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ISOTACHOPHORESIS OF ORGANIC ACIDS AFTER OXIDATION OF HYDROLYTIC PRODUCTS OF SOME MONOSACCHARIDES

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SUMMARY

One of the basic components of cultivation media for microorganisms at biotechnological processes are organic compounds as the source of organic acids. Optimal conditions were found for separation of organic acids by isotachophoresis. The products of the alkaline degradation of glucose and their oxidation products after successive glucose oxidation were tested.

INTRODUCTION

The hydrolytic degradation of wood produces large amounts of saccharinic acids of empirical formula $C_nH_{2n}O_n$. In general, they can originate from alkaline degradation and redox disproportionation of poly-, oligo- and monosacharides. Up to now the saccharinic acids have not been used economically, but it has been found that some of them can be utilized in the cultivation of microorganisms and some can be used to produce surface-active materials.

Organic acids can be identified and determined by various analytical methods. For the analysis of mixtures of organic acids, gas chromatography has mostly been used and less interest has been devoted to liquid and ion-exchange chromatography. In the determination of volatile aliphatic acids, it is necessary to convert the acids into suitable derivatives[1–6] in order to prevent interactions with the column filling[7,8]. Free non-derivatized acids were determined by Sakodinsky *et al.*[9] on Carbowax in combination with orthophosphoric acid. By using high-performance liquid chromatography (HPLC), organic aliphatic acids can be determined as suitable esters[10,11] or without preliminary modification of the sample by reversed-phase liquid chromatography[12] or ion-exchange chromatography[13]. For the analysis of complex mixtures, HPLC method has been used in combination with gas–liquid and thin-layer chromatography and mass spectrometry[14].

Isotachophoresis has been shown to be a suitable method for the identification and determination of organic acids. It possesses some advantages over chromatographic methods, especially the minimal consumption of the sample and the possibility of direct analysis without preliminary modification of the sample. The possibility of the direct determination of formic acid particularly favours this method over gas chromatography. Beckers *et al.*[15] described the separation of fatty acids in meth-

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TABLE I

OPERATING CONDITIONS

Electrolyte	*Parameter*	*I*	*II*
Leading	Electrolyte	HCl	HCl
	Concentration	10^{-2} *M*	10^{-2} *M*
	Counter ion	Creatinine	L-Histidine
	pH	5.00	5.95
	Metal ion	Ca^{2+}	–
	Concentration	$1 \cdot 10^{-3}$ *M*	–
	Additive	Mowiol	Mowiol
Terminating	Electrolyte	Capronate	Morpholinoethanesulphonic acid (MES)
	Concentration	$5 \cdot 10^{-3}$ *M*	$5 \cdot 10^{-3}$ *M*
	Counter ion	Tris	–

anol as a solvent. Boček *et al.*[16] studied the choice of suitable operating conditions for the analysis of typical Krebs cycle acids. Everaerts and Konz[17] studied the anionic products formed by homogeneous oxidation of sugars by isotachophoresis. Kaniansky *et al.*[18] studied the fermentation products of *n*-alkanes[18]. A computational method has been applied to the evaluation of mobility and pK_a values of organic and inorganic anions by Hirokawa *et al.*[19]. The possibility of separating some anions on the basis of the kinetically labile complex equilibria was studied and the composition of leading electrolytes suitable for this type of separation was discussed by Kaniansky and Everaerts[20].

EXPERIMENTAL

Hydrolysates of glucose were analysed by means of both capillary and two-capillary column isotachophoretic analysers. The hydrolysates of glucose were prepared in three ways: (1) alkaline hydrolysate by hydrolysis of glucose in an alkaline

TABLE II

RESULTS OF QUANTITATIVE ANALYSIS

Acid	*Concentration (g/l)*		
	I	*II*	*III*
Formic	0.4496	4.4726	57.3841
Glycolic	1.2627	0.2510	3.9298
Lactic	115.0267	4.6901	–
C_5-ISA	7.0715	–	–
C_6-ISA	22.2127	–	–
Oxalic	–	–	0.7879
Glyceric	–	–	3.9780
Hydroxybutyric	–	0.9925	4.5114
Hydroxyvaleric	–	0.0043	–

* I, Alkaline hydrolysate of glucose; II, alkaline hydrolysate of glucose after oxidation with hydrogen peroxide; III, oxidation of glucose in alkaline medium.

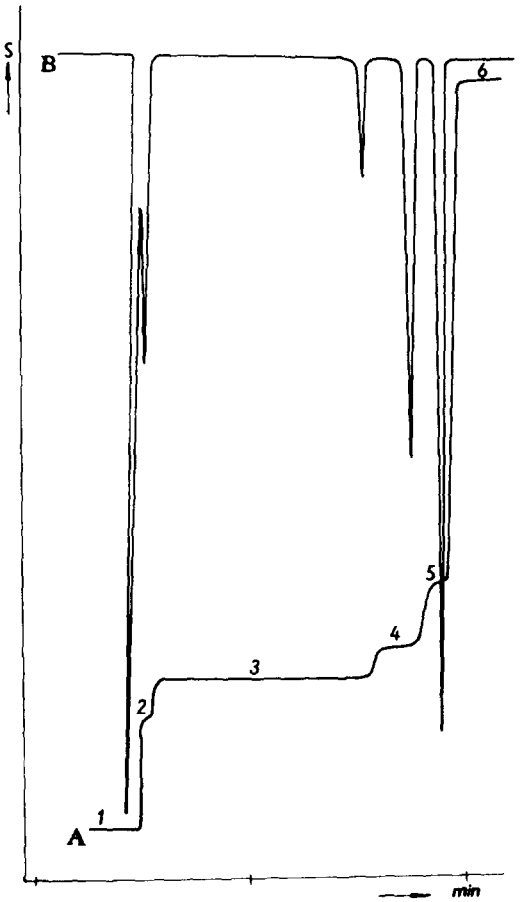

Fig. 1. Isotachopherogram of glucose hydrolysate in the electrolyte system leading electrolyte I–terminating electrolyte II. A, Conductivity; B, derivative. 1 = Cl^-; 2 = glycolic acid; 3 = lactic acid; 4 = C_5-ISA; 5 = C_6-ISA; 6 = MES.

medium in the absence of oxygen; (2) alkaline hydrolysate after oxidation with hydrogen peroxide; and (3) glucose after oxidation with hydrogen peroxide in ammonia solution. The products of the alkaline hydrolysis of glucose and their oxidation products after successive glucose oxidation were tested.

The operating conditions are given in Table I.

A conductometric detector was used. The current was 80 μA, with application of a two-column isotachophoretic instrument, the driving current being 250 μA in the pre-separation column and 45 μA in the analytical column. The time of analysis was 14–20 min.

RESULTS AND DISCUSSION

Individual acids were identified on the basis of the measurement and comparison of the characteristic constants of model mixtures and hydrolysates or their ox-

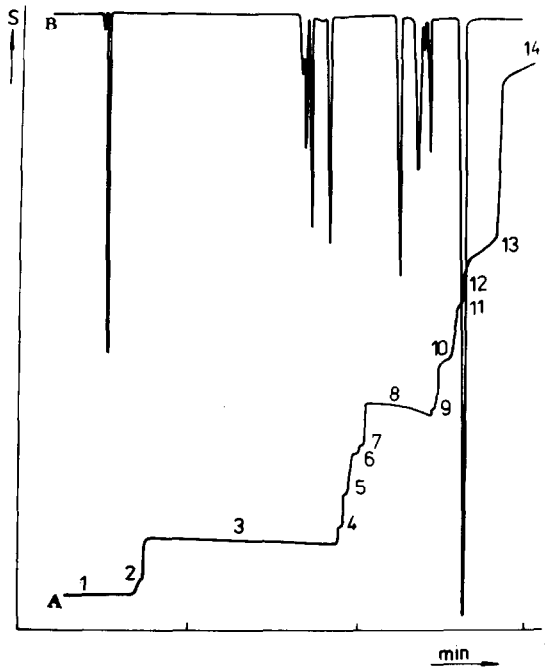

Fig. 2. Isotachopherogram of oxidation products of glucose hydrolysate. The analysis was performed in a two-column analyser in the electrolyte system leading electrolyte II–terminating electrolyte II. A, Conductivity; B, derivative. 1 = Cl^-; 3 = formic acid; 6 = glycolic acid; 8 = lactic acid; 10 and 11 = hydroxybutyric acid; 12 = hydroxyvaleric acid; 14 = MES; 2,4,5,7,9 and 13 = unidentified.

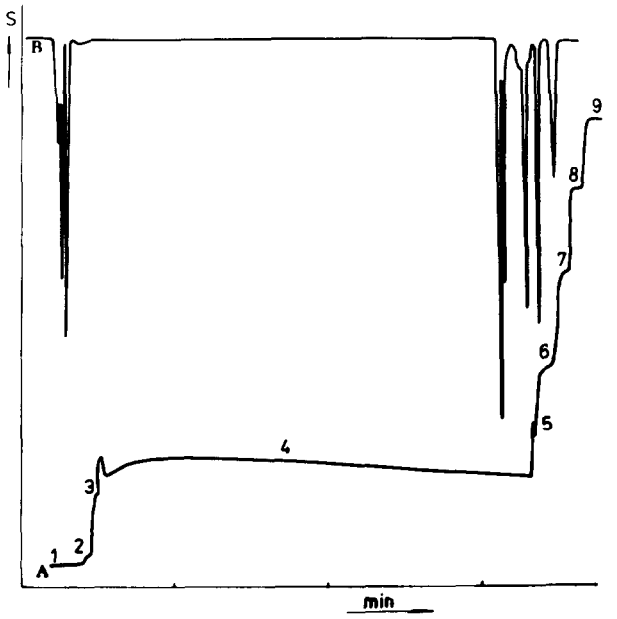

Fig. 3. Isotachopherogram of products of alkaline oxidation of glucose. The analysis was performed in a two-column analyser in the electrolyte system leading electrolyte II–terminating electrolyte I. A, Conductivity; B, derivative. 1 = Cl^-; 3 = oxalic acid; 4 = formic acid; 6 = glycolic acid; 7 = glyceric acid; 8 = hydroxybutyric acid; 9 = caproic acid; 2 and 5 = unidentified.

idation products. The identification was checked by standard additions of the acids in all instances. The following acids were identified and determined (Table II): formic, glycolic, lactic, C_5-isosaccharinic (C_5-ISA), C_6-isosaccharinic (C_6-ISA), oxalic, glyceric, hydroxybutyric and hydroxyvaleric. The relative standard deviation of the determination was less than 1%.

The composition of the individual hydrolysates depends on the various treatment procedures used. On alkaline hydrolysis glucose degrades mainly to lactic and saccharinic acids. Oxidation leads to formic, hydroxybutyric and oxalic acids. The qualitative differences among the individual mixtures analysed is evident from Figs. 1–3 and Table II.

Acid mixtures have been prepared in order to establish the possibility of utilizing individual components in alkaline wood hydrolysates by yeast culture microorganisms, which is the subject of future work.

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