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

### PART III. SEPARATION BY GEL PERMEATION CHROMATOGRAPHY AND CHEMICAL CHARACTERIZATION OF THE LOW MOLECULAR WEIGHT FRACTION

This is the concluding paper of a series of communications, published by the authors in this magazine [3, 4]. In the previous papers the separation of aqueous polysaccharide solutions, extracted from the activated sludge by means of a high performance gel permeation chromatography (GPC), has been described. In this paper a low molecular weight fraction separated by means of a high performance gel permeation chromatographic (GPC) system is analysed with respect to its chromatographic and chemical efficiency. It is essentially a saccharidic biopolymer of molecular weight about  $1 \times 10^4$ , containing in its chain D-galactosamine, L-arabinose and L-rhamnose. The character of this fraction depends on the nature of pollution fed into the activated sludge system.

#### 1. INTRODUCTION

In the first communication of this series [3], we have described the procedures of both extraction and isolation of polysaccharides from the activated sludge, treating the tannery effluents mixed with municipal sewage. The second communication [4] deals with the GPC separation of aqueous polysaccharide extracts. The GPC separation yielded three distinct chromatographic peaks; two high molecular weight fractions (peaks I and II) were subject to chemical analysis, resulting in chemical characteristics of the fraction denoted by peak I. The present paper deals with the analysis of the low molecular weight fraction denoted on the GPC record by peak III [4]. Using a more efficient GPC system this fraction was next separated in two fractions (peaks III A, III B) and attempts have been made to characterize chemically the fraction III A.

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## 2. EXPERIMENTAL

## 2.1. GPC SYSTEMS

Four modular GPC systems used for all analytical and preparative separations differed only in the lengths and packings of chromatographic column and the sample size. The apparatus was assembled according to schemes and knowledge generally adopted in up-to-date liquid chromatographs. Several functional elements were used in all the systems. A schematic lay-out of the GPC systems used is shown in fig. 1.

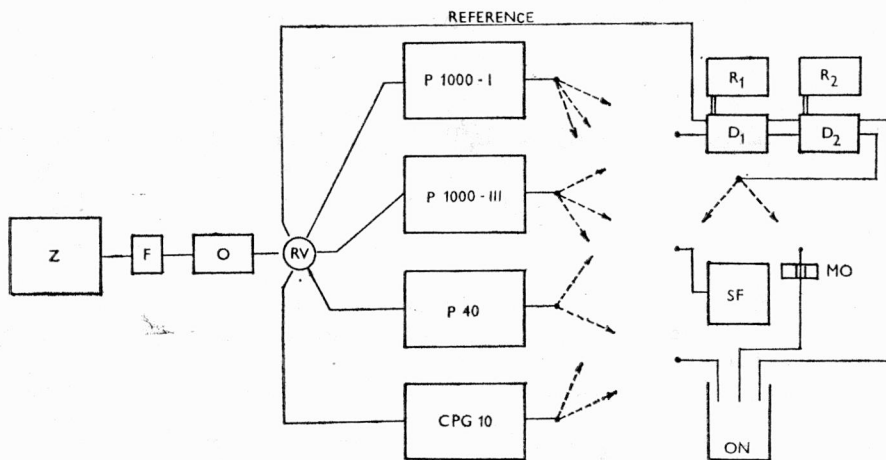


Fig. 1. Set-up of the GPC systems

*Z* – eluent storage tank (0.02% sodium azide solution in distilled water); *F* – filter; *O* – degasifier; *RV* – six-way distribution valve; *D*<sub>1</sub> – detector 1 (differential refractometer R-403, Waters Ass. Inc. Milford, Mass., U. S. A.); *D*<sub>2</sub> – detector 2 (differential UV analyzer 254 nm, Vyojové dílny ČSAV, Praha, ČSSR); *R*<sub>1</sub>, *R*<sub>2</sub> – recorders; *MO* – measuring device of elution volume (photometric siphon counter build in our laboratory, volume of about 5.5 cm<sup>3</sup>, signals to *R*<sub>1</sub> recorders); *SF* – fraction collector, type SF 62, Laboratorní přístroje, Praha, ČSSR (used in the case of preparative separations); *ON* – exhauster

Rys. 1. Schemat systemów GPC

*Z* – zbiornik zasobnikowy eluenta (0.02% roztwór azydku sodu w wodzie destylowanej); *F* – filtr; *O* – odgazowywacz; *RV* – sześciokanałowy zawór dozujący; *D*<sub>1</sub> – detektor 1 (refraktometr dyferencyjny R-403, Waters Ass. Inc. Milford, Mass., U.S.A.); *D*<sub>2</sub> – detektor 2 (analyzer dyferencyjny UV, 254 nm, Vyojové dílny ČSAV, Praha, ČSSR); *R*<sub>1</sub>, *R*<sub>2</sub> – rejestratory; *MO* – měřnick objemnosti eluenta (fotometryczny lewarowy licznik zbudowany w naszym laboratorium, objemność około 5,5 cm<sup>3</sup>, signály do rejestratorův *R*<sub>1</sub>); *SF* – kolektor frakcji, typ SF 62, Laboratorní přístroje, Praha, ČSSR (używany w przypadku rozdzielania przygotowanego), *ON* – ekshauster

## 2.2. SYSTEM P 1000-I

This system consists of three stainless steel columns (7.8 mm × 1200 mm) packed with the gel SPHERON P 1000 [1, 2] and a pump MC 706 (Mikrotechna, Praha; flow rate 41.85 cm<sup>3</sup>/h, volume of the sample injection loop 0.23 cm<sup>3</sup>). It was used only for preparative purposes aiming to obtain the III A fraction. The efficiency of this system was 21 200 theoretical plates (testing material – ethylene glycol).

## 2.3. SYSTEM P 1000-III

This system is analogical to the preceding system, used first for preparative purposes and later for analytical separations of polysaccharide extracts aiming at the determination of individual fraction volumes, molecular weights and molecular weight distributions. Testing and calibration of both systems were similar [4]. Application of the testing mixture Dextran T 2000 and ethylene glycol (flow rate 41.0 cm<sup>3</sup>/h, injection loop volume 0.083 cm<sup>3</sup>) provided by following values of efficiency parameters:  $N = 22059$ ,  $k = 1.047$ ,  $N_{\text{eff}} = 5753$ . The calibration relationship, derived for dextrane standards [4] in the form:

$$\log M_{\text{peak}} = 9.9646 - 0.05441 V_e,$$

was linear for molecular weights ranging within  $3 \times 10^3$ – $1.5 \times 10^6$ . For preparative purposes 2 cm<sup>3</sup> injection volume loops were used in both the systems (without substantial change of the separation efficiency). The outlets from both systems were connected with fraction collectors and the collection of individual fractions was programmed according to the previous chromatographic records.

## 2.4. SYSTEM P 40

Three stainless steel columns (7.8 mm × 610 mm) were packed with gel SPHERON P-40, size of swollen particle being 25–32 μm. At the flow rate of 37.5 cm<sup>3</sup>/h and injection loop volume of 0.01 cm<sup>3</sup>, the following values of the efficiency parameters have been obtained:  $N = 14333$ ,  $k = 0.995$ , and  $N_{\text{eff}} = 3565$ .

The calibration relationship in the form:

$$\log M_{\text{peak}} = 8.6877 - 0.0745 V_e,$$

derived by using dextrane standards [4], was linear for molecular weights ranging within  $2 \times 10^3$ – $10^5$ .

## 2.5. SYSTEM CPG 10

Stainless steel column (7.8 mm × 1220 mm) was filled with deactivated porous glass chromatographic packing CP6 000 75 C (Electro/Nucleonic, Inc. Fairfield, N. J., U.S.A.) according to the instructions provided by the producer. Test with Dextran T 2000 and ethylene glycol standard mixture (flow rate 30.0 cm<sup>3</sup>/h, injection loop volume 0.083 cm<sup>3</sup>) yielded the following values of separation efficiency parameters:  $N = 937$ ,  $k = 0.689$ ,  $N_{\text{eff}} = 162$ . Calibration with dextrane standards resulted in the following relationship:

$$\log M_{\text{peak}} = 7.3556 - 0.1077 V_e$$

which was linear within the range of molecular weights  $3 \times 10^3$  and  $3 \times 10^4$ .

## 2.6. SEPARATION OF EXTRACTED POLYSACCHARIDES BY GPC

The extraction of polysaccharides from activated sludge has been described in detail earlier [3]. It should be noted that the extracts obtained from 20 g sludge portions (referred to dry substance and one extract) were concentrated in a vacuum evaporator at 40° C to a volume of 50 cm<sup>3</sup> — denoted by *B*. The extracts from 40 g samples were concentrated to 80 cm<sup>3</sup> volumes — denoted by *B*<sup>2</sup>. This increase in sample weight was due to low contents of the fraction to be analyzed. Before the chromatographic analysis the polysaccharide solutions were subject to a two-step filtration, (first filter: pore size 1500 ± 400 nm, second filter: 600 ± 100 nm). The last filtration being made immediately before injection into the GPC system. The systems P 1000-I and P 1000-III were used for preparative separations aiming to obtain an adequate volume of the III A fraction. In both cases the injection loop volume was 2.0 cm<sup>3</sup>, flow rates ranging between 33.4 and 34.4 cm<sup>3</sup>/h.

The molecular weight and molecular weight distribution of this fraction were determined using the systems P 40 (sample volume 0.01 cm<sup>3</sup>, flow rate 37.5 cm<sup>3</sup>/h) and CPG 10 (sample volume 0.083 cm<sup>3</sup>, flow rate 29.9 cm<sup>3</sup>/h). For the determination of the total extraction yield and for the subsequent analytical investigation of the fraction III A, polysaccharides in solid state were required. Therefore the polysaccharide solutions, obtained by extraction or by GPC separation of extracts, were evaporated in vacuum (40° C) to a very small volume and precipitated by addition of a sixfold volume of acetone-alcohol mixture (1 : 1) and then by addition of sodium acetate solution (1/30 of the precipitation mix volume). The polysaccharides precipitated (total extract in the form of yellow-brown fibrous clutches or the III A fraction forming yellowish flocs) were washed successively with alcohol, acetone and vacuum dried to constant weight.

## 2.7. HYDROLYSIS OF THE POLYSACCHARIDE FRACTION III A

The polysaccharidic fraction was hydrolysed to monosaccharidic constituents by 4N H<sub>2</sub>SO<sub>4</sub> at 105° C for 12 hours. Detailed procedure has been described earlier [4]. Results of preliminary tests (positive ninhydrine test — proof of primary amines; negative isatine test — detection of the presence of amino acids; positive reactions with some agents like aniline-citric acid, aniline-diphenylamine, periodate-benzidine, confirming the presence of aminosaccharides) have shown that the analyzed fraction contains aminosaccharides. In such cases it is recommended in the bibliography to use hydrochloric acid for the hydrolysis, as it induces only slight destruction of glucoseamine. This method, however, appeared to be inefficient in our case.

## 2.8. IDENTIFICATION OF THE MONOSACCHARIDIC CONSTITUENTS OF THE III A FRACTION BY PAPER CHROMATOGRAPHY (PC)

After a series of preliminary experiments we have chosen two generally known solvent systems:

1) RS-1 (n-butanol-acetic acid-water 4:1:5; the mixture is thoroughly shaken and after minimum 24 hours standing the upper layer is used);

2) RS-2 (pyridine-ethyl acetate-acetic acid-water 5:5:1:3); the atmosphere in the chromatographic chamber is saturated with the mixture pyridine-ethyl acetate-water 11:40:6.

Chromatographic paper SS 2040 was used for the development of samples, with descending technique till overflow — due to low  $R_F$  values of the constituents. Two reagents were used for the detection: aniline-citric acid and solution of ninhydrine in ethanol (blue-violett colouring of aminosaccharide spots, non-substituted on nitrogen). The reagent aniline-citric acid enables resolution in colour between the aldopentoses and aldohexoses. Two chromatograms were performed for each sample using one of the above reagents respectively.

### 3. RESULTS AND DISCUSSION

Three sets of extracts were prepared for both analytical and preparative separations: B 23 — 27, B 30 — 35 and B<sup>2</sup> 38 — 49. Extracts within each set were obtained from one sample of activated sludge, treated by a parallel extraction procedures. The B 23 — 27 set was prepared simultaneously with the extracts B 20 and B 22. The latter were used solely for the determination of total yields of polysaccharides obtained by extractions with and without deproteinization. The results are the following:

B 20 yield without deproteinization 1258 mg,

B 22 yield after deproteinization 232 mg,

B 23 — 27 sum of yields of all the fractions separated by chromatography, obtained from one extract, equals 151 mg.

This comparison shows that the deproteinization of raw polysaccharide extracts is justified [3]. The difference between the total yield after deproteinization and the sum of yields of all the fractions from one extract, supposed to be the loss of polysaccharides during the preparative separation by GPC, is due to our attempts made to obtain the purest possible III A polysaccharide fraction.

#### 3.1. CHROMATOGRAPHIC SEPARATION

A typical chromatogram of the polysaccharide extract separation on the P 1000-III system is shown in fig. 2. The value of retention volume of the III A fraction ( $137.46 \text{ cm}^3$ ) shows clearly that this fraction is eluted beyond the linear range of the calibration curve (see the retention volume range of the calibration of system P 1000-III) and, moreover, beyond the range of simple monosaccharides. It should be noted that the dialytic membranes, used in the extraction procedure, are supposed to separate the molecules of the minimum size, corresponding to molecular weight of about 5000 (as determined by the test with dextrane standards). This, together with the symmetric shape of the chromato-

graphic peak, suggests the special character of this fraction. The prepared extracts were separated by chromatography using systems P 1000-I and P 10000-III working in parallel with a common fraction collector. The collection of fractions was performed automatically according to a programme based on retention volumes given in fig. 2.

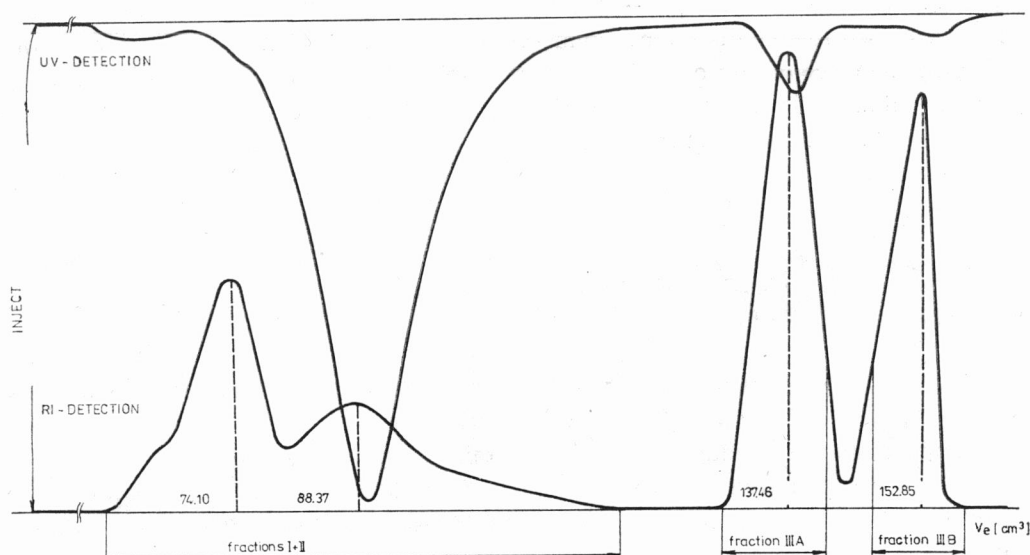


Fig. 2. Chromatogram of the polysaccharide extract from activated sludge

Rys. 2. Chromatogram ekstraktu polisacharydowego z osadu czynnego

Results obtained for individual sets of samples are summarized in tab. 1 (yields of the other fractions are given for comparison). It can be seen that the relative quantitative proportions of the individual fractions are practically constant.

Table 1

Yields of isolated polysaccharides  
Ilości wyizolowanych polisacharydów

Series	Fraction	<i>a</i> mg	<i>b</i> mg	<i>c</i> %
B 23-27	I+II	709.55	141.91	93.63
	III A	45.30	9.06	5.98
	III B	3.00	0.60	0.39
B 30-35	I+II	1 285.19	214.20	93.78
	III A	71.74	11.96	5.24
	III B	13.46	2.24	0.98
B <sup>2</sup> 38-49	I+II	5 033.70	419.48	94.67
	III A	276.54	23.05	5.01
	III B	210.99	17.58	0.32

*a* — total yield of the respective fraction within one series.

*b* — yield of the respective fraction from one extract of the series.

*c* — aliquot yield of the respective fraction from the total yield (i. e. from the sum of yields of all fractions) within one series.

## 3.2. QUANTITATIVE ELEMENTARY ANALYSIS

Because of the hygroscopic character of samples, all analyses were made simultaneously, immediately after the sealed tubes were opened. Results are listed in tab. 2. The high ash content is probably due to substantial adsorption of inorganic ions. This corresponds to relatively lower contents of organic matter and to considerable concentrations of sodium and sulfur. The adsorption of sodium azide from the GPC eluent was additionally confirmed by the results of IR spectroscopic analysis (paragraph 3.3), while there was no proof as to sulfur-containing functional groups.

Table 2

Quantitative elementary analysis of the III A fraction  
Elementarna analiza jakościowa frakcji III A

Series	C	H	N	S	P	Na	Ash
	%						
B 23-27	23.03	3.39	3.47	0.59	x	x	39.2
B 30-36	13.68	1.75	6.97	4.05	0.73	22.60	48.1
B <sup>2</sup> 38-49	9.30	1.78	18.18	20.3	1.11	27.33	57.7

x - not determined.

## 3.3. ANALYSIS BY IR SPECTROSCOPY

Aqueous polysaccharide solutions were prepared from the samples (stored in the sealed tubes). They were purified by centrifugation, cast on an AgCl glass to form films and analysed under usual conditions for quantitative measurements. Samples of the B<sup>2</sup> 38-49 formed discrete regions rather than a continuous film.

In this case spectra were measured on the original sample (without centrifugation). A schematic reproduction of the obtained IR spectra is given in fig. 3.

The spectra recorded show characteristic features of overlapping spectra of inorganic substances. The effect of inorganic constituents is the most pronounced in samples of the B<sup>2</sup> 38-49 series; it is less distinct in samples from both B 23-27 and B 30-35 series. Therefore, an unambiguous interpretation of results was impossible.

The overall character of the above spectra and particularly the unpronounced peak, corresponding to the C-H bond, are in agreement with the results of quantitative elementary analysis, indicating lower proportion of organic matter in the sample. Since it may be assumed that sodium azide is adsorbed on the polysaccharide investigated, spectrum of this substance was equally recorded. Therefore the resulting adsorption bands corresponding to those observed in the sample spectra are supposed to be sodium azide (see fig. 3). The following functional groups could be determined: OH, H<sub>2</sub>O, (NH<sub>2</sub>), -CH, -C=O, -C=O, NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup> or P-O-C.

|  
H

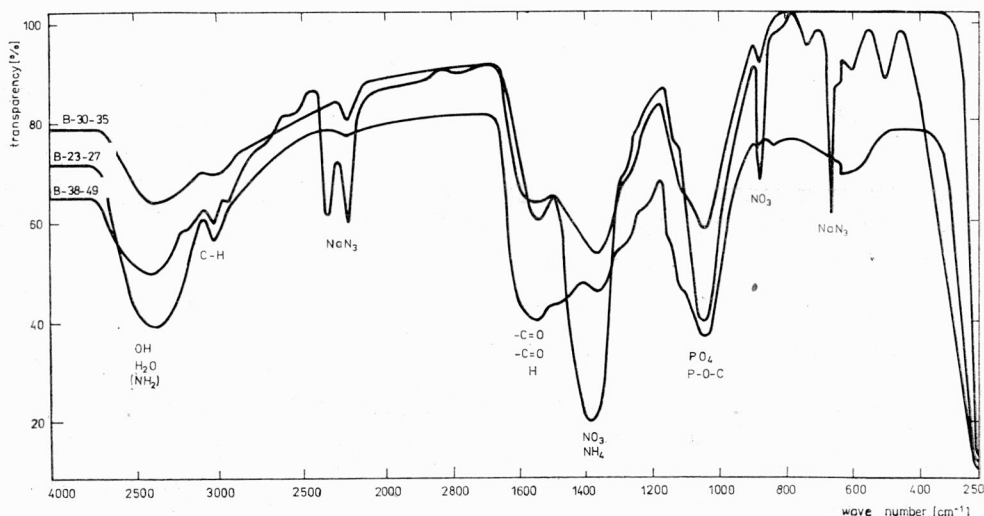


Fig. 3. Schematic reproduction of IR spectra of the III A fraction

Rys. 3. Schematyczne odtwarzanie widm podczerwieni frakcji III A

Peaks corresponding to the group  $\text{NH}_2$  (the presence of which is suggested by determination of monosaccharidic constituents — positive proof of aminosaccharides) are in the obtained spectra overlapped by peaks corresponding to OH or  $\text{H}_2\text{O}$  groups. As far as we know, analogous spectra have not been described in the literature yet.

#### 3.4. IDENTIFICATION OF MONOSACCHARIDIC CONSTITUENTS BY PC

Only samples of the B<sup>2</sup> 38-49 were available in quantities (separated polysaccharides) enabling additional analysis by PC. A series of chromatograms in preliminary experiments were evaluated using the hydrolysate of the polysaccharide investigated, standard monosaccharides being taken as references. As a result of these tests a reference mixture was designed to be applied to one path of each chart.

In the earlier experiments the following available mono-, di- and trisaccharides were run together with the sample: L-arabinose, cellobiose, 2-deoxy-D-glucose, 2-deoxy-D-ribose, D-erythrose, D-galacturonic acid, D-fructose, D-fucose, D-galactonic acid, D-galactosamine hydrochloride, D-glucosamine hydrochloride, D-galactose, D-glucose, glucurone lactone, D-glucuronic acid, lactose, D-maltose, D-mannose, D-raffinose, L-rhamnose, D-ribose, saccharose, L-sorbose, talose, D-tagatose, D-xylose, and N-acetyl-D-glucosamine.

The hydrolysate of the III A fraction gave seven spots corresponding to various monomeric units among which only three monosaccharidic constituents could be identified, despite the high number of the applied references. Positive proof was obtained of



the D-galactosamine presence while the identification of two other spots (corresponding to aminosaccharides) was not successful. The positive proof of L-arabinose is in agreement with the results reported in the previous communication [4]. L-rhamose was the third substance identified, while four monosaccharidic constituents could not be matched. They are assumed to be uncommon monosaccharides or perhaps nonsaccharidic substances which could explain the particular behaviour of the III A fraction.

### 3.5. DETERMINATION OF MOLECULAR WEIGHTS AND MOLECULAR WEIGHT DISTRIBUTIONS

Isolated polysaccharides were dissolved in the eluent (0.02% aqueous sodium azide solution) to obtain  $0.5 \text{ mg/dm}^3$  solutions. The dissolution was difficult and proceeded slowly. Most of the undissolved flocs disappeared as late as after 5 day swelling, followed by heating in a water bath ( $60^\circ \text{C}$ ) for one hour.

Considering the fact that the polysaccharide of the III A fraction on the gel SPHERON P 1000 is eluted beyond the linear region of the calibration curve, another GPC system was prepared for the molecular weight analysis (column packing gel SPHERON P 40, molecular weight separation ranging within  $3 \times 10^3$ – $4 \times 10^4$ ). This system appeared equally unsuitable for the determination of molecular weights of the polysaccharides of III A fraction, the retention volume of the respective peak being again beyond the linear range of the calibration curve. This observation supports the assumption that polysaccharide investigated has a certain affinity to the gels SPHERON. A third chromatographic

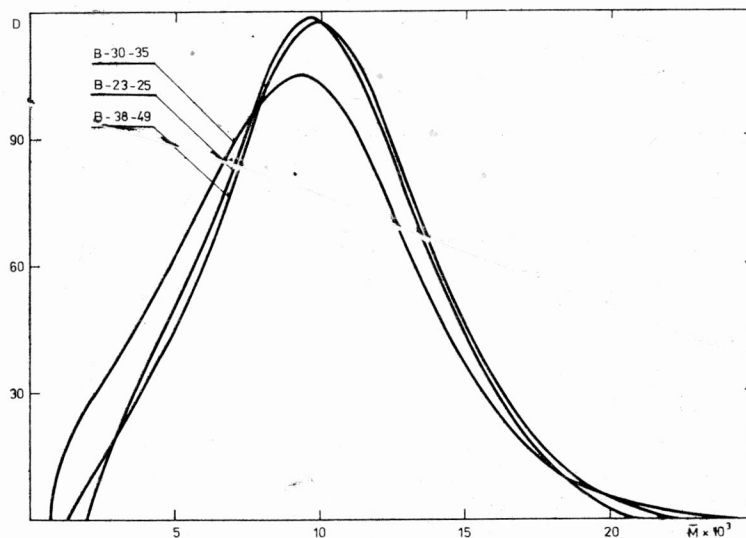


Fig. 4. Differential distribution curves of the III A fraction

Rys. 4. Rozkład różniczkowy krzywych frakcji III A

system (inert glass column packing CPG 10, separation range roughly equal to the one of the gel P 40) has been designed. Here the polysaccharidic III A fraction provided a main peak, eluted unmistakably in the linear region of the calibration curve, and an additional small peak, eluted at the upper limit of the molecular weight separation range (this fraction is assumed to be formed by agglomeration of several macromolecules from the original polysaccharide during swelling and dissolution).

Table 3

Average molecular weights and polydispersity  
of the III A fraction  
Średnie ciężary cząsteczkowe  
i polidispersyjność frakcji III A

Series	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_z$	$\bar{M}_w/\bar{M}_n$
B 23-27	8190	9609	10807	1.173
B 30-35	6663	8907	10521	1.337
B <sup>2</sup> 38-49	8231	9764	10996	1.186

Analysis of the refractive index (RI) record of the main peak, namely the computation of  $\bar{M}_w$ ,  $\bar{M}_n$ ,  $\bar{M}_z$  values as well as of the differential and integral distribution functions, was made using an automatic digital computer Odra 1204 with a special computing programme in the Algol language. The results are summarized in tab. 3 and the differential curves plotted in fig. 4. The differential distribution curves are similar in shape to the normal distribution, suggesting that the biopolymer is formed by a random natural condensation process.

### 3.6. DETERMINATION OF MOLECULAR WEIGHTS BY LIGHT SCATTERING

The measurements involved the samples from the three above series; contents of insolubles was about 10%. The prepared solutions (solvent H<sub>2</sub>O+0.1 M KCl) after centrifugation were brownish in colour which to some extent could contribute to lack of reliability of results.

Refractive index increments were measured on a differential refractometer Brice Phoenix using three different concentrations of a sample from the B<sup>2</sup> 38-49 series. The value of  $du/dc$  was 0.176.

Light scattering was measured on the Sofica apparatus in green light ( $\lambda = 546$  nm) at angles ranging between 30° and 150° using three concentrations in each case. Before measurements the samples were purified by centrifugation.

The results obtained with a Wang computer given in tab. 4 are interpreted as follows: The measurements of the samples from the B<sup>2</sup> 38-49 series can be considered as being reliable; the assymetry of samples from the B 30-35 series (proportional to the range

Table 4

Molecular weights of the III A fraction determined by light scattering  
Ciężary cząsteczkowe frakcji III A oznaczone metodą rozpraszania światła

Series	$M_w$	$A_2$
B 23-27	150000	—
B 30-35	270000	$16.2 \times 10^{-4}$
B <sup>2</sup> 38-49	80000	$-6.1 \times 10^{-4}$

$A_2$  — 2nd virial coefficient.

or particle swelling) decreases with the decreasing concentration (i.e. sample dilution), while the samples from the B 23-27 series shown opposite behaviour (the behaviour of this sample as such was anomalous). Therefore the  $M_w$  value determined is not reliable.

#### 4. CONCLUSIONS

Based on the analytical results, the III A fraction of the polysaccharide extract from the activated sludge, treating tannery effluents, which has been obtained by means of GPC system, can be characterized as follows:

The quantitatively yield of a saccharidic biopolymer fraction containing in its chain hexosamine, namely D-galactosamine, L-arabinose and L-rhamose, is very low amounting only to about 5 to 6% of the sum of yields of all the fractions. After precipitation from aqueous solutions it forms flocs and after drying a yellow-brown powder, dissolving very slowly in water (after several hours of swelling). Its molecular weight is about  $1 \times 10^4$ ; the extent of polydispersity considering the natural character of the polymer is very low (1.1-1.3). Unusual behaviour of this fraction is also manifested by considerable bonding of inorganic salts (low organic matter contents in the isolated samples) and by its affinity to the gel SPHERON packings.

All the above analyses (except for the identification of monosaccharidic constituents by PC) were performed using three samples of the III A fraction, which differed only in their origin (time of activated sludge sampling). Some differences in the analytical results between the individual samples can be attributed to the fact that the nature and properties of the matter investigated depend largely on the quality of pollution fed into the activated sludge process prior to sampling. Thus, it can be expected that the character of the III A fraction could provide some information on the constituents of the raw wastewater.

Individual analytical results are consistent with those obtained in the preceding stage of investigation [4], the except for the data obtained from comparative determination of molecular weights by the light scattering method.

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#### POLISACHARYDY OSADU CZYNNEGO WYTWORZONE PRZY OCZYSZCZANIU SCIEKÓW GARBARSKICH ŁĄCZNIE Z MIEJSKIMI CZEŚĆ III. ODDZIELANIE PRZEZ CHROMATOGRAFIĘ PRZENIKANIA ŻELU I CHEMICZNE OKREŚLENIE FRAKCJI NISKOCZĄSTECZKOWEJ

Jest to praca zamykająca serię komunikatów opublikowanych przez autorów w niniejszym czasopiśmie. W poprzednich artykułach opisano oddzielanie wodnych roztworów wielocukrów ekstrahowanych z osadu czynnego metodą wysokowydajnej chromatografii żelowej (GPC). W niniejszej pracy przeprowadzono analizę frakcji o małym ciężarze molekularnym wydzielanej w układzie chromatografii żelowej (GPC), wysokowydajnej pod względem chromatograficznym i chemicznym. Frakcja ta jest zasadniczo biopolimerem sacharydowym o masie cząsteczkowej około  $1 \times 10^4$ , zawierającym w swym łańcuchu D-galaktozoaminę, L-arabinozę i L-ramnozę. Charakter tej frakcji zależy od rodzaju zanieczyszczeń wprowadzonych do procesu osadu czynnego.

#### DARSTELLUNG DER BELEBUNGSSCHLAMMPOLYSACCHARIDEN BEI DER REINIGUNG VON GERBEREIABWÄSSERN ZUSAMMEN MIT KOMMUNALABWÄSSERN TEIL III. ABTRENNEN DURCH GELDURCHGANGCHROMATOGRAPHIE SOWIE CHEMISCHE BESTIMMUNG DER NIEDERMOLEKÖLFRAKTION

Diese Arbeit schließt eine Reihe von Berichten, die die Autoren in dieser Zeitschrift veröffentlicht haben. In den vorangegangenen Berichten wurde das Abtrennen der wässrigen Polysaccharidenlösungen, die aus Belebungschlamm extrahiert wurden, mit der Methode der Hochergieviger Gelchromatographie (GPC) beschrieben. In dieser Arbeit die Analyse der Niedermolekülfraction, die im Gelchromatographie-system (GPC) entstehen, und hochergievig chromatographisch und chemisch ist, beschrieben. Diese Fraktion stellt ein Saccharidbiopolymer mit Molekulargewicht von ca  $1 \times 10^4$  dar, der in seiner Kette D-Galaktosamin, L-Arabinose und L-Ramnose enthält. Die Beschaffenheit dieser Fraktion hängt von der Art der Verunreinigungen, die in den Belebungschlammprozeß eingeführt wurden, ab.

ПОЛИСАХАРИДЫ АКТИВНОГО ИЛА, ОБРАЗОВАННЫЕ ПРИ ОЧИСТКЕ  
КОЖЕВЕННЫХ СТОЧНЫХ ВОД ВМЕСТЕ С ГОРОДСКИМИ

ЧАСТЬ III. РАЗДЕЛЕНИЕ С ПОМОЩЬЮ ХРОМАТОГРАФИИ ПРОНИКАНИЯ ГЕЛЯ  
И ХИМИЧЕСКОЕ ОПРЕДЕЛЕНИЕ НИЗКОМОЛЕКУЛЯРНОЙ ФРАКЦИИ

Это работа, завершающая серию сообщений, опубликованных авторами в настоящем журнале. В предыдущих журналах описано разделение водных растворов полисахаридов, экстрагированных из активного ила методом высокопроизводительной гелевой хроматографии (GPC). В настоящей работе описан анализ фракции с малым молекулярным весом, выделяемой в системе гелевой хроматографии (GPC), высокопроизводительной в хроматографическом и химическом отношении. Эта фракция является, в основном, сахаридным биополимером с молекулярной массой около  $1 \times 10^4$ , содержащим в своей цепи D-галактозамин, L-арабинозу и L-рамнозу. Характер этой фракции зависит от вида загрязнений, введённых в процесс активного ила.