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Investigations on the biodegradation of alkylpolyglucosides by means of liquid chromatography–electrospray mass spectrometry

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Abstract The biodegradation of alkylpolyglucosides (APGs) was studied under the conditions of the OECD Screening Test with activated sludge as an inoculum. An influence of alkyl and sugar chain length on the biodegradation rate and a central scission pathway of the biodegradation were investigated. The liquid chromatography–electrospray mass spectrometry technique was used for alkylpolyglucoside analysis and for identification and semiquantitative determination of metabolites. It was found that APGs with a longer alkyl chain were biodegraded faster than those with a shorter one. However, a longer sugar chain caused slower biodegradation of APGs. The central scission pathway of biodegradation was also confirmed.

Keywords Alkylpolyglucosides · Biodegradation pathway · Central scission

Abbreviations

APG	Alkylpolyglucoside
OECD	Organization for Economic Co-operation and Development
ELSD	Evaporative light scattering detector
HPLC	High performance liquid chromatography
MS	Mass spectrometry
LOQ	Limit of quantitation
LOD	Limit of detection
RSD	Relative standard deviation

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Introduction

Alkylpolyglucosides (APGs) belong to non-ionic surfactants of growing popularity. Because of their good foaming properties as well as synergy with other surfactants they have found an application in laundry and dishwashing detergents (Balzer 1991; Kuhn and Neumbert 2004). Also, their good skin tolerance made them perfectly suitable for mild personal care products (Kuhn and Neumbert 2004). APGs can be used in many industrial processes. Among them the technology of micellar enhanced ultrafiltration seems to be very promising (Adamczak et al. 1999).

The industrial production of APGs is based on renewable materials. Fatty alcohols from plant oils and starch or glucose are converted to surfactants during the Fischer synthesis (Busch et al. 1993; Holmberg 2001). As a result, APGs are mixtures of homologues (having a different alkyl and sugar chain length), anomers (with an alkyl chain bonded to a sugar chain in α- or β-positions) and isomers

(furanosides and pyranosides). The properties of these surfactants depend on each of the parameters mentioned above.

The analysis of APGs can be performed by numerous techniques. Among them chromatography seems to give the best results allowing not only bulk product analysis, but also studies of biodegradation or environmental pollution. Gas chromatography analysis of APGs can be performed only after their derivatization due to low volatility of these analytes (Billian and Stan 1998). However, the derivatization procedure is usually complex and time consuming. High performance liquid chromatography allows direct analysis of underivatized APGs. However, use of the most popular UV absorbance detector is problematic due to lack of proper chromophores in the APGs' molecules. Although analysis can be performed below 200 nm, there is no paper describing the analysis of APGs in such conditions. The use of a refractive index detector (Qin et al. 2006) and evaporative light scattering detector (ELSD) (Elfakir and Lafosse 1997; Chaimbault et al. 1999; Czichocki et al. 2002) has been reported. However, sensitivity of a refractive index detector is low and its usage is limited to the control of APG synthesis or biodegradation studies. Similarly, the use of ELSD has been reported only in papers describing the control of APG synthesis or the chromatographic separation of APGs. Moreover, the analytical signal of particular APG homologues being analysed by ELSD is different (Czichocki et al. 2002). Here, liquid chromatography with mass spectrometric detection (HPLC-MS) was found to be a good alternative (Eichhorn and Knepper 1999; Kuhn and Neumbert 2004). This technique enables not only to perform analysis without derivatisation, but also to obtain high sensitivity and selectivity. Using HPLC-MS different homologues can be analysed without complete chromatographic separation. Degradation products can also be identified using this detector although no APG degradation products have been identified so far. Eichhorn and Knepper (1999) and Qin et al. (2006) suggested central scission pathway. However, they could not find the proposed biodegradation products. Eichhorn and Knepper (1999) suggested also a second biodegradation pathway, the ω-oxidation of alkyl chain with subsequent chain shortening during β-oxidation process. Theoretically possible biodegradation pathways of APGs are presented in Fig. 1. These pathways are a result of combining APG biodegradation pathways found in the literature (Eichhorn and Knepper 1999; Qin et al. 2006). Federle and Itrich (2006) suggested the ω-oxidation of alcohol oxyethylates with subsequent central scission leading to dicarboxylic acids. Taking into account similarity of alcohol oxyethylates and APGs, the above proposal of Federle and Itrich was also adopted as possible APG biodegradation pathway.

Biodegradability testing plays a key role in the assessment of the environmental behaviour of surfactants. Numerous tests are used for measuring biodegradation. Among them standardised tests exist which can be found, for example, in the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals (OECD 1992, 2001). Ready biodegradability of surfactants can be assessed using six OECD methods: 301A (DOC Die-Away), 301B (CO_2 Evolution), 301C (Ministry of International Trade and Industry, Japan), 301D (Closed Bottle), 301E (Modified OECD Screening) and 301F (Manometric Respirometry) (OECD 1992). The surfactants behaviour in sewage treatment plants can be simulated by OECD 303A test (Activated Sludge Unit) (OECD 2001). Here, a tested chemical with artificial sewage is introduced continuously into an appropriate test unit.

In this work biodegradation of two different commercially available APGs was investigated. Analysis of the influence of length of both alkyl and sugar chains on the biodegradation rate was performed. APG biodegradation products and possible degradation pathway were also identified.

Experimental

Reagents and chemicals

Alkylpolyglucosides–Glucopone 215 CS UP (APG 215) and Glucopone 600 CS UP (APG 600) were from Fluka (Switzerland). Octanoic, decanoic, dodecanoic and tetradecanoic acids, as well as glucose and maltose (all of analytical grade) were also from Fluka. HPLC-grade methanol from J.T. Baker (The Netherlands) was used for sample preparation and HPLC measurements. HPLC-grade water was prepared by reverse osmosis in a Demiwa system from Watek (Czech Republic), followed by double distillation

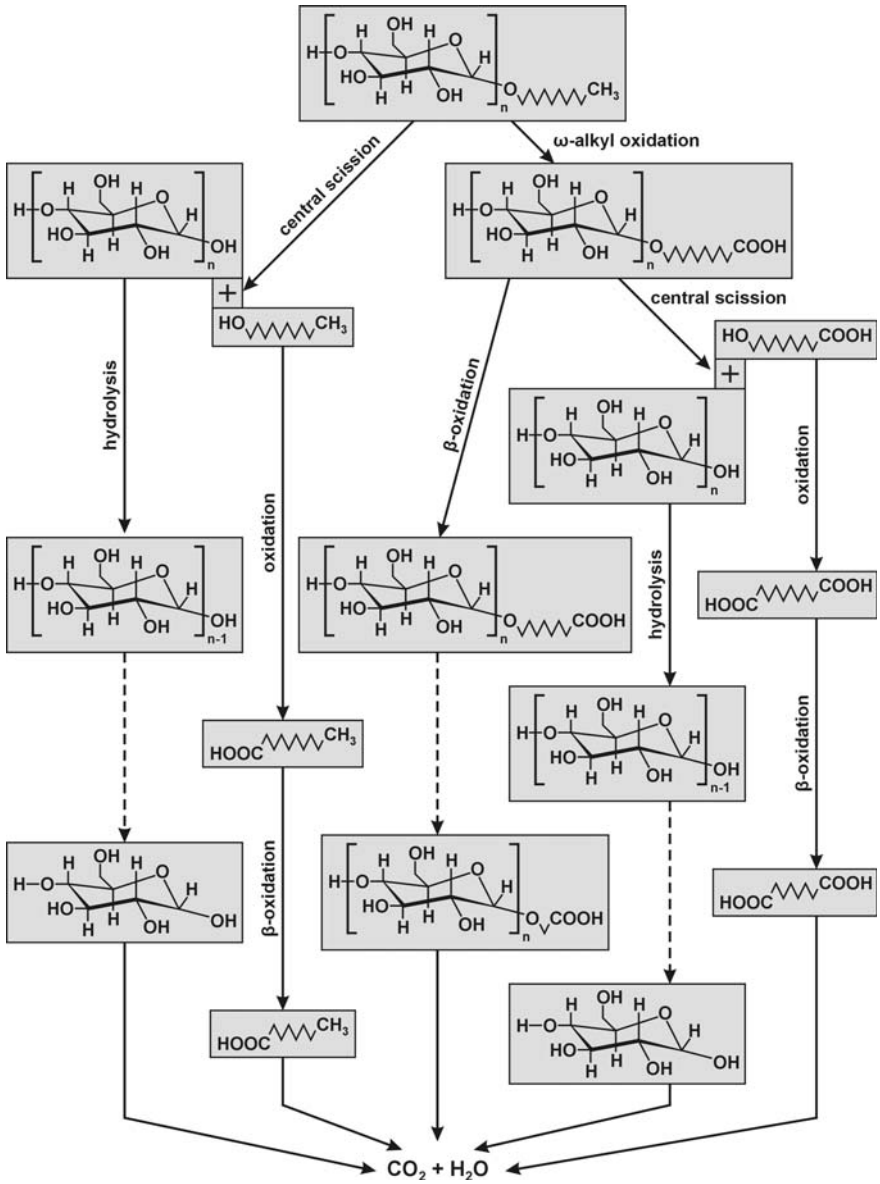

Fig. 1 Theoretically possible biodegradation pathways of alkylpolyglucosides

from a quartz apparatus. Only freshly distilled water was used. All reagents used for preparation of the test medium and synthetic sewage were purchased from POCh (Poland) except for peptone and beef extract, which were taken from BTL (Poland).

Liquid chromatography–mass spectrometery

The HPLC-MS analyses were performed using a Waters/Micromass (UK) ZQ mass spectrometer. The instrument was coupled to a Waters model 2690 HPLC pump (USA). Using an autosampler, the sample solutions were injected into the Atlantis C18 column (5 μm × 150 mm × 3.9 mm i.d., Waters). A gradient of water and methanol was applied. The linear gradient started from 30% of methanol reaching 100% of methanol after 10 min and the latter concentration was maintained for 10 min. The mobile phase flow rate was 0.5 ml min^{-1}. The electrospray source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage 30 V. The source temperature was 120°C and the desolvation temperature 300°C. Nitrogen was used as the nebulizing and

the desolvation gas at flow rates of 100 and 300 l h^{-1}, respectively. A quadrupole mass analyser was used.

Biodegradation studies (modified OECD screening test)

Static screening tests for ready biodegradability in aerobic conditions were performed according to the OECD method 301E (Modified OECD Screening Test) (OECD 1992). Two commercially available alkylpolyglucosides were tested: APG 215 and APG 600. Surfactant concentrations of 5 mg l^{-1} were applied in both tests. A medium used in the tests consisted of mineral components (KH_2PO_4, K_2HPO_4, $Na_2HPO_4 \cdot 2H_2O$, NH_4Cl, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, $MnSO_4 \cdot 4H_2O$, H_3BO_3, $ZnSO_4 \cdot 7H_2O$ and $(NH_4)_6Mo_7O_{24}$) in appropriate concentrations (OECD 1992). An activated sludge was used as an inoculum in the tests. The activated sludge was taken from the sewage treatment plant, which treats typical municipal sewage (predominantly domestic sewage). The sludge was placed in the Husmann plant (OECD 2001) for a 1-week cleaning process before use in the tests. During this time synthetic sewage was delivered to the Husmann plant to clean up the sludge. The synthetic sewage consisted of: peptone, beef extract, urea, NaCl, $CaCl_2$, $MgSO_4$ and K_2HPO_4 in appropriate concentrations (OECD 2001). After the cleaning process was finished, the activated sludge was taken to the tests. Both tests were performed in 200 ml bottles. One bottle was prepared for each experimental point. The biodegradation tests lasted for 10 days. The samples from the tests were subjected to HPLC-MS analysis and the biodegradation rate was determined.

Sample preparation

The samples were enriched using solid phase extraction. Polystyrene-divinylbenzene cartridges–ENVI Chrom P (6 ml, 250 mg) from Supelco (USA) were washed with 6 ml of methanol and conditioned with 7 ml of water. Without letting the cartridge dry, a water sample containing APGs was applied. A 20–50 ml aliquot of the sample was used. The cartridge was then dried and the sample eluted with 5 ml of methanol. The sample was taken for analysis directly or after concentration. During concentration, 2 or 3 ml of the sample were evaporated to 1 ml.

Results and discussion

Analytical method

Two commercially available APGs (APG 215 and APG 600) were studied in this work. Both these surfactants are mixtures of several homologues. The quantitative analysis of APGs is problematic as not every homologue standard can be purchased. However, during biodegradation studies only the percentage of initial concentration of the analytes is required. Thus, the same mixture of APGs, which was subjected to biodegradation, could be used as a standard for quantitative determination. However, calibration curves had to be constructed as a relationship between a recorded signal and overall concentration of APG 215 or APG 600. The results from calibration tests performed for APG 215 and APG 600 are presented in Fig. 2. The curves obtained for all the homologues are not linear. Such non-linear calibration curves in HPLC-MS measurements have already been reported in the literature (Nielsen et al. 2006). To facilitate the prediction of APG concentrations inverse regression was applied (Vander Heyden et al. 2007). Thus, appropriate curves were matched to the results obtained during calibration tests enabling quantitative analysis of APGs (Table 1). The obtained curves were used to calculate biodegradation of main homologues present in APG 215 and APG 600.

Accuracy of the method was tested for both APG 215 and APG 600. Relatively high recoveries were obtained for all the tested homologues (Table 1) allowing the use of the method in biodegradation studies of alkylpolyglucosides. The limit of quantitation (LOQ) and limit of detection (LOD) were calculated using the signal to noise method. The results obtained for LOQ and LOD are presented in Table 1. It is noteworthy that these results were calculated using standard solutions. Typical analytical procedure involved sample concentration. During the biodegradation tests real samples were concentrated from 4 to 20 times.

The analytical procedure used for the determination of alkylpolyglucosides was also applied in

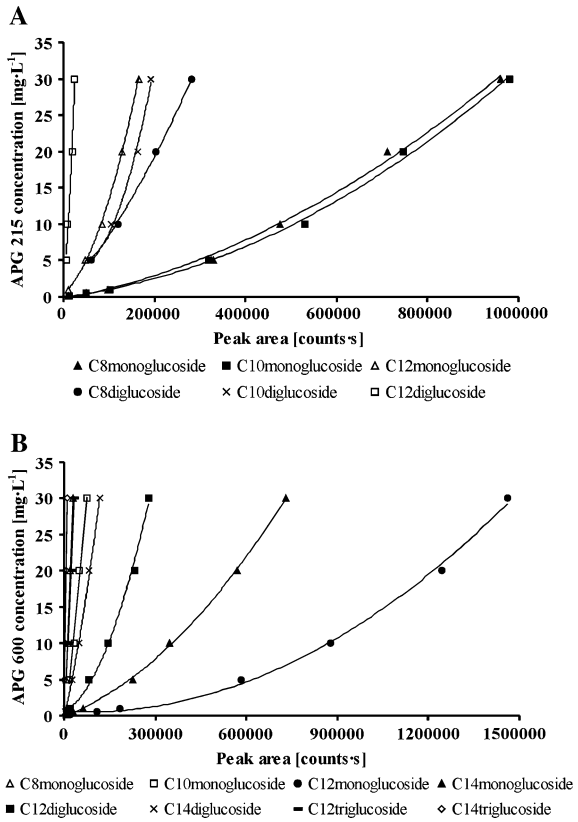

Fig. 2 Calibration curves of alkylpolyglucosides calculated as a relationship between peak area and total alkylpolyglucosides concentration. (**a**) APG 215, (**b**) APG 600

studies of APG biodegradation products. The probable biodegradation products suggested in the literature (Eichhorn and Knepper 1999; Qin et al. 2006) were tested. The biodegradation pathway suggested in the papers involves central scission leading to sugars and alcohols. The latter were to be oxidised to acids during further biodegradation. One of the aims of this work was to confirm or exclude the central scission pathway. As the analytical signal of octanol and higher alcohols in electrospray ionisation is low, it was decided to control only concentrations of sugars and carboxylic acids. The validation tests results for the method used for analysis of the biodegradation products are presented in Table 2. The method was tested for octanoic, decanoic, dodecanoic and tetradecanoic acids. All the parameters tested for these acids were satisfactory. Glucose and maltose were selected as mono- and disaccharide for monitoring central scission of alkylpolyglucosides. However, recoveries of both sugars were low. Thus, the method can be used only for semiquantitative analysis of these analytes.

Biodegradation tests

The OECD screening test 301E was performed for both APG 215 and APG 600. The tested compounds were the sole source of organic carbon. They were introduced to the biodegradation liquors at the beginning of the test at concentration of 5 mg l^{-1}. The biodegradation had been tested over 10 days. The results are presented in Figs. 3–5. It is noteworthy that both test were conducted with the use of more than 10 bottles. Each bottle was a separate biodegradation reactor. Thus, some experimental points depart from the biodegradation curves. It was found that the biodegradation rate depends on both alkyl and sugar chain lengths. The primary biodegradation of APGs with the longer alkyl chain is faster than with the shorter one (Figs. 3, 4). This difference in the biodegradation rates becomes more evident for APGs with higher alkyl chain lengths, namely dodecyl and tetradecyl. However, it can be observed only in first 3 days of the biodegradation tests. The above-mentioned difference becomes very small on the fourth day; most of the glucosides disappear and concentrations of the remaining ones are low. The analytical signal of these residual APGs is low and therefore difference in biodegradation rates should not be discussed at this time point of the test. Faster degradation of alkylpolyglucosides having longer alkyl chains has been reported by (Eichhorn and Knepper 1999). Qin et al. (2006) have reported faster biodegradation for decylglucosides than for octylglucosides. However, the biodegradation rates of dodecyl- and tetradecylglucosides reported by Qin et al. (2006) were much slower than for octyl- and decylglucosides. This can be ascribed to too high concentration of APGs used in the biodegradation tests. Qin et al. (2006) performed biodegradation tests for APGs at a concentration of 35 mg l^{-1}. APGs with dodecyl and tetradecyl alkyl chains are not soluble at this concentration. They form suspensions and only a small part of these APGs can be available to bacteria and subjected to biodegradation. This certainly was the reason why Qin et al. (2006) found the rate of biodegradation of APGs with longer alkyl chains slower than APGs with shorter alkyl chains.

Table 1 Validation tests results obtained for the method used for analysis of alkylpolyglucosides (RSD–relative standard deviation for injections of three samples from the recovery test)

APG 215	Monoglucosides			Diglucosides		
	Octyl	Decyl	Dodecyl	Octyl	Decyl	Dodecyl
Recovery [%]	87.3	95.8	91.2	87.4	95.8	65.9
Precision (RSD) [%]	0.5	1.7	2.3	3.8	1.3	6.7
Calibration curve range [mg l^{-1} of APG 215]	0.1–30	0.1–30	1–30	5–30	5–30	5–30
Correlation coefficient (r^2)	0.9968	0.9979	0.9995	0.9995	0.9960	0.9905
Limit of quantitation [mg l^{-1} of APG 215]	0.13	0.14	0.41	2.98	0.64	4.28
Limit of detection [mg l^{-1} of APG 215]	0.04	0.04	0.12	0.89	0.19	1.28

APG 600	Monoglucosides				Diglucosides		Triglucosides	
	Octyl	Decyl	Dodecyl	Tetradecyl	Dodecyl	Tetradecyl	Dodecyl	Tetradecyl
Recovery [%]	87.9	93.5	94.0	92.5	85.8	82.0	72.4	68.9
Precision (RSD) [%]	3.4	4.8	1.2	2.5	1.4	2.9	4.8	6.7
Calibration curve range [mg l^{-1} of APG 600]	5–30	5–30	0.1–30	0.1–30	0.5–30	0.5–30	10–30	5–30
Correlation coefficient (r^2)	0.9930	0.9882	0.9970	0.9996	0.9957	0.9994	0.9996	0.9964
Limit of quantitation [mg l^{-1} of APG 600]	5.33	4.06	0.06	0.13	0.25	0.21	1.34	2.76
Limit of detection [mg l^{-1} of APG 600]	1.60	1.22	0.02	0.04	0.07	0.06	0.40	0.83

Table 2 Validation tests results obtained for the method used for analysis of alkylpolyglucosides biodegradation products (RSD–relative standard deviation for injections of three samples from the recovery test)

Biodegradation products	Acids				Sugars	
	Octanoic	Decanoic	Dodecanoic	Tetradecanoic	Glucose	Maltose
Recovery [%]	58.7	94.3	77.6	36.6	35.0	11.1
Precision (RSD) [%]	17.1	9.1	11.4	10.8	6.2	8.6
Calibration curve range [mg l^{-1}]	0.3–8	0.3–8	0.3–8	0.3–12	0.25–2.5	0.5–5
Correlation coefficient (r^2)	0.9996	0.9998	0.9999	0.9997	0.9913	0.9941
Limit of quantitation [mg l^{-1}]	0.06	0.08	0.06	0.05	0.21	0.54
Limit of detection [mg l^{-1}]	0.02	0.02	0.02	0.01	0.06	0.16

The biodegradation rate of APGs for mono-, di- and triglucosides is different. A higher content of glucose in APG chains leads to slower primary biodegradation. This observation is in accordance with results obtained by Qin et al. (2006).

The biodegradation liquors were also tested for the presence of biodegradation products. As mentioned above Eichhorn and Knepper (1999) and Qin et al. (2006) suggested probable biodegradation pathways of APGs. However, those authors could not find any of the suggested biodegradation products. In the present paper both acids and sugars were found already at the beginning of the test, as these compounds were present in APG 215 and APG 600. The concentrations of acids after a 1 day lag phase decreased rapidly. A small increase of acid concentrations was observed at the beginning of the day 3 of biodegradation. However, on the following days of biodegradation the carboxylic acids were below the limit of detection.

Monosaccharides were not detected at the beginning of the test and disaccharides were detected at low concentration. During the first 2 days of biodegradation the concentration of disaccharides decreased rapidly in both tests. On day 3 of the tests an increase of concentrations of both mono- and disaccharides was observed (Fig. 5). The increase in sugars concentrations can be observed in the time when

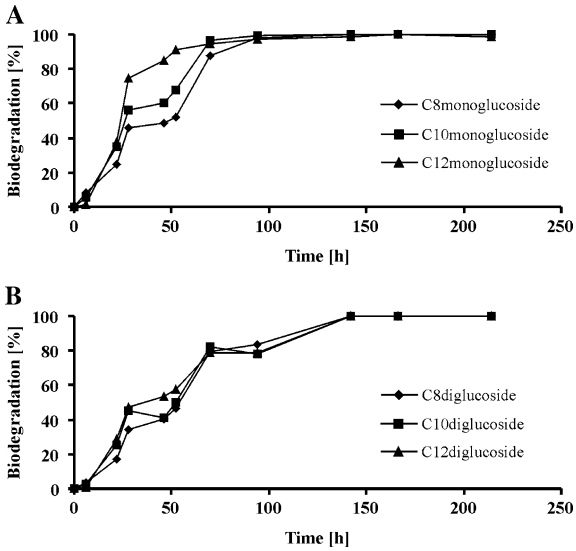

Fig. 3 Biodegradation of APG 215 in the modified OECD screening test. (**a**) monoglucosides, (**b**) diglucosides

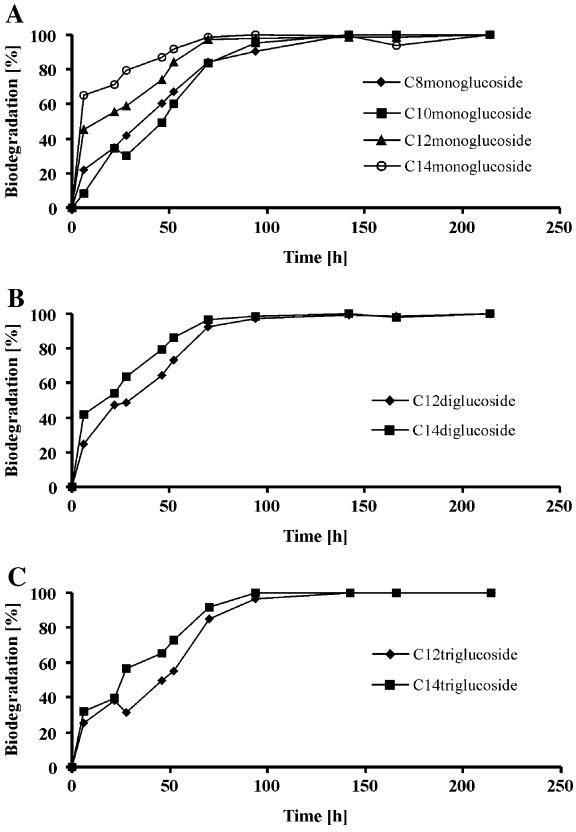

Fig. 4 Biodegradation of APG 600 in the modified OECD screening test. (**a**) monoglucosides, (**b**) diglucosides and (**c**) triglucosides

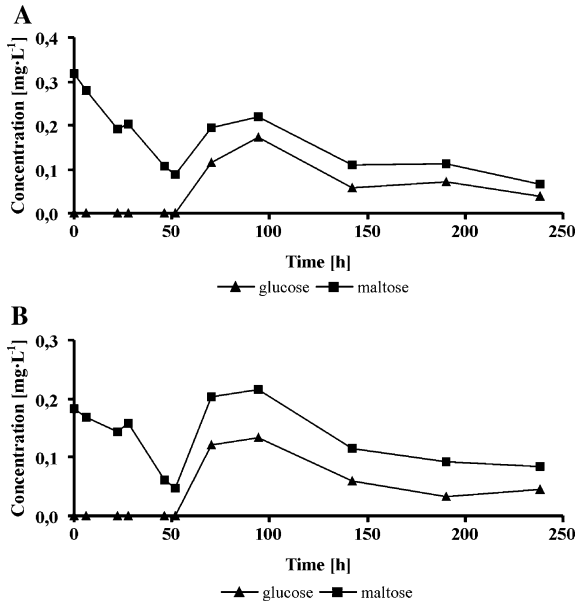

Fig. 5 Concentrations of sugars in the modified OECD screening tests of (**a**) APG 215, (**b**) APG 600

the fastest biodegradation of APGs takes place. This is direct evidence of central scission pathway in the biodegradation of APGs. The concentrations of both sugars were decreasing in the following days of biodegradation. However, both sugars were detectable even on day 10 of the test.

The alternative biodegradation pathway of APGs could not be confirmed. No ω-carboxylic APG acids or dicarboxylic acids were found in the biodegradation liquors. If this type of biodegradation is possible, it is certainly not the dominating pathway.

Conclusions

Under the used conditions both APG 215 and APG 600 undergo primary biodegradation to practically 100% in 1 week. APGs with the longer alkyl chain are biodegraded faster than those having a shorter one. However, a longer sugar chain leads to slower biodegradation of APGs than a shorter one. The central scission pathway of biodegradation was confirmed during the experiments. This pathway is in accordance with that suggested in the literature (Eichhorn and Knepper 1999; Qin et al. 2006).

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