Journal of Molecular Catalysis B: Enzymatic 69 (2011) 147–153

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Immobilisation of CGTase for continuous production of long-carbohydrate-chain alkyl glycosides
Control of product distribution by flow rate adjustment

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ARTICLE INFO

Article history:
Received 15 March 2010
Received in revised form 21 January 2011
Accepted 24 January 2011
Available online 28 January 2011

Keywords:
CGTase
Cyclodextrin glycosyltransferase
Immobilization
Packed-bed reactor
Alkyl glycosides

ABSTRACT

Bacillus macerans cyclodextrin glycosyltransferase (CGTase) (EC 2.4.1.19) was covalently immobilised on Eupergit C and used in a packed-bed reactor to investigate the continuous production of long-carbohydrate-chain alkyl glycosides from α-cyclodextrin (α-CD) and *n*-dodecyl-(1,4)-β-maltopyranoside ($C_{12}G_2\beta$). The effects of buffer ion strength and pH, and enzyme loading on the immobilisation yield and the enzyme activity were evaluated. Approximately 98% of the protein and 33% of the total activity were immobilised. At pH 5.15, the enzymatic half-life was 132 min at 60 °C and 18 min at 70 °C. The immobilised enzyme maintained 60% of its initial activity after 28 days storage at 4 °C. The degree of conversion was controlled by simple regulation of the flow rate through the reactor, making it possible to optimise the product distribution. It was possible to achieve a yield of the primary coupling product *n*-dodecyl-(1,4)-β-maltooctaoside ($C_{12}G_8\beta$) of about 50%, with a ratio between the primary and the secondary coupling product of about 10. *Thermoanaerobacter* sp. CGTase (Toruzyme 3.0 L) immobilised on Eupergit C had good operational stability at 60 and 70 °C thus showing the advantages of using more thermostable enzymes in biocatalysis. However, this enzyme was unsuitable for the production of $C_{12}G_8\beta$ due to extensive disproportionation reactions, giving a broad product range.

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1. Introduction

Biocatalysis has a promising future in the production of a wide variety of both fine chemicals and bulk chemicals. Biocatalysis is included in many commercial process involving carbohydrates owing to the need for selectivity. This is due to the polyfunctionality of the carbohydrates, several hydroxyl groups with equal or very similar reactivity, which is hard to manage with classical chemistry. The annual production of such products ranges from 170 million tons in beer and wine production, to 5000 tons of cyclodextrins, which are used in the food, pharmaceutical and cosmetic industries [1].

Biocatalyst stability is of major concern in virtually all processes because of its impact on process economics. Reduced biocatalyst stability results in longer reaction times, lower product yields and increased frequency of catalyst replacement. Biocatalyst inactivation is strongly associated with the process conditions, including extremes of temperature, pH and ionic strength and/or the solvents, reactants and products present [2]. Three main approaches have been used to enhance enzyme stability: screening for enzymes of extremophilic origin, protein engineering by site-directed mutagenesis or directed evolution, and stabilisation of existing enzymes (through chemical modification, immobilisation or the addition of stabilising agents) or a combination of two or more. Although the use of protein engineering is becoming more widespread to tailor-make catalysts, the stabilisation of existing enzymes through multipoint immobilisation is the most commonly used method in industry today, due to its simplicity, reliability and cost-effectiveness [2]. There are four principal methods of immobilisation: (i) adsorption onto a solid support, (ii) entrapment, (iii) membrane confinement and (iv) covalent immobilisation on a solid support. The immobilisation method used depends on the area of application. In aqueous systems and in systems where thermal inactivation is a problem, covalent immobilisation on a solid support is preferable. Not only does multipoint covalent immobilisation have a stabilising effect on enzymes but, more importantly, it also allows reuse of the enzyme, and provides better control of the operation, easier product recovery and greater flexibility of the reactor design [2].

Cyclodextrin glycosyltransferases (CGTases) (EC 2.4.1.19) represent one of the most important groups of microbial amylolytic enzymes catalysing four different reactions: cyclisation, coupling, disproportionation reactions and, to a limited extent, even hydrolysis. CGTases are used in the commercial production of cyclodextrins

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doi:10.1016/j.molcatb.2011.01.009

(CDs), which are used as an efficient complexing agent. However, there is increasing interest in developing efficient synthesis routes to modify natural glycosides, flavonoids, vitamins and surfactants [3–15]. Different approaches have been applied to scale up the production of such products, for example, immobilisation of CGTase based on adsorption and entrapment [16] or covalent binding [11,16,17].

The aim of the current investigation was to develop a method for continuous CGTase-catalysed synthesis of long-carbohydrate-chain alkyl glycosides using CGTase, and a packed-bed reactor to enable fast and simple reaction control by the adjustment of the flow rate and substrate concentrations.

2. Experimental

2.1. Materials

Bacillus macerans CGTase (EC 2.4.1.19) was purchased from Amano Enzyme Europe Ltd. (Milton Keynes, UK) and *Thermoanaerobacter* sp. CGTase (Toruzyme® 3.0 L) was kindly provided by Novozymes A/S (Bagsvaerd, Denmark). Two different solid supports, Eupergit C® (epoxy-activated acrylic beads with an average diameter of 150 μm) and silica were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Grace GmbH (Worms, Germany), respectively. 3-Aminopropyltriethoxysilane (APTS) and glutaraldehyde (25% (v/v)) were purchased from Janssen Chimica (Geel, Belgium), *n*-dodecyl-(1,4)-β-maltopyranoside (ANAGRADE®) ($C_{12}G_2\beta$) was purchased from Anatrace Inc. (Maumee, OH, USA) and alpha-cyclodextrin hydrate (α-CD) was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). All other chemicals were of p.a. grade and were obtained from VWR International (Stockholm, Sweden). MilliQ distilled water (dH_2O) was used throughout the study.

2.2. Methods

2.2.1. Immobilisation and evaluation of the enzyme preparation

An enzyme solution (0.14–5.6 ml, 1.99 mg protein/ml) in buffer (phosphate, sodium citrate or MOPS (3-(N-morpholino) propane sulphonic acid), 0.1–1.0 M, pH 6.2–8.0, 9.86–44.4 ml) was added to 0.1–1 g support material (Eupergit C or silica). Silica (Grace XWP silica gel number 1) was activated as described by Prousoontorn and Pantatan [11], using the silylation agent APTS (2% (v/v)) in dH_2O and further reacted using 0.5–1.0% (v/v) glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. The support (Eupergit C or silica) and enzyme solution were gently incubated for 17–63 h using either a tilt table or a shaking table. The preparations were collected by vacuum filtration using a porous glass filter. The preparations were thoroughly rinsed with 2× 10 ml immobilisation buffer, and finally 3× 10 ml 10 mM sodium citrate, pH 5.15, containing 2 mM $CaCl_2$. The preparations based on silica were washed an extra time with 1 M NaCl in 10 mM sodium citrate before they were washed as described above.

The sustainability of the preparations was evaluated by determining the degree of immobilisation and the immobilised activity. The degree of immobilisation was established by determining the protein content of the solutions before and after immobilisation using Bradford analysis [18] and the immobilised activity was determined using the CGTase activity assay. The results from the activity assay are for the *B. macerans* CGTase presented as the initial formation rate of primary coupling product ($C_{12}G_8\beta$) per minute and milligram of protein (μmol $C_{12}G_8\beta$/(min mg P)) and for *Thermoanaerobacter* sp. CGTase as the initial consumption rate of acceptor ($C_{12}G_2\beta$) per minute and milligram of protein (μmol $C_{12}G_2\beta$/(min mg P)). Preparations based on Eupergit C were proved to be superior and therefore used in the following experiments.

2.2.2. CGTase activity assay

The CGTase activity (free enzyme/immobilised preparation) was assayed by following the synthesis of $C_{12}G_8\beta$ and the consumption of $C_{12}G_2\beta$ at 60 °C using HPLC. Free enzyme (0.0219 mg/ml) or immobilised preparation (10–40 mg) was added to 1.0 ml assay solution (50 mM $C_{12}G_2\beta$, 400 mM α-CD, 2 mM $CaCl_2$, and 10 mM sodium citrate, pH 5.15) in 4 ml septum-capped vials using a HTMR-131 thermo-mixer (HLC, Bovenden, Germany) at 600 rpm. Six 30-μl samples were withdrawn from each assay at regular intervals during 10–30 min, and diluted in 970 μl 75% (v/v) methanol in water before heat inactivation for 1 min in boiling water. When immobilised enzyme was used, the samples were also filtered using 0.2 μm PTFE syringe filters (VWR collection, VWR International, Stockholm, Sweden) to remove any residual preparation before injected on the HPLC.

2.2.3. Process set-up for the glycosylation of $C_{12}G_2\beta$

A packed-bed reactor was used for the continuous glycosylation of $C_{12}G_2\beta$ (Fig. 1). It consisted of a glass cylinder with an inner diameter of 10 mm, fitted with adjustable cylindrical plugs at both ends, making it possible to vary the amount of enzyme preparation inside the reactor. The end plugs were equipped with a steel-mesh-covered cavity to retain the enzyme preparation and to ensure the even distribution of reaction mixture across the bed. Before the glass column was packed with suspended *B. macerans* CGTase preparation (~720 mg, 11 mg protein/g support with a specific activity of 62 μmol $C_{12}G_8\beta$/(min mg P) at 60 °C, in reaction buffer), small discs of Whatman filter paper (grade 1) were placed over the steel mesh to prevent the preparation from passing through the mesh. The packed column was firmly mounted in a clamp prior to connection to the system, and submerged in a temperature-controlled water bath. The reaction blend (50 mM $C_{12}G_2\beta$ and 200–400 mM α-CD dissolved in reaction buffer (10 mM sodium citrate buffer, pH 5.15, and 2 mM $CaCl_2$)) was pumped into

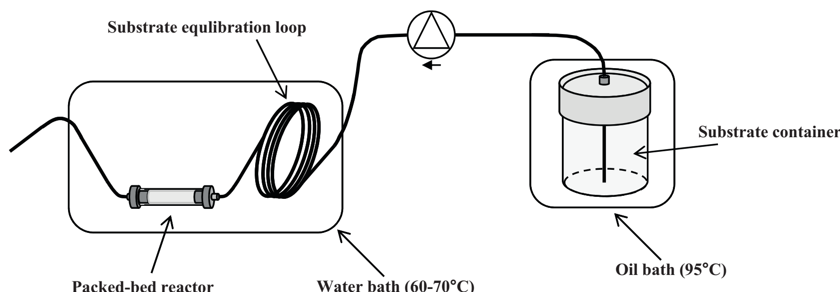

Fig. 1. Schematic picture showing the packed-bed reactor system for the continuous production of long-carbohydrate-chain alkyl glycoside using immobilised CGTase as catalyst.

the column from a reservoir using a peristaltic pump. Because the reaction media precipitates at temperatures ≤55 °C, the reservoir was maintained at 95 °C using a temperature-controlled oil bath (Heidolph MR 3001K/EKT 3001, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). To equilibrate the temperature of the reactants to 60 or 70 °C before entering the packed-bed reactor, a 1-m loop of silicone tubing was immersed in the water bath together with the reactor. All connecting tubing was insulated.

The process set-up was evaluated by varying the flow rate (0.325–1.4 ml/min) for both parameters investigated: α-CD concentration and temperature. Samples were collected, weighed and diluted in 970 μl 75% (v/v) methanol in dH_2O prior to analysis by HPLC.

2.2.4. Process and storage stability

To investigate the stability of the immobilised enzyme during the glycosylation process, reactions were performed in triplicate at 60 and 70 °C in 4 ml septum-capped vials using the same thermomixer and speed as described above. The enzyme preparation (30 mg, 11 mg protein/g support and 20 mg, 10.6 mg protein/g support of *B. macerans* CGTase and *Thermoanaerobacter* sp. CGTase respectively) was prewashed with reaction buffer to remove the smallest particle fraction (too small to sediment within a reasonable time), before 1 ml of reaction mixture was added (50 mM $C_{12}G_2\beta$, 400 mM α-CD, 2 mM $CaCl_2$ and 10 mM sodium citrate, pH 5.15). The reaction cycle time was varied depending on the reaction temperature and the degree of deactivation of the enzyme. After each cycle the reaction mixture was diluted with 3 ml reaction buffer before it was withdrawn, whereupon another 2 ml reaction buffer was added to wash the preparation. After the washing step, a new cycle was initiated by adding 1 ml fresh reaction medium. The remaining enzyme activity of *B. macerans* CGTase was measured as the initial coupling reaction rate (μmol $C_{12}G_8\beta$/(min mg P)) by withdrawing six samples during 30 min, which were immediately diluted in 75% (v/v) methanol in water, followed by heat treatment in boiling water for 1 min to inactivate the enzyme and solubilise the reaction products and finally filtered using 0.2 μm PTFE syringe filters (VWR collection, VWR International, Stockholm, Sweden) to remove any residual enzyme preparation before analysis on HPLC. In contrast to *B. macerans* CGTase, the residual enzyme activity of *Thermoanaerobacter* sp. CGTase was measured as the initial depletion rate of $C_{12}G_2\beta$ (μmol $C_{12}G_2\beta$/(min mg P)) by taking five samples during 10 min. The samples were treated and analysed as for the *B. macerans* CGTase samples although with a short heat treatment to solubilise the sample immediately followed by filtration.

To evaluate the storage stability of the *B. macerans* CGTase, the preparation was stored at 4 °C for 17 and 28 days and then tested using the activity assay described above.

2.2.5. High performance liquid chromatography analysis

Samples collected during the experiments were analysed using a Merck-Hitachi LaChrom L7000-series HPLC-system (Hitachi Ltd., Tokyo, Japan), equipped with a C-8 RP column (Kromasil 100-5C8, L: 25 cm, i.d.: 4.6 mm, Eka Chemicals AB, Separation Products, Bohus, Sweden). This was connected to an evaporative light scattering detector (Alltech 500 ELSD, Alltech Associates, Inc., Deerfield, IL, USA) operating at 82 °C, with a nebuliser gas flow of 2.03 standard litres per minute (SLPM). The column was eluted using a linear gradient (1 ml/min) of methanol (A) and 0.1% (v/v) acetic acid in water (B). The proportion of A was changed from 75 to 95% during a period of 10 min, followed by a hold period of 1 min at 95% A. Thereafter, the amount of A was reduced to 75% A during 1 min, and then kept constant for another 5 min, before the next sample was injected. Standard curves were constructed for $C_{12}G_2\beta$ and $C_{12}G_8\beta$. $C_{12}G_8\beta$ was produced and purified as described by Svensson et al. [19].

Table 1
Influence of buffer (phosphate, sodium citrate or MOPS), ion strength and pH on the immobilisation of CGTase from *B. macerans* on Eupergit C. The immobilisation yield is the proportion of added protein immobilised in percent, and the initial rate is the amount of coupling (C12G8β) product synthesised per minute per unit protein.

Buffer	Immobilisation yield (%)	Initial rate (μmol $C_{12}G_8\beta$/(min mg P))
MOPS 1.0 M, pH 7	14	56.2
Na-citrate 1.0 M, pH 6.2	~98	80.3
Na-citrate 0.1 M, pH 6.2	78	13.8
Phosphate 1.0 M, pH 7	~98	72.7
Phosphate 0.1 M, pH 7	39	47.2
Phosphate 1.0 M, pH 6	~98	71.1
Free enzyme		245.4

3. Results and discussion

3.1. Effects of protein loading and immobilisation buffer on the enzyme immobilisation

The initial immobilisation experiments using *B. macerans* CGTase showed that the degree of immobilisation was three times greater on Eupergit C than on silica. Therefore, Eupergit C was used in all further experiments.

The influence of the pH and ion strength of the buffer system on the immobilisation on Eupergit C is presented in Table 1, showing that the degree of immobilisation and the remaining activity of the immobilised enzyme are strongly dependent on the ion strength of the buffer system used. This has also been reported by Martin et al. [17], and can probably be explained by that the immobilisation is likely to occur through a two-step mechanism. Initially the enzyme is physically adsorbed onto the carrier by hydrophobic interactions, which are stronger at higher salt concentrations. This brings amino and thiol groups on the enzyme surface into close proximity to the epoxy groups of the carrier. The second step involves formation of very stable C–N and C–S bonds by nucleophilic attack of the reactive group of the enzyme on the epoxy group [20].

Varying the pH had little effect on the degree of immobilisation. The use of MOPS buffer (1.0 M, pH 7.0) decreased the degree of immobilisation, but improved the enzyme stability. The highest degree of immobilisation (≥98%) and remaining activity (33% compared to free enzyme) were achieved when using a sodium citrate buffer at 1.0 M and pH 6.2. Therefore, sodium citrate buffer was chosen to further characterise the preparation of the *B. macerans* CGTase on Eupergit C by varying the enzyme loading. Surprisingly, even at a relatively low enzyme loading (>3 mg/g support) substrate diffusion problems arose (Fig. 2). The protein immobilisation yield was still close to 98%, even at loads of 11 mg protein/g support. An activity yield of 33% was obtained at 3 mg protein/g support. However, the choice of buffer seems to be dependent on the CGTase used. Hence it was found optimal to use 1.0 M phosphate buffer pH 8 when immobilising the *Thermoanaerobacter* sp. CGTase (results not shown). Prousoontorn and Pantatan reported an activity yield of approximately 30% when immobilising of CGTase from *Paenibacillus* sp. A11 on glutaraldehyde activated alumina [11]. Other approaches based on adsorption and entrapment have been evaluated [16]. However, these methods are not suitable for use in aqueous systems as the enzyme will leak from the preparation during the reaction. Regardless of the method used, there are considerable losses in activity. This may be attributed to conformational modification of the enzyme, a disadvantageous microenvironment of the support, steric hindrances within the support pores, and/or limitations on diffusion. There would therefore be much to gain if a new method could be found that retains the activity of the CGTase to a higher degree.

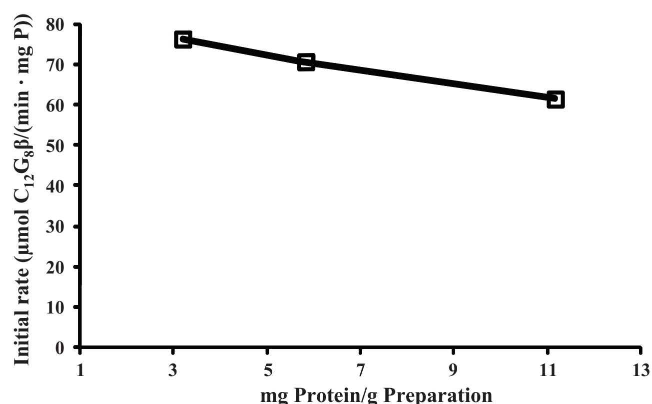

Fig. 2. Initial rate of coupling product formation versus protein loading of CGTase (*B. macerans*) on Eupergit C for the coupling reaction of α-CD and $C_{12}G_2\beta$ in Na citrate buffer, pH 5.15, at 60 °C.

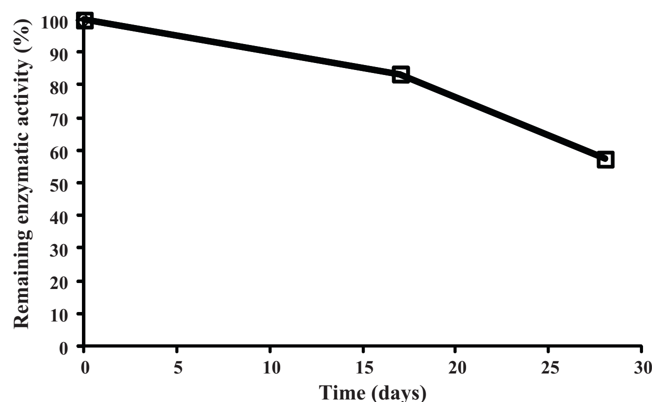

Fig. 4. Storage stability of CGTase from *B. macerans* immobilised on Eupergit C (11 mg protein/g carrier). 30 mg of preparation was used in the synthesis of $C_{12}G_8\beta$ through coupling of α-CD and $C_{12}G_2\beta$ in sodium citrate buffer, pH 5.15, at 60 °C.

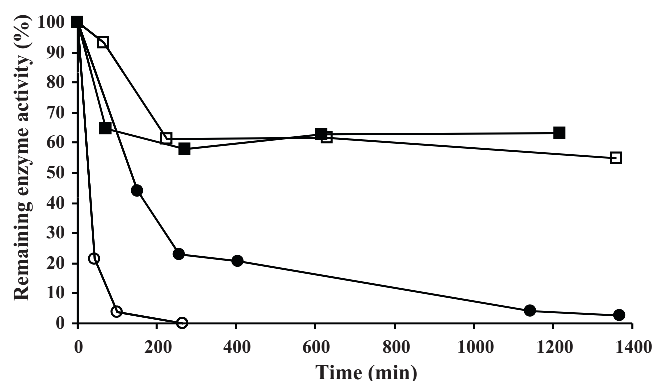

Fig. 3. Process stability of CGTase from *B. macerans* (circles) and *Thermoanaerobacter* sp. (squares) immobilised on Eupergit C (11 mg protein/g carrier) in the synthesis of $C_{12}G_8\beta$ through the coupling reaction of α-CD and $C_{12}G_2\beta$ in Na citrate buffer, pH 5.15, at 60 °C (filled) and 70 °C (unfilled).

3.2. Enzyme stability during processing and storage

Enzyme stability is crucial in large-scale biocatalysis. Initial studies of the operational stability of the immobilised CGTase were performed using small-scale batch reactions with product removal (rinsing of the enzyme preparation twice) between each batch. The initial synthesis rate of $C_{12}G_8\beta$ was followed for each cycle. The decrease in enzymatic activity was followed to complete inactivation to ensure that the true operational stability of the enzyme preparation was determined.

The operational stability of covalently immobilised CGTase of *B. macerans* was rather poor (Fig. 3). The half-life of the enzymatic preparation was 132 min at 60 °C and 18 min and at 70 °C. Lowering the temperature would improve the stability of this CGTase considerably, but this is not an option for this particular reaction, since the substrates would precipitate. On the contrary, using a more stable enzyme such as the thermostable *Thermoanaerobacter* sp. would be of great interest. The operational stability of this enzyme was in the present study superior to that of *B. macerans* CGTase (Fig. 3). The activity of *Thermoanaerobacter* sp. was more or less unaffected after almost 24 h at 70 °C. The initial loss of approx-

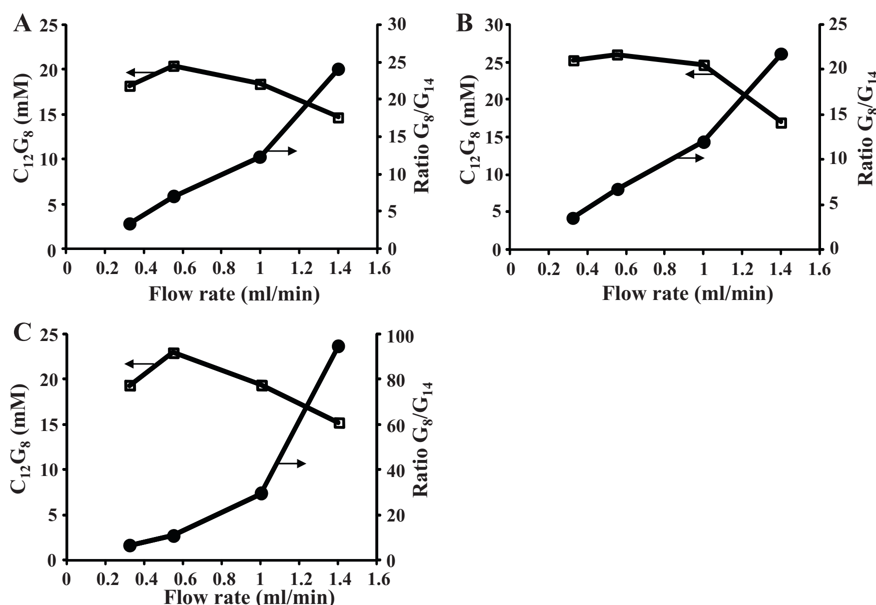

Fig. 5. Influence of flow rate, α-CD concentration and temperature on the continuous synthesis of $C_{12}G_8\beta$: (A) at 60 °C with 200 mM α-CD, (B) at 60 °C with 300 mM α-CD, and (C) at 70 °C with 200 mM α-CD. The $C_{12}G_2\beta$ was kept constant at 50 mM. The ratio G_8/G_{14} is the ratio of the first and second coupling products.

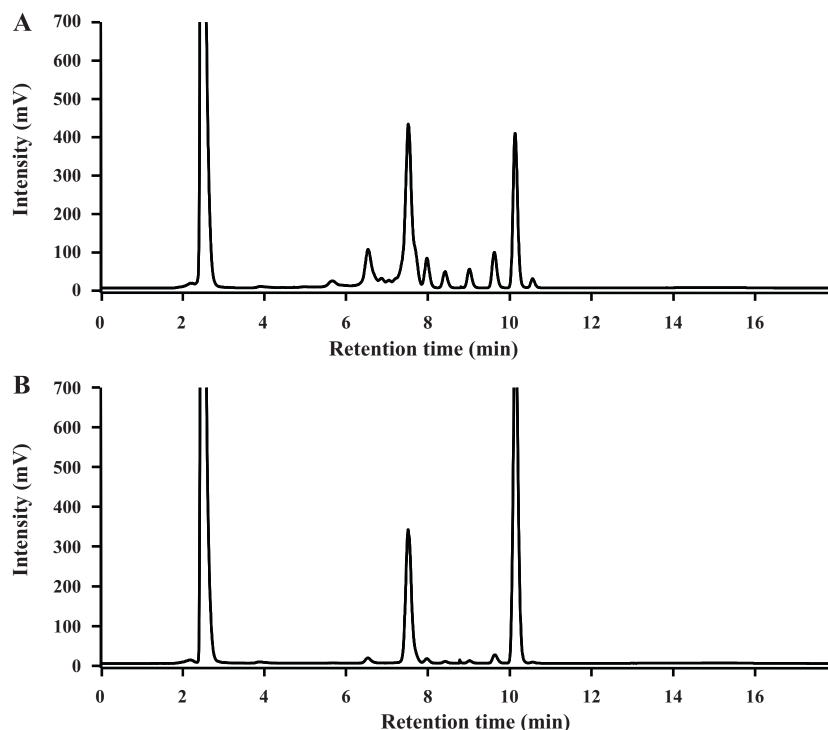

Fig. 6. Influence of the flow rate on the product composition at constant substrate concentration (200 mM α-CD and 50 mM $C_{12}G_2\beta$) and temperature (60 °C): (A) 0.325 ml/min and (B) 1.0 ml/min.

imately 40% of the activity was most probably due to leakage of the enzyme because of the presence of the surfactants. Initial investigations of the immobilisation of *Thermoanaerobacter* sp. CGTase indicated that the adsorption of the enzyme to the support was fast but the covalent binding between the enzyme and the matrix was very slow (data not shown). This leakage of enzyme in the case of *B. macerans* CGTase could probably be expected as well, but it was masked by the poor enzyme stability. A study that also showed good operational stability of *Thermoanaerobacter* sp. CGTase was performed by Martin et al. [21]. In this work the enzyme was subjected to batch synthesis cycles of 24 h of maltooligosaccharides at 60 °C and they reported that the remaining enzyme activity after

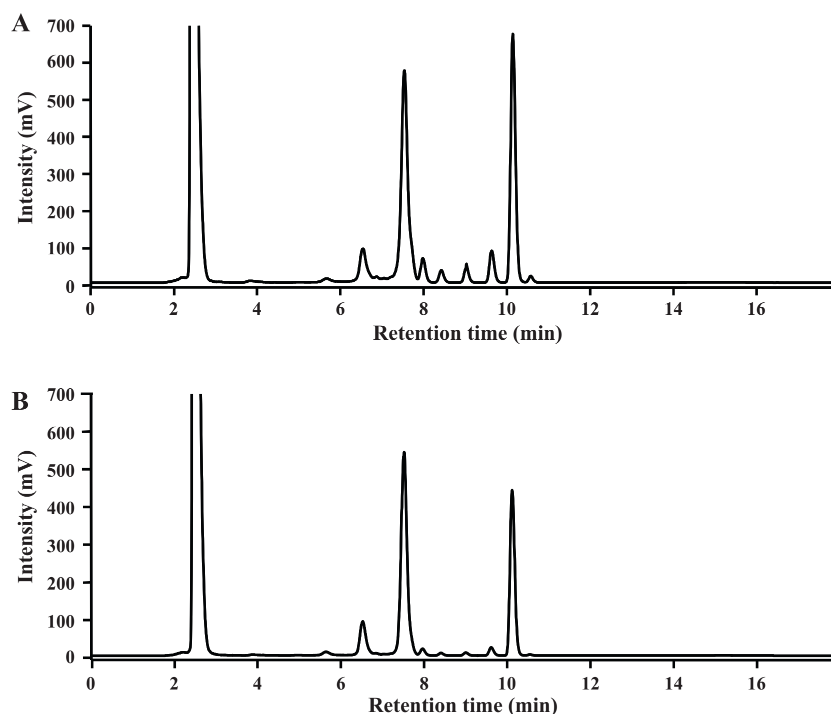

Fig. 7. Influence of α-CD concentration on the product composition at constant flow rate (0.55 ml/min), temperature (60 °C) and $C_{12}G_2\beta$ (50 mM): (A) 200 mM α-CD and (B) 400 mM α-CD.

10 cycles was 40% of the initial activity. Unfortunately, the *Thermoanaerobacter* sp. CGTase is not suitable for the synthesis of this particular long-carbohydrate-chain alkyl glycoside because disproportionation reactions dominate, giving a very broad product blend [19]. However, a higher degree of disproportionation may be beneficial in some applications.

The storage stability of immobilised enzymes is also an important factor determining their usefulness in industrial applications. Hence, the stability of the immobilised *B. macerans* CGTase was studied at 4 °C. It was found that after 17 days and 28 days the preparation had lost approximately 15% and 40% of its initial activity, respectively (Fig. 4).

3.3. Glycosylation of $C_{12}G_2\beta$ in a packed-bed reactor

The packed-bed reactor, containing on average 720 mg of immobilised CGTase from *B. macerans*, was tested at various flow rates, temperatures and concentrations of α-CD of the reaction mixture at a fixed concentration of 50 mM $C_{12}G_2\beta$. When evaluating the results, the amounts of coupling products (primary ($C_{12}G_8\beta$), secondary ($C_{12}G_{14}\beta$), etc.) and disproportionation products should be considered. The product composition can easily be controlled by adjusting the flow rate, which is a great advantage.

A low flow rate gave relatively low amounts of $C_{12}G_8\beta$ (Fig. 5), but favoured the synthesis of mainly double and triple coupling products, as well as some disproportionation products (Fig. 6A). Increasing the flow rate increased the synthesis of $C_{12}G_8\beta$ up to a certain point, at which the residence time became too short and the synthesis of $C_{12}G_8\beta$ declined (Figs. 5 and 6B). On the other hand, the ratio of $C_{12}G_8\beta/C_{12}G_{14}\beta$ (G_8/G_{14}) increased continuously as the increase in flow rate shortened the residence time in the column. An increase in α-CD concentration (Fig. 5B) led to a higher degree of coupling over a broad range of flows. Comparing Fig. 5A and B, it can be seen that higher amounts of $C_{12}G_8\beta$ were formed but a slightly lower G_8/G_{14} ratio was observed at the higher α-CD concentration. As expected, a higher concentration of α-CD gave higher amounts of coupling products and less disproportionation products (Fig. 7) [19]. When the temperature was increased to 70 °C, the G_8/G_{14} ratio increased dramatically (Fig. 5C). It appears that the primary coupling product $C_{12}G_8\beta$ was not further elongated. It may have aggregated, due to decreased solubility, making it inaccessible for further enzymatic reactions. Unfortunately, this interesting effect is concomitant with a considerable decrease in enzyme stability (Fig. 3).

4. Concluding remarks

The results of this study demonstrate that the use of immobilised CGTase in a packed-bed reactor makes it possible to control the product distribution so as to form either predominantly the primary coupling product $C_{12}G_8\beta$ in the pure form, or further coupling products and/or disproportionation products, simply by varying the flow rate through the reactor. This type of reactor is thus very convenient for controlling the extent of conversion compared to for example with a batch reactor. This is essentially because of the short reaction time, which in the case of free enzyme in batch mode results in difficulty to achieve an efficient heat inactivation within reasonable time without affecting the product composition. In the packed-bed reactor it was possible to achieve a yield of the primary coupling product *n*-dodecyl-(1,4)-β-maltooctaoside of about 50% with a ratio between the primary and the secondary coupling products of about 10 at a flow rate of 1 ml/min (Fig. 5B). If desired, this ratio can be increased to above 90 by increasing the flow rate to 1.4 ml/min and the reaction temperature to 70 °C, but the yield of the primary coupling product then decreases to 30% (Fig. 5C).

The reaction studied should be considered a model reaction. Because of the high temperature needed to avoid precipitation in the reaction mixture, enzyme stability was poor, and thus the advantages of immobilisation could not be fully exploited. However, in processes that can be carried out at lower temperatures or in processes catalysed by more thermostable CGTases, such as the *Thermoanaerobacter* sp. enzyme, further benefits of immobilisation, such as increased productivity, could be obtained.

The substrates used in this study, α-CD and $C_{12}G_2\beta$, are too expensive for most commercial applications. However, we have recently found it to be possible to carry out the same type of reaction starting with a technical grade alkyl polyglycoside, with dodecyl β-D-glucopyranoside and tetradecyl β-D-glucopyranoside as the main components (manuscript). A suitable procedure for product isolation is of great importance in practical applications. At present, downstream processing is carried out using reversed-phase chromatography, which is time consuming and rather expensive [19]. Alternative procedures are under development. Furthermore, in some applications the presence of carbohydrates such as cyclodextrins and maltooligosaccharides in the product may be acceptable, thus greatly simplifying downstream processing.

Previously published methods of preparing alkyl glycosides with long carbohydrate chains involve protection/deprotection steps and chemical catalysis [22,23]. These methods are unfavourable with respect to atom economy, waste generation, use of organic solvents and other principles of green chemistry. In this respect, the method presented here is quite attractive, although further improvements are needed for large-scale applications to be feasible.

Acknowledgements

This work was performed within the framework of Greenchem, a research programme supported by the Foundation for Strategic Environmental Research (Mistra). We like to thank Dr. Pär Tufvesson for providing the packed-bed column.

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