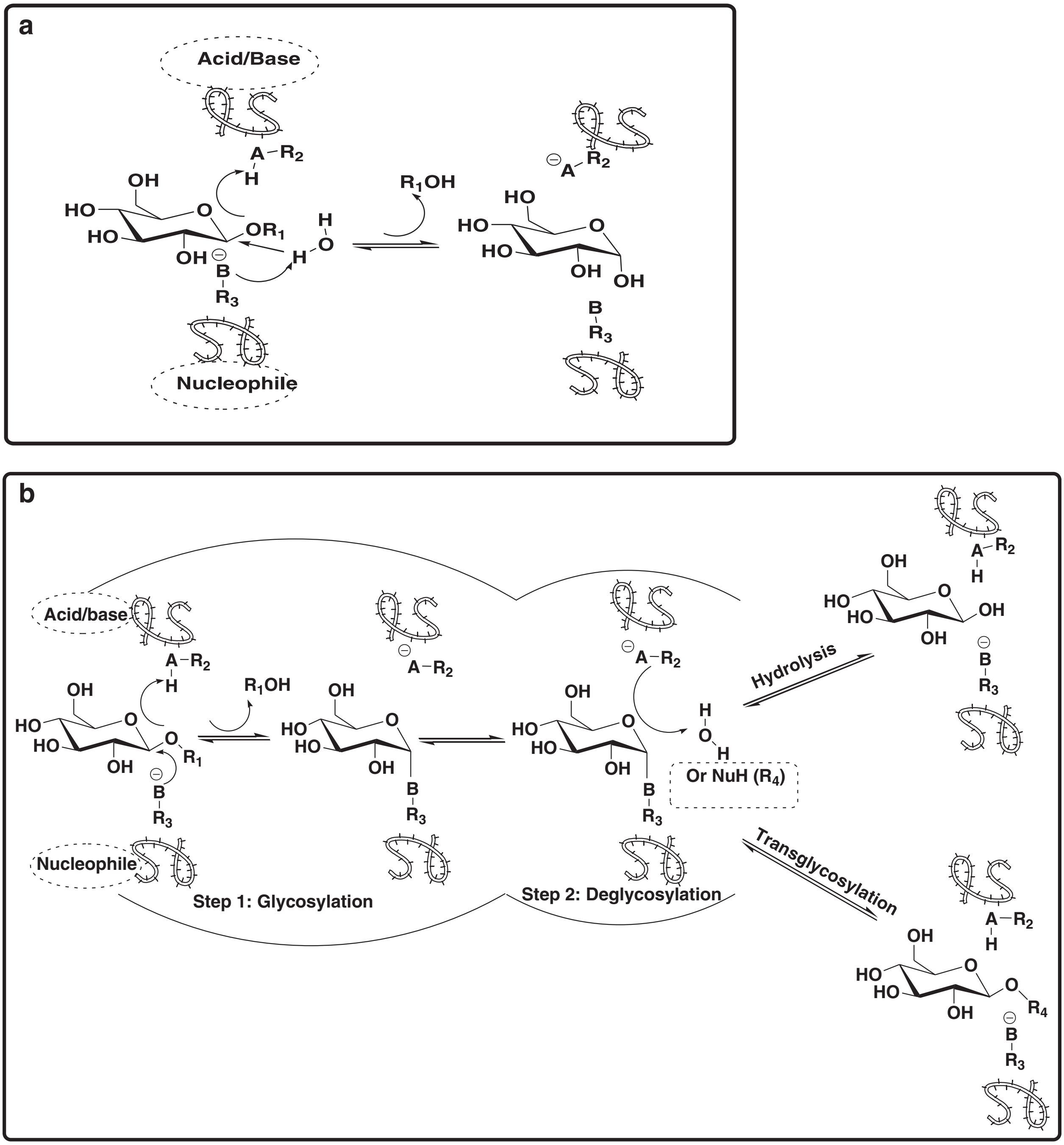

Figure 2 General reaction mechanism for glycosidases. (**a**) Inverting enzymes, (**b**) Retaining enzymes. The reaction cycle is explained in the text.

Advantages of β-glycosidase driven synthesis

The synthesis of well-defined sugars and sugar linked derivatives by β-glycosidases and other glycosyl hydrolases has resulted in a boom in the area of Green chemistry. Other enzymes, namely, transglycosidases, glycoside phosphorylases and 'Leloir' glycosyl transferases, each with specific characteristics, may also be used in glycosylation reactions [43]. Although 'Leloir' glycosyl transferases are highly efficient Nature's catalyst for glycosylation reactions but they require very expensive nucleotide-activated sugars as donors like UDP-glucose, UDP-galactose which is the major bottleneck in their application for the commercial production of glycosides [43]. However, glycoside phosphorylases and transglycosidases are active with low cost donors that can be obtained in large quantities. Our discussion in the following paragraph focuses on β–glycosidases.

β-Glycosidases have gained wide acceptance in the area of biocatalysis. In the CAZy database (Carbohydrate Active Enzymes, http://www.cazy.org/) glycosidases are structurally divided in to 132 families [44]. Most of the well characterized β–glycosidases are from GH1, GH3 and GH30 families and are retaining enzymes, whereas those belonging to GH9 are inverting enzymes. GH1 includes the largest number of characterized β-glucosidases, which are of interest in biomass conversion [45]. Under unnatural conditions they are able to form the glycosidic linkages. The glycosidases used for the synthetic purposes are exo-glycosidases.

The two essential parameters for the use of glycosidases in industry are space-time yield (STY) and *E* factor (product/waste ratio) [46]. Under optimized conditions, the enzymatic glycosylation reactions have been reported to generate 5-fold less waste and 15-fold higher space-time yield [47]. One of the major problems in the fine chemical industry is generation of non-recyclable waste. In case of the classical Koenigs-Knorr procedure, nearly half of the waste consists of acetic acid. In the modified procedure of glycosylation [16] nearly 5g of mercuric salts are formed for each gram of the synthesized product. In the enzymatic methods, on the other hand, sugars are the only wastes formed. Although the cost of the substrate is high, the process is cleaner as enzymes operate naturally at ambient temperature and do not require toxic catalysts. A comparison between the chemical and the enzymatic method [13] indicates that while the yields are better in the former, it is accompanied by generation of a lot of waste (Table 1). In the different methods employed [48-52], transglycosylation route resulted in the highest space-time yield [49].

Another fundamental parameter in glycosidase catalyzed synthesis is the cost and availability of reactants, especially the glycosyl donor. The most commonly used glycosyl donors in bulk are starch, sucrose, cellulose, glucose and fructose. Although there are other active glycosyl donors, which are more effective in transglycosylation reaction, the main drawback is the high cost of these compounds which restricts their use on large scale. Some commonly used donors in the glycosidase catalyzed reactions are listed in Figure 3.

β-Glycosidase driven synthesis can be performed using either the reverse hydrolysis or the transglycosylation approach. As shown in Table 2, moderate yields (up to 62%) have been reported for lower chain length AGs, namely, methyl glucoside/xyloside and ethyl glucoside/xyloside, in the reverse hydrolysis approach over prolonged reaction times [5,53,54]. The yield of many of these compounds could be increased substantially (even up to ~90%) in the transglycosylation approach [5,53-58] (Table 3). A yield of over 90% has been reported for methyl-β-D-fucoside using the *Paenibacillus* enzyme [59].The yields (Table 2) for higher chain alkyl glucosides range from 1.9% (decyl glucoside) to 36% (for butyl glucoside) in the reverse hydrolysis approach [19,21,54,55]. In the transglycosylation route (Table 3), the reported yield ranges from 4-97% [5,16,17,19,20,24,26-29,53,55-70]. As observed in Tables 2 and 3, majority of these synthesis reactions have been carried out using purified enzymes. A list of the microbial sources of these enzymes is provided in Figure 4. It can also be seen that much of the reported work has involved the use of bacterial or the almond enzyme (which is commercially available). Whole cell catalysis is a relatively newer area but given the high yields reported with some microbial cells [20,24,26], this approach requires an in-depth study.

Process parameters affecting enzymatic synthesis

Water activity

A key process parameter which affects the glycosidase-catalyzed synthesis is the water activity of the reaction medium; a sufficiently high water activity ($a_w = 0.6$) is required by glycosidases to retain activity. This is unlike lipases which can be active at water activity close to zero [71]. However, a high water content promotes hydrolysis thus decreasing the overall product concentration. The yield in kinetically controlled reactions depends on the concentration of water and other competing nucleophiles like alcohols and the selectivity of the enzyme. Large alcohols need higher water activity to be active as glycosyl

Table 1 Comparison of enzymatic and chemical glycosylation in terms of Space-Time Yield and *E*-factor

Glycosylation method	Glycosyl donor	Product	*E* factor (kg waste per kg product)	Space-time yield (g/l/d)	Reference
Direct glycosylation (by glycosidase)	Glucose	Glcβ-O$(CH_2)_6$OH	0.5	5.6	[48]
Transglycosylation (by β-galactosidase)	Lactose	Galβ-O$(CH_2)_6$OH	3	**2380**	[49]
Koenigs-Knorr method	Galactose	Galβ-OPh-2-NO_2	**10**	26	[50]
Direct glycosylation (by glycosidase)	Glucose	Glcβ-OCH(CH_3)CHCH_2	2	10	[51]
Koenigs-Knorr method	Glucose	Glcβ-OCH(CH_3)CHCH_2	**20**	36	[52]

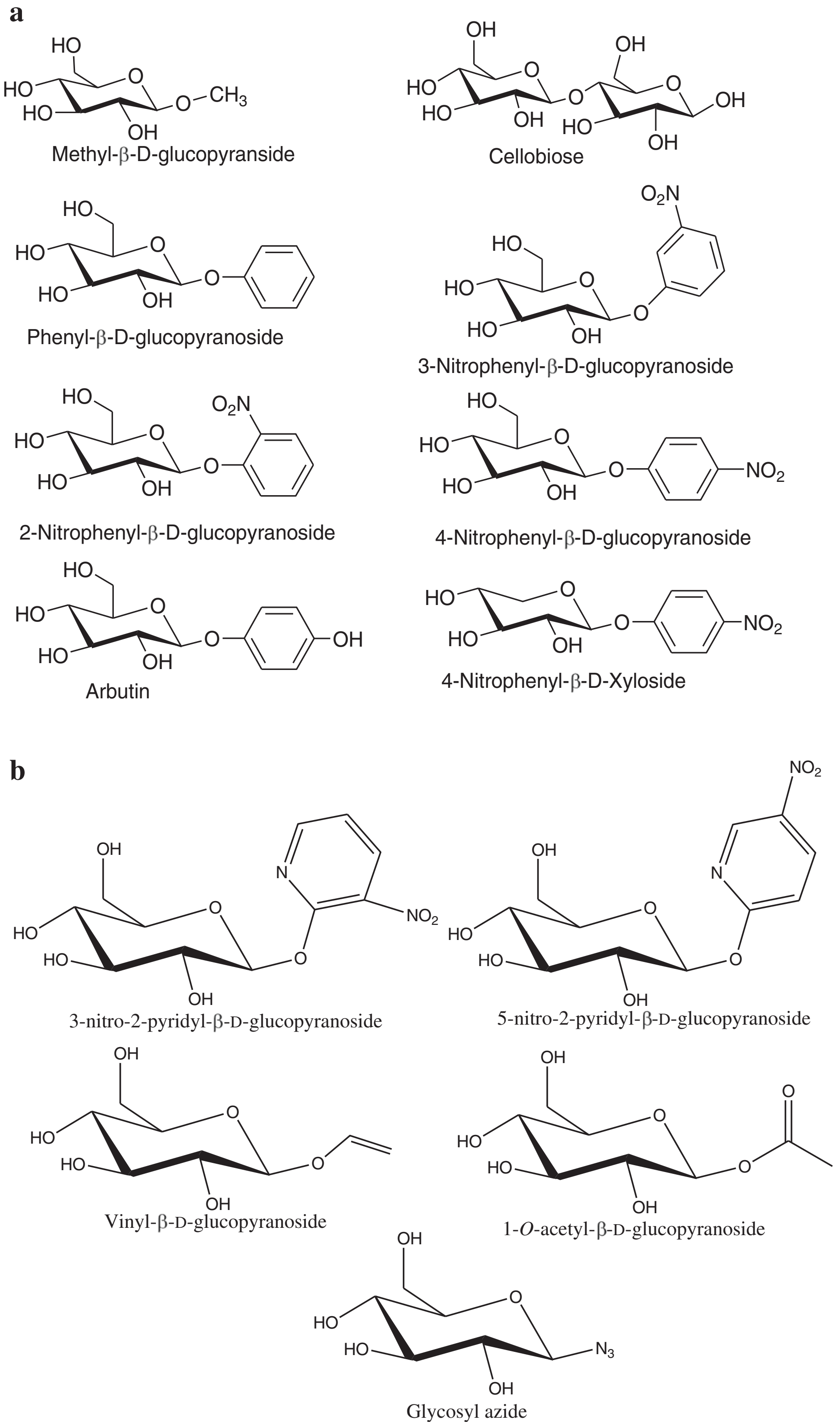

Figure 3 Glycosyl donors used for enzymatic synthesis of alkyl glycosides. (**a**) Commonly used substrates such as cellobiose, methyl-β-D-glucoside and aryl glucosides. (**b**) Novel substrates.

Table 2 Synthesis of alkyl glycosides by β-glycosidases via reverse hydrolysis route

Source of enzyme	Glycosyl donor	Glycosyl acceptor	Product	Yield (%)	Time(h)	Ref
Almond β-glucosidase	Xylose	Methanol	Methyl-β-D-xylopyranoside	Poor Yields	6 Days	[5]
		Ethanol	Ethyl-β-D-xylopyranoside			
		Propanol	Propyl-β-D-xylopyranoside			
		Butanol	Butyl-β-D-xylopyranoside			
		Pentanol	ND			
		Hexanol	ND			
		Heptanol	ND			
		Octanol	ND			
Almond β-glucosidase	Glucose	Hexanol	Hexyl-β-D-glucopyranoside	11	144	[55]
Almond β-glucosidase	Glucose	Methanol	Methyl-β-D- glucopyranoside	58	54	[53]
Almond β-glucosidase	Glucose	Butanol	Butyl-β-D- glucopyranoside	30	Not Given	[21]
Almond β-glucosidase	Glucose	Octanol	Octyl-β-D-glucopyranoside	20	200	[19]
Almond meal	Glucose	Methanol	Methyl-β-D-glucopyranoside	27.6	168	[54]
		Ethanol	Ethyl-β-D-glucopyranoside	62.5		
		Propanol	Propyl-β-D-glucopyranoside	58.7		
		Butanol	Butyl-β-D-glucopyranoside	36.1		
		Pentanol	Pentyl-β-D-glucopyranoside	13.7		
		Hexanol	Hexyl-β-D-glucopyranoside	9.17		
		Heptanol	Heptyl-β-D-glucopyranoside	4.57		
		Octanol	Octyl-β-D-glucopyranoside	4.05		
		Nonanol	Nonyl-β-D-glucopyranoside	2.92		
		Decanol	Decyl-β-D-glucopyranoside	1.93		

ND: not detected.

acceptors compared to the small ones as this increases the flexibility of the active site for accommodating the large nucleophiles. The optimal water activity has been determined for different chain length alcohols and indicated water activity (a_w) of 0.43, 0.71 and 0.81 for propanol, hexanol and octanol respectively [16]. An optimal water activity of 0.67 was also reported for XAD-4 immobilized β-glucosidase for octyl-β-D-glucoside synthesis [71]. An elaborate study on five different glycosidase-catalyzed transglycosylation reactions in hexanol showed that the best conditions for transglycosylation were higher water activity but without a separate aqueous phase and for all the enzymes the selectivity for alcohol increased with an increase in water activity [16]. In the reverse hydrolysis mode, a decrease in water activity resulted in an increase in equilibrium yield of hexyl glucoside but this was not the case in the kinetically controlled reaction wherein the ratio between transglucosylation and hydrolysis increased with an increase in water activity [72]. Since water activity and water content show close correlation, studies on the effect of water content in the organic reaction are of crucial significance. Effect of water content (5-30%, v/v) on the ratio of transglycosylation rate to hydrolysis rate (r_T/r_H) has been studied during the synthesis of hexyl glucoside and octyl glucoside using *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) and methyl glucoside as donors respectively. For hexyl glucoside synthesis, a ratio of r_T/r_H of 5.1 at 16% water content was found to be optimum and a maximum ratio of r_T/r_H 2.28 at 8% water content was optimum for octyl glucoside synthesis. Above this optimum water content, a drastic decrease in r_T/r_H was observed possibly because of mass transfer limitation of *p*NPG or methyl glucoside from organic to aqueous phase [24,66]. Thus, for every enzyme/cell system, the conditions need to be optimized.

Nature of the substrate

As mentioned in the previous sections, the yield of alkyl glycoside is generally lower in the reverse hydrolysis mode (Table 2) as the equilibrium constant of the reaction lies strongly in favour of hydrolysis. Thus, higher concentration of both the monosaccharide and other nucleophile is required in this route of synthesis. Since the transglycosylation route is directly under kinetic control and utilizes a pre- formed activated glycoside donor; the glycosyl donor must be cleaved more rapidly than the

Table 3 Synthesis of alkyl glycosides by β–glycosidases via transglycosylation route

Source of enzyme	Glycosyl donor	Glycosyl acceptor	Product	Yield (%)	Time (h)	Reference
Trichoderma reesei β-xylosidase	Methyl-β-D-xyloside	Ethanol, Propanol	Ethyl-β-D-xyloside, Propyl-β-D-xyloside	52-55	12	[5]
Candida molischiana35M5N β- glucosidase	Cellobiose	1-decanol	Decyl-β-D-glucopyranoside	Lower yields	20	[60]
Almond β-glucosidase	Methyl-β-D-glucoside	Hexanol	Hexyl-β-D-glucopyranoside	25	24	[55]
	Cellobiose			28	48	
β-glucosidase II *Aspergillus niger*	Cellobiose	Methanol	Methyl-β-D-glucopyranoside	82	0.5	[56]
		Ethanol	Ethyl-β-D- glucopyranoside	55		
Almond β-glucosidase	Cellobiose	Methanol	Methyl-β-D-glucopyranoside	37	5	[53]
β-glucosidase from *Fusarium oxysporum*	Cellobiose	Methanol	Methyl-β-D-glucopyranoside	54.4		[57]
	Gentiobiose			51.3		
	Amygdalin			63.4		
	Arbutin			72.2		
	Salicin			58		
	*p*NPG			74.2		
Aspergillus niger β-glucosidase II	Cellobiose	Methanol	Methyl-β-D-glucopyranoside	83	6	[58]
		Ethanol	Ethyl-β-D- glucopyranoside	53	3	
Hyperthermophilic β-glycosidase	Lactose	Heptanol	Heptyl-β-D-glucopyranoside	22.5	25	[61]
β-Glucosidase from Thai Rosewood	*p*NPG	C1-C4 1° alcohols	β-alkyl glucosides	C1 = 97	7	[62]
				C2 = 93		
				C3 = 81		
				C4 = 89		
β-Galactosidase *Penicillium canescencs*	Lactose	Heptanol	Heptyl-β-D-galactopyranoside	43	80	[19]
		Octanol	Octyl-β-D- galactopyranoside	45		
		Nonanol	Nonyl-β-D-galactopyranoside	37		
Almond β- glucosidase	*p*NPG	Octanol	Octyl-β-D- glucopyranoside	42	NA	[63]
β-glucosidase from *C. Saccharolyticum*	*p*NPG	Octanol	Octyl-β-D- glucopyranoside	45		
β-glycosidase from bitter almond	Methyl-β-D-glucoside	Hexanol	Hexyl-β-D- glucopyranoside	21	155	[64]
β-galactosidase from *Aspergillus oryzae*	Lactose		Hexyl-β-D- galactopyranoside	4		
β-glycosidases from *Sulfolobus solfataricus* (*LacS*)/ *Pyrococcus furiosus* (*CelB*).	Lactose	Hexanol	Hexyl-β-D- glucopyranoside/ Hexyl-β-D- galactopyranoside	29/28	10	[16]
				41/63	20	
Almond β- glucosidase adsorbed on Celite R-640®	*p*NPG	Octanol	Octyl-β-D- glucopyranoside	37.2	650	[17]
Almond β- glucosidase	*p*NPG	Primary alcohols (C1-C6)	β-alkyl glucosides	C1 = 81	24	[65]
				C2 = 64		
				C3 = 48		
				C4 = 25		
				C5 = 22		
				C6 = 18		
Whole cells of *Bacillus pseudofirmus*	Lactose	Hexanol	Hexyl-β-D-galactopyranoside	50	3	[26]
		Octanol	Octyl-β-D-galactopyranoside	26	20	

Table 3 Synthesis of alkyl glycosides by β–glycosidases via transglycosylation route *(Continued)*

Thermotoga neapolitana β-glucosidase B	*p*NPG	Hexanol	Hexyl-β-D- glucoside	80.3	3	[66]
β-galactosidase from *Aspergillus aculeatus* displayed on Yeast cells	*p*NPG	Hexanol	Hexyl-β-D- glucoside	27.3	12	[27]
β-glucosidase *Sclerotinia sclerotium*	Cellobiose	Primary alcohols (C4-C8)	β-alkyl- glucosides	Moderate yields	30	[38]
β-galactosidase displayed on *Bacillus spores*	Lactose	Octanol	Octyl-β-D- galactopyranoside	27.7	24	[29]
Pseudoaltermonas 22b β- galactosidase	Lactose	1° alcohols (C5-C9)	Pentyl-β-D-galactopyranoside	31	48	[68]
			Hexyl-β-D- galactopyranoside	28		
			Heptyl- β-D-galactopyranoside	18		
			Octyl- β-D- glactopyranoside	9		
			Nonoyl- β-D-galactopyranoside	5		
β-D-Galactosidase from *Paenibacillus thiaminolyticus*	*p*NP-β-D-Fucose	Methanol	Methyl-β-D-fucoside	94	2.5	[59]
		Ethanol	Ethyl-β-D-fucoside	72		
		Propanol	Propyl-β-D-fucoside	56 4		
		Octanol	Octyl-β-D-fucoside	15 2		
β-D-galactosidase from *Aspergillus niger*	Lactose	Hexanol	Hexyl-β-D-pyranoside	10	3	[69]
		Heptanol	Heptyl-β-D-galacto pyranoside	8		
Cell bound β-glucosidase of pyranoside *Pichia etchellsii*	pNPG	Octanol	Octyl-β-D-glucopyranoside	70	4	[20]
β-Galactosidase on cell surface of *Bacillus subtilis*	Lactose	Octanol	Octyl-β-D-galactopyranoside	33.7	24	[28]
Xylanase from *Thermobacillus xylanilyticus*	Birchwood xylan	Pentanol	Pentyl-β-D-xylobioside	85	1	[70]
		Octanol	Octyl-β-D-xyloside	8		
			Octyl-β-D-xylobioside	14		
			Octyl-β-D-xylotrioside	21		
Cell bound β-glucosidase of *Pichia etchellsii*	Methyl-β- D-glucoside	Hexanol	Hexyl-β-D-glucopyranoside	48	6	[24]
		Octanol	Octyl-β-D-glucopyranoside	53		
		Decanol	Decyl-β-D-glucopyranoside	27		
		Dodecanol	Dodecyl-β-D-glucopyranoside	13		

product formed. Several compounds have been used as glycosyl donors, the predominant ones being the aryl glycosides and disaccharides (Figure 3a).The transglycosylation approach gives higher yields (Table 3), especially, when aryl donors are used in the reaction. The use of nitro-phenyl glycoside donors may have many drawbacks like limited solubility, auto-condensation of the donor substrate and difficulty in removal of nitrophenol from the reaction mixture [12]. Glycosyl donors with other leaving groups (Figure 3b) have been proposed as a solution to this problem [12]. Besides these, glycosyl fluoride donors, which have poor stability in water, have also been used with glycosynthases. For β-glucosidases and β-galactosidases, glycosyl azides are considered to be good alternative substrates [73].

Different chain length alcohols, mostly primary alcohols, have been used as acceptors for the synthesis of AGs (Tables 2 and 3). These are also found to be better acceptors compared to the secondary or tertiary alcohols. This is attributed to their increased reactivity on account of favorable steric factors during nucleophilic attack. The inability of β-glycosidases to use secondary or tertiary alcohols as acceptors appears to be a general phenomenon with almost all glycosidases [13]. However, an exception is reported and this is the β-glucosidase from cassava [74].

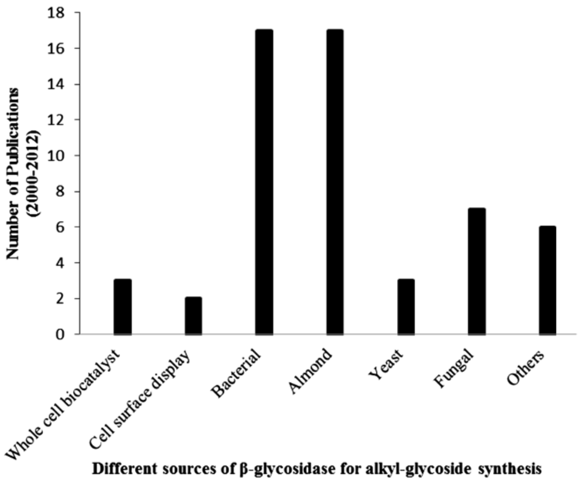

Figure 4 Publications on use of β-glycosidase from different sources for synthesis of various alkyl-glycosides in the period of 2000–2012. Source: Web of Science database (http://www.scopus.com/home.url).

Effect of pH, temperature and shaking

To carry out enzymatic reactions in aqueous solutions, it is a common practice to add buffering agents to influence the state of ionization of important functional groups at the active site of the enzyme. Likewise, ionization of functional groups at active site is also important while carrying out reactions in presence of organic solvents. Addition or removal of proton at the active site can turn the enzyme on or off. Enzymes in organic solvents retain the ionization state of active site residues of its natural environment (water) even after lyophilizing the enzyme, this is called as the 'pH memory' of enzymes [75]. Most of the glycosidases are active in the entire pH range of 4–7 and have highest hydrolytic activity at pH 6. Therefore, most of the synthesis work has been carried out in this pH range leading to maximum yield of the final product.

Effect of temperature on direct alkylation of glucose or in transglycosylation reactions has also been investigated and indicates no significant improvement in final yields with an increase in temperature [15,20]. Although higher temperature is favourable for increasing the solubility of the substrates, it can inactivate the enzymes. Thus, thermostable enzymes would be desirable in synthesis. Generally, β-glycosidase have temperature optima between 40-50°C [76] and most of the synthesis work has been carried out in this temperature range. With some thermophilic β-glucosidases, such as those from *Thermotoga neapolitana* and *Pyroccocus furiosus*, synthesis of hexyl and octyl glucoside using *p*NPG and pentyl glucoside respectively as glucosyl donor has been reported. *T. neapolitana* β-glucosidases catalyzed 100% conversion of *p*NPG in hexanol/water mixture with increase in temperature up to 90°C with a yield of 80.3% [66]. Mutants from *P. furiosus* showed higher selectivity and resulted in increased hexyl glucoside yield from 56 to 69% over a temperature range of 50-95°C compared to the wild type [16]. These differences may be explained by two factors, namely enzyme denaturation and variation in media composition [77]. Thus, higher temperature can lead to better conversion efficiencies.

For the synthesis of long chain AGs, an important limitation is the poor water solubility of the glycosyl acceptor thus preventing an efficient contact with the glycosyl enzyme intermediate. For minimizing this physical effect, strategies such as vigorous shaking, use of co-solvents and use of different chain length glycosyl acceptors (like methanol, butanol, pentanol, hexanol, heptanol, octanol, decanol and dodecanol) in bulk can be employed [70]. Not much work has been done to study the effect of shaking but it is expected to enhance the conversion efficiencies by removing the products from the enzyme active site. An increase from 200 to 1000 rpm has been reported to enhance the yield from 80 mg/g to 362 mg/g [70] in the transglycosylation route.

Available strategies for improving the yield of alkyl glycosides

A number of strategies have been developed to increase the yield in enzyme driven synthesis of AGs and these are summarized below.

Immobilization, whole cell biocatalysis and cell surface display of enzymes

The use of immobilized enzymes is justified if the biocatalyst can be reused efficiently. For improving the yield of the glycosidase catalyzed reactions, hydration level of the media has to be carefully maintained because the presence of water is necessary for enzyme activity but at the same time water causes parasitic hydrolysis of the substrate and product [17]. Celite R-640® is a chemically inert silica-based matrix and has shown promising potential for maintaining hydration level of media during enzyme catalyzed peptide and amide synthesis [78]. The application of Celite R-640® as a support for immobilization of β-glucosidase for synthesis of AGs has been demonstrated [17]. This preparation exhibited good stability of the enzyme even in co-solvents like dimethyl formamide compared to enzymes immobilized on Amberlite XAD-4. Other materials such as DEAE-sepharose and polyacrylamide have also been used to immobilize β-glucosidases for synthesis of AGs. More than increased yield, this lead to improved stability of the enzyme when compared to the free enzyme [18]. Another support, namely, XAD-4/16 Celite has also been used and was shown to increase β-glucosidase activity. No effect on yield was reported with these preparations [16]. There is only one report on whole cell immobilization and that is of *Bacillus pseudofirmus*

AR 199 cells on polyvinyl alcohol cryogel beads and on Celite for synthesis of alkyl galactosides. A decrease in reaction rate was observed with final yield of 26% for octyl galactoside in both cases after 72 h of reaction in comparison to 20 h with free cells [26].

Microencapsulation of β-glucosidase in permeable matrices prepared from polyallylamine hydrochloride/1-6, hexanediamine and dodecanedioyl chloride has been attempted to overcome mass transfer limitations in aqueous organic two phase system; the aqueous phase containing the enzyme and the substrate being contained in the hydrophobic phase [65]. The enzyme in the micro capsules prepared from polyallylamine hydrochloride performed significantly better presumably due to enhanced permeability emphasizing the importance of optimizing the polymer composition. At the same enzyme loading, the formation of hexyl glucoside was 2.5 times faster in microencapsulated system than with the enzyme immobilized on XAD-4 polymeric adsorbent. The potential for improved productivity using microencapsulated β-glucosidase has been further explored by operating a batch reactor at 50 ml scale with similar reaction kinetics over a period of two weeks by recharging microcapsules with glucose [65].

Whole cell biocatalysis approach has also been attempted, albeit, by only a few laboratories, for synthesis of AGs. The principal advantage of using whole cells is that the process can be economical and exhibit less sensitivity to denaturation as the cells can act as natural immobilized systems [20,24,26]. Cell permeabilization using membrane disrupting agents was suggested as a means to overcome mass transfer limitations but no effect was observed on the final yield of the desired products [24,26]. Another advantage of using whole cell biocatalysts is that cells can be lyophilized without the aid of lyoprotectants unlike purified enzymes. Lyophilization presumably locks the enzyme molecules on the cell surface in their right conformation so that activity is not lost. On the other hand, with purified enzymes it can lead to significant denaturation of activity. Using a bacterial system, 26% yield was reported using lactose as glycosyl donor [26]. Promising yields of 53% and 70% were reported for a medium chain length alkyl glycoside (octyl glucoside) using methyl glucoside and *p*NPG as donors respectively [20,24]. Interestingly, no side reactions were observed with this conversion. The whole cell system was also operated at 50 ml scale using methyl glucoside as donor for octyl glucoside synthesis. The reactor performance was tested in three reaction cycles run semi continuously over a period of 18 h with 100% substrate conversion and improvement in yield [24]. Given the economic viability of using whole cells, such transformations need to be studied with other systems as well. Another recent methodology attempted to make whole cell biocatalysis more attractive is to display recombinant enzymes on the cell surface. This can serve as an immobilized enzyme preparation with cell-wall-anchored proteins [27]. A yield of 27.3% of hexyl glucoside was obtained with this system using *p*NPG as donor. Recently, β-galactosidase was expressed on *Bacillus subtilis* spores by fusion with the spore coat proteins. This immobilized enzyme showed better solvent stability, enhanced thermal stability and transgalactosylation efficiency in non-aqueous media resulting in overall yield of 27.7 to 33.7% of octyl galactoside [28,29].

Two-phase, micro-aqueous and mono-phasic system

The success of glycosidic bond formation depends on whether the glycosyl-enzyme intermediate is trapped by glycosyl acceptors other than water. Alcohols are better bound at enzyme active site than water, but at the same time water acts as a competing nucleophile thereby decreasing the overall transglycosylation yield [71]. The yields can be improved if these reactions are carried out predominantly in organic solvents but glycosidase lose activity in such media. Unlike lipase, glycosidase require a minimum amount of water to catalyze transglycosylation reactions. A water activity (a_w) of 0.4-0.5 is required by almond β-glucosidase for lower alcohols (C3-alcohols) while for higher chain alcohols (C8) a water activity in the range of 0.67-0.8 was required [13,71]. While using whole cells of *Pichia etchellsii*, again a high water activity was required for synthesis of octyl glucoside [20]. While it is desirable to shift the reactions to lower water activity environment to shift the reaction equilibrium towards synthesis, this will result in lowered activity of the enzyme and also poor solubility of long chain alcohols affecting the overall yield. To address these problems, aqueous organic two phase system has been suggested and used for synthesis of AGs [15]. The problems associated with the two phase system were the instability of glycosidases and competitive hydrolysis of substrate and products [13]. Organic one phase liquid system has also been used to address the problems associated with the two phase system, but some amount of water is always found to be necessary for catalytic activity of glycosidases and they fail to catalyze reactions in completely anhydrous organic media [21,24]. It has been suggested that adding co-solvents increases the solubility of sugar substrates and decreases the water activity [15]. The co-solvents generally used should be biocompatible, water miscible, of low boiling point and must maintain high enzyme activity and low water activity. The most suitable co-solvents for β–glycosidases were found to be monoglyme, diglyme, 1,4-dioxane, methanol, acetonitrile, dimethyl sulfoxide, tetrahydrofuran, dimethyl formamide, *tert*-butanol. These can be used up to a concentration of 10 to 20% (v/v) of the total reaction volume [79]. The yields obtained under such conditions were 52, 26, 21 and 17% for hexyl-, octyl-, decyl and dodecyl glucoside respectively [79]. It is also important to note that low aqueous systems are feasible only with hydrophobic glycosyl donors like methyl-β-D-glucopyranoside, phenyl-β-D-glucopyranoside, and 4-nitrophenyl-β-D-glucopyranoside [20,24].

Reverse micelles

Reverse micelles or micro emulsions can be considered as individual nano bioreactors and these have added new properties to biocatalysts in a variety of biotransformations [19]. Reverse micelles in organic medium are formed by association of surfactant molecules with outer layer formed of hydrocarbon tails and inner layer by polar heads. The most remarkable property of reverse micelles is their capacity to solubilize water and hydrophilic substrates with retention of catalytic activity. As observed in the preceding paragraph, much of the synthesis work on AGs has been reported in two-phase systems, with the aqueous phase containing the enzyme and the substrate. A major bottleneck in two-phase system is the poor solubility of long chain alcohols in the aqueous phase thereby displaying limited accessibility to the glycosyl enzyme intermediate. This leads to reduced rate of transglycosylation. Elevated temperatures can be used to improve yields but it leads to denaturation of enzyme [20,21]. Due to these disadvantages in the two phase system, reverse micelles have been used and shown to enhance the yield significantly [19]. Reverse micelle system has also been used to catalyze synthesis of octyl-β-D-glucopyranoside and octyl-β-D-galactopyranoside with maximum yields of 40 and 45% using glucose and lactose as donors respectively exceeding the yields of enzymatic synthesis in two phase system where synthesis proceeds in extremely low yields (6-9%) [19]. The major concern in this reaction system remains the recovery of the final product from the surfactant in the reaction system [80].

Use of ionic liquids as a novel medium for yield improvement

An elegant new strategy to enhance yield in glycosidase catalyzed reactions has been the use of ionic liquids in place of organic solvents to suppress/lower the water activity of the system. These are salts that do not crystallize at room temperature. Because of the outstanding thermal stability (up to 300°C), near to zero vapor pressure and an excellent miscibility property, they are considered as green solvents for future [81,82]. Typical ionic liquids are mixtures of dialkylimidazolium cations with weakly coordinating anions such as methyl sulfate or hexafluorophosphate. Over the past decade, ionic liquids have gained increased attention as reaction media for enzyme catalyzed reactions with promising results by replacing organic co-solvents. They can be used as a pure solvent, as a co-solvent in aqueous systems or in a biphasic system. To date use of ionic liquids (1-Butyl-3-methylimidazolium hexafluorophosphate, 1-Butyl-3-methylimidazolium tetrafluoroborate) has shown excellent results with lipase, esterases and protease enzymes by retaining activity and selectivity as in the original medium [83]. Very little research has been done on synthesis of alkyl glycosides and oligosaccharides in ionic liquids using glycosidases [84]. The yields are often disappointing as the formation of the glycosidic bond is considered to be thermodynamically less favourable than formation of an ester or an amide bond. In contrast to esterases and lipases, most glycosidases lose activity in ionic liquids, although solubility of their substrates (sugars) is reported to be increased [85]. Use of ionic liquid 1,3-dimethyl-imidazol methyl sulfate for the enzymatic synthesis of *N*-acetyllactosoamine from lactose and *N*-acetylglucosamine using β-galactosidase from *Bacillus circulans* has shown promising results by doubling the yield from 30% to 60% by suppressing secondary hydrolysis of the synthesized product [86]. Another glycosidase from *P. furious* has shown 10% increase in galactosyl transfer from lactose to glycerol using 45% (v/v) 1,3-di-methyl-imidazol methyl sulfate ionic liquid [84]. Recently, Yang and his co-workers have examined various ionic liquids for the enzymatic synthesis of aryl-, alkyl-β-D-glucopyranosides catalyzed by crude enzyme preparation (prune seed meal, *Prunus domestica*). Among the examined ionic liquids, the novel 1-butyl-3-methylimidazole iodide [BMIm]I at 10% concentration proved to be the best and was used first time for the synthesis of aryl-, alkyl-β-D-glucopyranosides with yields ranging from 15-28% [87]. Use of ionic liquid 1-butyl-3-methyl-imidazolium chloride has been reported by synthetic chemists in conversion of cellulose to isomers of alkyl glucosides with yields of 82-86% in presence of an acid catalyst [88]. This reaction can be replaced by an enzymatic route to make the process more attractive. From the initial investigation on use of ionic liquids, it is obvious that they offer a useful method in enzymatic transglycosylation and are effective in reducing water activity and increase solubility of the hydrophilic substrates. The polar nature of ionic liquids makes them suitable candidates for glycosidase catalyzed synthesis, if one has to use cheap polar substrates like glucose, lactose, cellobiose, maltose, cellulose hydrolysate as glycosyl donors. In order to make the ionic liquid system more competitive and better than the water co-solvent systems, recovery and reuse of ionic liquids will play a major role. The issues of cost, toxicity and biodegradability of these compounds also needs to be addressed.

Protein engineering

Recent technological and scientific achievements in DNA recombinant technology has broadened the scope of biocatalysis both in the laboratory as well as on industrial scale [89]. Major bottleneck for using biocatalyst is its poor stability and optimizing the biocatalyst for the non-natural substrates. Currently the molecular biology methods are used to rapidly modify enzymes via *in vitro* version of Darwinian evolution, commonly known as directed evolution. This involves creation of smarter libraries by random amino acid changes in protein with improvement in its stability and broad substrate specificity [89]. Since glycosidases are often limited by their tight donor specificities, several groups have developed glycosidases with altered and broad substrate specificity. Promiscuous glycosidases have been developed by site directed mutagenesis in *Sulfolobus solfataricus* by mutating two key residues which resulted in broad substrate specificity capable of processing many sugars namely lactose, xylose and glucose. The mutant enzymes from *S. solfataricus* showed good synthetic activity and produced methyl-β-galactoside, methyl-β-xyloside and methyl-β-glucoside in 100, 50 and 87% yield [90].

Conclusions

Alkyl glycosides are used in wide range of applications particularly in personal care products, membrane protein research, as boosters for antibacterial agents. They have several attractive properties like biodegradability, low toxicity and can be prepared from renewable sources. The chemical route of synthesis is followed currently for large scale production of these compounds. The enzymatic routes can be followed using glycosidases which use either the `reverse hydrolysis' or the `transglycosylation' approach. The major advantage of using β-glycosidases for alkyl glycoside synthesis is that these operate at neutral pH, ambient temperature, atmospheric pressure and catalyze enantio- and regio-specific reactions. While significant progress has been made in increasing the yield in enzyme catalyzed synthesis the cost of production still remains high. Further, the enzyme based synthesis works well for the synthesis of lower chain length alkyl glycosides. With higher chain length alcohols (above C8) low yields are obtained due to poor miscibility of long chain alcohols with water. Medium engineering has been used as a successful approach for increasing the yield. Although solubility can be improved by using higher temperature but that leads to denaturation of the enzymes. Development of thermophilic and organic solvent tolerant enzymes by protein engineering approach can address some of these issues.

Future prospects

Nearly 30% more people will occupy Earth in the year 2050, increasing the demand for day-to-day services. This is likely to result in ecosystem degradation, reduced biodiversity and of course changing climate. One of the major concerns is to reduce use of chemical routes for synthesis of common products and shift towards enzyme based technologies. New strategies need to be developed as enzymatic methods provide an excellent eco-friendly alternative. Coupling the enzymatic routes to continuous product removal strategies (for instance, by use of special matrices that selectively bind products) can lead to efficient conversion efficiencies. While such systems can be operated at laboratory scale, a bioreactor for commercial scale production is in high demand. Thermophilic enzymes (or cell sources of these enzymes) could also be used in such conversions. Whole cell catalysis is a novel approach and requires an in-depth study. Development of genetically engineered enzymes with increased organic solvent tolerance and selectivity towards nucleophile other than water would be desirable. Ionic liquids offer many advantages over conventional organic solvents because of their excellent physico chemical properties like thermal stability, near-zero vapour pressure, tunable properties like polarity/hydrophobicity/solvent miscibility. Some success has been reported with these in alkyl glucoside synthesis. These also allow use of a large range of compounds which can be solubilized by adjusting the cation/anion ratio. Another important feature of using ionic liquids is their ability to enhance in some cases enantio-, stereo-, and regio selectivity. Very little work has been reported using this solvent for β–glycosidases and needs to be investigated extensively.

Competing interests

The authors declare that they do not have any competing interests.

Authors' contributions

Both the authors have contributed to writing of the manuscript. The final version was read and approved by both the authors.

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References

1. Yakimchuk OD, Kotomin AA, Petel'skii MB, Naumov VN: **Cleaning action and surfactant properties of alkyl glucosides.** *Russ J Appl Chem* 2004, **77:**2001–2005.
2. Lin JT, Riedel S, Kinne R: **The use of octyl-β-D-glucoside as detergent for hog kidney brush border membrane.** *Biochim Biophys Acta* 1979, **557:**179–187.
3. Svensson D, Ulvenlund S, Adlercreutz P: **Enzymatic route to alkyl glycosides having oligomeric head groups.** *Green Chem* 2009, **11:**1222–1226.
4. Izawa S, Sakai-Tomito Y, Kinomura K, Kitazawa S, Tsuda M, Tsuchiya T: **Introduction of a series of alkyl thiomaltosides, useful new non-ionic detergents, to membrane biochemistry.** *J Biochem* 1993, **113:**573–576.

5. Drouet P, Zhang M, Legoy MD: **Enzymatic synthesis of alkyl β-D-xylosides by transxylosylation and reverse hydrolysis.** *Biotechnol Bioeng* 1994, **43:**1075–1080.
6. Milkerert G, Garamus VM, Veermans K, Willumert R, Vill V: **Strutures of micelles formed by dynthetic alkyl glycosides with unsaturated alkyl chains.** *J Coll Int Sci* 2005, **284:**704–713.
7. VanAken T, Foxall-vanAken S, Castleman S, Ferguson-Miller S: **Alkyl glycoside detergents: synthesis and applications to the study of membrane proteins.** *Methods Enzymol* 1986, **125:**27–35.
8. Von Rybinski W, Hill K: **ChemInform Abstract: Alkyl polyglycosides — properties and applications of a new class of surfactants.** *Angew Chem Int Ed* 1998, **37:**1328–1345.
9. Tesmann H, Kahre J, Hensen H, Salka BA: **Alkyl polyglycosides in personal care products. Alkyl Polyglycosides.** *Wiley-VCH Verlag GmbH* 2008, **ch5:**71–98.
10. Corma A, Iborra S, Miquel S, Primo J: **Preparation of long-chain alkyl-glucoside surfactants by one-step direct Fischer glucosidation, and by transacetylation of butyl glucosides, on beta zeolite catalysts.** *J Catal* 1998, **180:**218–224.
11. Igarashi K: **The Koenigs-Knorr reaction.** *Adv Carbohydr Chem Biochem* 1977, **34:**243–283.
12. Bojarová P, Křen V: **Glycosidases: a key to tailored carbohydrates.** *Trends Biotechnol* 2009, **27:**199–209.
13. Van Rantwijk F, Woudenberg-van Oosterom M, Sheldon RA: **Glycosidase-catalysed synthesis of alkyl glycosides.** *J Mol Catal B: Enzym* 1999, **6:**511–632.
14. Crout DHG, Vic G: **Glycosidases and glycosyl transferases in glycoside and oligosaccharide synthesis.** *Curr Opin Chem Biol* 1998, **2:**98–111.
15. Vulfson EN, Patel R, Law BA: **Alkyl-β-glucoside synthesis in a water-organic two-phase system.** *Biotechnol Lett* 1990, **12:**397–402.
16. Hansson T, Adlercreutz P: **Enhanced transglucosylation/hydrolysis ratio of mutants of *Pyrococcus furiosus* β-glucosidase: Effects of donor concentration, water content, and temperature on activity and selectivity in hexanol.** *Biotechnol Bioeng* 2001, **75:**656–665.
17. Basso A, Ducret A, Gardossi L, Lortie R: **Synthesis of octyl glucopyranoside by almond β-glucosidase adsorbed onto Celite R-640®.** *Tetrahedron Lett* 2002, **43:**2005–2008.
18. Gargouri M, Smaali I, Maugard T, Legoy MD, Marzouki N: **Fungus β-glycosidases: immobilization and use in alkyl-β-glycoside synthesis.** *J Mol Catal B: Enzym* 2004, **29:**89–94.
19. Kouptsova OS, Klyachko NL, Levashov AV: **Synthesis of alkyl glycosides catalyzed by β-glycosidases in a system of reverse micelles.** *Russ J Bioorg Chem* 2001, **27:**380–384.
20. Rather MY, Mishra S, Chand S: **β-Glucosidase catalyzed synthesis of octyl-β-D-glucopyranoside using whole cells of *Pichia etchellsii* in micro aqueous media.** *J Biotechnol* 2010, **150:**490–496.
21. Ismail A, Soultani S, Ghoul M: **Enzymatic-catalyzed synthesis of alkyl-glycosides in monophasic and biphasic systems I. The transglycosylation reaction.** *J Biotechnol* 1999, **69:**135–143.
22. Lang M, Kamrat T, Nidetzky B: **Influence of ionic liquid cosolvent on transgalactosylation reactions catalyzed by thermostable β-glycosylhydrolase CelB from *Pyrococcus furiosus*.** *Biotechnol Bioeng* 2006, **95:**1093–1100.
23. De Carvalho CCCR: **Enzymatic and whole cell catalysis: finding new strategies for old processes.** *Biotechnol Adv* 2011, **29:**75–83.
24. Rather MY, Mishra S, Verma V, Chand S: **Biotransformation of methyl-β-D-glucopyranoside to higher chain alkyl glucosides by cell bound β-glucosidase of *Pichia etchellsii*.** *Bioresour Technol* 2012, **107:**287–294.
25. Rather MY, Mishra S, Aravinda S: **Exploring the synthetic potential of cell bound β-glycosidase of *Pichia etchellsii*.** *J Biotechnol* 2013, **165:**63–68.
26. Das-Bradoo S, Svensson I, Santos J, Plieva F, Mattiasson B, Hatti-Kaul R: **Synthesis of alkylgalactosides using whole cells of *Bacillus pseudofirmus* species as catalysts.** *J Biotechnol* 2004, **110:**273–286.
27. Ito J, Ebe T, Shibasaki S, Fukuda H, Kondo A: **Production of alkyl glucoside from cellooligosaccharides using yeast strains displaying *Aspergillus aculeatus* β-glucosidase 1.** *J Mol Catal B: Enzym* 2007, **49:**92–97.
28. Bae J, Choi EH, Pan JG: **Efficient synthesis of octyl-β-D-galactopyranoside by *Bacillus* spore-displayed β-galactosidase using an amphiphilic 1,2-dimethoxyethane co-solvent.** *Enzyme Microb Technol* 2011, **48:**232–238.
29. Kwon SJ, Jung HC, Pan JG: **Transgalactosylation in a water-solvent biphasic reaction system with β-galactosidase displayed on the surfaces of *Bacillus subtilis* spores.** *Appl Environ Microbiol* 2007, **73:**2251–2256.
30. Ly HD, Withers SG: **Mutagenesis of glycosidases.** *Annu Rev Biochem* 1999, **68:**487–522.
31. Mackenzie LF, Wang Q, Warren RAJ, Withers SG: **Glycosynthases: mutant glycosidases for oligosaccharide synthesis.** *J Am Chem Soc* 1998, **120:**5583–5584.
32. Villandier N, Corma A: **Transformation of cellulose into biodegradable alkyl glycosides by following two different chemical routes.** *ChemSusChem* 2011, **4:**508–513.
33. Roy B, Mukhopadhyay B: **Sulfuric acid immobilized on silica: an excellent catalyst for Fischer type glycosylation.** *Tetrahedron Lett* 2007, **48:**3783–3787.
34. Bornaghi LF, Poulsen SA: **Microwave-accelerated Fischer glycosylation.** *Tetrahedron Lett* 2005, **46:**3485–3488.
35. Wimmer Z, Pechova L, Saman D: **Koenigs-Knorr synthesis of cycloalkyl glycosides.** *Molecules* 2004, **9:**902–912.
36. Richel A, Laurent P, Wathelet B, Wathelet JP, Paquot M: **Microwave-assisted conversion of carbohydrates. State of the art and outlook.** *Comptes Rendus Chimie* 2011, **14:**224–234.
37. Panintrarux C, Adachi S, Araki Y, Kimura Y, Matsuno R: **Equilibrium yield of n-alkyl-β-D-glucoside through condensation of glucose and - alcohol by β-glucosidase in a biphasic system.** *Enzyme Microb Technol* 2005, **17:**32–40.
38. Chahid Z, Montet D, Pina M, Bonnot F, Graille J: **Biocatalyzed octylglycoside synthesis from a disaccharide.** *Biotechnol Lett* 1994, **16:**795–800.
39. Rye CS, Withers SG: **Glycosidase mechanisms.** *Curr Opin Chem Biol* 2000, **4:**573–580.
40. Withers SG: **Mechanisms of glycosyltransferases and hydrolases.** *Carbohydr Polym* 2001, **44:**325–337.
41. Davies G, Henrissat B: **Structures and mechanisms of glycosyl hydrolases.** *Structure* 1995, **3:**853–859.
42. McCarter JD, Stephen Withers G: **Mechanisms of enzymatic glycoside hydrolysis.** *Curr Opin Struct Biol* 1994, **4:**885–892.
43. Desmet T, Soetaert W, Bojarová P, Křen V, Dijkhuizen L, Eastwick-Field V, Schiller A: **Enzymatic glycosylation of small molecules: challenging substrates require tailored catalysts.** *Chem Euro J* 2012, **18:**10786–10801.
44. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B: **The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics.** *Nucleic Acids Res* 2009, **37:**D233–D238.
45. Ketudat CJ, Esen A: **β-Glucosidases.** *Cell Mol Life Sci* 2010, **67:**3389–3405.
46. Weignerova L, Bojarova P, Kren V: **Glycosidases in synthesis.** *Carbohyd Chem Chem Biol Appro* 2009, **35:**310–332.
47. De Roode BM, Franssen MCR, Padt A, Boom RM: **Perspectives for the industrial enzymatic production of glycosides.** *Biotechnol Prog* 2003, **19:**1391–1402.
48. Vic G, Hastings JJ, Crout DHG: **Glycosidase-catalysed synthesis of glycosides by an improved procedure for reverse hydrolysis: application to the chemoenzymatic synthesis of galactopyranosyl-(1–4)-O-α-galactopyranoside derivatives.** *Tetrahed Asymm* 1996, **7:**1973–1984.
49. Stevenson DE, Stanley RA, Furneaux RH: **Optimization of alkyl β-D -galactopyranoside synthesis from lactose using commercially available β-galactosidases.** *Biotechnol Bioeng* 1993, **42:**657–666.
50. Concie J, Levy GA, Whistler RL, Wolfrom ML, BeMiller JN: *Methods in Carbohydrate Chemistry,Vol. II.* New York: Academic Press; 1963:335.
51. Gibson RR, Dickinson RP, Boons GJ: **Vinyl glycosides in oligosaccharide synthesis. 4. Glycosidase-catalyzed preparation of substituted allyl-glycosides.** *J Chem Soc Perkin Trans* 1997, **1:**3357–3360.
52. Boons GJ, Isles S: **Vinyl glycosides in oligosaccharide synthesis. 2. The use of allyl and vinyl glycosides in oligosaccharide synthesis.** *J Org Chem* 1996, **61:**4262–4271.
53. Millqvist-Fureby A, Gill IS, Vulfson EN: **Enzymatic transformations in supersaturated substrate solutions: I. A general study with glycosidases.** *Biotechnol Bioeng* 1998, **60:**190–196.
54. Wang Q, Yu H, Zhao N, Li C, Shang Y, Liu H, Xu J: **Significantly improved equilibrium yield of long-chain alkyl glucosides via reverse hydrolysis in a water-poor system using cross-linked almond meal as a cheap and robust biocatalyst.** *Chinese J Catal* 2012, **33:**275–280.
55. Vic G, Thomas D, Crout DHG: **Solvent effect on enzyme-catalyzed synthesis of β-D-glucosides using the reverse hydrolysis method:**

Application to the preparative-scale synthesis of 2-hydroxybenzyl and octyl β-D-glucopyranosides. *Enzyme Microb Technol* 1997, **20**:597–603.
56. Yan TR, Liau JC: **Synthesis of alkyl β-glucosides from cellobiose with *Aspergillus niger* β-glucosidase II.** *Biotechnol Lett* 1998, **20**:653–657.
57. Makropoulou M, Christakopoulos P, Tsitsimpikou C, Kekos D, Kolisis FN, Macris BJ: **Factors affecting the specificity of β-glucosidase from *Fusarium oxysporum* in enzymatic synthesis of alkyl-β-D-glucosides.** *Int J Biol Macromol* 1998, **22**:97–101.
58. Yan TR, Lin YH, Lin CL: **Purification and characterization of an extracellular β-glucosidase II with high hydrolysis and transglucosylation activities from *Aspergillus niger*.** *J Agr Food Chem* 1998, **46**:431–437.
59. Benešová E, Lipovová P, Dvořáková H, Králová B: **β-D-Galactosidase from *Paenibacillus thiaminolyticus* catalyzing transfucosylation reactions.** *Glycobiol* 2010, **20**:442–451.
60. Gueguen Y, Chemardin P, Pommares P, Arnaud A, Galzy P: **Enzymatic synthesis of dodecyl β-D-glucopyranoside catalyzed by *Candida molischiana 35M5N* β-glucosidase.** *Bioresour Technol* 1995, **53**:263–267.
61. García-Garibay M, López-Munguía A, Barzana E: **Alcoholysis and reverse hydrolysis reactions in organic one-phase system with a hyperthermophilic β-glycosidase.** *Biotechnol Bioeng* 2000, **69**:627–632.
62. Lirdprapamongkol K, Svasti J: **Alkyl glucoside synthesis using Thai rosewood β-glucosidase.** *Biotechnol Lett* 2000, **22**:1889–1894.
63. Ducret A, Trani M, Lortie R: **Screening of various glycosidases for the synthesis of octyl glucoside.** *Biotechnol Bioeng* 2002, **77**:752–757.
64. Papanikolaou S: **Enzyme-catalyzed synthesis of alkyl-β-glucosides in a water-alcohol two-phase system.** *Bioresour Technol* 2001, **77**:157–161.
65. Yi Q, Sarney DB, Khan JA, Vulfson EN: **A novel approach to biotransformations in aqueous-organic two-phase systems: Enzymatic synthesis of alkyl β-D-glucosides using microencapsulated β-glucosidase.** *Biotechnol Bioeng* 1998, **60**:385–390.
66. Turner P, Svensson D, Adlercreutz P, Karlsson EN: **A novel variant of *Thermotoga neapolitana* β-glucosidase B is an efficient catalyst for the synthesis of alkyl glucosides by transglycosylation.** *J Biotechnol* 2007, **130**:67–74.
67. Smaali I, Maugard T, Limam F, Legoy M-D, Marzouki N: **Efficient synthesis of gluco-oligosaccharides and alkyl-glucosides by transglycosylation activity of β-glucosidase from *Sclerotinia sclerotiorum*.** *World J Microb Biotechnol* 2007, **23**:145–149.
68. Makowski K, Białkowska A, Olczak J, Kur J, Turkiewicz M: **Antarctic, cold-adapted β-galactosidase of *Pseudoalteromonas sp. 22b* as an effective tool for alkyl galactopyranosides synthesis.** *Enzyme Microb Technol* 2009, **44**:59–64.
69. Bilanikova D, Mastihuba V, Mastihubova M, Balesova J, Schmidt S: **Improvements in enzymatic preparations of alkyl glycosides.** *Czech J Food Sci* 2010, **28**:69–73.
70. Ochs M, Muzard M, Plantier-Royon R, Estrine B, Remond C: **Enzymatic synthesis of alkyl β-D-xylosides and oligoxylosides from xylans and from hydrothermally pretreated wheat bran.** *Green Chem* 2011, **13**:2380–2388.
71. Ljunger G, Adlercreutz P, Mattiasson B: **Enzymatic synthesis of octyl-β-D-glucoside in octanol at controlled water activity.** *Enzyme Microb Technol* 1994, **16**:751–755.
72. Andersson M, Adlercreutz P: **A kinetic study of almond β-glucosidase catalysed synthesis of hexyl-glycosides in low aqueous media. Influence of glycosyl donor and water activity.** *J Mol Catal B: Enzym* 2000, **607**:1–8.
73. Bojarová P, Petrásková L, Ferrandi EE, Monti D, Pelantová H, Kuzma M: **Glycosyl azides – an alternative way to disaccharides.** *Adv Synth Catal* 2007, **2007**(349):1514–1520.
74. Svasti J, Phongsak T, Sarnthima R: **Transglucosylation of tertiary alcohols using cassava β-glucosidase.** *Biochem Biophys Res Commun* 2003, **305**:470–475.
75. Zaks A, Klibanov AM: **Enzymatic catalysis in nonaqueous solvents.** *J Biol Chem* 1998, **263**:3194–3201.
76. Wallecha A, Mishra S: **Purification and characterization of two β-glucosidases from a thermo-tolerant yeast *Pichia etchellsii*.** *Biochim Biophys Acta-Protein Struct Mech* 2003, **1649**:74–84.
77. Zou ZZ, Yu HL, Li CX, Zhou XW, Hayashi C, Sun J: **A new thermostable β-glucosidase mined from *Dictyoglomus thermophilum:* Properties and performance in octyl glucoside synthesis at high temperatures.** *Bioresour Technol* 2012, **118**:425–430.
78. Basso A, De Martin L, Ebert C, Gardossi L, Linda P: **High isolated yields in thermodynamically controlled peptide synthesis in toluene catalysed by thermolysin adsorbed on Celite R-640.** *Chem Commun* 2000, **6**:467–468.
79. Park DW, Kim HS, Jung JK, Haam S, Kim WS: **Enzymatic synthesis of alkyl-glucosides by amphiphilic phase enzyme reaction.** *Biotechnol Lett* 2000, **22**:951–956.
80. Klyachko NL, Levashov AV: **Bioorganic synthesis in reverse micelles and related systems.** *Curr Opin Colloid In* 2003, **8**:179–186.
81. Earle MJ, Esperanca JMSS, Gilea MA, Canongia Lopes JN, Rebelo LPN, Magee JW: **The distillation and volatility of ionic liquids.** *Nature* 2006, **439**:831–834.
82. Van Rantwijk F, Sheldon RA: **Biocatalysis in ionic liquids.** *Chem Rev* 2007, **107**:2757–2785.
83. Kragl U, Eckstein M, Kaftzik N: **Enzyme catalysis in ionic liquids.** *Curr Opin Biotechnol* 2002, **13**:565–571.
84. Earle M, Wasserscheid P, Schulz P, Olivier-Bourbigou H, Favre F, Vaultier M, Kirschning A, Singh V, Riisager A, Fehrmann R, Kuhlmann S: **Organic synthesis**. Edited by Wasserscheid P, Wetton T. Weinheim: Wiley-VCH; 2008:265–568.
85. Gorke J, Srienc F, Kazlauskas R: **Toward advanced ionic liquids. Polar, enzyme-friendly solvents for biocatalysis.** *Biotechnol Bioprocess Eng* 2010, **15**:40–53.
86. Kaftzik N, Wasserscheid P, Kragl U: **Use of ionic liquids to increase the yield and enzyme stability in the β-galactosidase catalysed synthesis of N-acetyllactosamine.** *Org Process Res Dev* 2002, **6**:553–557.
87. Yang RL, Li N, Zong MH: **Using ionic liquid co-solvents to improve enzymatic synthesis of arylalkyl β-D-glucopyranosides.** *J Mol Catal B: Enzym* 2012, **74**:24–28.
88. Villandier N, Corma A: **One pot catalytic conversion of cellulose into biodegradable surfactants.** *Chem Comm* 2010, **46**:4408–4410.
89. Bornscheuer UT, Huisman GW, Kazlauskas RJ, Lutz S, Moore JC, Robins K: **Engineering the third wave of biocatalysis.** *Nature* 2012, **485**:185–194.
90. Hancock SM, Corbett K, Fordham-Skelton AP, Gatehouse JA, Davis BG: **Developing promiscuous glycosidases for glycoside synthesis: residues W433 and E432 in *Sulfolobus solfataricus* β-glycosidase are important glucoside- and galactoside-specificity determinants.** *ChemBioChem* 2005, **6**:866–875.

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