Journal of Environmental Polymer Degradation, Vol. 3, No. 4, 1995

Structure–Biodegradation Relationships of Polymeric Materials. 1. Effect of Degree of Oxidation on Biodegradability of Carbohydrate Polymers

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The biodegradability of oxidized starch and inulin has been studied in relation to the degree of periodate oxidation to dialdehyde derivatives, by measuring oxygen consumption and mineralization to carbon dioxide. A higher degree of oxidation of dialdehyde starch and dialdehyde inulin results in a lower rate at which the polymers are biodegraded. It is demonstrated that the biodegradation rate of dialdehyde inulin derivatives decreases more than that of equivalent starch derivatives. The differences in biodegradation behavior between dialdehyde starch and dialdehyde inulin, resulting from comparable modifications, are discussed in terms of conformational structure.

KEY WORDS: Biodegradation; starch; inulin; dialdehyde derivatives; mineralization.

INTRODUCTION

As a result of the growing attention during the last decades to environmentally friendly products, including legislation [1], a number of said biodegradable materials are being developed. These materials vary from biodegradable surfactants [2] and new builder materials for detergents [3] to various plastics for packaging applications, claimed to be biodegradable [4–11].

These materials will, sooner or later, end up in the environment. To predict the degradation behavior of these materials after disposal, a research program has been started at our institute aimed at the development of a model for studying relationships between polymer structure and biodegradability. In the scope of this program, the biodegradability of a number of polymeric materials in which molecular structure and conformation are varied by chemical modification is being tested. The knowledge obtained in this way, regarding the effect of these higher-order structures on biodegradability, can result in accurate insight on structure–biodegradability relationships. Models based on these relationships are very useful in designing new materials with specific degradation behavior.

In this work we consider biodegradation as complete mineralization of the organic material to water, carbon dioxide, and other naturally occurring gasses and minerals. In an aerobic environment an organic compound will be mineralized following the equation:

$$\text{compound} + O_2 \rightarrow CO_2 + H_2O + \text{minerals } (HNO_3,\ H_3PO_4, \text{ etc.}) \quad (1)$$

To study biodegradation defined in this way, two tests were used with aerobic aquatic conditions. One is a *modified Sturm test*, in which biological mineralization is followed by measuring the amount of CO_2 evolution. The other test, the *two-phase BOD test*, focuses on the other side of the equation by measuring the O_2 consumption by microorganisms, which can be related to the amount of oxygen needed to oxidize the compound completely to carbon dioxide, water, and mineral components.

Starch and inulin are both naturally occurring carbohydrate polymers but have different properties, re-

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1064-7546/95/1000-0235$07.50/0

sulting partially from differences in higher-order conformational structures. Complete biodegradation of these biopolymers can be expected. Modification of chemical structure and conformation can be achieved by glycol cleavage of the diol systems with periodate. In this paper, the biodegradation behavior of dialdehyde starch and dialdehyde inulin derivatives thus obtained is compared and discussed in terms of conformational structure.

EXPERIMENTAL

Materials

Native potato starch was purchased from Avebe n.v., Foxhol, The Netherlands. Dialdehyde starch (DAS) was prepared by periodate oxidation of native potato starch as described by Veelaert *et al.* [12]. Chicory inulin was purchased from Suiker Unie b.v., Roosendaal, The Netherlands. Precipitation steps with water/ethanol and water/acetone were performed to obtain inulin with an average DP of 16. Dialdehyde inulin (DAI) was prepared by the same periodate oxidation process as used to produce dialdehyde starch.

The degree of oxidation to dialdehyde moieties was determined by HPLC analyses using a Dionex DX 300 HPLC unit, equipped with a 4 × 250-mm CarboPac MA1 column, an advanced gradient pump module, and a pulsed electrochemical detector. Prior to analysis the (partially) oxidized starch was reduced with sodium borohydride and sodium hydroxide. Subsequently, the polyalcohol was hydrolyzed at increased temperature by adding sulfuric acid. The resulting sample was injected onto the HPLC column after dilution, with mannitol as internal standard [12].

The amount of free dialdehyde functions in oxidized materials was determined with an oxime reaction. Aqueous hydroxylamine hydrochloride (4 ml, 1 *M*, pH 5) was added to a suspension of material (500 mg in 30 ml water, 25°C, pH 5). The pH was kept constant by automatically adding 1 *M* sodium hydroxide solution, using a pH controller (Metrohm 614) and a motor burette (Metrohm 665; 10 ml). The amount of sodium hydroxide consumed was measured until completion of reaction.

In biodegradation experiments, materials were used in powdered form (fractions with sizes between 125 and 150 μm). All materials were analyzed for dry weight (remaining mass after heating at 105°C until constant weight), ash content (remaining mass after heating at 550°C until constant weight), and elemental composition, using a Carlo-Erba Instruments CHNS-O EA 1108 elemental analyzer (Milan, Italy).

Biodegradation Test Methods

Two-Phase (Semi-) Closed-Bottle BOD Test

The two-phase BOD test was carried out as described previously [13]. The inoculum was obtained by mixing raw settled sewage at a 1 : 1 ratio with effluent of an aerobic wastewater treatment plant operated by the Department of Environmental Technology of the Wageningen Agricultural University. The mixture was filtered on arrival at the lab (< 125 μm), diluted two times with the mineral medium, and aerated for 16 h. Ten milliliters of this mixture was used per liter to inoculate the mineral medium.

Per sample, three replicate glass bottles with approximately 50 mg of polymeric material (approx. 50 mg TOD) were incubated with 350 ml of inoculated mineral medium, together with three blank bottles. Bottles were closed with butyl rubber stoppers and aluminium caps. The air phase was approx. 215 ml.

The oxygen concentration in the incubation medium was determined by penetrating the butyl rubber stoppers with syringe needles and circulating the medium through a flow cell equipped with two YSI microoxygen electrodes, Model 5357 (Yellow Springs, OH) connected in series to a YSI Biological Oxygen Monitor (Model 5300). When the oxygen concentration in one of the sample bottles dropped below 50% of saturation level, the bottles were purged with air for 15 min. After conditioning for 2 h, dissolved oxygen concentrations were measured again.

The biological oxygen demand (BOD) was calculated as milligrams of oxygen consumed per milligram of polymeric material added to the test bottles and is expressed as a percentage of the theoretical oxygen demand (TOD).

Modified Sturm Test

The modified Sturm test was performed as described previously [13]. Per sample, approximately 200 mg of polymeric material (approx. 80 mg C) was incubated in duplicate with 300 ml of mineral medium, together with two blank bottles and at least one reference material.

The inoculum was prepared as follows: 100 g of forest soil was suspended in 1000 ml of 0.01 *M* pyro-

phosphate solution. After stirring and sieving (< 125 μm), the mixture was left for 30 min to allow total sedimentation. The supernatant was mixed at a 1 : 1 ratio with activated sludge derived from the aerobic wastewater treatment plant operated by the Department of Environmental Technology of the Wageningen Agricultural University. The mixture of soil extract and activated sludge was aerated for 16 h, after which it was blended for 2 min in a Waring Blendor at medium speed. The mixture was allowed to settle for 30 min and the supernatant was decanted. This supernatant was allowed to settle for another 30 min. The remaining supernatant was decanted and used as a 1% inoculum.

The CO_2 trapping system to absorb the biologically produced gas consisted of a series of three gas-washing bottles, each with 100 ml of a 0.0125 *M* $Ba(OH)_2$ solution. Carbon dioxide was precipitated as $BaCO_3$. Titration of the remaining $Ba(OH)_2$ was carried out with 0.05 *M* hydrochloric acid. The evolved amount of CO_2 was calculated as a percentage of the theoretical CO_2 production (TCO_2) of the polymeric materials.

Calculations

Theoretical oxygen demand (TOD) was defined as the amount of oxygen needed to oxidize a compound to $CO_2 + H_2O + NH_4OH + H_2SO_4 + HCl + HNO_3 + H_3PO_4 + NaOH$ (mg O_2/mg) [14, 15]. The TOD of the compound $C_cH_hCl_{cl}N_nS_xP_pNa_{na}O_o$ with molar weight M_w was calculated using Eq. (2).

$$\mathrm{TOD} = \frac{8 \cdot [4c + (h - cl - 3n) + 6s + 5p + na - o]}{M_w} \tag{2}$$

For compounds with elemental composition *s*% C, *t*% H, *u*% Cl, *v*% N, *w*% S, *x*% P, *y*% Na, and *z*% O, this amounts to Eq. (3).

$$\mathrm{TOD} = \frac{2.67s + 8t - 0.23u - 1.71v + 1.5w + 1.29x + 0.35y - z}{100} \tag{3}$$

Theoretical CO_2 production (TCO_2) was defined as the amount of CO_2 obtained from oxidizing all carbon to carbon dioxide (mg CO_2/mg). For compounds with *s*% C, this is calculated with Eq. (4).

$$\mathrm{TCO_2} = 0.01s \cdot \frac{M_{CO_2}}{M_C} = 3.667 \cdot 10^{-2} \cdot s \tag{4}$$

RESULTS AND DISCUSSION

Biodegradation Studies

A convenient way to compare the biodegradation behavior of polymeric materials is to determine the mineralization to carbon dioxide in the modified Sturm test. In this test, starch was almost completely converted to carbon dioxide, as can be expected from a naturally occurring glucopyranose polymer (Fig. 1). The reference material sucrose was mineralized to the same extent (although slightly faster) in the same test (data not shown). In practice, mineralization of 100% will not be reached in the time frame of this test, because some of the degradation products will be used in the formation of new biomass (growth of microorganisms).

Cleavage of the C2–C3 diol in starch with the formation of dialdehyde starch had a significant effect on the conversion of these compounds to carbon dioxide. Increasing the amount of glucopyranose moieties oxidized to dialdehydes slowed down the mineralization process. Completely oxidized starch (DAS-100%) was only very slowly mineralized to CO_2. For example, after 50 days of incubation, only 30% of the dialdehyde starch had been converted to carbon dioxide (Fig. 1).

Determination of biodegradability can also be based on measurement of biological oxygen consumption due to microbial oxidation of materials during the degradation process. This so-called biological oxygen demand (BOD) of partially oxidized carbohydrates was assessed using the two-phase BOD test. The BOD of native potato starch was equal to that of the reference substance sucrose (approximately 80% of TOD in 28 days), confirming complete biodegradation. Partial oxidation of starch to dialdehyde starch resulted in lower oxygen demands (Fig. 2). For example, after 50 days of aerobic incubation, the BOD of completely oxidized starch (DAS-100%) was less than 20% of its theoretical oxygen demand (TOD).

As the degree of starch oxidation had the same effect on the conversion of carbon to CO_2 in the modified Sturm test as on the BOD in the two-phase BOD test, it is concluded that the biodegradation of dialdehyde starch is clearly influenced by the relative amount of dialdehyde groups. Taking into account that DAS-100% showed some mineralization to CO_2 and induced biological oxygen consumption, it is expected that the polymer eventually will be completely mineralized to CO_2 and water and thus may be called "ultimately" biodegradable. Therefore, the degree of starch oxidation

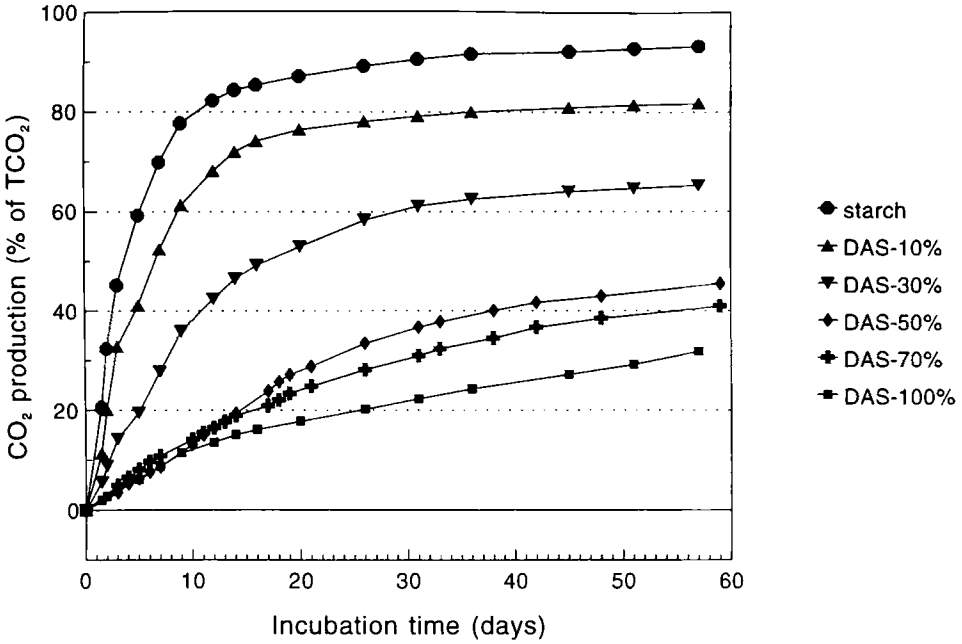

Fig. 1. Mineralization of native potato starch and several (partially) oxidized dialdehyde starches (DAS; degree of oxidation is 10, 30, 50, 70, and 100%) to CO_2 as determined with the modified Sturm test.

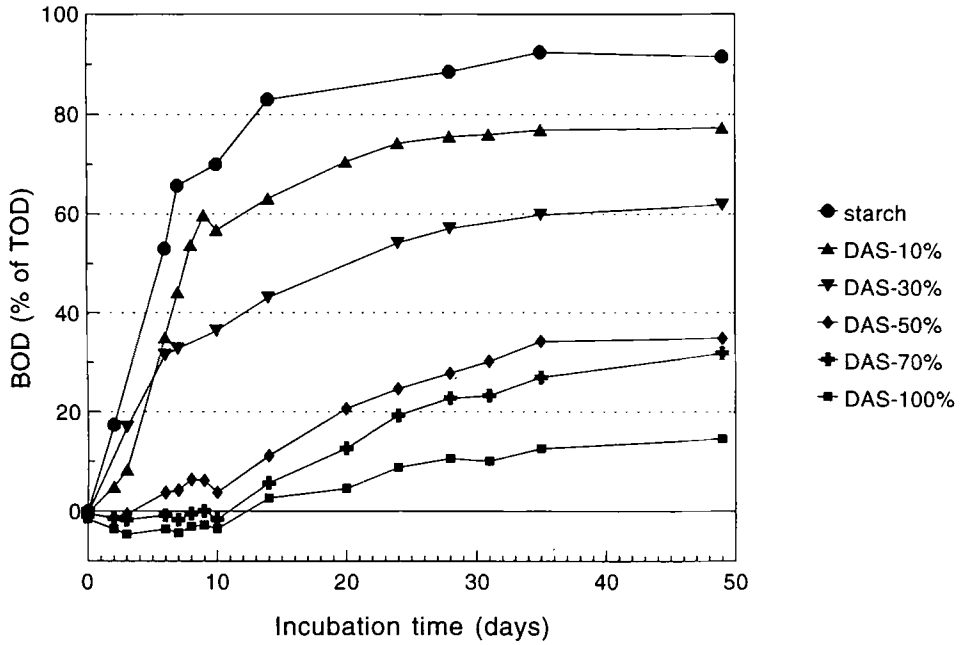

Fig. 2. Biological oxygen demand (BOD) of native potato starch and several (partially) oxidized dialdehyde starches (DAS; degree of oxidation is 10, 30, 50, 70, and 100%) as determined with the two-phase BOD test.

merely effects the rate of biodegradation, and not the final extent of mineralization. Long-term biodegradation experiments of more than 6 months are currently in progress to support this hypothesis.

Biodegradability of inulin and partially oxidized dialdehyde inulin was also determined with the modified Sturm test and the two-phase BOD test. Inulin was rapidly mineralized to CO_2 in the modified Sturm test (Fig. 3) as can be expected from a naturally occurring fructose polymer. However, with 10% of its fructofuranose moieties converted to dialdehyde fructose, the biodegradation rate decreased considerably. Dialdehyde inulin with a degree of oxidation (DO) higher than 30% showed an even lower biodegradation rate (Fig. 3). For example, after 50 days of incubation, inulin was almost completely mineralized. In the same time DAI-10% was mineralized for 55% of TCO_2, and DAI-30%, DAI-40%, and DAI-100% for not more than 20% of TCO_2. The same effect was observed with inulin derivatives in the two-phase BOD test (Fig. 4).

To exclude possible inhibitory effects from the inulin derivatives or its degradation products on the inoculum, some extra inulin was added to the incubation flasks after 3 weeks of incubation. The supplementary inulin, however, was rapidly and completely mineralized in both the modified Sturm test and the two-phase BOD test (data not shown). This indicates that the oxidized inulins do not have inhibitory effects on the inoculum at the concentrations used. Therefore, the results obtained have to be ascribed to the materials themselves.

In Fig. 5 the mineralization of starch and inulin derivatives after 14 and 58 days of incubation is shown as a function of the degree of periodate oxidation. Native starch and inulin are biodegraded at approximately the same rate. However, a significant difference was observed between the dialdehyde starch and the dialdehyde inulin derivatives in the effect the degree of oxidation has on their biodegradation rate. For example, when 10% of the diol groups in starch is oxidized to dialdehyde (DAS-10%), a small effect is observed in the degree of degradation after 14 days of incubation (10% decrease compared to nonoxidized starch). However, when 10% of the diol groups in inulin is oxidized in a similar way (DAI-10%), the degree of degradation after 14 days drops a substantial 40% compared to nonoxidized inulin (Fig. 5A).

At longer incubation times (e.g., 58 days), the degree of oxidation of starch and inulin still has a large effect on the capability of microorganisms to degrade the molecules (Fig. 5B). Again, this effect is more pronounced for dialdehyde inulin than for dialdehyde starch.

The decrease in degradation rate is not linear with the increase in degree of oxidation. This effect therefore can be attributed to conformational changes in the poly-

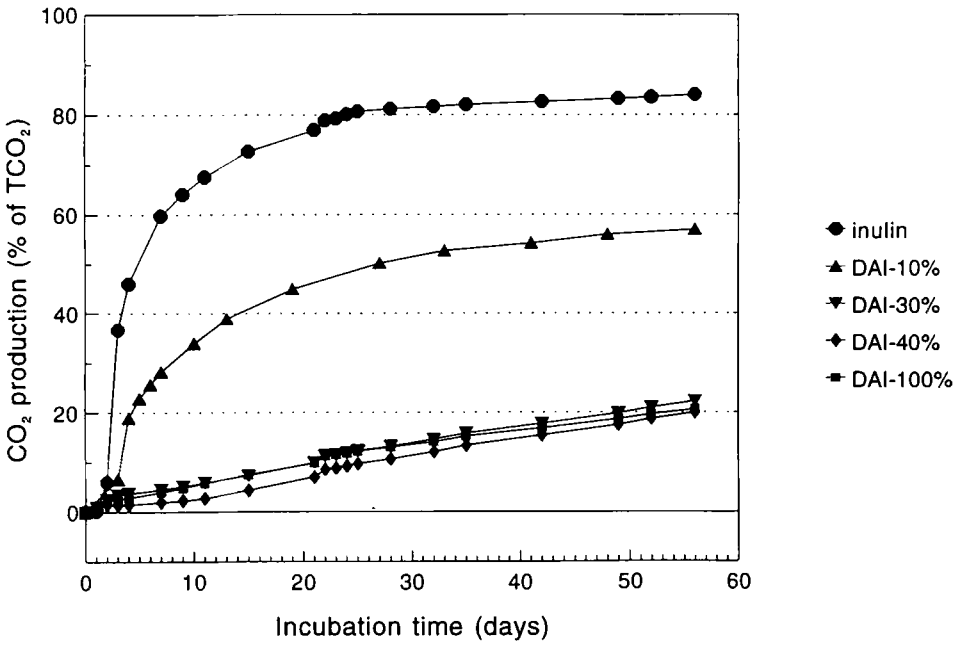

Fig. 3. Mineralization of inulin and several (partially) oxidized dialdehyde inulins (DAI; degree of oxidation is 10, 30, 40, and 100%) to CO_2 as determined with the modified Sturm test.

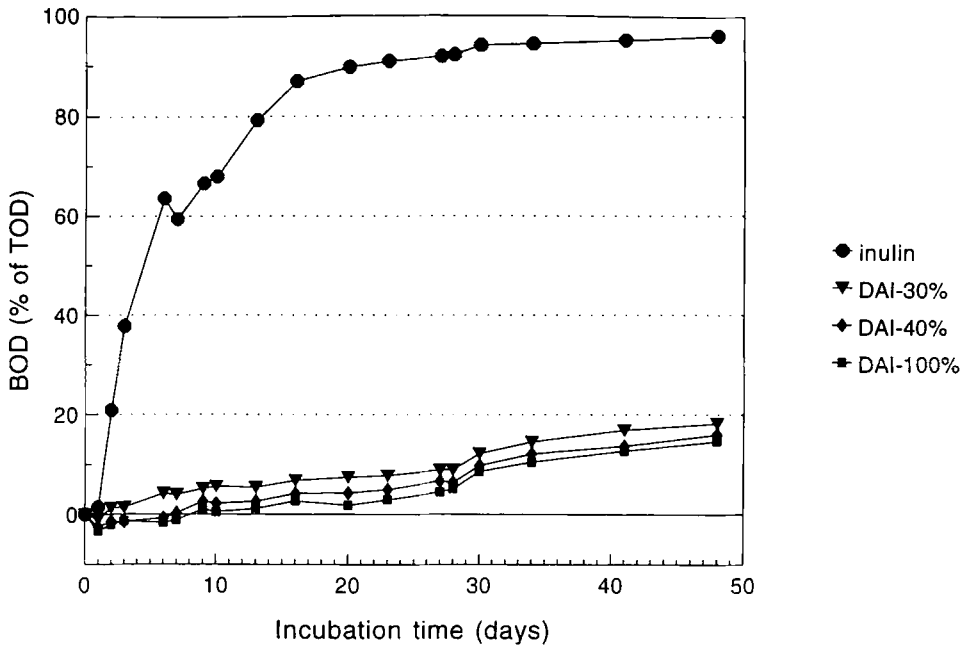

Fig. 4. Biological oxygen demand (BOD) of inulin and several (partially) oxidized dialdehyde inulins (DAI; degree of oxidation is 30, 40, and 100%) as determined with the two-phase BOD test.

mers, leading to lower susceptibility to enzymatic actions. Long-range ordering and crystallinity are known to slow down biodegradation of polymeric materials [16–18]. However, it has been reported previously that within a starch granule, crystallinity disappears upon periodate oxidation at an early stage of reaction, due to random oxidation [19]. To interpret the decrease in degradability, another phenomenon must predominate, which is supposed to be inter- and intramolecular cross-linking.

A model has been proposed for dialdehyde starch, in which the dialdehyde functions are involved in cross-linking reactions (Fig. 6) [12]. This has been confirmed with the oxime reaction, in which the ability of the aldehyde groups to form oximes with hydroxylamine is tested. From the results, it is concluded that not all aldehyde groups are free for reaction because of acetal formation. This intramolecular cross-linking results in a more hydrophobic polymer, which is proposed to cause conformational changes within the starch molecule, reducing the susceptibility to microorganisms.

Because the fructofuranose units are assumed to be less rigidly involved in the polymer backbone [20], it is expected that, when oxidized, they have a different freedom of movement compared to the oxidized glucose moieties in starch, which are embedded in the linear chain. Therefore, differences in higher-order structure between dialdehyde starch and dialdehyde inulin derivatives have to be expected, with consequently different susceptibilities to microbial attack.

CONCLUSIONS

A higher degree of oxidation of dialdehyde starch and dialdehyde inulin derivatives results in a lower biodegradation rate of these polymers. This effect can be attributed to changes in the polymer structure, due to intra- and intermolecular acetal formation. The decrease in biodegradation rate due to oxidation of inulin is more pronounced than in the case of equivalent oxidation of starch. Apparently, the oxidized starch and inulin derivatives adopt different conformations, resulting in a different susceptibility to microbial attack.

ACKNOWLEDGMENTS

We wish to thank Sarah Veelaert for the stimulating discussions. We are also indebted to Marieke Polling and Ted Slaghek for the oxidized starches and inulins.

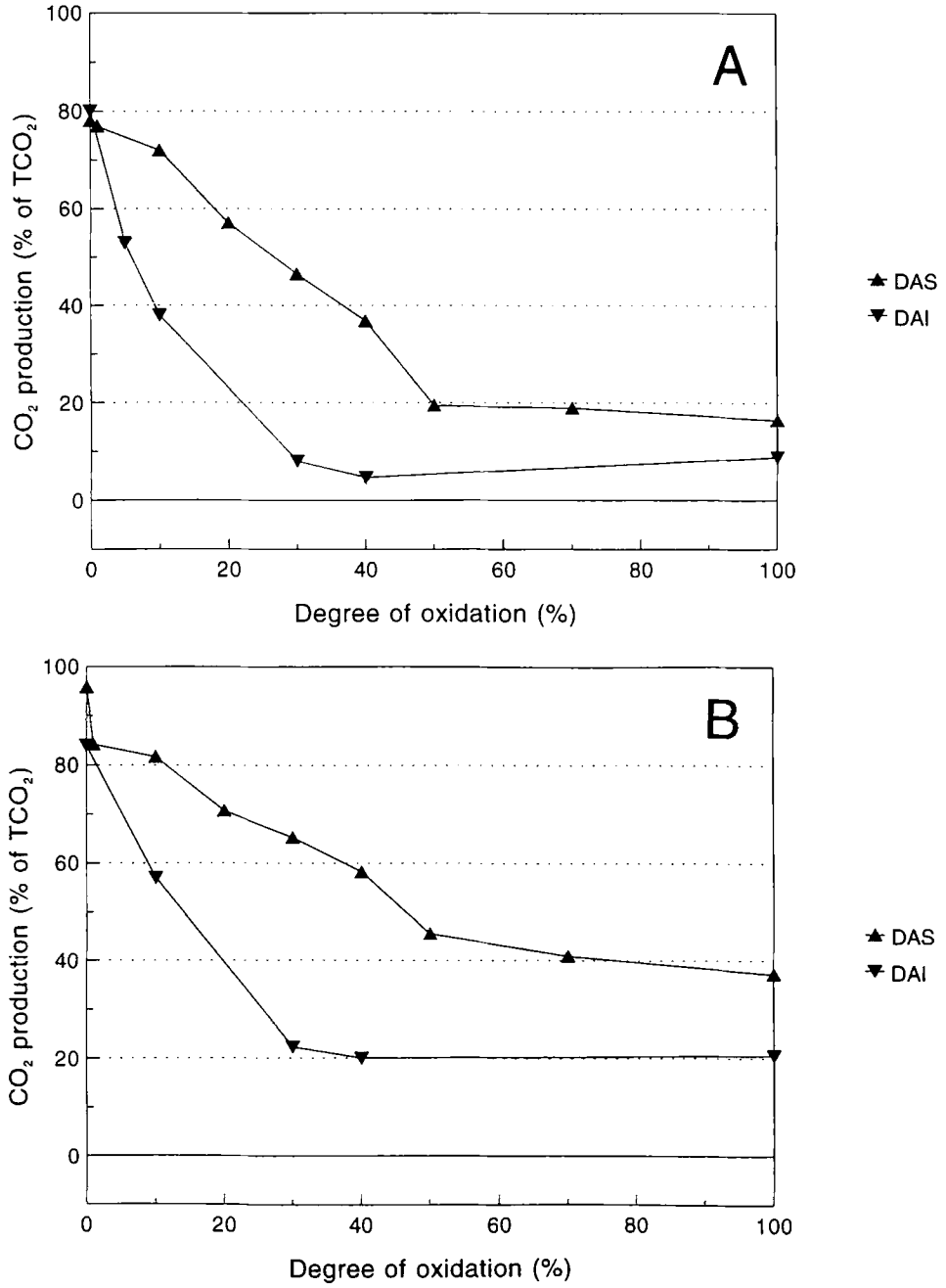

Fig. 5. Mineralization of dialdehyde starch and dialdehyde inulin to CO_2 in the modified Sturm test as a function of the degree of periodate oxidation: (A) after 14 days of incubation and (B) after 58 days of incubation.

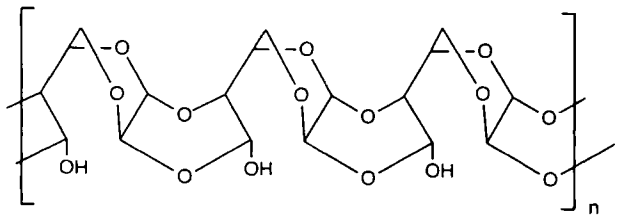

Fig. 6. Scheme of hemiacetal and acetal formation in partially oxidized dialdehyde starch [12].

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