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A COMPARATIVE STUDY OF BIOPOLYMERS FROM A CONVENTIONAL AND AN ADVANCED ACTIVATED SLUDGE TREATMENT PLANT

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ABSTRACT

Biopolymer characteristics from a traditional and an advanced activated sludge treatment plant performing biological N and P removal were compared. The biopolymers were extracted using an ion exchange resin (DOWEX in Na-form). Differences between chemical compositions of the total sludges were observed by measuring protein, polysaccharide and uronic acids whereas differences in the same compounds were not found in the extracted biopolymers. High Pressure Size Exclusion Chromatography was performed on the two biopolymer matrixes and differences were found in the biopolymer matrixes. Biopolymers from the advanced treatment plant contained two fractions of large and hydrophobic compounds which contributed to a major fraction of the chromatogram area. These peaks could only to a minor extent be found in the extracted biopolymers from the traditional treatment plant.

KEYWORDS

Activated sludge; Biopolymers; Carbohydrate; Protein; Uronic acid; HPSEC.

INTRODUCTION AND BACKGROUND

Biopolymers are one of the major components of activated sludge flocs (Li and Ganczarczyk, 1990). Biopolymers influence activated sludge properties like dewaterability (Kajornatiyudh, 1986) and sludge settleability (Forster, 1985). There is an ongoing expansion of treatment plants from the traditional removal of organic carbon also to include biological nitrogen and phosphorus removal. These expansions may alter the composition of the activated sludge biopolymer matrix and thereby change the activated sludge properties. A comparison between biopolymer matrixes from a treatment plant performing only biological carbon removal (Aså treatment plant, sludge age of 8 days) and a treatment plant performing biological nitrogen and phosphorus removal (Aalborg East treatment plant, sludge age of 36 days) was performed in order to reveal possible differences.

MATERIALS AND METHODS

Immediately after the sludges were collected from the aeration tanks they were sedimented for 1.5 hour at 4 °C. Thickened sludges were centrifuged for 15 minutes at 2000xg at 4 °C. The sludge pellets were resuspended in a phosphate buffer at pH 7 (9 mM NaCl, 1 mM KCl, 2 mM Na_3PO_4 and 4 mM NaH_2PO_4). 60 gr. DOWEX in Na-form was added to 100 ml resuspended sludge and the suspensions were stirred at 4 °C for 1 hour. The extracted sludges were centrifuged for 15 minutes at 12000xg and 4 °C twice in order to harvest the extracted biopolymers. High Pressure Size Exclusion Chromatography (HPSEC) measurements were done immediately after extraction. Proteins, polysaccharides and uronic acids were determined in the activated sludges and in the biopolymers according to the method of Lowry (Lowry et al. 1951), Anthrone (Dreywood, 1946) and a slightly modified m-hydroxydiphenyl method as described by Kintner and Van Buren (1982).

The extracted biopolymers were characterized using HPSEC in order to get a fingerprint of the biopolymer matrixes. A chromatographic setup was developed using a Chrompack P 1000 GFC column with a column material of polystyrenedivinylbenzene and a size separation range of 10000 to $2x10^6$ dalton. The mobile phase was 10 mM NaCl, 0.333 mM Na_3PO_4 and 0.666 mM NaH_2PO_4 at pH 7. On-line detection was carried out by UV absorbance at 280 nm. All measurements were done using mobile phase flow of 1 ml per minute and 0.1 ml sample volume. The total exclusion limit was estimated by glucose and found to be 9.8 ml. The column was investigated for non size exclusion effects using model compounds according to Pfankoch et al. (1980). The column was found to exhibit ion adsorption and ion exclusion effects due to a negative charge of the column packing material. The packing material showed hydrophobic adsorption as well. The non size exclusion effects could be controlled for the individual model compounds by altering the ionic strength of the mobile phase.

RESULTS AND DISCUSSION

The presented results are all from one data set. HPSEC analyses have shown only minor variation on a day to day basis. Thus, the presented results can be taken as representative for the investigated sludges. The results of the chemical analysis of the sludges showed that the amount of polysaccharides was higher in the sludge from Aså than in the sludge from Aalborg East (Table 1). The ratios of proteins to polysaccharides were 2.1 and 1.4 for Aalborg East and Aså sludge, respectively. Similarly, the ratios of polysaccharides to uronic acids are 8.5 and 13.4 for Aalborg East and Aså sludge, respectively. The chemical analysis of the extracted biopolymer showed only minor differences between the two plants (Table 2). The ratios for proteins to polysaccharides were 7.5 and 8.5 for Aalborg East and Aså, respectively. These ratios of proteins to polysaccharides were significantly higher for the extracted biopolymers than for the sludges. For both sludges this finding can be explained as less efficiency in polysaccharide extraction than in extraction of the fraction of uronic acids and proteins.

TABLE 1. Chemical Composition Of Activated Sludge

Treatment plant	Protein mg/g VSS	Polysaccharide mg/g VSS	Uronic acid mg/g VSS
Aså	441.3	310.8	23.2
Aalborg East	435.9	207.0	24.3

TABLE 2. Chemical Composition Of Extracted Biopolymers

Treatment plant	Protein mg/g VSS	Polysaccharide mg/g VSS	Uronic acid mg/g VSS
Aså	53.8	6.3	1.6
Aalborg East	48.8	6.5	2.0

The high ratio of protein to polysaccharide found in the extracted biopolymers might indicate that intracellular material was released due to the extraction method. According to the literature the ratio of protein to polysaccharide is generally between 3 and 4 which is lower than found in this study. Extraction experiments on activated sludge from Aalborg East have indicated that induced cell lysis due to the extraction method does not take place. Extractions using smaller amounts of DOWEX have shown ratios of protein to polysaccharides in the same range as reported in this study. Furthermore, two other extraction methods have been used to extract biopolymers from Aalborg East activated sludge. The pH 11 method described by Kajornatiyudh (1986) revealed a protein to polysaccharide ratio in the biopolymers of 11.5 whereas extraction at 80 °C for 1 hour yielded a ratio of 5.6. Kajornatiyudh (1986) tested the pH 11 extraction method for possible lysis by measuring DNA in the biopolymers and did not find significant amounts. Kajornatiyudh (1986) concluded that significant induced cell lysis did not occur due to the pH 11 extraction method. This indicates that the high ratios of proteins to polysaccharides found in this study represent a picture of extracted biopolymers and not induced cell lysis.

Differences and similarities between the biopolymer matrixes of the sludges were shown using HPSEC (Figure 1 and Figure 2).

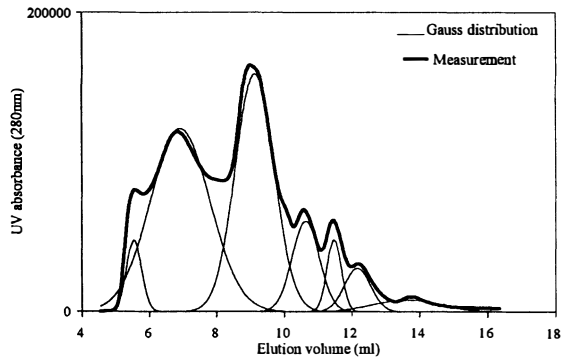

Fig.1. Chromatogram of Aalborg East biopolymers. Correlating the sum of the Gauss distributions with the measured chromatogram gives R^2 of 0.995.

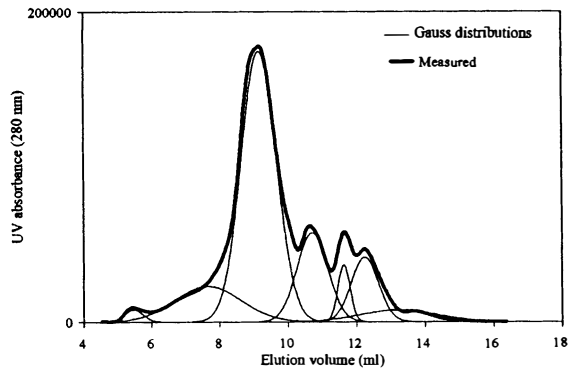

Fig. 2. Chromatogram of Aså biopolymers. Correlating the sum of the Gauss distributions with the measured chromatogram gives R^2 of 0.998.

In the biopolymers from the Aalborg East treatment plant peak 1 and peak 2 (numbered in direction of elution) were found to contribute to a large part of the chromatogram area. The first two peaks from the Aså biopolymers were eluted almost at the same elution volume but, in contrast to the Aalborg East chromatogram, these two peaks corresponded only to a minor part of the chromatogram area. In each case the two chromatograms were modelled by fitting seven Gauss-distributions (Figure 1 and 2). From the fitted Gauss-distributions as it was found that the two peaks eluted first in the chromatogram from Aalborg East biopolymers make up 45.5 % of the total area in contrast to the first two peaks in the Aså chromatogram which only contribute 14.2 % of the total chromatogram area. A comparison between the two chromatograms for the remaining peaks revealed no major differences between the biopolymers from the two types of sludge. The last four peaks in both chromatograms were eluted after the total exclusion limit and were hence retained in the column due to non size exclusion effects. These compounds were shown to have a molecular weight of less than 10000 dalton by HPSEC separation after dialysis of the samples against distilled water. Information of the biopolymer chemical properties was achieved by HPSEC separations Frølund and Keiding (1993). HPSEC separations were made using biopolymers from Aalborg East and systematically varying the mobile phase ionic strength. The separations were performed with the same relative chemical composition of the mobile phase. The compounds from the first two peaks in the Aalborg East chromatogram exhibited hydrophobic properties. Thus, the biopolymers from the Aalborg East treatment plant contained a large amount of molecules with hydrophobic properties in contrast to the biopolymers from the Aså treatment plant. The HPSEC results illustrated that HPSEC can be a useful tool in finding and characterizing the differences between biopolymer matrixes thereby getting information on which peaks to investigate further.

CONCLUSIONS

Biopolymers were extracted from activated sludge from an advanced treatment plant and from a traditional operating treatment plant in order to see whether differences occurred. Chemical analyses of the full sludge showed higher polysaccharide content in the young sludge (310.8 mg/g VSS) as compared to the old sludge (207.0 mg/g VSS). Chemical analyses of the biopolymer matrixes revealed no differences in content of protein, polysaccharides and uronic acids. In general, a high ratio of protein to polysaccharide was found in the biopolymers, which suggested the proteins to be a major fraction of the biopolymers. HPSEC measurements showed a significant difference in the biopolymers. The biopolymers from the advanced treatment plant contained two peaks of large, and in comparison to the other peaks, very hydrophobic compounds which made up a large portion of the chromatogram area (45.1 %). The same peaks contributed only 14.2 % of the chromatogram area for the biopolymer matrix from the traditional treatment plant. Thus, the HPSEC setup was found suitable to provide information both on the fingerprint of the biopolymer matrixes and on the hydrophobic properties of the biopolymers.

REFERENCES

Dreywood R. (1946). Qualitative test for carbohydrate material. Industrial and Engineering Chemistry. **18** 449.

Forster C. F. (1985). Factors involved in the settlement of activated sludge - I. Nutrients and surface polymers. Wat. Res. **14** 1257 - 1264.

Frølund B. and Keiding K. (1993). Characterization and separation of activated sludge biopolymers using a polystyrenedivinylbenzene HPSEC column. In prep.

Kintner P. K. and Van Buren J. P. (1982). Carbohydrate interference and its correction in pectin analysis using m-hydroxydiphenyl method. Journal of Food Science **47** 756 - 760.

Kajornatiyudh S. (1986). Bacterial extracellular polymers and flocculation of activated sludges. Ph.D. Thesis. Polytechnic Institute and State University, Blacksburg, Virginia.

Li D. -H. and Ganczarczyk J. J. (1990). Structure of activated sludge flocs. Biotechnology and Bioengineering. **35** 57 - 65.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951). Protein measurement with the folin reagent. J. Biol. Chem. **193** 265 - 275.

Pfannkoch E., Lu K. C., Regnier F. E. and Barth H.G. Characterization of some commercial High Performance Size-Exclusion Chromatography Columns for water-soluble polymers. Journal of Chromatographic Science **18** 430 - 441.