Journal of Chromatography, 546 (1991) 297–309
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2162

Determination of saccharides in biological materials by high-performance anion-exchange chromatography with pulsed amperometric detection

D. A. MARTENS and W. T. FRANKENBERGER, Jr.*
Department of Soil and Environmental Sciences, University of California, Riverside, CA 92521 (U.S.A.)

ABSTRACT

High-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) under alkaline conditions (pH 9–13) separates aminosaccharides, neutral saccharides and glycuronic acids based upon their molecular size, saccharide composition and glycosidic linkages. Carbohydrates were extracted by utilizing 0.5 *M* H_2SO_4 (neutral monosaccharides), 0.25 *M* H_2SO_4 coupled with enzyme catalysis (glycuronic acids) and 3 *M* H_2SO_4 (aminosaccharides). Solid-phase extraction with strong cation and strong anion resins was used to partition the cationic aminosaccharides and anionic glycuronic acids and to deionize acid extracts for neutral saccharides. Separation was conducted on a medium-capacity anion-exchange column (36 mequiv.) utilizing sodium hydroxide (5–200 m*M* and sodium acetate (0–250 m*M*) as the mobile phase. The saccharides were detected by oxidation at a gold working electrode with triple-pulsed amperometry. HPAEC–PAD was found superior to high-performance liquid chromatography with refractive index (RI) detection for neutral monosaccharides and aminosaccharides and to low-wavelength UV detection for glycuronic acids in terms of resolution and sensitivity. HPAEC–PAD was not subject to interferences as was the case for low UV detection (210 nm) or RI analyses and was highly selective for mono- and aminosaccharides and glycuronic acids. The use of HPAEC–PAD was applied for the determination of the saccharide composition of organic materials (plant residues, animal wastes and sewage sludge), microbial polymers and soil.

INTRODUCTION

Saccharides play a major role as structural (*e.g.*, cellulose and hemicellulose) components of plants and provide a major source of energy for microbial processes when cycled in soil. Aminosaccharides have been reported in plants [1], microorganisms [2], crustaceae and insects [3] and an important source of organic nitrogen in soil [4]. Uronic acids occur in animal tissue [5], microbes [6] and plant [7] structures. In addition to energy and structural roles in animals, insects, microbes and plants, some of the more important microbial ecological functions of saccharides include conferring virulence of pathogenic bacteria, protecting organisms from dessication, regulating ionic traffic at the cell surface, concentrating nutrients, protecting microbes from toxic heavy metals or antibacterial agents and attachment to solid surfaces [8].

Detection of a wide diversity of monosaccharides, aminosaccharides and glycuronic acids in nature has lead to the development of many analytical techniques.

0021-9673/91/$03.50

Current methods for quantification of polysaccharides in biological materials include colorimetric assays [9–13], planar chromatography [14], gas chromatography (GC) [1,15,16] and high-performance liquid chromatography (HPLC) [18,19]. Colorimetric assays do not provide information on the composition of saccharides in biological materials and are often subject to many interferences. Planar chromatography has limitations in separation of similar saccharides. GC analyses involve the necessity of making derivatives. The most common method for quantification of saccharides with HPLC analysis is refractive index (RI) detection, which has several drawbacks. RI detection monitors the bulk property of the LC eluent, thus is non-selective, and cannot easily be used with gradient elution. It also lacks high sensitivity. Uronic acids can be analyzed by UV detection, although the use of low UV wavelengths (210 nm) is subject to interference from other low UV absorbing compounds. The use of high-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) has several advantages over RI and low-UV detection. PAD equipped with a gold electrode is selective only to compounds containing oxidizable functional groups including hydroxyl, amine and sulfide groups. Carboxylic acids and inorganic species do not interfere with detection by PAD. Glycosidic monomers derived from polysaccharides are weak acids with pK_a values in the range 9–13.5 and thus can be separated as anions by controlling the pH of the mobile phase.

Analysis of glycosidic units comprising polysaccharides presents many problems. The susceptibility of the glycosidic linkage to acid hydrolysis varies greatly along with the stability of the resulting monosaccharide unit in the acid solution. Pyranose forms of neutral monosaccharides exhibit the greatest stability to acid conditions, while the furanoses are more susceptible [20]. Polysaccharides containing aminosaccharides or uronic acid units are resistant to mild acid hydrolysis because of the amino and carboxylic moieties.

The objective of this study was to evaluate saccharide extraction, purification and detection of aminosaccharides, neutral saccharides and glycuronic acids present in various biological materials. HPAEC–PAD was used to detect the saccharide composition of various organic materials including plant residues, animal wastes, sewage sludge and soil extracts.

EXPERIMENTAL

Reagents

All neutral monosaccharide and aminosaccharide standards were obtained from Supelco (Bellefonte, PA, U.S.A.). D-Glucuronic acid, D-galacturonic acid and the enzymes, pectolyase from *Aspergillus japonicus* [reported to contain both endopolygalacturonase (EC 3.2.1.15) and endopectin lyase (EC 4.2.2.10)] and β-D-glucuronidase (EC 3.2.1.31) were obtained from Sigma (St. Louis, MO, U.S.A.).

Chromatographic instrumentation

HPAEC–PAD. The HPAEC–PAD analysis was performed on a Dionex (Sunnyvale, CA, U.S.A.) LC gradient pump module and a Model PAD2 detector. Sample injection was via a Dionex autosampler equipped with a 200-μl sample loop. Neutral monosaccharides, aminosaccharides and glycuronic acids were separated on a CarboPac PA1 pellicular anion-exchange resin (250 × 4 mm I.D.) and a CarboPac PA

guard column (25 × 3 mm I.D.) at a flow-rate of 0.8 ml min^{-1} at ambient temperature. Neutral monosaccharides were separated with the following gradient [21]:

Eluent A: 18 MΩ 0.22-μm filtered water; Eluent B: 50 m*M* sodium hydroxide containing 1.5 m*M* sodium acetate

	Eluent	
Time (min)	A (%)	B (%)
0	93	7
15	93	7
25	0	100
30	93	7

Aminosaccharides were separated with the following gradient [22]:

Eluent A: 18 MΩ 0.22-μm filtered water; Eluent B: 100 m*M* sodium hydroxide; Eluent C: 200 m*M* sodium hydroxide

	Eluent		
Time (min)	A (%)	B (%)	C (%)
0	95	5	0
15	95	5	0
20	0	0	100
25	0	0	100
30	95	5	0

Sodium hydroxide (200 m*M*) was used to elute interfering species that may act as displacing ions and shorten the retention times of subsequent runs. Uronic acids were separated with the following gradient [23]:

Eluent A: 18 MΩ 0.22-μm filtered water; Eluent B: 200 m*M* sodium hydroxide; Eluent C: 100 m*M* sodium hydroxide containing 250 m*M* sodium acetate

	Eluent		
Time (min)	A (%)	B (%)	C (%)
0	25	25	50
10	25	25	50
25	8	8	84
30	25	25	50

All mobile phases were degassed to prevent absorption of carbon dioxide producing carbonate. Carbonate will act as a displacing ion and shorten retention times. Detection was by triple-pulsed amperometry with a gold working electrode [24]. The following working pulse potentials (E) and durations (t) were used for the detection of saccharides: $E_1 = 0.10$ V ($t_1 = 300$ ms); $E_2 = 0.60$ V ($t_2 = 120$ ms); $E_3 = -0.60$ V ($t_3 = 60$ ms). The CHOH groups are oxidized at E_1, E_2 removes the reaction prod-

ucts, while E_3 cleans the electrode at a negative potential. Cyclic voltammetry was used to choose the three potentials. The PAD response time was set to 1 s. Chromatographic data were collected and plotted using the Dionex AutoIon 300 software. HPLC-RI and UV determinations were described in previous studies for aminosaccharides [22], neutral monosaccharides [21] and glycuronic acids [23].

Saccharide extraction

Neutral monosaccharides and glycuronic acids were extracted by the methods described by Martens and Frankenberger [21,23]. Briefly, the samples were pretreated with 0.4 ml 6 *M* H_2SO_4 for 2 h at room temperature and then refluxed with 0.5 *M* H_2SO_4 for 16 h at 80°C. Neutral saccharides in poultry manure and sewage sludge were extracted as described above and, in addition, refluxed with 3 *M* H_2SO_4 for 5 h at 80°C. Aminosaccharides were extracted by the method of Martens and Frankenberger [22] with a 6 *M* H_2SO_4 pretreatment and a 3 *M* H_2SO_4 reflux for 16 h at 90°C. The neutral monosaccharides, aminosaccharides and glycuronic acid samples were then treated with 1 ml 0.1 *M* EDTA, titrated to pH 4 with 5 *M* KOH and centrifuged at 10 000 rpm (RCF = 12 062).

Purification of acidic extracts

*Neutral monosaccharides.*The colored materials present in the refluxed organic materials (poultry manure, sewage sludge, straw and alfalfa) and soil extracts were removed by filtration through a Supelco solid-phase extraction column system composed of a 3-ml strong cation (SCX, 3-propylsulfonic acid, H^+) and a 3-ml strong anion (SAX, 3-quaternary propylammonium, Cl^-) column [21].

Aminosaccharides. A 1-ml aliquot of the refluxed organic amendments, microbial polymers, or soil extracts was diluted to 5 ml with water and passed through a Supelco 3-ml SCX solid-phase extraction column. The SCX column was then rinsed with 3 ml of water to elute all non-retained compounds. The aminosaccharides were eluted with 5 ml of 0.3 *M* HCl [22].

Glycuronic acids. A 1-ml aliquot of the acid-hydrolyzed sample was diluted to 5 ml and passed through a SCX column (Supelco). Pectolyase (3 units ml^{-1}; pH 5.5) or β-D-glucuronidase (30 units ml^{-1}; pH 6.8) were added to the SCX eluent and the mixture was incubated at ambient temperatures overnight (16 h). The enzyme–extract mixture was passed through a SAX column (Supelco) and rinsed with several ml of water. The glycuronic acids were then eluted with 0.1 *M* NaCl (pH 8.0) [23].

All samples were filtered before analysis through a Millipore type GS 0.22-μm filters (Bedford, MA, U.S.A.).

RESULTS AND DISCUSSION

Extraction

The following solvents have been reported for extraction of saccharides in biological materials: water, aqueous buffers, complexing reagents, mineral acids (0.25–12 *M*), organic reagents, alkali and combinations of the above [25]. The association between saccharides and soil constituents is believed to involve hydrogen and covalent bonds [26]. Sulfuric acid is often used as the extracting reagent to break these bonds.

Several reports have shown that aminosaccharides can be extracted from biological materials by treatment with hot mineral acids [1,27,28]. Isolation of uronic acids from polysaccharides, however, is very difficult because their glycosidic linkages are more stable than that of the neutral saccharides. Higher concentrations of mineral acids (3–6 M H^+) have been employed to release glycuronic acids [16,29,30], but these concentrations can also decarboxylate the free glycuronic residues [31] and convert them into lactones which are difficult to separate from the neutral saccharides [17].

Various extraction procedures have been used for quantifying saccharides in soils and organic amendments with different degrees of effectiveness. Seven monosaccharides in soil were detected by GC [15]. Angers *et al.* [19], using a lead-loaded cation-exchange column with HPLC–RI, detected five monosaccharides in soil. They recognized problems with coelution of certain monosaccharides with their technique. Coelution of saccharides with HPLC-RI is a common problem as illustrated in Fig. 1. A sample of barley straw was separated on a calcium-based resin column [21]. The solutes were collected with a fraction collector and analyzed with HPAEC–PAD. This work clearly shows that glucose and galactose coelute during the analysis. Retention times of standards analyzed by HPLC–RI indicated that complex materials may be prone to coelution of xylose, mannose and rhamnose with galactose and fucose with arabinose [21]. These saccharides must be resolved because they are important constituents of soils. HPAEC–PAD analyses indicated that in addition to these saccharides, inositol, mannitol and/or ribitol are also present in soils, plant residues, animal wastes and sewage sludge. A HPAEC–PAD chromatogram of ten saccharide standards shows the potential of ion-exchange chromatography for the analysis of saccharides with high resolution (Fig. 2).

Recent reports have suggested that saccharides determined in hot water extracts of soil samples by the anthrone–sulfuric acid analysis were significantly correlated

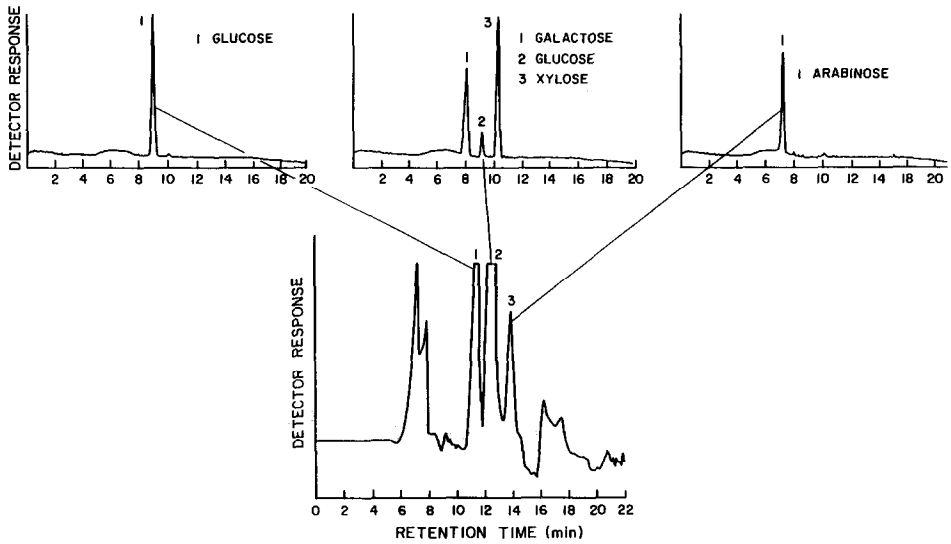

Fig. 1. Chromatogram of an acidic extract of barley straw detected by HPLC–RI. Insets are HPLC–RI fractions collected and then analyzed by HPAEC–PAD.

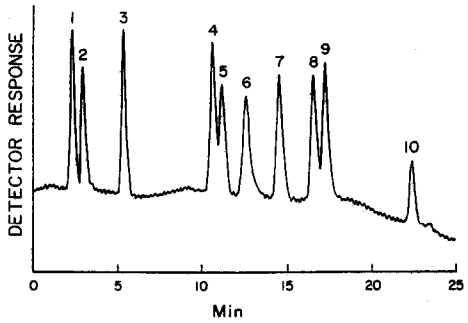

Fig. 2. Chromatogram of saccharides detected by HPAEC–PAD. Peaks: 1 = inositol; 2 = ribitol; 3 = fucose; 4 = arabinose; 5 = rhamnose; 6 = galactose; 7 = glucose; 8 = xylose; 9 = mannose; 10 = lactose (internal standard).

with the structural improvement in soil [32–33]. Martens and Frankenberger [34] found that little or no saccharides were extracted from organic-amended soil with hot (80°C) or cold (25°C) water and that the anthrone–sulfuric acid analysis of these extracts was prone to many interferences. However, the combination of heat (80°C) with H_2SO_4 concentrations as low as 0.13 *M* was effective in extracting saccharides (Fig. 3). The optimum concentration of H_2SO_4 for extraction of saccharides in soils and plant materials was determined to be 0.5 *M* (Table I). However, this $[H^+]$ is not

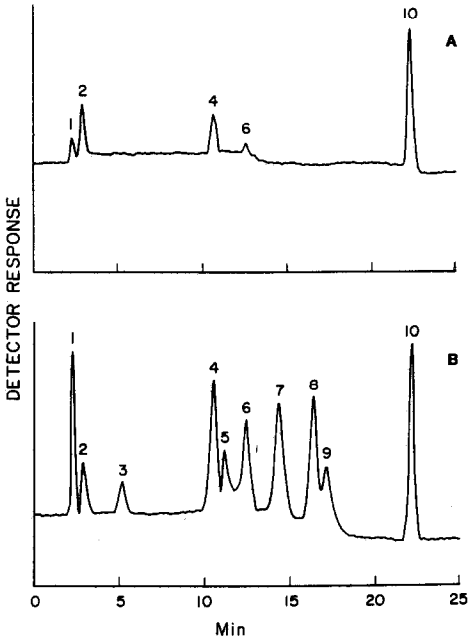

Fig. 3. HPAEC–PAD chromatograms of the acidic extracts of (A) straw-amended soil extracted with 2.5 *M* H_2SO_4 (25°C) and (B) straw-amended soil extracted with 0.13 *M* H_2SO_4 (80°C). Peaks as in Fig. 2.

TABLE I

COMPARISON OF H_2SO_4 CONCENTRATIONS AND HEAT ON EXTRACTION OF SACCHARIDES FROM BIOLOGICAL SAMPLES DETECTED BY HPAEC–PAD

Treatment	Saccharides extracted (mg kg^{-1})									
	Inositol	Ribitol	Fucose	Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose	Total
Soil										
0.13 *M* H_2SO_4 (80°C)	35	53	20	279	48	265	192	242	62	1196
0.5 *M* H_2SO_4 (80°C)	38	46	30	303	45	365	423	208	157	1616
2.5 *M* H_2SO_4 (25°C)	13	25	ND[a]	133	ND	Trace	ND	ND	ND	172
Poultry manure										
0.5 *M* H_2SO_4 (80°C)	213	232	160	2313	ND	ND	172	ND	ND	3090
3.0 *M* H_2SO_4 (80 °C)	300	104	231	2889	349	2055	2906	6425	488	15644
Earthworm casts										
0.5 *M* H_2SO_4 (80°C)	38	39	288	2210	396	1736	1099	2951	658	9518
3.0 *M* H_2SO_4 (80°C)	59	32	ND	318	ND	189	1196	320	462	2547

[a] ND = not determined.

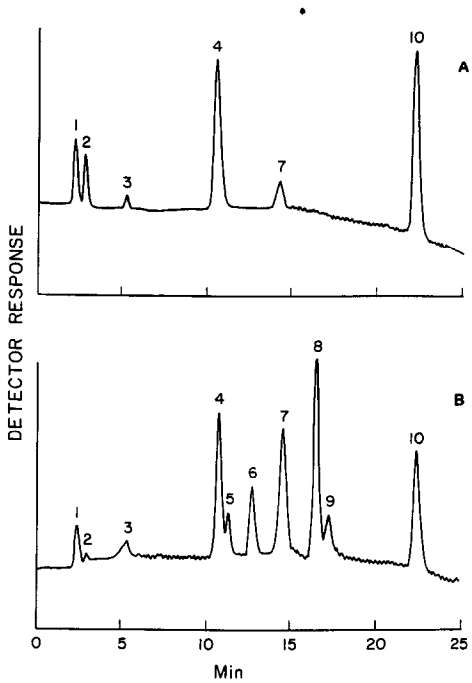

Fig. 4. HPAEC–PAD chromatograms of the acidic extracts of (A) poultry manure extracted with 0.5 *M* H_2SO_4 (80°C) and (B) poultry manure extracted with 3.0 *M* H_2SO_4 (80°C). Peaks as in Fig. 2.

effective for extracting saccharides in sewage sludge or poultry manure. Fig. 4 indicates that low levels of saccharides were extracted by 0.5 *M* H_2SO_4 from poultry manure and that much higher H_2SO_4 concentrations were required. The optimum H_2SO_4 concentration for saccharide determination in animal waste and sludge was 3.0 *M* H_2SO_4.

Research has shown that earthworm (*Lumbricus terrestris*) activity can have a beneficial effect on soil. Part of this benefit may be due to the breakdown of organic residues and deposition of saccharide-rich worm casts on the soil surface (Fig. 5).

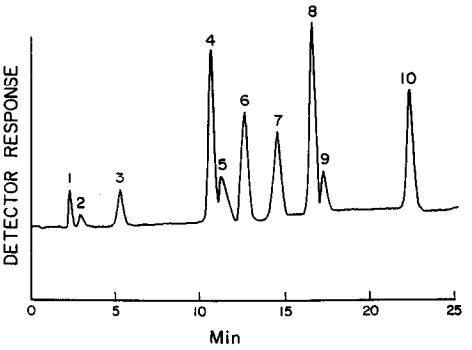

Fig. 5. HPAEC–PAD chromatogram of the acidic extract of worm casts (0.5 *M* H_2SO_4; 80°C). Peaks as in Fig. 2.

These casts were found to be 7.5 × richer in saccharide content than the surrounding soil (Table I). The optimum H_2SO_4 concentration for saccharide extraction from earthworm casts was determined to be 1 *M*.

Saccharide analysis of acid extracts of various biological materials by HPAEC–PAD has revealed new information on the composition of these materials. The saccharide composition of plant residues appears to be composed mainly of arabinose, galactose, glucose and xylose (Table II). The composition of poultry manure, sewage sludge and soil, in addition to the above four listed saccharides, included fucose, rhamnose and mannose units (Tables II and III). The analysis of worm casts showed the same trend as soil and animal wastes but tended to be richer in the saccharides arabinose, galactose, glucose and xylose, reflecting the decomposition of plant residues (Table I).

TABLE II

COMPOSITION AND QUANTITY OF AMINO-, MONOSACCHARIDE AND URONIC ACIDS EXTRACTED FROM ORGANIC AMENDMENTS

Polysaccharide material	Organic amendment (mg kg^{-1})			
	Poultry manure	Sewage sludge	Straw	Alfalfa
Monosaccharides				
Inositol	514	556	1042	11842
Ribitol	ND[b]	196	271	ND
Mannitol	337	ND	490	ND
Fucose	391	1320	ND	ND
Arabinose	5203	10503	26407	40961
Rhamnose	350	1846	ND	46
Galactose	2296	4409	8754	20222
Glucose	3079	3338	12457	31337
Xylose	6575	2599	154035	58045
Mannose	734	2930	ND	373
Total	19479	27697	203456	162826
Aminosaccharides				
Galactosamine	49	188	1405	1825
Mannosamine	39	90	230	685
Glucosamine	38	115	905	1212
N,N′-Diacetylchitobiose	187	84	13140	6040
Acetylgalactosamine	18	35	149	245
Acetylmannosamine	67	73	390	1565
Acetylglucosamine	60	59	968	1950
Total	450	643	17195	13533
Glycuronic acids				
Galacturonic acid	212	740	39790	34900
Glucuronic acid	195	987	58890	43880
Total	407	1787	98680	78780
Total[a]	20336	30127	319331	255139

[a] Sum of monosaccharides + aminosaccharides + glycuronic acids.
[b] ND = Not determined.

TABLE III

COMPOSITION AND QUANTITY OF AMINO-, MONOSACCHARIDE AND URONIC ACIDS EXTRACTED FROM ORGANIC AMENDED AND UNAMENDED SOIL

Polysaccharide material	Organic-amended soil (mg kg^{-1})				
	Poultry manure	Sewage sludge	Straw	Alfalfa	Check (unamended)
Monosaccharides					
Inositol	189	212	238	232	187
Ribitol	64	65	97	61	49
Fucose	91	88	88	94	82
Arabinose	421	386	419	404	288
Rhamnose	120	169	109	128	89
Galactose	407	413	391	332	292
Glucose	282	307	308	237	201
Xylose	708	342	497	335	252
Mannose	62	67	74	78	55
Total	2344	2049	2221	1901	1495
Aminosaccharides					
Galactosamine	4	5	5	13	1
Mannosamine	3	3	3	2	<1
Glucosamine	4	5	5	34	2
N,N′-diacetylchitobiose	6	3	4	5	1
Acetylgalactosamine	<1	1	13	1	1
Acetylmannosamine	2	3	3	0	1
Acetylglucosamine	3	2	3	1	<1
Total	24	23	36	56	7
Glycuronic acids					
Galacturonic acid	94	20	2	7	2
Glucuronic acid	28	132	16	42	11
Total	122	170	218	49	13
Total[a]	2490	2242	2275	2006	1515

[a] Sum of monosaccharides + aminosaccharides + glycuronic acids.

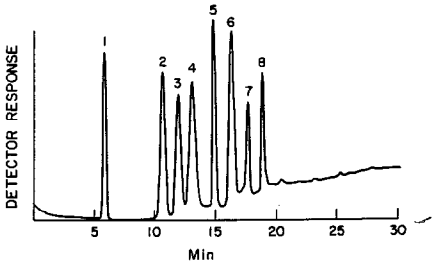

Fig. 6. Chromatogram of aminosaccharides detected by HPAEC–PAD. Peaks: 1 = 2-deoxyribose (internal standard); 2 = galactosamine; 3 = mannosamine; 4 = glucosamine; 5 = N,N′-diacetylchitobiose; 6 = acetylgalactosamine; 7 = acetylmannosamine; 8 = acetylglucosamine.

Many of the oligosaccharides found in plant, microbial and animal tissues consist of various combinations of neutral monosaccharides, aminosaccharides and uronic acids [20]. Fig. 6 shows the separation of seven aminosaccharides by HPAEC–PAD. Cation-exchange chromatography (SXC) [22] separates the aminosaccharides from the neutral monosaccharides and glycuronic acids released by the hydrolysis process. Aminosaccharides, principally chitin (acetyl-D-glucosamine repeating units), have been reported to be present in the cell walls of algae and fungi but have not been found as a component in higher plants [35]. Both straw (Fig. 7) and alfalfa were found to contain large amounts of aminosaccharides (Table II), with the majority of aminosaccharides present in straw as chitobiose (N,N'-diacetylchitobiose) [22]. In lower plants, chitin frequently replaces cellulose as the structural entity in cell walls and may have the same role in maintaining the structural integrity of straw and alfalfa. The aminosaccharide-N fraction in straw was found to account for about 20% of the total N content [22]. Aminosaccharides were also found to compose about 8% of a bacterial polymer produced by *Chromobacterium violaceum* [22] (Fig. 7). Incorporation of straw and alfalfa residues into soil and incubation for eight months resulted in decomposition of nearly all of the added aminosaccharides (Table III).

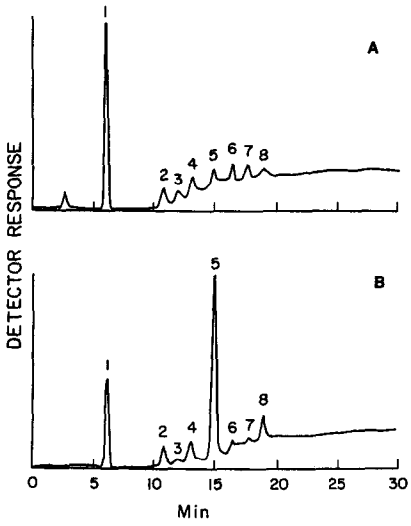

Fig. 7. HPAEC–PAD chromatograms of the acidic extracts (3.0 *M* H_2SO_4; 90°C) of (A) a bacterial polymer produced by *Chromobacterium violaceum* and (B) barley straw. Peaks as in Fig. 6.

The use of pectolyase and β-D-glucuronidase enzymes can be a powerful tool in the preparative separation and elucidation of the structure of polysaccharides. The use of mild acid hydrolysis (0.25 *M* H_2SO_4) to fragment the saccharide polymers coupled with enzyme catalysis released large amounts of uronic acids from the plant materials but released less from the animal waste or sewage sludge and only trace amounts from the organic-amended soil (Tables II and III). Fig. 8 shows a chromatogram of galacturonic acid and glucuronic acid as detected by HPAEC–PAD. Structural analysis of a hemicellulose fraction of barley straw indicated that uronic acids

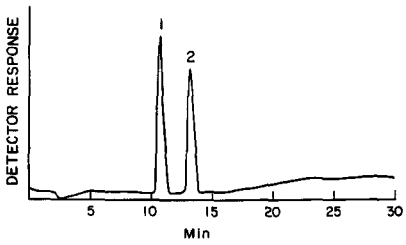

Fig. 8. Chromatogram of glycuronic acids detected by HPAEC–PAD. Peaks: 1 = galacturonic acid; 2 = glucuronic acid.

and L-arabinose monomers link the xylose chains to form the structural polymers [36]. Treatment of the barley straw extract with pectolyase resulted in the release of low levels of uronic acids but use of β-D-glucuronidase with the same extract resulted in a 10× increase in the release of uronic acids (Fig. 9). Incorporation of the straw residue into soil resulted in a rapid decomposition of added uronic acids and after eight months, only trace amounts were present (Table III).

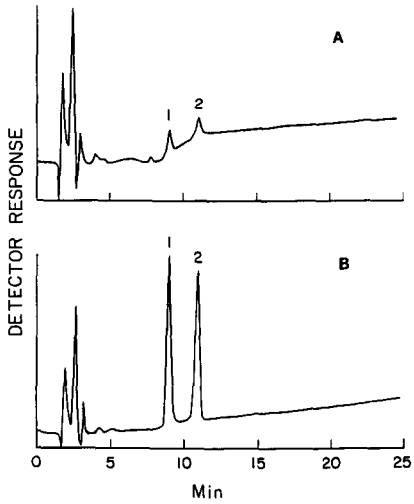

Fig. 9. HPAEC–PAD chromatograms of the acidic-enzyme extracts of (A) barley straw treated with pectolyase and (B) barley straw treated with β-D-glucuronidase. Peaks as in Fig. 8.

CONCLUSIONS

The work presented here showed that plant materials, animal waste, sewage sludge and soil contain neutral monosaccharides, aminosaccharides and glycuronic acids. The saccharide composition of each of these materials was quantified by optimizing the conditions for extraction, purification and chromatography. The use of HPAEC–PAD for separation of saccharides in these complex materials was superior to HPLC–RI and HPLC–UV in terms of sample preparation, resolution and sensitivity.

REFERENCES

1 L. Benzing-Purdie, *Soil Sci. Soc. Am. J.*, 48 (1984) 219.
2 H. R. Perkins, *Bact. Rev.*, 27 (1963) 18.
3 G. Ledderhose, *Berichte*, 9 (1876) 1200.
4 J. M. Bremner and K. Shaw, *J. Agr. Sci.*, 44 (1954) 152.
5 M. Stacey and S. A. Barker, *Carbohydrates of Living Tissues*, Van Nostrand, London, 1962, p. 1.
6 M. Stacey and S. A. Barker, *Polysaccharides of Microorganisms*, Oxford University Press, London, 1960, p. 8.
7 G. O. Aspinall, *Adv. Carbohyd. Chem. Biochem.*, 24 (1969) 333.
8 W. F. Dudman, in I. W. Sutherland (Editor), *Surface Carbohydrates of the Prokaryotic Cell,* Academic Press, New York, 1977, p. 357.
9 R. H. Brink, Jr., P. Dubach and D. L. Lynch, *Soil Sci.*, 89 (1960) 157.
10 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, 28 (1956) 350.
11 L. A. Elson and W. T. J. Morgan, *Biochem. J.*, 27 (1933) 1824.
12 A. H. Brown, *Arch. Biochem.*, 11 (1946) 269.
13 Z. Dische, *J. Biol. Chem.*, 167 (1947) 189.
14 M. V. Cheshire, C. M. Mundie and H. Shepherd, *Soil Biol. Biochem.*, 1 (1969) 117.
15 J. M. Oades, M. A. Kirkman and G. H. Wagner, *Soil Sci. Soc. Amer. Proc.*, 34 (1970) 230.
16 L. Benzing-Purdie and J. H. Nikiforuk, *Soil Sci.*, 145 (1988) 264.
17 S. A. Fazio, D. J. Uhlinger, J. H. Parker and D. C. White, *Appl. Environ. Microbiol.*, 43 (1982) 1151.
18 A. G. J. Voragen, H. A. Schols, J. A. DeVries and W. Pelnik, *J. Chromatogr.*, 244 (1982) 327.
19 P. A. Angers, P. Nadeau and G. R. Mehuys, *J. Chromatogr.*, 454 (1988) 444.
20 J. H. Pahur, in W. Pigman and D. Horton (Editors), *The Carbohydrates*, Vol. IIA, Academic Press, New York, 1970, p. 78.
21 D. A. Martens and W. T. Frankenberger, Jr., *Chromatographia*, 29 (1990) 7.
22 D. A. Martens and W. T. Frankenberger, Jr., *Talanta*, in press.
23 D. A. Martens and W. T. Frankenberger, Jr., *Chromatographia*, 30 (1990) 651.
24 S. Hughes and D. C. Johnson, *Anal. Chim. Acta,* 132 (1983) 11.
25 G. D. Swincer, J. M. Oades and D. J. Greenland, *Aust. J. Soil Res.*, 6 (1969) 211.
26 J. W. Parson and J. Tinsley, *Soil Sci.*, 92 (1961) 46.
27 L. Benzing-Purdie, *Soil Sci. Soc. Am. J.*, 45 (1981) 66.
28 J. M. Bremner, *J. Sci. Food Agr.*, 9 (1958) 528.
29 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484.
30 O. Raunhardt, *Dissertation*, ETH Zürich, Zürich, No. 4161, 1968.
31 O. Theander, in W. Pigman and D. Horton (Editors), *The Carbohydrates*, Vol. IB, Academic Press, New York, 1980, p. 1032.
32 R. J. Haynes and R. S. Swift, *J. Soil Sci.*, 41 (1990) 73.
33 R. S. Kinsbursky, D. Levanon and B. Yaron, *Soil Sci. Soc. Am. J.*, 53 (1989) 1086.
34 D. A. Martens and W. T. Frankenberger, Jr., *Soil Biol. Biochem.*, 22 (1990) 1173.
35 K. Ward, Jr. and P. A. Seib, in W. Pigman and D. Horton (Editors), *The Carbohydrates*, Vol. IIA, Academic Press, New York, 1970, p. 435.
36 R. L. Whistler and E. L. Richards, in W. Pigman and D. Horton (Editors), *The Carbohydrates,* Vol. IIA, Academic Press, New York, 1970, p. 447.