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POLYSACCHARIDES OF ACTIVATED SLUDGE PRODUCED BY TREATMENT OF THE MIXTURE OF TANNERY EFFLUENTS WITH SEWAGE

II. THE GEL PERMEATION CHROMATOGRAPHIC SEPARATION THE CHEMICAL CHARACTERIZATION OF THE HIGH-MOLECULAR WEIGHT FRACTION

The aqueous solution of polysaccharides of the activated sludge, obtained by extraction and deproteinization procedures described in detail (including sludge specification) in a preceeding communication [1], has been separated and analysed by the gel permeation chromatography method (GPC). The chromatographic separation has been performed using 0.02% aqueous sodium azide solution as eluent and the gel SPHERON P-1000 The detection system included a differential refractometer and an UV analyser-254 nm, connected in series. For the calibration of the chromatographic column system dextrane standards have been used; the plot of log M_{peak} vs. V_e shows a linear relationship in the molecular weight range between 10^4 and 3.4×10^6. The separation resulted in three distinct peaks. The high molecular weight fractions, i.e. the peaks I and II, have been characterized by both molecular weights (peak I: $\overline{M}_n = 1.30\times10^6$, $\overline{M}_w = 1.56\times10^6$; peak II: $\overline{M}_n = 8.55\times10^4$, $\overline{M}_w = 1.43\times10^5$) and their respective distributions. Polysaccharides have been, moreover, separated by preparative GPC. The yield of the high molecular weight fraction has been high enough to enable its more detailed chemical characterization.

By the identification using the paper chromatography method after hydrolysis, the presence of six simple monosaccharides has been proved. There was no proof of either ribose or desoxyribose which, in connection of a low phosphorus contents established by quantitative elementary analysis and a low UV-254 nm absoprtion, excludes the possibility of presence of nucleic acids, probably entrained by extraction. On the other hand, the fraction corresponding to peak II shows a strong absorption at 254 nm and the proof of ribose in its hydrolysates was very significant; this can be explained by the presence of the ribonucleic acid. A comparative determination of the weight average molecular weight of the high molecular weight polysaccharide fraction by the light scattering method resulted in a value of 1.50×10^6. This result confirms the value obtained by the GPC method verifying simultaneously the correctness of the entire chromatographic analysis. The determination of IR spectra showing a character similar to the one of standard cellulose and starch spectra, provided

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evidence of a range of functional groups and thus confirmed that the biopolymer investigated is a polysaccharide — a heterosaccharide including in its molecular structure several constituents different as to their nature.

The final confrontation of all analytical results obtained provided a range of important and interesting relationships; some discrepancies established have been explained by the sorption of inorganic ions on the polysaccharides investigated.

1. INTRODUCTION

During several past years the GPC has become doubtless the most efficient method of polymer separation on both analytical and preparative scale. It is well known that in principle any molecules differing as to their dimensions can be separated by GPC provided that they are soluble in some solvent. Substantial results of the analytical separation, i.e. molecular weights (MW) and their distributions (MWD) can be obtained relatively easily and rapidly in comparison with other techniques. If the standards used for the calibration of the GPC system are equivalent from point of view of their character to the polymers investigated, both precision and accuracy of results are very good.

In a preceeding paper [1] a detailed extraction and isolation procedure of polysaccharides contained in the activated sludge produced by the treatment of tannery effluents with sewage have been reported. In the present work the GPC separation of aqueous polysaccharide solutions obtained by extraction is described providing on the analytical scale both MW and MWD values and on the preparative scale adequate yields of high molecular weight fractions. This yield of high molecular weight fractions of polysaccharides is high enough to enable their detailed chemical characterization. As a base for the chromatographic separation of polysaccharides investigated a detailed literature review concerning the GPC separations of various polysaccharides has been set up. This review, being beyond the scope of the present work, will not be discussed here.

2. EXPERIMENTAL

2.1. GPC SYSTEM

All the separations described have been performed using a modular chromatograph including in its assembly the following essential parts: pump, model MC 706 (Mikrotechna, Prague, Czechoslovakia); sample injector, a six port valve with interchangeable sample injection loops, volumes 0.23 ml and 1.0 ml; detectors, i.e. differential refractometer R-403 (Waters Assoc., Milford, Mass., U.S.A.) and differential UV analyser, 254 nm (Development Shops of ČSAV, Prague, Czechoslovakia); recorders, models EZ 11 and TZ 21S (Laboratorni přistroje, Prague, Czechoslovakia); flow measuring device, photo-

electric siphon counter; inlets and connections; stainless steel capillaries, int. diameter 1 mm; fraction collector SF 62 (Laboratorni přìstroje, Prague, Czechoslovakia).

Both analytical and preparative separations have been performed using four stainless steel columns (dimensions of 7.8 mm×1220 mm) packed with SPHERON P-1000, produced on commercial scale (Lachema Brno, Czechoslovakia) by the copolymerization of 2-hydroxyethyl-methacrylate and ethylen-di-methacrylate in a non-polar dispersive medium [2, 3]. A 0.02% sodium azide solution in distilled water (bacteriostat) was applied as the eluent.

It is generally known that the azide exerts no interaction with saccharides and/or proteins and does not affect their chromatographic behaviour. Details concerning the packing and the overall chromatographic characteristics of the columns used have been reported previously [4]. The separation efficiency of the entire four column system has been tested by repeated injections of 0.5% ethylene glycol solution in the eluent; at a sample volume of 0.23 ml and a flow rate of 20 ml / h^{-1}, the average value of the number of theoretical plates (before starting separations) was 10.650 (i.e. 2200 theoretical plates per one meter of the gel column length).

2.2. CALIBRATION

The assembled GPC system has been calibrated using dextran standards (Pharmacia Fine Chemicals, Uppsala, Sweden). Dextrans are polysaccharides based on glucose molecules, produced as a result of bacterial activity. Considering the object investigated in these studies, their application appears to be particulary suitable. Specifications of the abovementioned standards, provided by the producer, include the average molecular weight ($\overline{M}_w$), the number of average molecular weight ($\overline{M}_n$) and differential and integral distri-

Table 1

Calibration of the GPC system
Cechowanie systemu chromatografii (GPC)

Dextran	$\overline{M}_w \times 10^{-3}$	$\overline{M}_n \times 10^{-3}$	$M_{peak} \times 10^{-3}$	log M_{peak}	V_e, cm^3
T 2000	2000	—*	—	—	113.0
T 500	516	212	330.7	5.51943	130.0
T 250	240	121	170.4	5.23147	137.3
T 150	154	86	115.1	5.06108	139.2
T 110	100.5	68	82.7	4.91751	144.4
T 70	71.7	41.6	54.6	4.73719	148.1
T 40	39.5	29.5	34.1	4.53275	155.4
T 20	22.3	15	18.3	4.26245	161.4
T 10	10.4	6.2	8.0	3.90309	169.8

* Values not given by the producer.

bution curves. As the polydispersity of the standards supplied was not low enough ($\overline{M}_w/\overline{M}_n > 1.1$), not even an approximate validity of the equality $\overline{M}_w = \overline{M}_n$ could be assumed and thus it was necessary to calculate for each individual standard the molecular weight corresponding to the top value of its chromatographic peak M_{peak}. The above-mentioned relationships have been applied in these calculations [5, 6]. Results together with the calibration data obtained are listed in table 1 and plotted in figure 1. The calibration

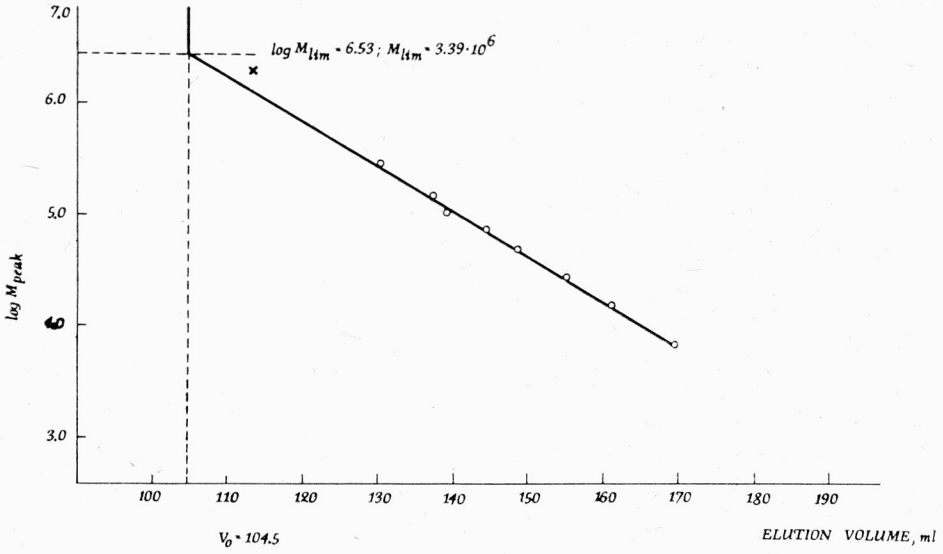

Fig. 1. Calibration of the GPC system
○ — dextran standards, × — dextran T-2000 (log $\overline{M}_w$)

Rys. 1. Cechowanie systemu chromatografii (GPC)
○ — wzorce dekstranowe, × — dekstran T-2000 (log $\overline{M}_w$)

has been performed by injecting 0.23 ml portions of the standard solution in the eluent at a flow rate of 34.5 ml/h^{-1}. The elution volume values (V_e) listed are the ones corresponding to peak maxima on the refractometer record. The calibration data of dextrans given in table 1 have been further processed by the least square method and as a result the calibration curve (straight line) of the GPC system has been obtained; the analytical expression of the calibration curve is:

$$\log M_{peak} = 10.6404 - 0.0396\, V_e. \quad (1)$$

Moreover, the value of the free volume (V_0) and the respective value of the limiting molecular weight (M_{lim}), by which the exclusion limit of the applied gel is determined, are marked in figure 1. The accepted value of free volume was the elution volume on the chromatographic record marked by starting of elution of the high molecular weight por-

tions of T 2000 and T 500 dextrans which are eluted simultaneously, their dimensions being considerably in excess of the exclusion limit of the gel applied. The elution volume of glucose, i.e. the monosaccharide determining by its dimensions the permeation limit of the gel utilized for saccharide separation, is 197. 1 ml.

2.3. PREPARATIVE ASSEMBLY

For preparative separation purposes the sample volume injected has been increased by inserting a sample injection loop of 1 ml in volume. By repeated testing with ethylene glycol it has been established that the separation efficiency was only slightly reduced. At the column exit a fraction collector has been fitted; the collection of the individual polysaccharide fractions was time-programmed according to a series of preliminary chromatograms (see fig. 2). The sample injection was obviously controlled by the time-programming.

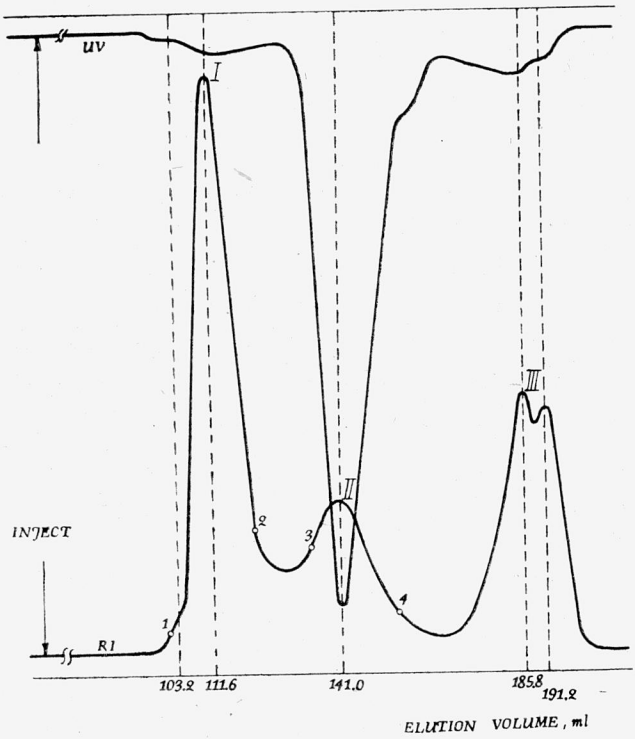

Fig. 2. Chromatogram of the GPC separation of activate sludge polysaccharides

RI — refractometric detection; UV — detection by UV-254 nm absorption, applying SPHERON P-1000 gel, four columns 7.8×1220 mm; 1 cm³ volume of sample injected; flow rate of 34.5 cm³/h

Rys. 2. Chromatogram rozdzielania polisacharydów osadu czynnego

RI — wykrywanie refraktometryczne, UV — wykrywanie przez adsorbcję UV-254 nm stosując żel SPHERON P-1000, cztery kolumny 7,8×1200 mm, objętość wtryskiwanych próbek — 1 cm³, a prędkość przepływu 34,5 cm³/h

2.4. GPC SEPARATION PROCEDURE OF POLYSACCHARIDES INVESTIGATED

The dialysed polysaccharide solutions samples collected from individual parallel extractions have been concentrated to 80 ml volumes in a vacuum evaporator at a temperature of about 40 °C and filtered through a filter with pore size of 1500±400 nm. The filtrates were stored at a temperature of about 5 °C and successively injected in 1 ml portions into the GPC system. The injected portions were subject to additional filtration through a filter inserted in the injection valve (pore size of 600±100 nm). As a result of a series of preliminary experiments this filter pore size has been established as the optimum for the final filtration before injection. The use of a filter with the pores, e.g. of 170±30 nm, increased the proportion of macromolecules trapped to such a level that any chromatographic peaks could be hardly observed on the record of GPC. This is in agremeent with the published data reporting the diameters of the helixes of various polysaccharides in aqueous solutions as being equal up to 200 nm and the length of the rod-shaped polysaccharide macromolecules as equalling 350 nm. The collected polysaccharide fractions corresponding to the individual chromatographic peaks from 80 injections were concentrated to volumes 20 to 25 ml, precipitated and isolated using the method previously described for the isolation of the initial extract [1].

2.5. DEGRADATION OF POLYSACCHARIDES

The isolated polysaccharides have been subject to hydrolysis by 4 N sulphuric acid in a sealed glass test tubes (about 60 mg of polysaccharide dry mass plus 3 ml of acid). After cooling the test tubes have been opened and the hydrolysate neutralized by addition of $BaCO_3$, using Congo red as indicator; the precipitated barium sulphate has been removed by centrifugation. According to our experience and data reported in the bibliography, concerning direct hydrolysis of the activated sludge [7], the use of a more diluted acid is inefficient.

2.6. PAPER CHROMATOGRAPHY

Monosaccharides in the hydrolysates of the isolated polysaccharides have been identified using paper chromatography method (PC). The best chromatograms have been obtained by using the elution system n-butanol–acetic acid–water (4 : 1 : 5). The blend is thoroughly mixed, allowed to stand for at least 24 hours and then the upper layer is used. Chromatograms have been developed on a SS 2040 paper (Schleicher-Schuell A.C., Feldbach, Switzerland) during 31 hours using descending technique till overrun. Two reagents were used for the detection: aniline–citric acid mixture and triphenyltetrazolium chloride.

2.7. LIGHT SCATTERING

For comparative purposes, the $\overline{M}_w$ value of the high-molecular weight polysaccharide fraction, obtained by the preparative GPC separation, has been determined by the light scattering method using the SOFICA apparatus ($\lambda = 546$ nm, $\Theta = 30$–$150°$, $t = 25°C$) in a 0.1 M aqueous KCl solution. The refractive index increment ($dn/dc = 0.153$) has been measured on the differential refractometer Brice-Phoenix. Analytical solutions of the high-molecular weight fraction (six concentrations, the highest being 3×10^{-3} g · ml^{-1}) have been centrifugated to obtain optical clarity.

2.8. IR SPECTROSCOPY

Films prepared from the high-molecular weight polysaccharide fractions, dissolved in formic acid, have been measured on an AgCl prism (apparatus Perkin Elmer Model 283) under conditions commonly used for quantitative measurements.

3. RESULTS AND DISCUSSION

The repeated verifications of the extraction procedure involving activated sludge samples and covering a time interval of almost one year provided an important information, that there are no significant variations in the composition of the activated sludge extracts as to their character and that differences exist in quantitative composition of the individual extracts. No changes have been observed in the number and character of peaks in the individual GPC records, the size of peaks was, however, variable. Elution volumes varied in a certain range, being, however, quite narrow.

All the chromatographic separations have been performed from extracts obtained by five parallel extractions of a single activated sludge sample; the resulting polysaccharide solutions have been subsequently mixed (thorough homogenization of the five extracts).

3.1. ANALYTICAL GPC SEPARATION

GPC analysis of polysaccharides of activated sludge resulted in chromatographs recorded from two detectors — a differential refractometer and an UV analyser-254 nm. Examples of both chromatographic records are shown in fig. 2. The GPC analysis provided essentially three distinct chromatographic peaks (I, II and III) which can be particularly well observed on the refractometric record. The *MW* and *MWD* values have been determined for the fractions corresponding to peaks I and II of the refractometric record. The both peaks are included in the high-molecular weight range. Chromatograms have been evaluated according to the procedure described by PICKETT et al. [8]. Since a computer program based on the above procedure does not includ the correction for spreading

of chromatographic zones, that is why the obtained polydispersity values are higher than the actual polydispersities of polymers analysed. Some problems were caused by the fact that peaks I and II are not completely separated (till the base line of the chromatographic record). This difficulty has been eliminated by usual procedure (taking in consideration the apparent distribution), i.e. by graphical approximation of both peaks to the base line. The final results of the GPC analysis are given in tab. 2. The differential

Table 2

Molecular weights of activated sludge polysaccharides determined by the GPC method
Ciężary cząsteczkowe polisacharydów osadu czynnego oznaczone metodą chromatograficzną (GPC)

Fraction	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$*	$\overline{M}_w/\overline{M}_n$
Peak I	1303812	1560512	1825865	1.197
Peak II	85459	143299	226068	1.677

* $\overline{M}_z$ — average value of high-molecular weight portions.

distribution curves of the both high-molecular weight fractions of polysaccharides are shown in figs. 3 and 4. The shapes of the distribution curves being in agreement with the Poisson distribution confirm the assumption that the polysaccharides investigated are for-

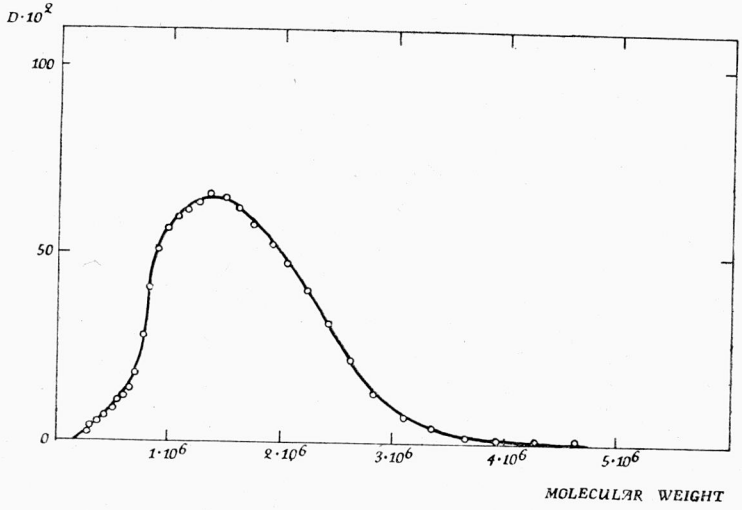

Fig. 3. Differential curve of a molecular weight distribution of the high-molecular weight fraction of polysaccharides of activated sludge (chromatographic peak I)

Rys. 3. Krzywa różniczkowa rozkładu ciężaru cząsteczkowego wielkocząsteczkowej frakcji wagowej polisacharydów osadu czynnego (pik chromatograficzny I)

med by a really natural polycondensation process providing the most probable distribution of molecular weight. The distributions obtained provide a proof of adequacy of the method applied for MWD determination.

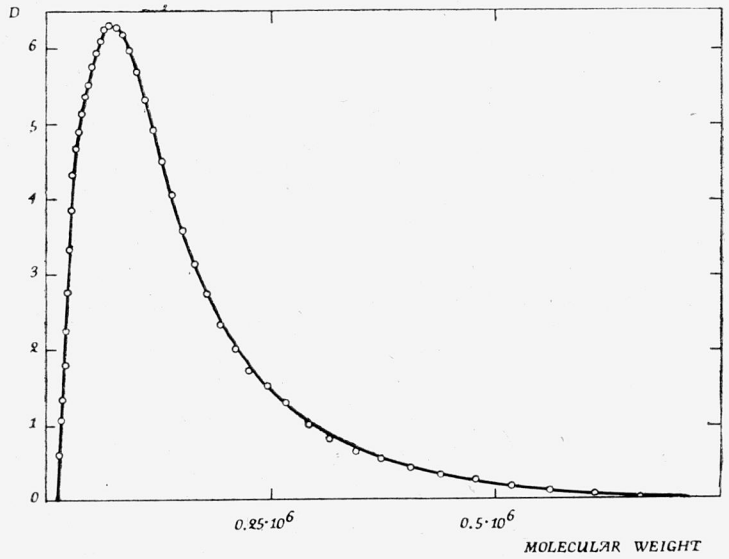

Fig. 4. Differential curve of molecular weight distribution of polysaccharides of activated sludge (chromatographic peak II)

Rys. 4. Krzywa różniczkowa rozkładu ciężaru cząsteczkowego wielkocząsteczkowej frakcji wagowej polisacharydów osadu czynnego (pik chromatograficzny II)

The chromatographic peak III lies on the dextran calibration curve in the region of elution volumes (fig. 1) corresponding to molecular weights below the permeation limit of the GPC system applied and thus the analysis could not be evaluated. The chromatographic separation and chemical characterization of the polysaccharide fraction recorded as the peak III will be described and discussed in the next paper.

3.2. PREPARATIVE GPC SEPARATION

The collected fractions I and II have been marked by points I to 4 on the chromatogram in fig. 2. The time required for separation of one injected portion was almost 6 hours. In sum, complete preparative separations of 255 extracted polysaccharide portions have been performed within this study. A survey of respective yields is given in tab. 3. The isolated matter is hygroscopic and swelling before being solved in water.

Table 3

Yields of the preparative GPC separation of activated sludge polysaccharides
Wydajność wstępnego oddzielenia polisacharydów osadu czynnego metodą chromatograficzną (GPC)

Peak	Sampling	Yield, mg	Isolate appearance
I	1– 80	185.7	After precipitation white fibrous matter, after drying becoming a brittle and breakable beige-coloured mass
	81–160	187.9	
	161–240	150.0	
	241–255	42.45	
II	1– 80	57.62	Light-brown flocculated matter
	81–160	28.71	
	161–240	25.57	
	241–261	—*	

* The fraction collected has not been isolated.

3.3. IDENTIFICATION OF MONOSACCHARIDIC CONSTITUENTS

Results of identification of constituents of individual hydrolysates are given in tab. 4.

Hydrolysates of the polysaccharide fraction corresponding to the peak I have been further chromatographed using several solvent systems recommended for saccharides in the bibliography. Application of well-known methods of sugar detection yielded at most six spots. Detection with ninhydrine and isatin aiming to trace the eventual presence of amino sugars or amino acids provided no evidence of that.

Table 4

Identified monosaccharide constituents of activated sludge polysaccharides
Oznaczone monosacharydowe składniki polisacharydów osadu czynnego

Hydrolysate of the total extract	Hydrolysate of the peak I	Hydrolysate of the peak II
D-galactose	D-galactose	D-galactose
D-glucose	D-glucose	D-glucose
D-mannose	D-mannose	D-mannose
D-arabinose	—	—
D-xylose	D-xylose	D-xylose
D-ribose	—	D-ribose
L-fucose	L-fucose	L-fucose
L-rhamnose	L-rhamnose	L-rhamnose

3.4. STUDIES OF HIGH-MOLECULAR WEIGHT POLYSACCHARIDE FRACTION

From the above-mentioned results the conclusion can be drawn that the polysaccharide fraction corresponding to the chromatographic peak I is that with the highest MW value and usually present in the highest amounts. Yields obtained by the preparative GPC separation were high enough as to enable its more detailed chemical characterization.

$\overline{M}_w$ values determined for comparative purposes using the light scattering method (Zimm's graph) have shown polydispersity of the fraction investigated and, moreover, the presence of a small amount of much larger particles in addition to the main portion. The $\overline{M}_w$ value, including the effect of the larger particles (extrapolation based on the entire angular range), is equal to 3.2×10^6, while that in which it is eliminated (by neglecting values measured in angles 30°–60°) equals to 1.5×10^6. This result appears to be in surprisingly good agreement with the value obtained by GPC method. Small amounts of the large particles present in the high-molecular weight fraction seem to have no substantial effect on the shape as well as on the elution volume of the peak I and thus neither MWD nor $\overline{M}_w$ values are influenced. On UV record from GPC separation the presence of those large particles is probably registered as a peak with elution volume 103.2 ml. On the RI record this peak can be hardly observed, there is just a slight noise at the foot of the peak I. It appears thus to be a very low portion of an accompanying macromolecular substance different as to its nature, exerting a substantially higher UV-254 nm absorption than the main portion. Although according to light scattering results those particles are much larger in size than the main portion, they could not be separetad by GPC method, the peak I being quite proach ($V_e = 111.6$) to the exclusion limit of the gel applied ($V_0 = 104.5$) above which the separation does not work. Results of the quantitative elementary analysis performed under consideration of the hygroscopic character of the sample are given in tab. 5.

Table 5

Quantitative elementary analysis of the high-molecular weight polysaccharide fraction
Elementarna analiza ilościowa wielkocząsteczkowych frakcji wagowych polisacharydów

Constituent	C	H	N	P	S	Incombustible residue
Contents, %	38.29	5.82	6.02	0.12	0.90	14.15

In figure 5 IR spectra of the high-molecular weight polysaccharide are reproduced including functional groups determined, i.e. OH group (associated), as well as NH, CH, C=O (possible alternatives being —COOR, COOH—, —CO—, —COH), CO—NH, CH_3 or CH_2, CO—C and CO groups, the latter present as C—OH. To enable a more detailed specification of the C=O group providing an absorption band at the wave num-

ber of 1725 cm^{-1}, the sample in the form of film has been converted into its sodium salt. A shift of the absorption maximum in the new IR spectrum to the wave number of 1605 cm^{-1} confirms that the involved C=O group belongs to the carboxyl one.

Results of evaluation by electron microscopy and X-ray analysis provided mutual confirmation that the high-molecular weight polysaccharide fraction, isolated by the procedure described, is a biopolymer which is neither crystalline nor oriented.

3.5. CONFRONTATION OF RESULTS

The *MW* and *MWD* values determined for the high-molecular weight polysaccharide fraction have been checked using an independent method, the light scattering one. An excellent agreement of the results of the both methods confirms good separation properties of the gel SPHERON P-1000 for the given purpose on one hand and the expected suitability of dextran standards for the calibration of the GPC system assembled on the other.

By comparing the results of analyses of the high-molecular weight polysaccharide fraction, a range of important and interesting relationships and also some discrepancies have been established; for the latter it has been possible to find only a questionable explanation. From the ratio of the respective areas on RI and UV-254 nm records an unequivocal conclusion can be drawn, that the fraction corresponding to the peak I exerts, in comparison with the peak II, a very low UV-254 nm record. Significance of the statement is supported by the respective quantities of both fractions in the sample (see results of preparative separation). This means that this particular biopolymer is unlikely to contain any groups absorbing in UV-254 nm range and, what is important, any accompanying nucleic acids which could possibly be coextracted and could exert absorption maxima in the proximity of the wavelenght used for measurements. These conclusions are in agreement with the results of identification of the monosaccharide constituents in this fraction, providing no evidence of either ribose or desoxyribose, as well as with the low contents, of phosphorus which have been established. On the other hand, the fraction corresponding to the peak II shows a high absorption at UV-254 nm wavelength which could be possibly explained by presence of ribonucleic acid; this is in agreement with a distinct proof of ribose and with a substantial phosphorus contents (2.02%) stated in the original extract before separation.

The IR spectra of the high-molecular weight fraction are, in general, similar to the spectra of cellulose or starch, confirming that the compound involved is a polysaccharide (fig. 5). Relatively high number of functional groups established suggests the presence of a heterosaccharide which is in agreement with the number of monosaccharidic constituents identified. The established functional groups are not fully consistent with the results of the quantitative elementary analysis. No functional groups have been established to explain the sulphur contents determined (0.9%). The nitrogen contents (6.02%) seem to be related to the presence of functional groups —NH— and —CONH—. Identification by the paper chromatography method, to which has been given particular attention, pro-

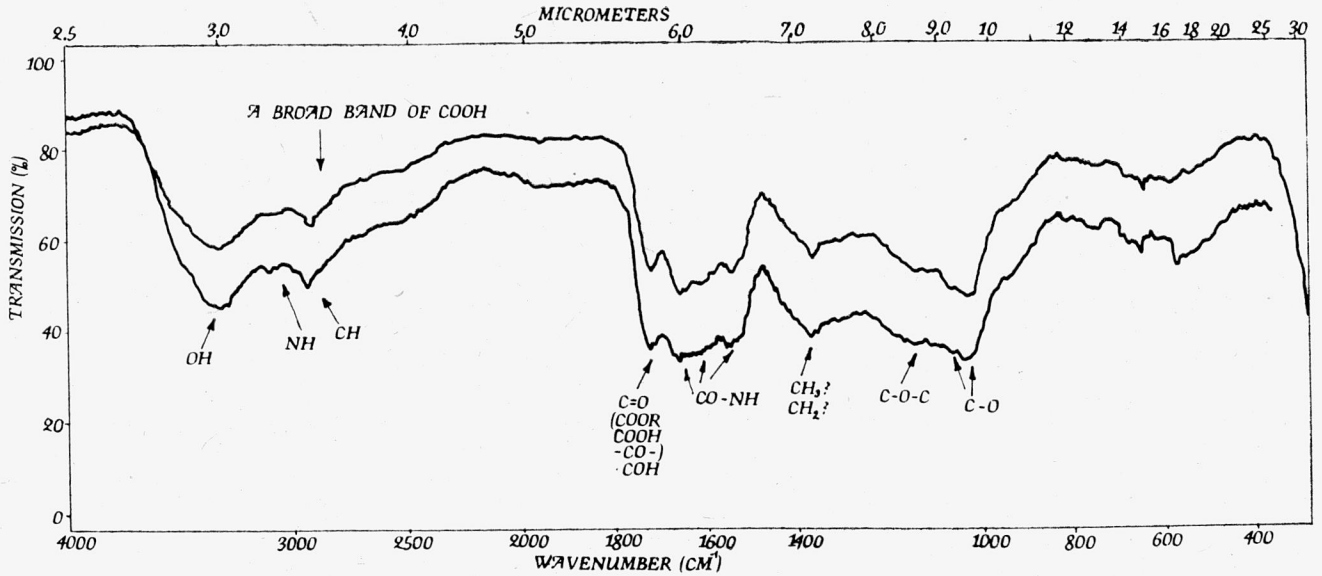

Fig. 5. Infrared spectra of the high-molecular weight fraction of activated sludge polysaccharides (two parallel measurements)

Rys. 5. Widma IR wielkocząsteczkowych frakcji wagowych polisacharydów osadu czynnego (dwa równoległe pomiary)

vided, however, no proof of any nitrogen-containing monosaccharide. Considering this fact (note that the stechiometric contents of nitrogen in e.g. N-acetylglucosamine is as low as 6.3%) the nitrogen contents is far too high as to be explained by the sensitivity limit of aminosaccharide detection. The sulphur contents together with an unadequately high nitrogen contents and a substantial non-relative solids are probably due to the sorption of inorganic ions on the polysaccharide analysed which is not negligible. This sorption has been tested by increasing doses of sodium azide in to the aqueous solution of extracted polysaccharides. This has been manifested in GPC chromatograms by increasing azide peaks and, moreover, by an increase in the respective areas of all the peaks involved (particulary of the peak I). The sorption of inorganics (e.g. of Na_2S which is present in tannery effluents in fairly high quantities) might be the only explanation of the established sulphur contents.

Presence of —COOH group should be reasonably connected with the presence of some monosaccharide constituent containing this group. Here are to be considered D-glucuronic and/or D-galacturonic acids which are quite often found in natural matter. Acidic hydrolysis, even if performed under quite mild conditions, induces, however, decomposition (particulary decarboxylation) of the uronic acids originated; e.g. when D-glucuronic acid is boiled in 12% hydrochloric acid, decarboxylation occurs with simultaneous formation of the respective pentose (D-xylose). In fact, D-xylose has been found in the hydrolysates of the high-molecular weight fraction and also in hydrolysates of the original extract and of the fraction corresponding to peak II. Its chromatographic spot was quite distinct in comparison with other spots detected.

4. CONCLUSIONS

The GPC separation of biopolymers contained in the aqueous phase of the phenol–water extract of the tannery activated sludge provided three distinct chromatographic peaks. Calibration of the GPC system using dextran standards enabled a fast and accurate determination of both molecular weights and their distributions; the values found being checked by the light scattering method. Separations carried out on a preparative scale qrovided adequate yields of both first and second fractions of the biopolymer extracted, from which particulary the highest molecular weight fraction could be chemically characterized on a conclusive level. The results have shown unambiguously that the fraction investigated is a polysaccharide — heterosaccharide having in its molecular structure six simple monosaccharides and that it is not contaminated by nucleic acids. Thereby the assumption of selectivity of the extraction procedure applied has been confirmed.

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POLISACHARYDY OSADU CZYNNEGO OTRZYMANE PRZEZ OCZYSZCZENIE MIESZANINY ŚCIEKÓW GARBARSKICH I KANALIZACYJNYCH

II. CHARAKTERYSTYKA CHEMICZNA WIELKOCZĄSTECZKOWEJ FRAKCJI WAGOWEJ

Wodny roztwór polisacharydów osadu czynnego rozdzielano i analizowano za pomocą metody chromatograficznej. Zastosowano 0,2% wodny roztwór azydku sodu jako eluent i żel Spheron P-1000. W wyniku rozdziału otrzymano trzy różne piki. Piki I i II odpowiadały frakcjom wielkocząsteczkowym. Ilość wysokocząsteczkowej frakcji wagowej była wystarczająco duża, by wykonać dokładną analizę chemiczną. Wykryto obecność sześciu monosacharydów. Nie stwierdzono rybozy ani dezoksyrybozy, wykluczając przez to obecność kwasów nukleinowych, które prawdopodobnie były usunięte podczas ekstrakcji. We frakcji odpowiadającej pikowi II stwierdzono rybozę i jej hydrolizaty, co można tłumaczyć obecnością kwasu rybonukleinowego. Porównawcze badania średniego ciężaru cząsteczkowego wielkocząsteczkowej frakcji polisacharydów metodą rozproszenia potwierdziły wyniki analizy chromatograficznej.

POLYSACCHARIDE IM BELEBTSCHLAMM VON GERBEREI- UND KOMMUNALABWÄSSER

II. CHEMISCHE CHARAKTERISTIK EINER MAKROMOLEKULAREN WÄGEFRAKTION

Eine wässrige Lösung von Polysacchariden des Belebtschlammes wurde chromatographisch getrennt. Angewandt wurde eine 0,2% Lösung des Natriumazides als Elutionsmittel und Gel Spheron P-1000. Als Resultat der Trennung kamen drei verschiedene Ausschlag-Spectren zum Vorschein. Ausschläge I und II entsprechen den großmolekularen Fraktionen. Die Menge der hochmolekularen Wägefraktion war entsprechend groß, um eine genaue chemische Analyse zu ermöglichen. Ermittelt wurden sechs Monosaccharide. Nicht nachweisbar waren Ribose und Desoxyribose was auf die Abwesenheit von Nukleinsäuren deutet (welche möglicherweise durch die Extraction entfernt wurden). In der Fraktion II war sowohl Ribose wie deren Hydrolysate feststellbar, was auf die Anwesenheit von Ribonukleinsäure deutet. Vergleichende Untersuchungen des durchschnittlichen Molekulargewichts der großmolekularen Polysaccharide-Fraktion mittels der Dispersionsmethode, haben die Ergebnisse der chromatographischen Analyse voll bestätigt.

ПОЛИСАХАРИДЫ АКТИВНОГО ИЛА, ПОЛУЧЕННЫЕ ПУТЁМ ОЧИСТКИ СМЕСИ КОЖЕВЕННЫХ И КАНАЛИЗАЦИОННЫХ СТОЧНЫХ ВОД

II. ХИМИЧЕСКАЯ ХАРАКТЕРИСТИКА ВЫСОКОМОЛЕКУЛЯРНОЙ ВЕСОВОЙ ФРАКЦИИ

Водный раствор полисахаридов активного ила разделялся и анализировался с помощью хроматографического метода. Был применён 0,2% водный раствор азида натрия в качестве элюента и гель Сферон Р-1000. В результате разделения были получены три разные пики. Пики I и II соответствовали высокомолекулярным фракциям. Количество высокомолекулярной весовой фракции было достаточно большим, чтобы произвести точный химический анализ. Было выявлено наличие шести моносахаридов. Не обнаружено рибозы, ни дезоксирибозы, исключая тем самым присутствие нуклеиновых кислот, которые вероятно были удалены во время экстракции. Во фракции, соответствующей пику II, было выявлено наличие рибозы и её гидролизата, что можно объяснить присутствием рибонуклеиновой кислоты. Сравнительные исследования среднего молекулярного веса высокомолекулярной фракции полисахаридов методом рассеяния подтвердили результаты хромотографического анализа.