ANJOU RECHERCHE

VEOLIA Water

Technische Universität Berlin

Application of Liquid Chromatography-Online Carbon Detection (LC-OCD) to the understanding of organic fouling in membrane bioreactors (MBRs)

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Contents

Terminology and abbreviations

c	concentration [mg/L]
C	carbon
d	days
D	Dalton
DOC	dissolved organic carbon
EfOM	effluent organic matter
feed	water sample used for stirred cell experiments
filtrate	aqueous sample from the membrane reactor filtered over paper filter to separate the activated sludge from the water phase
g	gram
h	hour
HRT	hydraulic retention time
IMS	integrated membrane systems
J	flux [L/hm²]
k	kilo
L	liter
LC-OCD	liquid chromatography-organic carbon detection
m	milli
m	meter
M	molar
MBR	membrane bio-reactor
MF	microfiltration
MW	molecular weight
MWCO	molecular weight cut-off
N	normal
OC	organic carbon
P	pressure [kPa]
Pa	Pascal
PEG	polyethylene glycol
perm	permeate collected during stirred cell experiments
permeate	aqueous sample taken after the membrane of the MBR pilot plants
PP 1	pilot plant 1 operated under pre-denitrification conditions
PP 2	pilot plant 2 operated under post-denitrification conditions without additional dosing of an external carbon source
PVDF	polyvinylidene fluoride
R	resistance
RC	regenerated cellulose
S	Siemens
SEC	size exclusion chromatography
SMP	soluble microbial products
SRT	sludge retention time
TMP	transmembrane pressure
TOC	total organic carbon
UF	ultrafiltration
UV	ultraviolet
WWTP	wastewater treatment plant
µ	micro

Chapter 1 Introduction

At the Ruhleben wastewater treatment plant (WWTP) two membrane bioreactor (MBR) pilot plants have been operated since September 2001 by Veolia Water and Berliner Wasserbetriebe. The primary aim of the piloting is the investigation of biological phosphorus removal in conjunction with nitrification/denitrification in MBRs for later use in remote areas and small scale applications (WWTP serving a few thousand inhabitants) [Gnirss et al 2003a].

Both plants are fed with the same raw wastewater as it is treated in the conventional wastewater treatment plant. Instead of the mechanical treatment of the conventional plant, the raw wastewater passes a 1 mm punch hole screen prior to the biological treatment in the two MBR pilot plants. The two pilot plants are operated under parallel operating conditions (same raw wastewater, same sludge age and sludge concentration , etc.), but there are two different biological process configurations: pre-denitrification and post-denitrification without addition of a carbon source. Over the first year of operation, it has been observed that the unit with post-denitrification exhibited more rapid membrane fouling than the one with pre-denitrification. Preliminary LC-OCD (liquid chromatography-organic carbon detection) measurements carried out with the permeate compared to paper filtered sludge showed differences between the two units regarding the concentration of colloids and large macromolecules (as measured in the polysaccharide peak). Hence, an assessment and investigation of the fouling behaviour of the two MBR pilot plants was commenced. The results are presented in this report.

Chapter 2 Objectives

The filtration capacity of membrane bio-reactors (MBR) is influenced by suspended solids in the activated sludge as well as colloidal and soluble microbial products produced by the microorganisms during biological treatment. Since the suspended solids are identical in both pilot plants, colloidal and dissolved organic matter in the filtered activated sludge are investigated and correlated to the fouling rate of the membranes in this study. The objectives of this project are:

1) to identify the components responsible for the fouling of both MBR pilot plants,
2) to investigate the influence of the process configurations of pre-denitrification versus post-denitrification without additional carbon source, and
3) to compare the LC-OCD method with a photometric method for polysaccharides.

In order to achieve these objectives, the following tasks are carried out:
- development of a sampling procedure including the optimisation of pretreatment, validation of the methods with regards to reproducibility, accuracy of samples, sample storage (section 4.1-4.3),
- comparison of the results from the LC-OCD with the photometric method (section 4.4),
- survey of the seasonal variation in the two membrane reactors (chapter 5),
- characterisation of polysaccharides (chapter 6),
- stirred cell experiments to confirm fouling behaviour of the pilot plants (chapter 7).

Chapter 3 Experimental Set-up and Analyses

3.1 Membrane bioreactor pilot plants

Two membrane bio-reactor (MBR) pilot plants are operated at the wastewater treatment plant Berlin-Ruhleben (WWTP Ruhleben) by Veolia Water and Berliner Wasserbetriebe. Both plants are fed with the same raw wastewater as it is treated in the conventional wastewater treatment plant. Instead of the mechanical treatment of the conventional plant, the raw wastewater passes a 1 mm punch hole screen prior to the biological treatment in the two MBR pilot plants. The primary aim of the piloting is the investigation of biological phosphorus removal in conjunction with nitrification/denitrification in MBRs for later use in remote areas and small scale applications (WWTP serving a few thousand inhabitants) [Gnirss et al 2003a].

The two pilot plants are equiped with a hollow fiber module from USF Memcor, Australia, made of polyvinylidene fluoride (PVDF) and a pore size of 0.1 – 0.2 µm. The operating conditions (e.g. loading rate , hydraulic retention time, sludge retention time, temperature…) are identical for the two pilot plants. The pilot plants have been operated since September 2001 under different sludge retention times (SRT) ranging from 8 to 26 days [Gnirss et al 2003a]. All experiments carried out for this study have been performed between August 2002 and November 2003.

The difference between the two pilot plants is the placement of the anoxic zone (see Figure 3.1): pilot plant 1 (PP 1) is operated in the conventional pre-denitrification mode where the aerobic zone is preceded by the anoxic zone, while the aerobic zone is followed by the anoxic zone in pilot plant 2 (PP 2). Thus, pilot plant 2 operates in a post-denitrification mode without additional dosing of a carbon source.

During this study, the two pilot plants are operated at two constant sludge retention times (SRT): eight days from January 2003 – May 2003 and fifteen days from July 2003 – November 2003. The contact time in the membrane reactor is four minutes in both pilot plants. Due to the different configurations, the two pilot plants have slightly different volumes: 2.1 m³ for pilot plant 1 (pre-denitrification) and 1.9 m³ for pilot plant 2 (post-denitrification). The operating conditions for the two sludge retention times and both pilot plants are summarized in Table 3.1. On 29th July 2003 the module A3 (flat sheet membrane) has been connected in by-pass to pilot plant 1. The reactor volume and throughflow are kept proportional and therefore, the load for the Memcor hollow fiber membrane has remained identical. For further information on operating conditions and results of the MBR see Gnirss et al [2003a and 2003b].

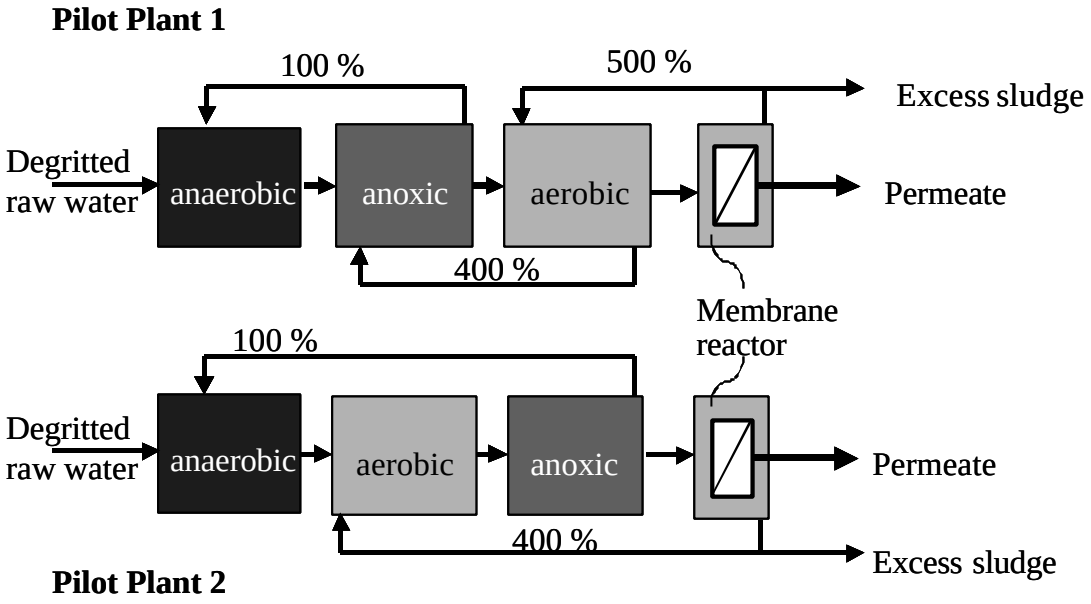

Figure 3.1 Schematic of the two MBR pilot plants operated at the municipal wastewater treatment plant Berlin-Ruhleben, Germany

Table 3.1 Operating conditions for MBR pilot plants at SRT of 8 d and 15 d

	Pilot plant 1		Pilot plant 2	
SRT	8 d	15 d	8 d	15 d
HRT (h)	11.4-12.4	9.9-12.4	12.1-12.6	12.3-14.4
Throughflow (L/h)	182.7-203.2	180.3-313*	161.2-174.5	128.2-167.4
Permeate flux (L/m²/h)	22.5-25.0	18-22.5	20.9-21.6	14.9-21.6
MLSS (g/L)	7.3-12.9	9.2-16.5	7.1-11.3	10.4-14.7

* includes flow through module A3 of 107-129 L/h from 29th July 2003

3.2 Stirred cell testing

The fouling potential is determined as flux decline over time using Amicon 8200 dead-end stirred cells (Millipore, USA) as shown in Figure 3.2. The cells have a volume of 200 mL and the effective membrane filtration area is 28.7 cm². Due to an attached feed reservoir it is possible to filter samples of up to 4 L. The water reservoir is filled with the sample and pressurized using nitrogen gas (5.0 grade). The sample in the filtration cell is stirred over the entire experiment to minimize concentration polarization and the membrane flux is measured using a volumetric cylinder and stopwatch. All experiments are run at room temperature (~ 22°C) and constant pressure. Experiments are carried out at 0.3 bar (MF) or 1.0 bar (UF) depending on the membrane used (see Table 3.2).

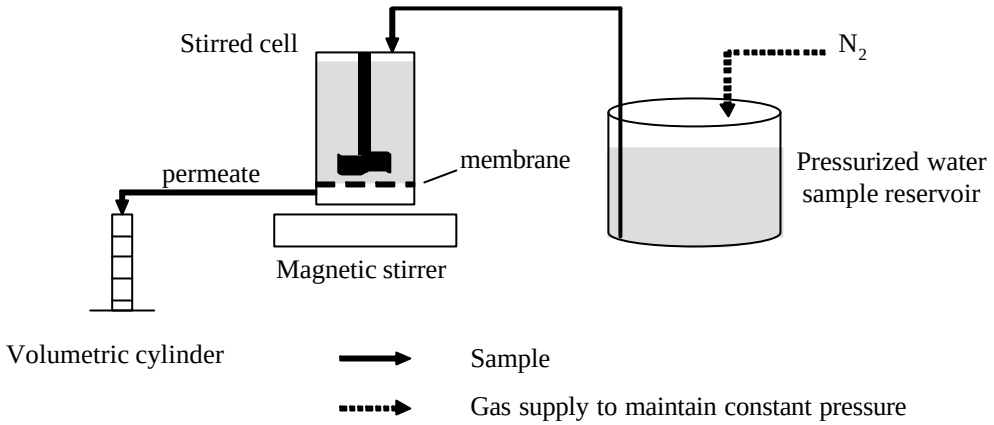

Figure 3.2 Experimental set-up for stirred cell testing

A new membrane is used for each experiment unless stated otherwise. Prior to use each membrane is placed in ultra-pure water for at least 24 h to remove wetting agents and production residues. Immediately before the stirred cell test the pure water flux of the membrane is determined by filtering ultra-pure water through the membrane until a stable permeate flux is reached.

Two different membranes are used to test different materials and pore sizes / molecular weight cut-offs (see Table 3.2). The first membrane (VVLP) is chosen due to its similarity to the membrane used in the pilot plants in Berlin-Ruhleben, Germany. The pore size lies between 0.1 µm and 0.2 µm and the membrane material is polyvinylidene fluoride which is hydrophilized during its fabrication. The applied pressure during the stirred cell experiments is 0.3 bar. This corresponds to the transmembrane pressure (TMP) threshold in the pilot plants which are operated under constant flux conditions, i.e. when a TMP of 0.3 bar is reached a chemical cleaning of the membrane is commenced.

The second membrane (YM100) is chosen to be able to discern different fouling mechanisms such as cake formation, pore blockage, and adsorption. As a loose ultrafiltration membrane, the YM100 membrane, has a nominal molecular weight cut-off (MWCO) of 100000 Dalton (D) and is made of regenerated cellulose. This membrane is very hydrophilic (contact angle 18°) and stirred cell experiments are carried out at 1 bar.

Table 3.2 Characteristics of flatsheet membranes used in stirred cell units

Membrane	Material	Pore size MWCO	Contact angle & hydrophobicity	Manufacturer	applied pressure
VVLP	PVDF	0.1-0.2 µm	Hydrophilized	Millipore	0.3 bar
YM100	RC	100 000 D	18°, hydrophilic	Amicon	1 bar

PVDF: Polyvinylidene fluoride RC: regenerated cellulose

3.3 Feed water for stirred cell testing

3.3.1 Conventional wastewater treatment plant Berlin-Ruhleben

The wastewater treatment plant Ruhleben is the largest of five plants treating municipal wastewater from Berlin, Germany. Raw wastewater, including 20-30 % industrial wastewater, is treated by conventional activated sludge (CAS) with biological nitrification/ denitrification and biological phosphorous removal. The plant's capacity is 240.000 m³/d and annual average effluent quality is summarized in Table 3.3.

Table 3.3 Annual average values of the conventional effluent of the WWTP Ruhleben for 2002 [Gnirss 2004, Gnirss et al 2003b]

COD mg/L	BOD mg/L	TOC mg/L	AOX mg/L	TKN mg/L	$NH_4^+ - N$ mg/L	$NO_3^- - N$ mg/L	TP mg/L	Cl^- mg/L
43	3.7	14	76.5	12	< 0.4	7.7	0.3	142

Samples are taken as grab samples. For a comparison of the pilot plants with the conventional WWTP, activated sludge from the conventional WWTP is filtered over paper filter (Schwarzband, Schleicher & Schuell GmbH, Germany) to get the aqueous phase only. These samples are referred to in this text as "filtrate CAS".

3.3.2 MBR pilot plants

The two pilot plants are described in section 3.1. Samples are taken from the membrane reactors and filtered over paper filter (Schwarzband, Schleicher & Schuell GmbH, Germany) in order to separate the sludge from the water phase. The paper filters are rinsed with 200 mL permeate. The filtered activated sludge from the membrane reactors is referred to as "filtrate PP 1" or "filtrate PP 2", depending on the pilot plant sampled. To minimize daily and weekly variations, samples are taken on the same weekday between 8 am and 9 am, seven minutes after the last backwash. Additionally, permeate grab samples are used for experiments (= "permeate PP 1" or " permeate PP 2").

3.4 Size exclusion chromatography

3.4.1 Size exclusion chromatography systems

Size exclusion chromatography (SEC) with UV and online organic cabon detection is used for the characterisation of the organic carbon in the samples. The system in Berlin is an LC-OCD (manufacturer DOC-LABOR Dr. Huber, Karlsruhe, Germany). LC-OCD stands for Liquid Chromatography – Organic Carbon Detection.

The system consists of a size exclusion chromatography column in order to separate organic molecules according to their molecular size. The underlying principle is the diffusion of molecules into the resin bead pores. This means that larger molecules elute

first as they can not penetrate the pores very deeply, while smaller molecules take more time to diffuse into the pores and out again.

Table 3.4 shows the characteristics of the two columns (columns are 250 mm long and have a diameter of 20 mm) used for this research, such as pore size of resin and range of molecular weight discernable with each column. The resin consists of semi-rigid, spherical beads with a hydrophilic surface and is synthezised by co-polymerisation of ethylene glycol and methacrylate-type polymers. The supplier (GROM Analytik + HPLC GmbH, Herrenberg, Germany) states the molecular size separation range detectable according to polyethylene glycols (PEG), dextrans, and globular proteins as standard molecules.

Table 3.4 Columns used in the LC-OCD system at the Technical University Berlin (TU); characteristics according to the supplier GROM Analytik + HPLC GmbH [GROM 2003]

Toyopearl TSK	particle size [µm]	pore size [Å]	PEG [D]	Dextran [D]	Globular Proteins [D]
HW-50S	20 - 40	125	100 – 18 000	500 – 20 000	500 – 80 000
HW-55S	20 - 40	300	100 – 150 000	1 000 – 200 000	1 000 – 700 000

The system is operated with a flowrate of 1 mL/min using a phosphate buffer as eluent. Sample preparation consists solely of a filtration step if particles are present (which is not the case here as all samples are prefiltered, see section 3.3). Samples are not adjusted to the pH and ionic strength of the eluent (I=0.18 mol/L, pH=6.6, κ=3.1 µS/cm). However, the samples have to be diluted to a TOC of 2-5 mg/L. This is done with ultra pure water. The organic carbon content of the bulk sample is measured at the beginning of each run (injection at 0 minutes) using a by-pass around the column in the LC-OCD set-up. This results in the by-pass peak. After approximately 10 minutes the sample is injected into the column.

Figure 3.3 provides an overview of the system set-up. The separated compounds are detected by UV absorption at 254 nm (WellChrom fixed wavelength detector K-200, Knauer, Berlin, Germany) followed by dissolved organic carbon (DOC) detection. In order to eliminate any inorganic carbon, phosphoric acid is added after the UV detector. In the LC-OCD the organic carbon is oxidised in the Gräntzel thin-film reactor by radiolytically produced oxygen radicals (aqueous sample + 185 nm UV light under nitrogen atmosphere). The produced carbon dioxide is detected by non-dispersive infra-red absorption (Ultramat 6, Siemens, Munich, Germany) [Huber and Frimmel 1991].

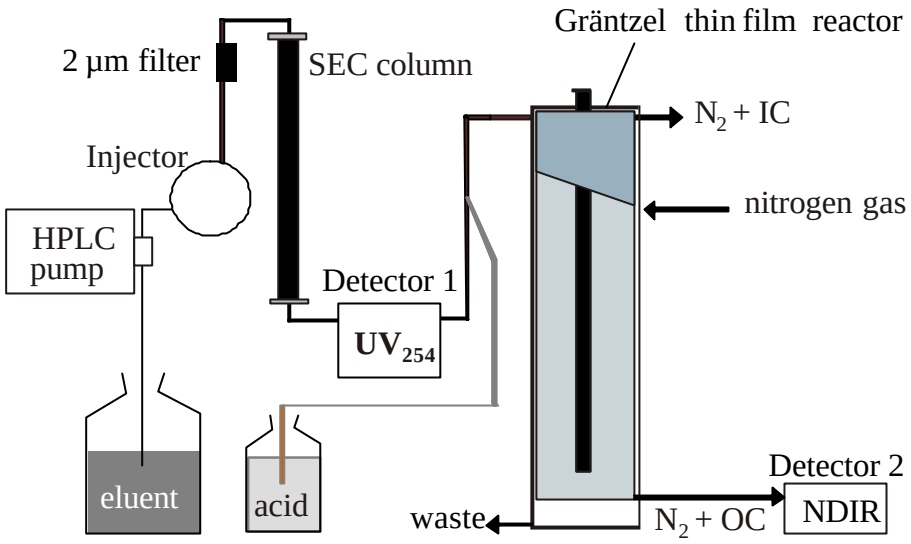

Figure 3.3 Set-up of the LC-OCD; the system has an automated data acquisition for the detectors (not shown)

Because of the TOC by-pass measurement by the LC-OCD, the first 10 min (by-pass peak) do not pertain to the chromatogram itself. These first ten minutes are, however, not substracted from the chromatograms in this study. This is legitimate as by-pass measurements are performed on both calibration standards and samples.

3.4.2 Analysis of chromatograms

In an ideal chromatography the molecules are retained due to this diffusion only. Very hydrophobic or neutral molecules, however, may interact with the resin. Due to this non-ideal chromatography effects, these molecules do not elute according to their size. Instead they elute after long retention times, i.e. after the salt boundary. Hence, no conclusions may be drawn on the molecular size of such hydrophobic substances.

Figure 3.4 shows the relevant boundaries: (i) the void volume peak which can be measured using Blue Dextran 2000 with a molecular weight of ~ 2000000 Dalton, and (ii) the salt boundary which is determined with sodium nitrate ($NaNO_3$) and the UV detector. Within these boundaries one can assume a more or less ideal size exclusion chromatography.

Figures 3.5 and 3.6 each show the organic carbon (OC) chromatogram of a typical WWTP effluent sample using the 50S column and the 55S column, respectively. The first peak of the organic carbon chromatogram on column 50S (Figure 3.5) after 38 min (peak maximum) is the so called polysaccharide peak (= PS). Besides polysaccharides, proteins and organic colloids elute in this peak too. It is followed by the humic substances peak (HS) at 53 min which often shows a more or less distinct shoulder/peak attributed to humic hydrolysates or building blocks (BB) around 57 min. The acid peak (62 min) is due to the way the system is operated (non buffered samples) and contains organic acids. After this

distinct acid peak neutral and amphiphilic[1] compounds may show. Nevertheless, it should be kept in mind that anything eluting after the salt boundary at approximately 70 minutes, is interacting with the column resin, and therefore should be disregarded although its organic carbon content can be reported.

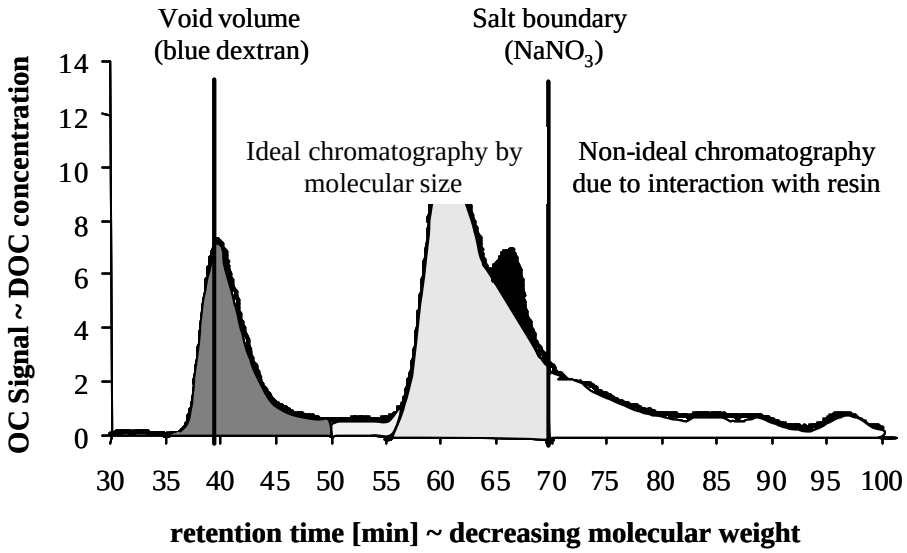

Figure 3.4 Ideal versus non-ideal chromatography as defined through the void volume using Blue Dextran 2000 and the salt boundary using $NaNO_3$

The first peak of the organic carbon chromatogram using column 55S after 40 minutes (peak maximum) is the polysaccharide peak (= PS). Contrary to column 50S, only organic colloids would also elute in this peak as the molecular size separation spans a wider range (see Table 3.4). This allows for a separation of proteins, e.g. the standard protein bovin serum albumin (BSA) elutes after 52.9 minutes. However, the Ruhleben effluent does not exhibit a distinct peak in this area (after 53 min). Instead the next distinct peak is the humic substances peak (HS). It includes the humic hydrolysates as resolution is lost in the lower molecular weight range in exchange for a better resolution in the larger molecular size range (see above). The third distinct peak in this chromatogram is the organic acids peak.

The UV chromatograms show similar distributions with one exceptions: polysaccharides are not detectable with UV because they do not have double bounds necessary for the absorption of light with a wavelength of 254 nm. Nevertheless, a UV absorption peak is often found slightly before the PS peak in the OC chromatogram. This may be due to UV absorption by organic colloids present in the sample or light scattering due to inorganic colloids such as silica, ferric or alum colloids as suggested by Huber and Frimmel [1996].

[1] amphiphilic = molecules with hydrophilic and hydrophobic ends

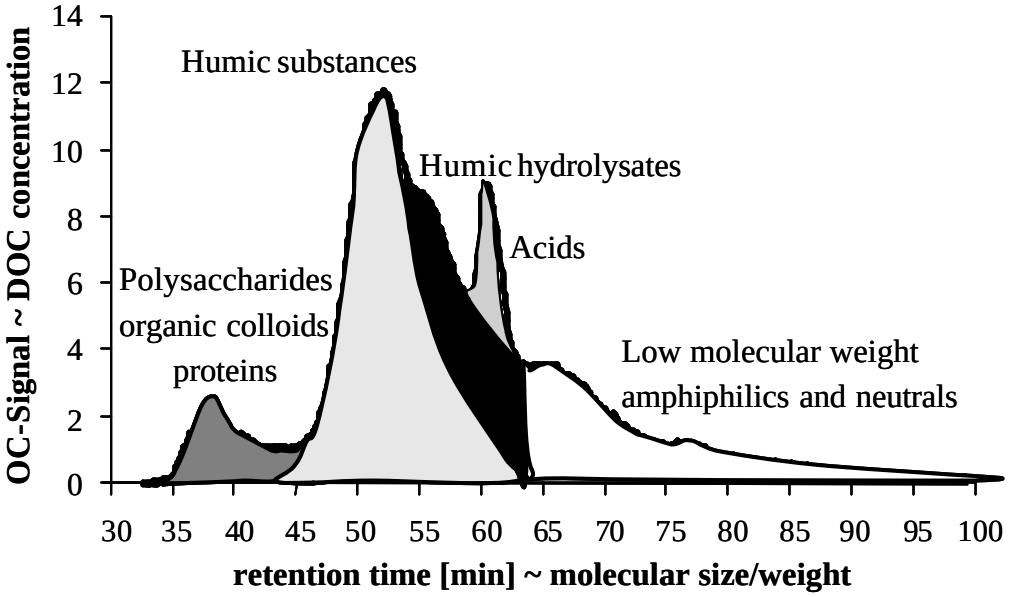

Figure 3.5 Attribution of different organic compounds to the main peaks detected during size exclusion chromatography (column 50S)

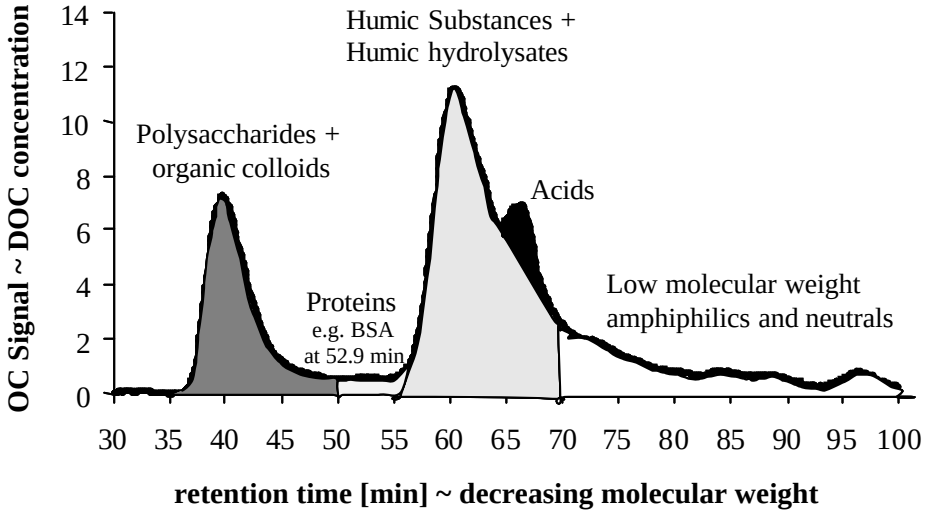

Figure 3.6 Attribution of different organic compounds to the main peaks detected during size exclusion chromatography (column 55S)

3.4.3 Calibration of OC detector with potassium phthalate

A calibration of the organic carbon detector is mandatory in order to be able to calculate the organic carbon content from the peak area. Potassium hydrogen phthalate ($KHC_8H_4O_4$) is used as organic carbon source. All samples are measured using the by-pass mode since this calibration aims at the infra-red detector itself and how sensitive the CO_2 detection is. Figure 3.7 depicts the resulting calibration curve using injection volumes of 50 µL, 100 µL, and 200 µL. The resulting equation to convert peak area into ng C is:

$$y = 0.0432x + 0.3383 \ (R^2 = 0.9986).$$

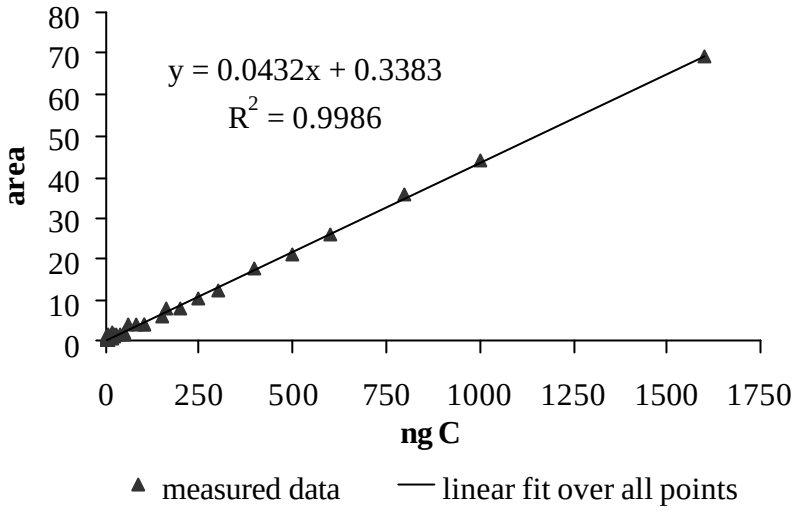

Figure 3.7 Calibration curve for organic carbon detector as measured in the by-pass mode with potassium hydrogen phthalate (injection volumes: 50 µL, 100 µL, and 200 µL)

Regarding the calibration of the UV detector the DOC-LABOR Dr. Huber (manufacturer) has calculated a conversion factor of 0.0554 for the LC-OCD system at TU Berlin (for a sample injection volume of 2000 µL). As standards, Suwannee River Humic and Fulvic Acid (IHSS HA and FA) are used or, more precisely, the ratio of UVA_{254} and OC for each of them: UVA_{254}/OC (FA) = 4.56 L/(mg*m) and UVA_{254}/OC (HA) = 7.85 L/(mg*m). These values have been cross-checked with other analysis methods by the DOC-LABOR Dr. Huber.

3.4.4 Calibration of SEC columns with known substances

In order to get an approximate idea of the retention time of various molecular weights/sizes a calibration with polyethylene glycols as well as with dextrans is made. Figure 3.8 shows the OC chromatograms of ten polyethylene glycols ranging from 194 Dalton to 182000 Dalton for the LC-OCD system using column 55S. The differing height of the peaks is due to different organic carbon concentrations of the standards. Of more interest is the fact that all PEGs elute according to their molecular weight and size. A similar picture is found with dextrans ranging from 1080 D to 123600 D (see Figure 3.9).

In Figure 3.10 the retention time corresponding to the peak maximum of each PEG and dextran standard is plotted against its molecular weight. Additionally, the retention time for Blue Dextran 2000 (~ 2 Mio. D), bovine serum albumin (~ 67000 D), humic acid and fulvic acid are shown. The peak maximum of Blue Dextran 2000 elutes after 40 minutes and determines the void volume. The diagram makes it clear that a calibration with PEGs is not absolute since other molecular structures behave differently, e.g. albumin elutes after the 40000 D PEG although it has a higher molecular weight. The obtained calibration conforms to the separation range given by the manufacturer of the columns (see Table 3.4).

Similar results are obtained for the calibration of column 50S with the exception that the two largest PEG standards (116 kD and 182 kD) are out of the separation range (Figure 3.11).

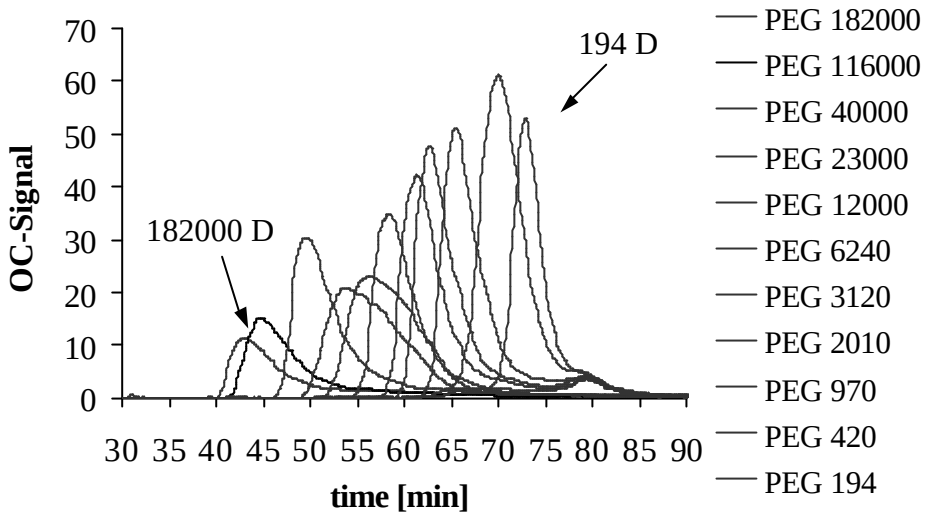

Figure 3.8 Superposed chromatograms of various polyethylene glycols (PEGs), from left to right (graph) and top to bottom (legend): large to small

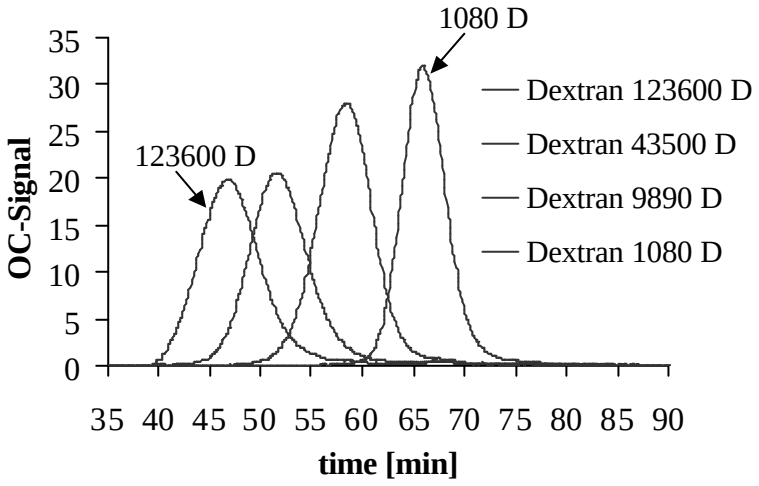

Figure 3.9 Superposed chromatograms of various dextrans, from left to right (graph) and top to bottom (legend): large to small

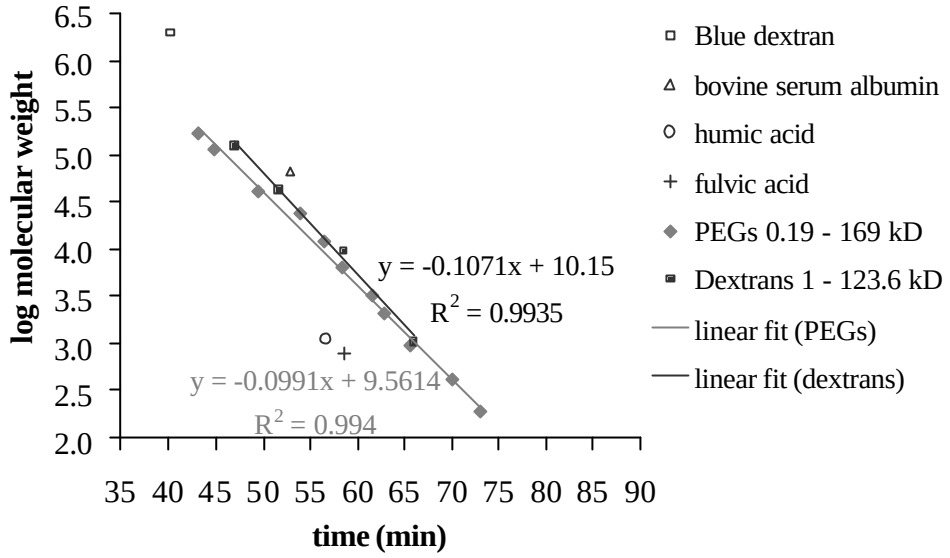

Figure 3.10 Calibration of molecular weight versus retention time with various standard compounds for column HW-55S

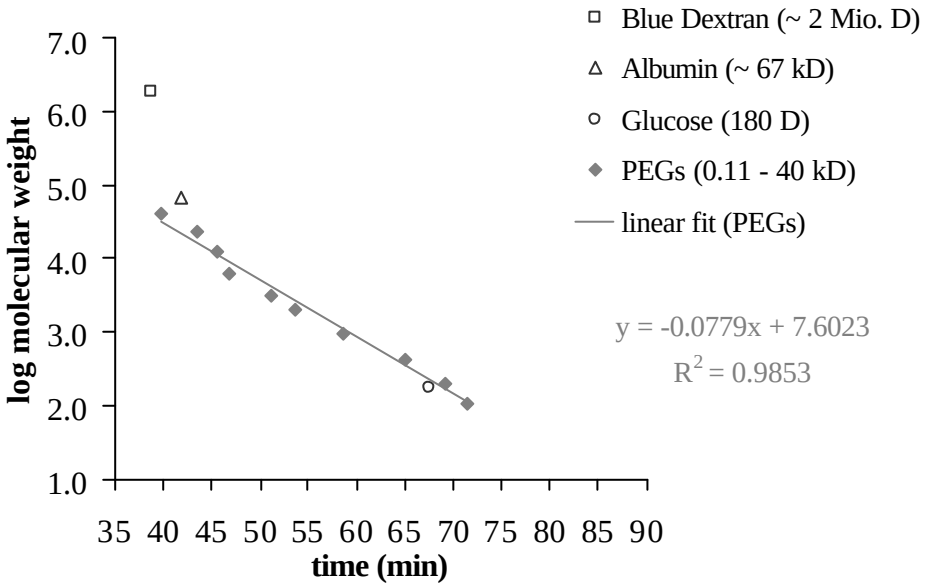

Figure 3.11 Calibration of molecular weight versus retention time with various standard compounds for column HW-50S

3.5 Turbidity measurements

Turbidity measurements of filtrate and permeate samples are done with a 2100N Turbidimeter from HACH Company, Loveland, Colorado, USA. For the calibration the Gelex® Secondary Turbidity Standards Kit is used. For each measurement, the turbidity

cuvette is rinsed with ultra pure water followed by a rinse with the sample. The analysis is made with 40 mL of sample.

3.6 Photometric measurements and EPS extraction

The photometric analyses for polysaccharides and proteins and the EPS extraction are carried out by Dr. Sandra Rosenberger according to the procedures explained in Rosenberger [2003]. The photometric analysis of polysaccharides follows the method of Dubois et al [1956]. The method of Lowry et al [1951], as modified by Frolund et al [1996], is used for the photometric analysis of proteins. The extraction of bound extracellular polymeric substances (EPS) from the cell surface is performed with a cation exchange resin (DOWEX) according to the method of Frolund et al [1996]. The activated sludge sample is diluted to 10 g suspended solids /L (the washing step has been omitted here). Approximately 70-75 g ion exchanger are needed per g of organic suspended solids. After two hours extraction time, the extracted EPS is separated from the solid phase by centrifugation [Rosenberger 2003].

Chapter 4 Method development

4.1 Pretreatment procedure

For this research feed and permeate samples from the two membrane bio-reactor pilot plants are investigated. The feed samples are taken directly out of each membrane reactor. However, these samples consist of a mixture of activated sludge and water. Since only soluble and colloidal compounds are of interest for this study, a pretreatment step has to be established to separate the sludge from the water phase. Two different pretreatment procedures are compared: paper filtration over Schwarzband filter (Schleicher & Schuell GmbH, Germany) and centrifugation at different speeds.

One grab sample of 1 L is taken from the membrane reactor of PP 2 on 19th November 2002 at 8:00 AM and is filtered over paper filter in the laboratory on-site at the WWTP Ruhleben. Half of the sample is filtered over a paper filter that has been previously rinsed with 200 mL pure water (H_2O_{dest}) while the second half is filtered over a paper filter that has been rinsed with 200 mL permeate from pilot plant 2. A second grab sample of 2 L is taken at 8:30 AM from the membrane reactor of pilot plant 2. The sample is transferred to the laboratory at the Technical University Berlin and three aliquots are centrifugated at different speeds (2000 rpm, 3000 rpm, and 3800 rpm) for 10 minutes.

Figure 4.1 gives the results for all five samples with regards to the paramaters: COD (chemical oxygen demand), TOC (total organic carbon), turbidity, polysaccharides, and proteins. The COD, polysaccharides and proteins are analysed using photometric methods by S. Rosenberger. All parameters are clearly decreasing with increasing centrifugation speed. Nevertheless, paper filtration results in even smaller values for all parameters. This is true regardless of whether the paper filter has been rinsed with pure water or permeate. The former one (water rinse) gives slighter smaller values than the latter one (permeate rinse). The reason for this could be a small dilution of the sample by water remaining in the filter as it is not possible to discard the first few milliliter of the sample due to the rapid clogging of the filter by the activated sludge.

Figure 4.2 and 4.3 depict the chromatograms of the LC-OCD analysis for the organic carbon response and UV absorption at 254 nm, respectively. Only four out of the five samples have been analysed because the turbidity of the sample being centrifugated at 2000 rpm has been too high for direct injection in the LC-OCD. For the other samples, the organic carbon response as well as the UV absorption follow the pattern seen with the previously discussed parameters, i.e. higher values (meaning higher peak heights or larger peak areas) for the centrifugated samples than for the paper filter samples. The distribution of the peaks is identical for both pretreatment steps which would be expected as the underlying sample is the same.

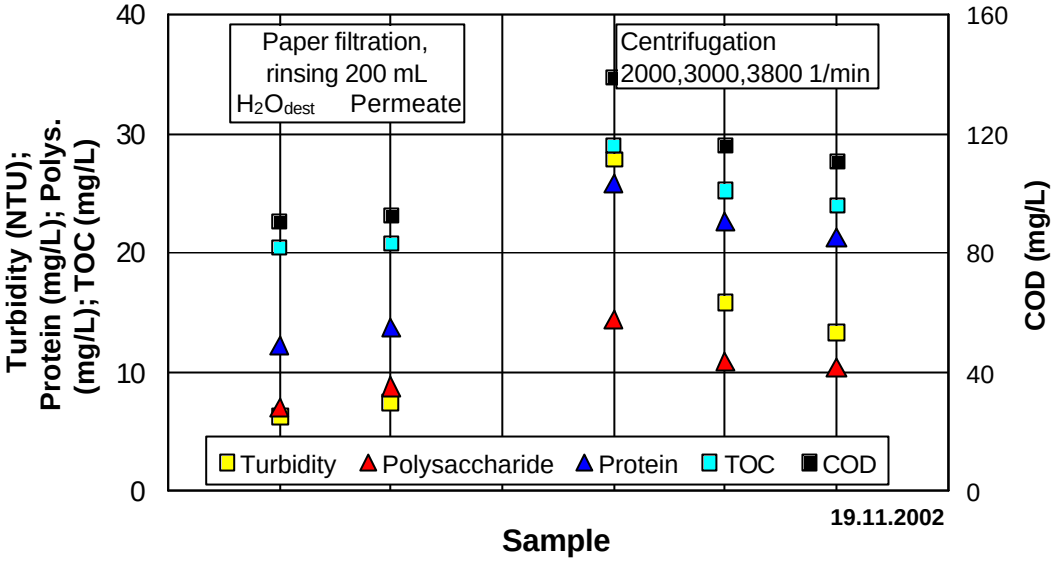

Figure 4.1 Results for paper filtered and centrifuged activated sludge sample from the membrane reactor of PP 2 with regards to COD, TOC, turbidity, and polysaccharide and protein concentrations (both measured photometrically by S. Rosenberger)

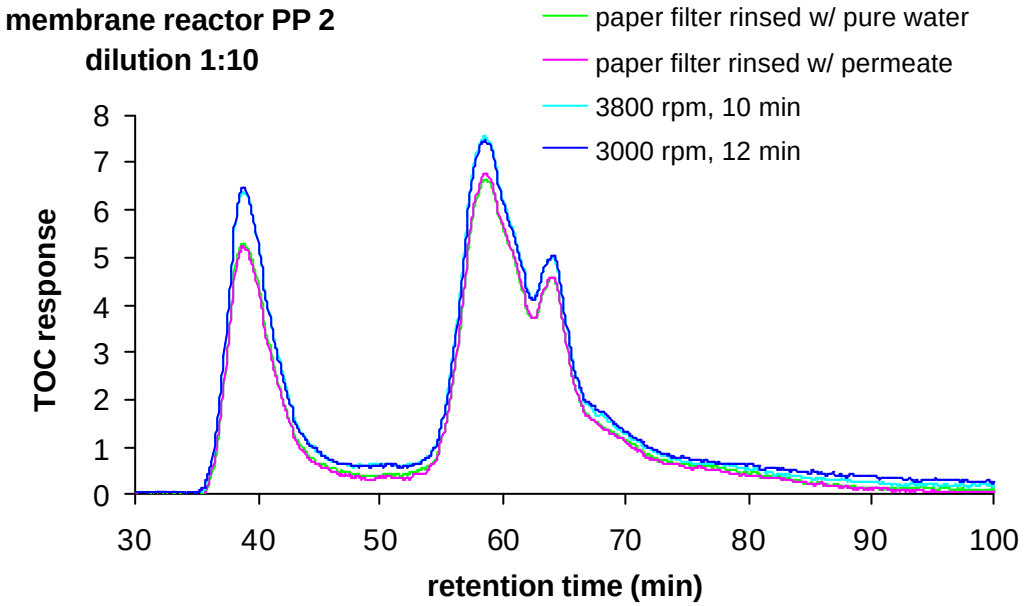

Figure 4.2 Organic carbon chromatograms for four of the five samples (too many particles have remained in the sample being centrifuged at 2000 rpm for 10 min for direct analysis on the LC-OCD)

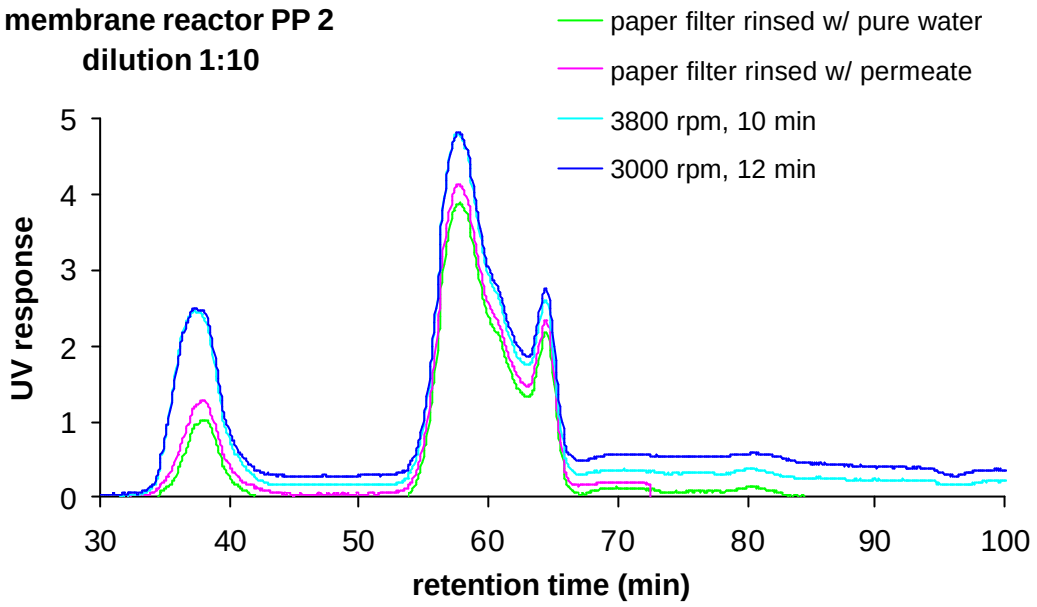

Figure 4.3 UV absorption (254 nm) chromatograms for four of the five samples (too many particles have remained in the sample being centrifuged at 2000 rpm for 10 min for direct analysis on the LC-OCD)

It is suggested to use the paper filtration with permeate rinsing as pretreatment step as it separates the water phase better from the sludge (lower turbidity values). However, a possible explanation for the difference between paper filtered and centrifuged samples could also lie in the fact that the paper filtered samples have been taken 30 minutes before the centrifuged samples. Hence, a second sampling is done on 29th November 2002 to prove or disprove this possibility.

A grab sample (3 L) of activated sludge is taken out of the membrane reactor of pilot plant 2 at 7:45 AM on 29th November 2002. For each sample, an aliquot of 200-300 mL is taken from the grab sample. Samples 1-6 are filtered over paper filter that have been rinsed with permeate from pilot plant 2. A new paper filter is used for each sample. Samples 1 and 2 are filtered in parallel immediately upon arrival of the activated sludge sample in the laboratory on-site in Ruhleben. Once these two samples are filtered, samples 3 and 4 are paper filtered (see Table 4.2). Thus, the first four samples (1-4) are paper filtered as soon as possible after sampling. Samples 5 and 6 are filtered 45 min after sampling in order to have a direct comparison with the centrifuged samples.

Approximately half of the grab sample is transferred to the laboratory at the Technical University Berlin which takes 30-45 min. Hence, centrifugation (3000 rpm, 20 min) of samples 7 and 8 starts at 8:30 AM, i.e. 45 min after sampling. Once these two samples are centrifuged, samples 9 and 10 are centrifuged at 2000 rpm for 10 min (start is 65 min after sampling). After the centrifugation an additional glass fiber filtration is performed on samples 8 and 10 (see Table 4.2).

Table 4.2 Sample preparation of activated sludge from the membrane reactor of PP 2 taken on 29th November 2002

Sample ID	Start of sample preparation	type of sample preparation
1	7 :47	paper filtration
2	7 :47	paper filtration
3	7 :56	paper filtration
4	7 :56	paper filtration
5	8 :29	paper filtration
6	8 :29	paper filtration
7	8 :30	Centrifuge, 3000 rpm, 20 min
8	8 :30	sample 7 + glass fiber filtration*
9	8 :50	Centrifuge, 2000 rpm, 10 min
10	8 :50	Sample 9 + glass fiber filtration*

*1 µm glass fiber filters (GF52, Schleicher & Schuell, Germany)

Figure 4.4 gives the results of the COD, TOC, turbidity, polysaccharide, and protein measurements for all 10 samples. The paper filtered samples (1-6) have overall lower values than the centrifuged samples (7, 9, 10), thus confirming the results from 19th November 2002. Sample 8 (centrifugation at 3000 rpm for 20 min followed by glass fiber filtration) shows similar results as the paper filtered samples. Considering that the paper filtration is much faster than centrifuging and glass fiber filtering the sample, the paper filtration appears to be the more viable option. It has the additional advantage that it can be done on-site and thus, as soon as possible after sampling.

The LC-OCD analysis supports these findings. Figure 4.5 and 4.6 depict the organic carbon chromatograms of the ten samples. A comparison of the six paper filtered samples provides very similar results with a slightly higher PS peak for sample 4 and a some what smaller HS peak for sample 2 (see Figure 4.5). As for all the other parameters the sample that has been only centrifuged at 2000 rpm for 10 min (sample 9) exhibits the highest PS peak (see Figure 4.6) while the other three centrifuged samples show similar chromatograms. The small additional peak at 82 min in the two glass fiber filtered samples (sample 8 & 10) is probably due to some leaching from the filters. This suggests that the permeate rinse of the filters has not been sufficient.

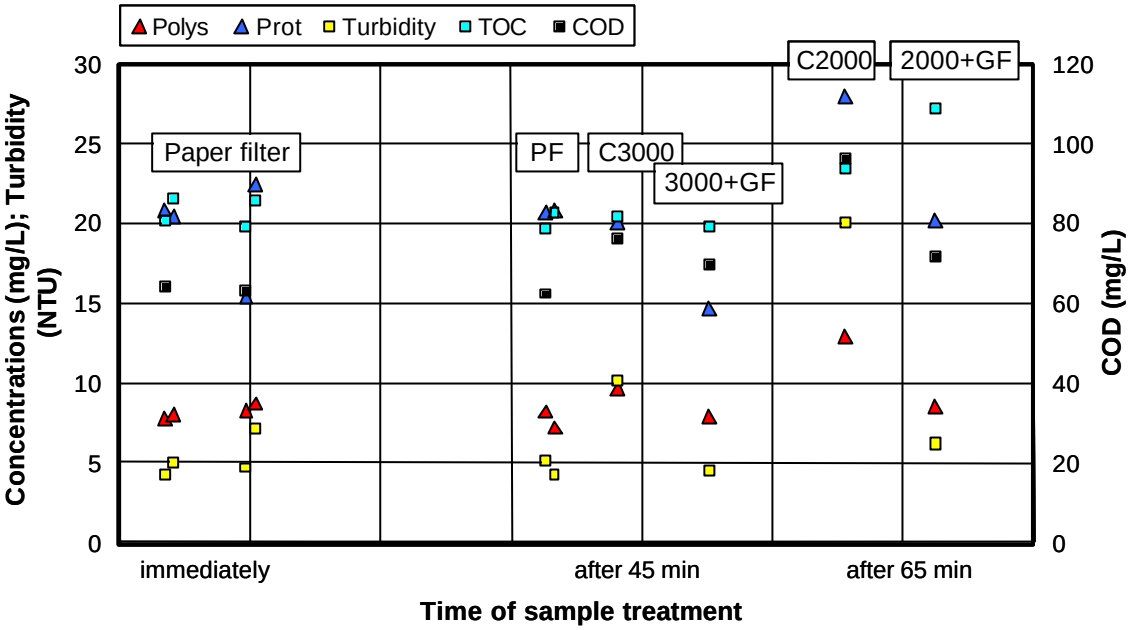

Figure 4.4 Results of experiments regarding sample preparation for the paramaters COD, TOC, turbidity, and polysaccharides and proteins as measured by the photometric method (sample: activated sludge from the membrane reactor of PP 2; sampling date: 29.11.02); PF=paper filter, GF=glass fiber filter

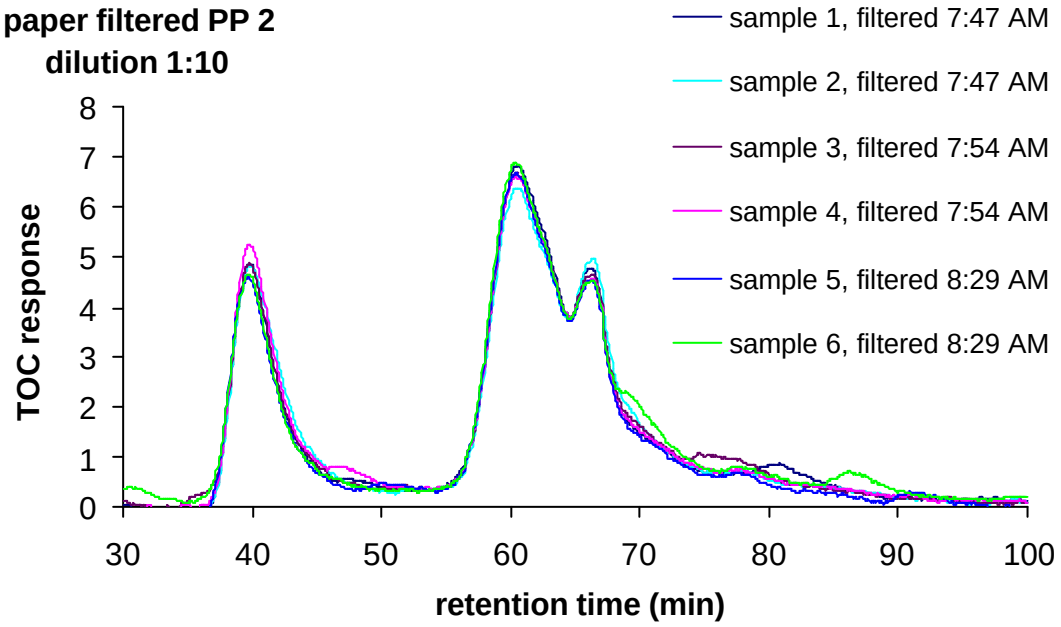

Figure 4.5 Organic carbon chromatograms for the replicate experiment using paper filtration as sample preparation (29.11.02)

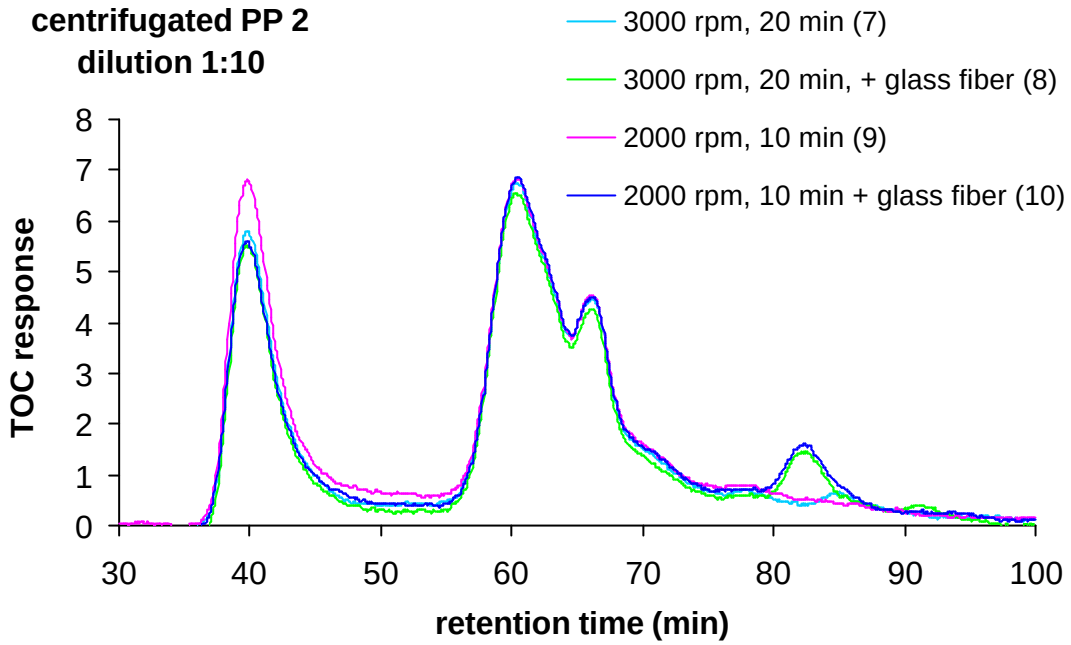

Figure 4.6 Organic carbon chromatograms for centrifugated samples (29.11.02)

A comparison of the six paper filtered samples gives as maximal deviation of the mean values:

COD	5 %	
TOC	5 %	
Polysaccharide	10 %	
Protein	23 % (6 % without sample 3)	values appear to scatter
Turbidity	40 % (16 % without sample 4)	values appear to scatter
LC-OCD (PS peak)	10 %.	

The protein measurement is prone to interferences with humic substances [Frolund et al 1996]. As the protein concentration does not show any correlation with the fouling rate, the analysis of this parameter is disregarded after the first few month of the study. The turbidity measurement of the paper filtered samples is primarily used to control the sample pre-treatment. Hence, the overall error of sampling, paper filtration, and sample analysis amounts to 10 %.

4.2 Sample storage

Although samples should always be measured on the sampling day, this is sometimes not possible. In order to find out how long samples can be stored under refrigeration (4° C) without losses, a sample storage experiment has been carried out. The filtrate sample taken on 11th December 2002 from each pilot plant has been paper filtered on-site and analysed the same day. The paper filtered samples are then stored in the refrigerator and further analyses are performed on 20th December 2002 and 8th/9th January 2003. Unfortunately, one third of the sludge from pilot plant 1 has been lost the weekend before the sampling,

i.e. on 7th and 8th December 2002. This resulted in a high turbidity of the filtrate PP 1 sample. With 8 NTU the turbidity is four times the value measured for filtrate PP 2 which had 2 NTU (see Figures 4.7 and 4.8).

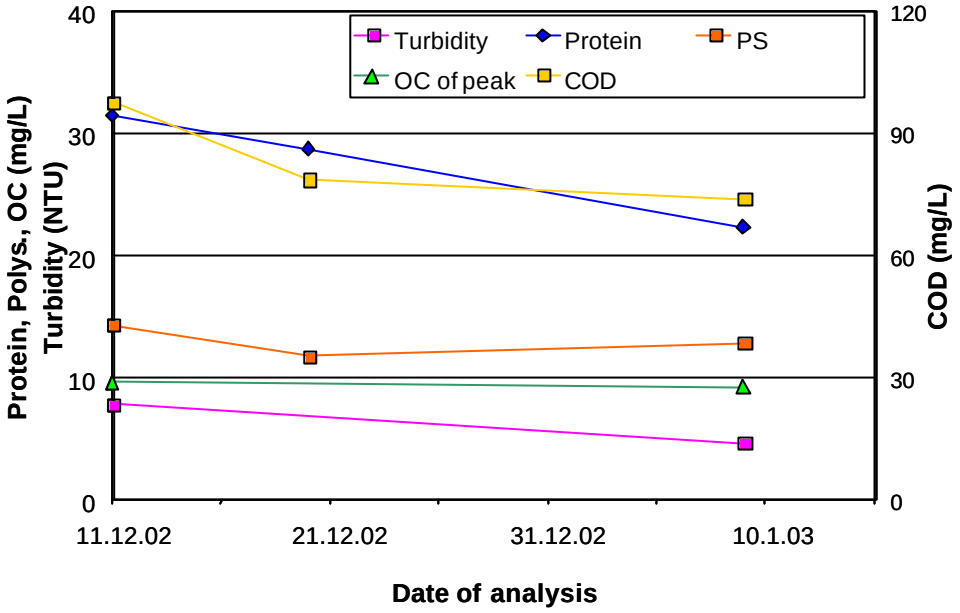

Figure 4.7 Results of sample storage experiment for filtrate PP 1 (OC of peak = organic carbon concentration of PS peak as measured by LC-OCD; PS = polysaccharide concentration measured photometrically)

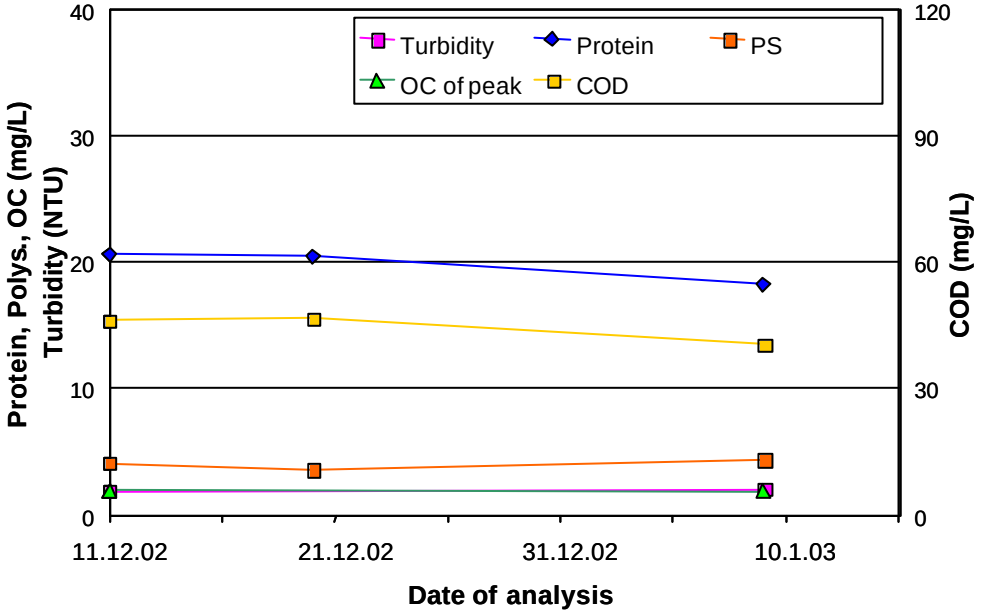

Figure 4.8 Results of sample storage experiment for filtrate PP 2 (OC of peak = organic carbon concentration of PS peak as measured by LC-OCD; PS = polysaccharide concentration measured photometrically)

In all parameters a general decrease in concentration can be observed. In PP 2, this is a very slow process with nearly no changes for the polysaccharides concentration (measured photometrically as well as with the LC-OCD) and for the turbidity. The decrease in COD and protein concentrations occur only after a storage time of more than 10 days. In PP 1, the changes are severe, indicating either a microbiological activity or a removal of the organic carbon through aggregation. In addition to the decrease of all parameters, a change in the chromatograms is observed (Figure 4.9 and 4.10). The first peak in the UV chromatogram (inorganic colloids) is significantly lower after storage of one month (Figure 4.10) supporting the possibility of aggregation. The peaks in the TOC chromatogram remain at approx. the same height, but an additional peak appears after 89 min. retention time (amphiphilic and neutral compounds).

In conclusion, a storage of "normal" samples under refrigeration appears to be reasonable for two or three weeks. If longer storage times are necessary, freezing the samples should be considered. However, storage of highly turbid samples is not possible. The filtration step should be repeated, if the sample shows obvious turbidity after sampling. If the turbidity remains, it should be analysed as soon as possible.

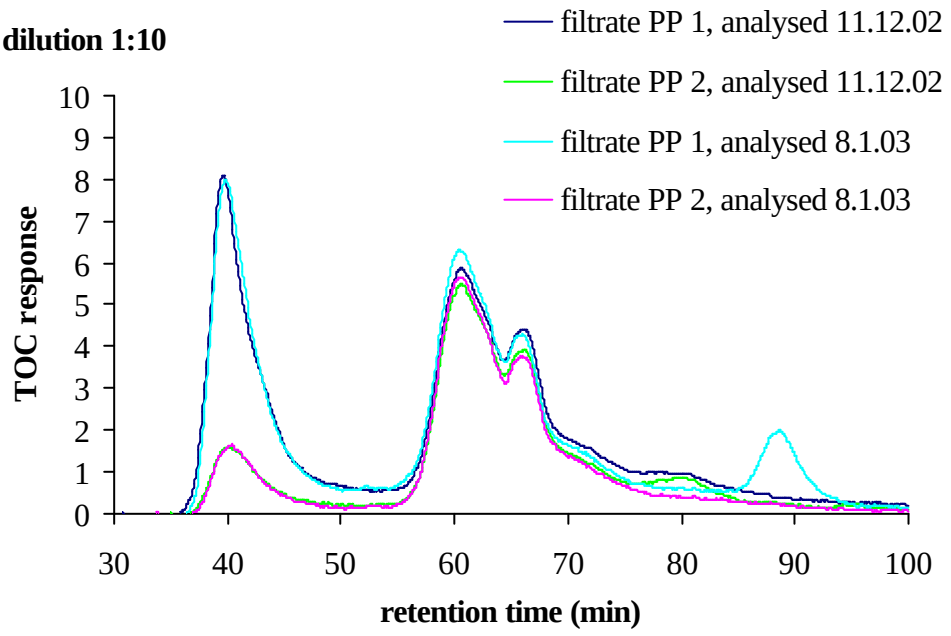

Figure 4.9 Organic carbon chromatograms for filtrate PP 1 sampled 11th December 2002, analysed 11th December 2002 and 8th January 2003

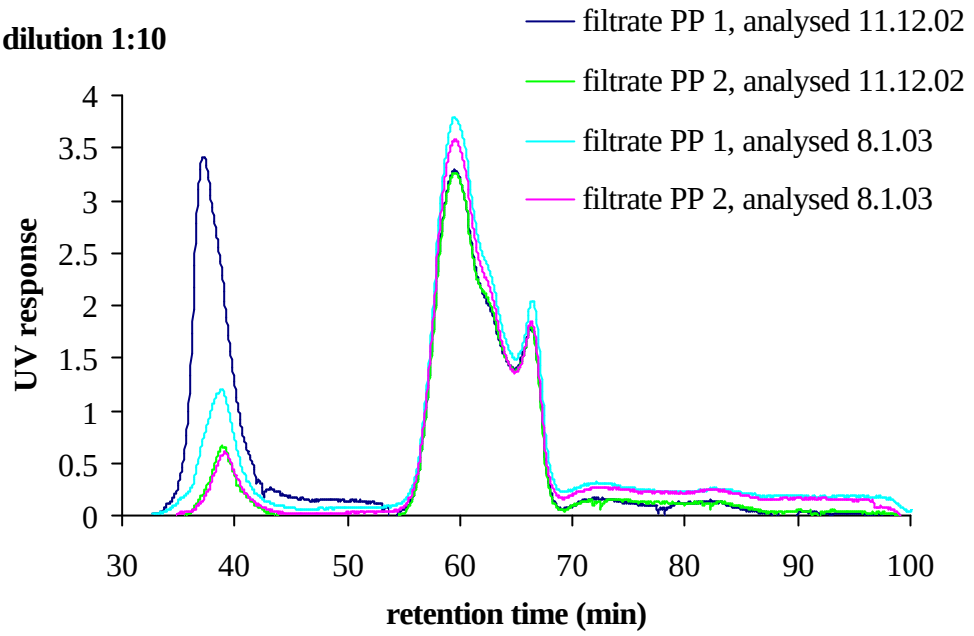

Figure 4.10 UV absorption chromatograms for filtrate PP 1 sampled 11th December 2002, analysed 11th December 2002 and 8th January 2003

4.3 Procedures for sampling, pre-treatment and sample analyses

The following procedures for sampling, sample pre-treatment and handling as well as sample analyses result from the method development experiments as described in sections 4.1 and 4.2. Samples are taken every week. Photometric analysis are done on all samples by Dr. Sandra Rosenberger (separate report) while LC-OCD analysis are done every two weeks (see results section 6).

4.3.1 Sampling

Samples are taken 7 min after the last backwash, i.e. in the middle of a filtration cycle between two backwashes (a backwash is performed every 12 minutes). The time of sampling within one filtration cycle could have an impact on the samples due to the concentration of rejected solutes on the feed side of the membrane, i.e. in the sludge. Furthermore, any influence of the backwash procedure should be minimized to avoid any possible problems when comparing data.

In contrast to the time of sampling within one filtration cycle, it is known that the time of sample within a week is very important. Besides strong daily variations, weekly variations of the wastewater influent exist. Hence, samples are taken at approximately 8:00 AM on Wednesdays (December 2002 – July 2003) / Tuesdays (July 2003 – November 2003). The change of day from Wednesday to Tuesday is made because further measurements are done on Tuesday, enabling better comparison to other studies on the pilot plants.

4.3.2 Sample pre-treatment

Paper filtration with permeate rinsing is used as pre-treatment step in order to separate the water phase from the activated sludge. The advantages of this procedure are that it is a standard protocol for sludge separation, considers colloids and solutes, and can be done on-site in Ruhleben. The latter one guarantees that the pre-treatment step is done as soon as possible, although no clear influence has been detected within one hour of sampling.

4.3.3 Sample analyses

The results from the storage experiments indicated that only minimal changes of the samples occur within two weeks when stored in the refrigerator. Samples should, therefore, be analysed as soon as possible but within two weeks at the latest. Strong changes of the samples have been detected after four weeks of storage, i.e. somewhere between two and four weeks samples start to change even when kept in the refrigerator.

4.4 Comparison of LC-OCD and photometric methods

Besides establishing good sampling and analysis routines, another objective of this research study is to compare the results obtained by the LC-OCD technique with the results derived from the photometric method according to Dubois. The exact procedure of the photometric method is explained in Rosenberger [2003].

In order to be able to compare the two methods, the organic carbon concentration (mg C/L) is calculated from the respective results obtained by each method. More specifically, the organic carbon concentration of the PS peak eluting between 35 min and 50 min retention time is used for the LC-OCD method. The results of the photometric method are usually given as mg glucose equivalent/L. This value is thus multiplied by a factor of 0.36 which corresponds to the carbon content of the glucose standard used (monohydrate glucose).

Figure 4.11 shows the correlation between the two methods. A general correlation exists, however, the correlation factor is two instead of one, with the LC-OCD showing higher values than the photometric method. Additionally, the correlation factor appears to be slightly below two for an SRT of 15 d compared to the SRT of 8 d. This might be due to a possible change in the sludge characteristics at different SRT.

A calibration of the two methods with the same standards can not explain the correlation factor of two: when using glucose (=standard of photometric method), the same amount of organic carbon is found by both methods; when using dextrans (=standard of LC-OCD method), both methods give similar organic carbon concentrations although the results of the photometric method are approximately 15 % lower. It is, therefore, supposed that the nature of the polysaccharides from the membrane bio-reactors is of a complex nature leading to an underestimation by the photometric method. On the other hand, the LC-OCD

method leads to an overestimation of the polysaccharides as organic colloids and probably proteins elute in the PS peak, too.

In conclusion, both methods are suited to assess the evolution of the polysaccharides in the two pilot plants as well as their influence on the fouling of the membranes.

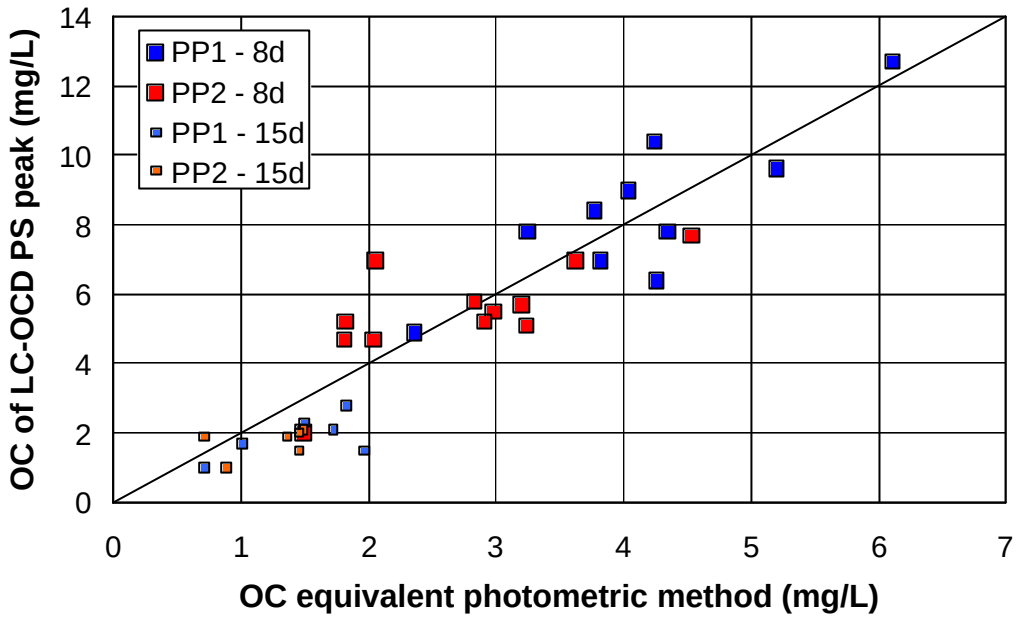

Figure 4.11 Comparison of organic carbon content as measured in the PS peak of the LC-OCD chromatogram and by the photometric method (method following Dubois; photometric measurements done by S. Rosenberger)

Chapter 5 Results of bi-monthly baseline measurements

5.1 Identification of main foulants

Two membrane bioreactors (MBRs) are studied over a period of one year from December 2002 to November 2003. Samples are taken every two weeks from the membrane reactors. These samples are paper filtered (see section 3.3) and analysed with LC-OCD. The activated sludge from the membrane reactor is separated from the water sample through this pre-filtration step. This enables the analysis of the dissolved/colloidal microbial products. Additionally, permeate samples are used for comparison purposes. Figure 5.1 depicts representative organic carbon chromatograms of a sample from the membrane reactor (filtrate) and the permeate for pilot plant 2. The main difference is the absence of the polysaccharide (PS, after 40 min) peak in the permeate. Thus, large macromolecules such as organic colloids, polysaccharides and proteins with molecular sizes of roughly 50000 to > 150000 D are retained by the membrane. On the other hand, smaller macromolecules, i.e. humic substances, their hydrolysates, and organic acids, are able to pass the membrane barrier. Only small amounts of these molecules are rejected by the membrane as can be seen in Figure 5.1 by the slightly smaller peak height in these peaks for the permeate sample. These molecules are likely trapped in the fouling layer or adsorbed onto activated sludge flocs during the membrane filtration. Hence, organic colloids and polysaccharides can be seen as main foulants in membrane bioreactors. During the twelve month monitoring period of the two pilot plants, the evolution of the PS peak in the chromatograms is investigated and correlated with operating conditions such as sludge retention time (SRT) or temperature.

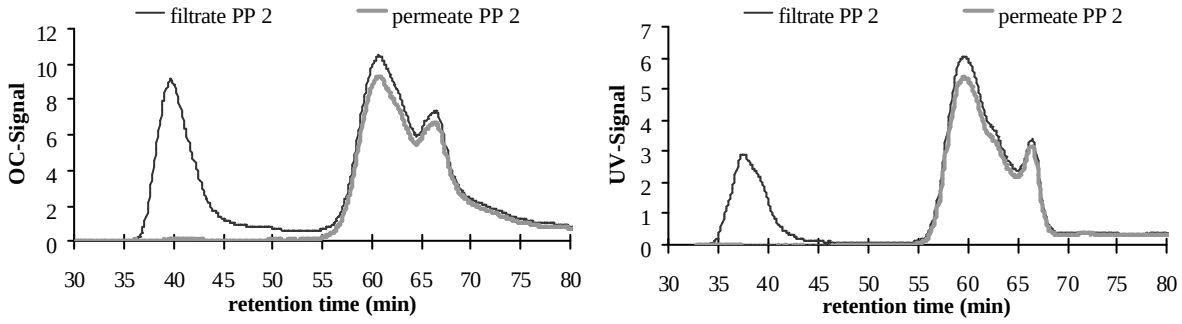

Figure 5.1 LC-OCD chromatograms of filtrate and permeate for pilot plant 2; left for organic carbon content, right for UV absorption at 254 nm

Besides the molecular size distribution of the organic carbon, the total organic carbon (TOC) of each sample is measured in the by-pass peak (see section 3.4 for details). Figure 5.2 shows the evolution of both TOC and organic carbon content of the PS peak for the filtrate samples of pilot plant 1. A clear evolution over the year can be seen for both, with higher values during winter and lower values in summer. In general, the TOC and PS evolutions follow the same pattern. This suggests that an increase in total organic carbon is

primarily due to an increase in large macromolecules (organic colloids and polysaccharides) as detected in the PS peak. In comparison, the humic substances and organic acids peaks in the LC-OCD chromatograms remain constant throughout the year. The organic carbon content present in both peaks together is also depicted in Figure 5.2 (HS + acids). The mean value for the humic substances plus organic acids is 11.0 ± 1.2 mg C/L. Pilot plant 2 exhibits a similar trend showing a good correlation between TOC and PS (data not shown) while the mean value of humic substances plus acids amounts to exactly the same value as for pilot plant 1 (11.0 ± 1.2 mg C/L). Thus, a difference in the performance of the two pilots plants can be fully attributed to the amount of organic colloids, polysaccharides, and proteins as far as dissolved/colloidal molecules are concerned.

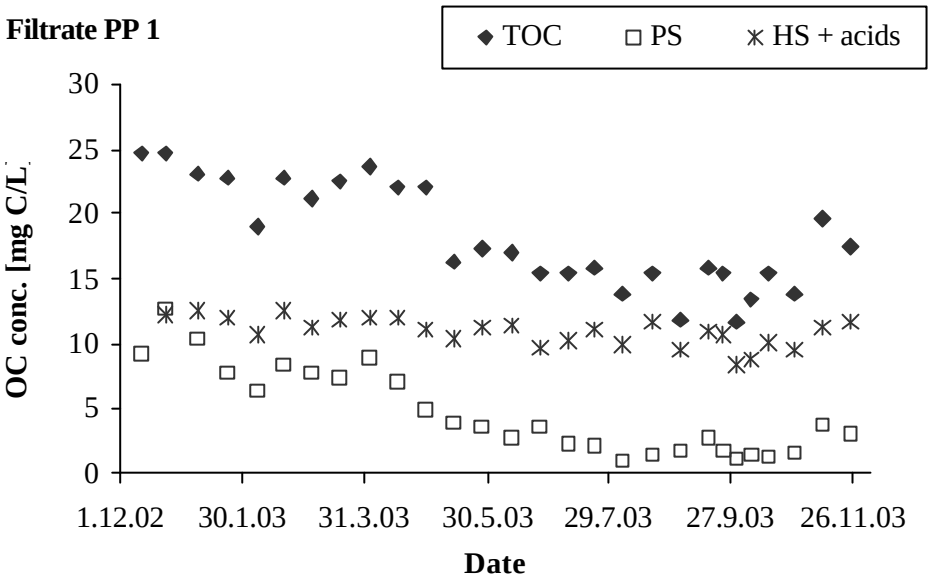

Figure 5.2 Evolution of total organic carbon (TOC), polysaccharides and organic colloids (PS), and humic substances and organic acids (HS + acids) over a 12 month period for the filtrate of pilot plant 1

Since the large macromolecules such as polysaccharides, proteins, and organic colloids are retained completely by the MBR, the organic carbon in the permeate of both pilot plants mimics the course of the humic substances and organic acids in the filtrate samples. The total organic carbon concentration in the permeate is, therefore, stable at around 12.5 ± 1.2 mg C/L for pilot plant 1 and 12.6 ± 1.6 mg C/L for pilot plant 2, regardless of the incoming wastewater. The difference of these values to the humic substances and organic acids concentrations given above for the filtrate sample is due to amphiphilic compounds which elute after the organic acids peak (see section3.4.2).

Furthermore, the analysis of the by-pass peak in conjunction with the chromatograms reveals informations on two more fractions: i) the total organic carbon rejected by the membranes and ii) hydrophobic compounds such as lipids which adsorb onto the SEC resin and therefore, are not accessible to an evaluation using size exclusion

chromatography. For the first fraction (i), the difference between filtrate and permeate samples (=by-pass $TOC_{filtrate}$ – by-pass $TOC_{permeate}$) is calculated as it equals the total organic carbon retained by the membranes. Of the total organic carbon present in the filtrate samples an average of 4.6 mg C/L are retained in PP 1 and an average of 4.8 mg C/L in PP 2. On the other hand, the hydrophobic compounds (fraction ii) are assessed by comparing the TOC measured in the by-pass mode and the sum of the peaks in the chromatogram (CDOC[2]). The hydrophobic compounds would be equal to the difference between by-pass TOC and CDOC. However, no significant amounts of hydrophobic compounds could be detected for the Ruhleben wastewater in both pilot plants (TOC and CDOC values are approximately identical within the measuring error of the instrument).

5.2 Influence of operational parameters

Pre-trials to this study in August 2002 are showing a higher polysaccharide peak for pilot plant 2 as depicted in Figure 5.3. This is in accordance with the higher fouling of the membrane in pilot plant 2 (post-denitrification) at that time. One of the objectives of the MBR pilot plant study is, therefore, to find out whether the pre-denitrification process design induces less fouling of the membrane than the post-denitrification or vice versa. However, by December 2002, when regular sampling started on both pilot plants for this study, the situation has been inversed with pilot plant 1 (pre-denitrification) exhibiting higher fouling and higher polysaccharide concentrations. Hence, a more important fouling potential can not be linked solely to either one of the two configurations tested, i.e. pre-denitrification (PP 1) or post-denitrification (PP 2), as apparently other parameters are of importance, too.

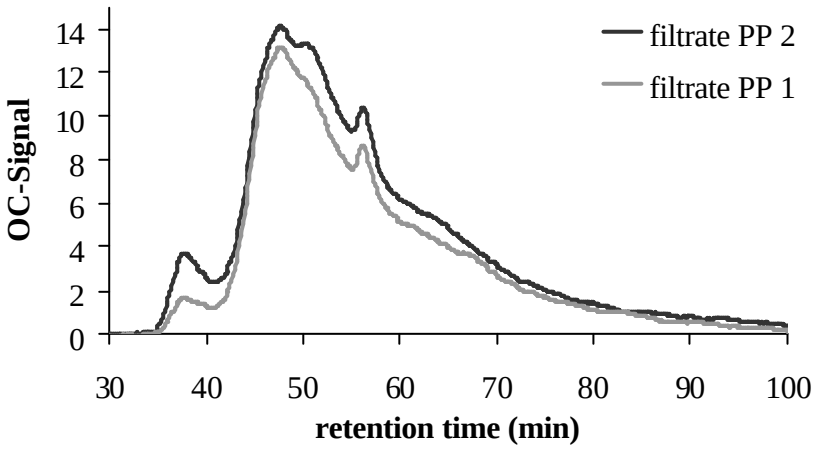

Figure 5.3 Organic carbon chromatograms (column HW-50S) of filtrate samples from 27th August 2002 for pilot plant 1 (bottom) and 2 (top)

During this research the pilot plants have been operated at two different sludge retention times (SRT): an SRT of eight days from January until June 2003 and an SRT of fifteen

[2] CDOC = dissolved organic carbon accessible by size exclusion chromatography

days from July until November 2003. In December 2002, the SRT has been decreased continuously to reach the eight days by January 2003 as earlier research has been done at 26 days down to 20 days sludge retention time [Gnirss et al 2003a and 2003b]. A large amount of fibers has been cut out of the membrane unit in pilot plant 2 for autopsy purposes on 15th September 2003. Aeration problems in the aerobic zone of pilot plant 2 have occurred in October and November 2003 leading to a complete failure of nitrification from 23rd Oct. - 7th Nov. 2003. Hence, only data from 8th January 2003 until 16th September 2003 are taken into consideration for the following analysis and evaluation.

For this timeframe, the average overall correlation between the TOC of the permeate and the TOC of the filtrate has been: $TOC_{filtrate} = TOC_{permeate} + 1.25 * PS_{filtrate}$ (see Figure 5.4). Thus, the substances eluting in the PS peak account for 80 % of the TOC that is retained by the membrane. The remaining 20 % correspond to the rejection of small amounts of humic substances,organic acids, and amphiphilic compounds.

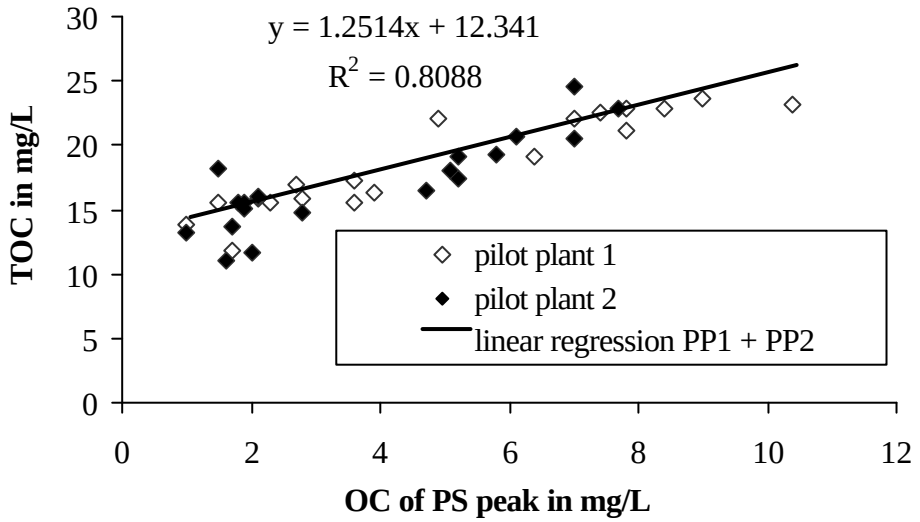

Figure 5.4 Correlation between TOC and PS in the filtrate samples for both pilot plants

Since the compounds of the PS peak (organic colloids, polysaccharides, proteins) are the only ones being completely retained in the membrane bioreactors apart from hydrophobic substances, a good correlation with the fouling rate should be found. The fouling rate is defined here as the slope of the membrane resistance (for a more detailed description on how to calculate the fouling rate see Rosenberger et al [2003]). However, a correlation between fouling rate and polysaccharide concentration in the filtrate samples of both pilot plants is only discernable for a sludge retention time (SRT) of eight days, while for an SRT of 15 days only a poor correlation can be found, i.e. scattered points (see Figure 5.5). However, for an SRT of 15 days, all values (polysaccharide concentrations as well as fouling rate) are in the low range and therefore, can have higher errors (less sensitivity of the photometric method used by Rosenberger in the low concentration range; the detection limit of the method is 2 mg/l as glucose equivalent). Additionally, the database for the SRT

of 8d is larger as a total of six months (January-June 2003) is assessed while only the first three months of data are used for the SRT of 15d (July-September 2003).

It must be mentioned that Figure 5.5 depicts the correlation between fouling rate and polysaccharide concentration measured photometrically as glucose equivalent by S. Rosenberger [Rosenberger and Laabs unpublished results]. The photometric measurements have been done in parallel to the LC-OCD analysis but samples are analysed weekly instead of every two weeks as for the LC-OCD. Hence, clearer correlations can be extracted from the photometric analyses due to more data points.

In their review of soluble microbial products (SMP), Barker and Stuckey [1999] state that the amount of SMP produced is influenced by sludge retention time, organic loading rate, and temperature. For sludge retention time and organic loading rate an optimal operation point / range seems to exist depending on the specific system (aerobic, anaerobic) and the wastewater characteristics.

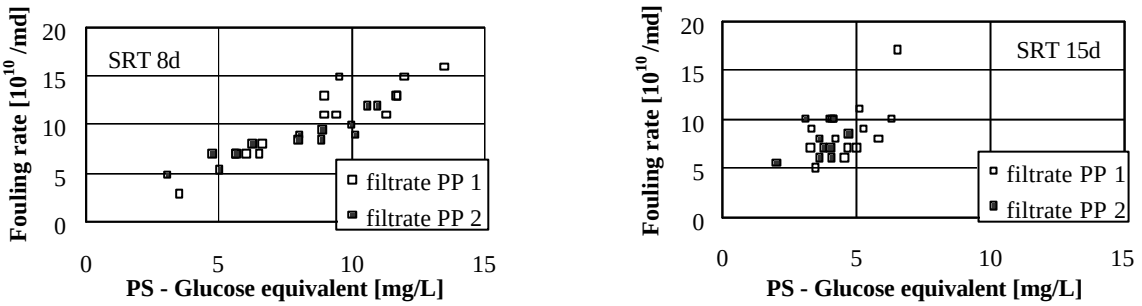

Figure 5.5 Correlation between fouling rate and polysaccharides measured photometrically as glucose equivalent [Rosenberger and Laabs unpublished results] for a sludge retention time of eight days (left) and fifteen days (right)

Nevertheless, a clear evolution of the PS peak in accordance with the change of SRT from 8 days to 15 days can not be found (see Figure 5.6): the PS peak starts to decrease from nearly 8 mg C/L in April 2003 to around 2 mg C/L by June 2003. During this time the SRT is held constant at approx. 8 d. It is only changed at the end of June 2003, reaching a constant SRT of 15 days by mid July 2003.

Besides the sludge retention time, temperature plays an important role in the production of extra-cellular polymeric substances (EPS) / soluble microbial products (SMP). In general, an increase in SMP is seen with decreasing temperature [Barker & Stuckey 1999]. For the two MBR pilot plants in Ruhleben the temperature evolution of the wastewater corresponds much better to the evolution of the PS peak than the sludge retention time. Simultaneously to the decrease of the PS peak an increase in the wastewater temperature from 17° C in mid April 2003 to 23° C in June 2003 is observed (see Figure 5.6 for pilot

plant 2; pilot plant 1 shows similar trends). Other operation parameters such as MLSS and F/M ratio exhibit no clear correlation with the fouling rate in the studied range.

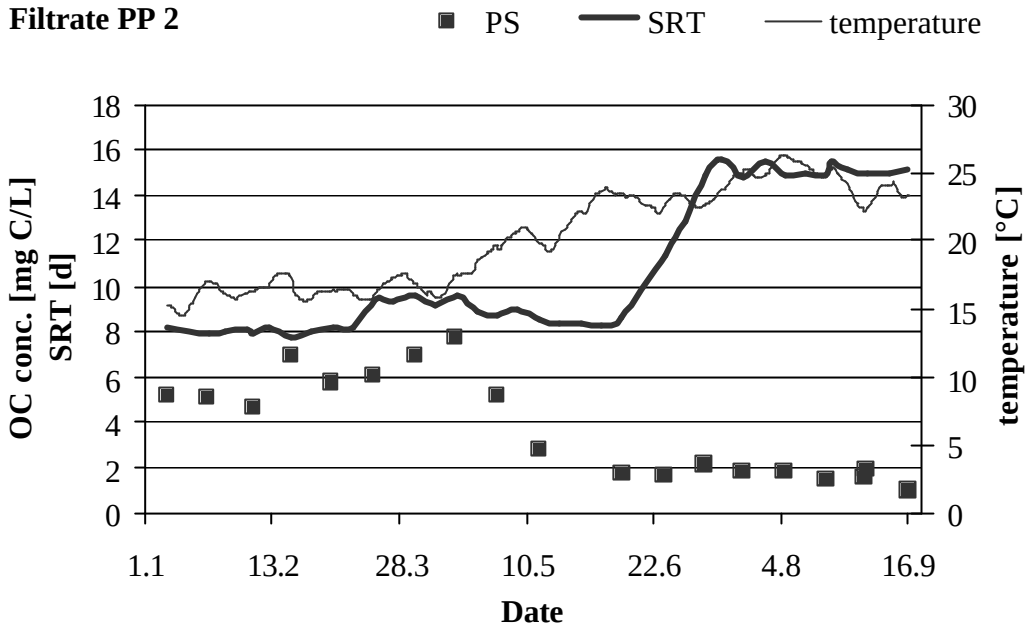

Figure 5.6 Evolution of PS peak (as organic carbon concentration), sludge retention time (SRT in days), and wastewater temperature (as 7-day moving average) from 8.1.-16.9.2003 for pilot plant 2

Although no direct measurement has been done on the amount of stress the microorganisms have been exposed to, environmental stress turned out to be the most important factor in correlation to the PS concentration. Two occurences lead to this impression:

1) The weekend of 7th/8th December 2002, one third of the sludge of pilot plant 1 has been lost resulting in an overload of organic carbon and nutrients for the remaining microorganisms in conjunction with a sudden decrease of SRT. Unfortunately, the first routine sampling of PP 1 has been on 11th December 2002. At this sampling, the PS concentration in the filtrate PP 1 sample is 4.5 times the PS concentration in filtrate PP 2. It takes nearly four months for the PS concentration to come back to the same level as PP 2 (Figure 5.7).
2) In October 2003, pilot plant 2 has aeration problems in the aerobic zone which eventually leads to a complete loss of the nitrification process. A strong and immediate increase in the PS concentration is detected congruently to the decrease of dissolved oxygen in the aerobic reactors. Once the problem is fixed at the beginning of November, the PS concentrations decrease again.

Furthermore, it is not clear how the biological phosphorous removal with the anaerobic reactor impacts the PS production. More in-depth studies would be of interest to resolve this issue.

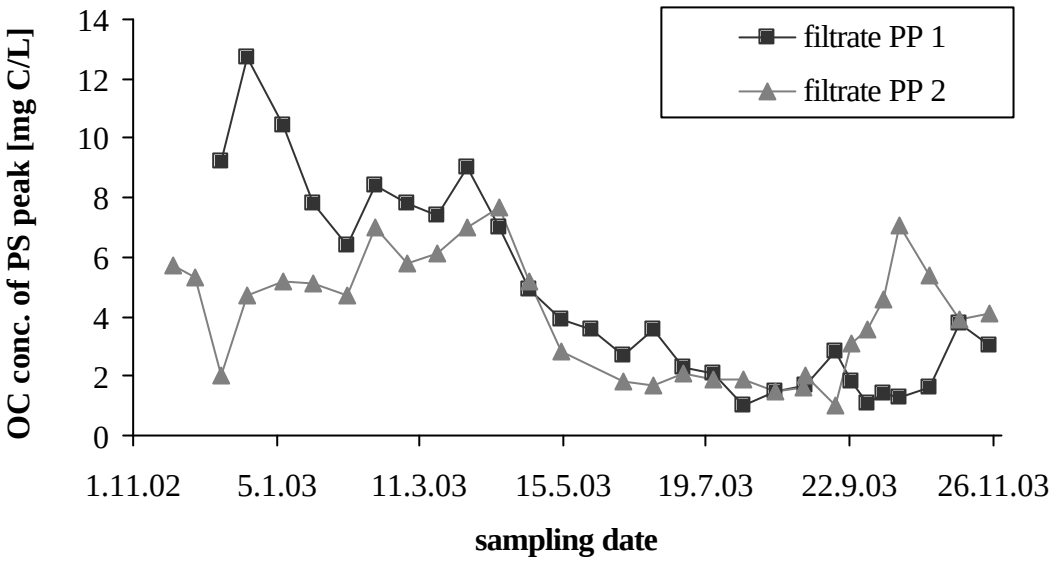

Figure 5.7 Evolution of the organic carbon concentration in the PS peak as measured with the LC-OCD for all filtrate PP 1 and filtrate PP 2 samples

In summary, the following operating conditions have been found to be influential for the fouling behaviour of the two membrane pilot plants:

- stress situation for the microorganisms,
- temperature, and
- sludge retention time

regardless of the nitrification/denitrification process configuration.

Chapter 6 Character of polysaccharides

6.1 Molecular size of PS peak

One characteristic of the polysaccharides and organic colloids as present in the PS peak is the molecular size of these compounds. The molecular size can be assessed from the LC-OCD analyses in conjunction with the stirred cell experiments / pilot plant monitoring. Depending on the calibration standards used, the HW-55S column of the LC-OCD can separate molecules between a few hundred Dalton and approximately 200000 D (see section 3.4). An exact correlation between molecular weight (D or g/mol) and molecular size does not exist, but a rough approximation is that 10000 D correspond to 1 nm. Since the PS peak elutes very close to the void volume it can not be ruled out that the compounds of this peak have larger molecular sizes/ weights than the separation range. This is supported by the shape of the peak which looks slightly off-centered as it goes straight up to the maximum. Hence, the molecular size of the compounds eluting in the PS peak is at least 15-20 nm according to the separation range of the column (HW-55S). Secondly, it can be seen from the stirred cell experiments and pilot plant monitoring that part of the polysaccharides are retained by the membranes. If these compounds are retained by the microfiltration membranes by sieving then a molecular size of more than 100-200 nm could be expected. To get a better idea of the molecular weight range of these substances other SEC columns have to be used, i.e. spanning separation ranges up to a few million Dalton.

6.2 Comparison with extra-cellular polymeric substances (EPS)

In order to support the hypothesis that the polysaccharides are produced during the biological wastewater treatment process some additional analyses are performed. The aim is to see whether extra-cellular polymeric substances (EPS) that are extracted from the biomass of the activated sludge have the same organic carbon distribution as the paper filtered samples of the pilot plants (filtrate PP 1 and filtrate PP 2) when injected into the LC-OCD. In the literature, several methods are described to extract extra-cellular polymeric substances (EPS) from microorganisms [Rosenberger 2003, Späth 1998]. Oftentimes, chemical extraction is used in conjunction with centrifugation. The methods differ in the strength of extraction and possible damage caused to the bacterial cells which is undesirable because lysis products would then be measured as EPS [Rosenberger 2003].

An activated sludge sample from the membrane reactor of pilot plant 2 (sampling date: 7. Oct. 2003; MLSS concentration: 12.5 g/L) is used for the analyses. The EPS is extracted from the biomass of the activated sludge sample by ion-exchanger which is separated from the extracted EPS by sedimentation and centrifugation after 2 hours (experiments are carried out by Sandra Rosenberger using the method described in Rosenberger [2003]).

Parallel to the EPS extraction from the biomass, a filtrate PP 2 sample is mixed with the ion-exchanger (filtrate PP 2 + IX) as well as a sample of pure water (=reference sample).

The results of the LC-OCD analysis for the three samples are depicted in Figure 6.1 (organic carbon response) and Figure 6.2 (UV absorption). The chromatograms of the reference sample (H_2O + IX) represent the amount of organic carbon and UV absorption, respectively, caused by bleeding of the ion-exchanger as pure water itself gives no organic carbon or UV signal. The filtrate PP 2 + IX sample is identical to the filtrate PP 2 sample (filtrate PP 2 as measured in the routine analyses, i.e. without addition of ion-exchanger). The higher HS peak and acid peak and the additional peak at 82 min retention time for the filtrate PP 2 + IX sample compared to the filtrate PP 2 sample are due to the bleeding of the ion-exchanger (see reference sample, Figure 6.1).

The most important organic carbon peak for the extracted EPS sample (sludge + IX, Figure 6.1) is the PS peak. Hence, polysaccharides and organic colloids make up a large part of the extra-cellular polymeric substances. Proteins, on the other hand, do not seem to play such an important role as there is no clear peak around 53 min retention time where the bovine serum albumin (BSA) standard elutes. Of course, this can also mean that the proteins are larger than BSA and are eluting in the PS peak. Overall, the distribution of organic carbon is similar for the extracted EPS (sludge + IX) and filtrate PP 2 (with and without addition of IX). This is not the case for the UV absorption chromatograms where the first peak of the extracted EPS clearly dominates the whole chromatogram. The first peak in the UV chromatogram is usually attributed to inorganic colloids [Huber and Frimmel 1996] although it is not clear where these would stem from in this case. Other possible explanations would be i) that the UV absorption is caused by organic colloids present in the PS peak of the organic carbon chromatogram which are scattering the light or ii) that proteins are present in the PS peak of the organic carbon chromatogram which absorb UV light meaning that the two peaks do correspond or iii) a combination of the two aforementioned things. Further research is needed in this area to investigate the cause of the UV absorption and whether the first peak of the OC and UV chromatograms correspond to one another or not.

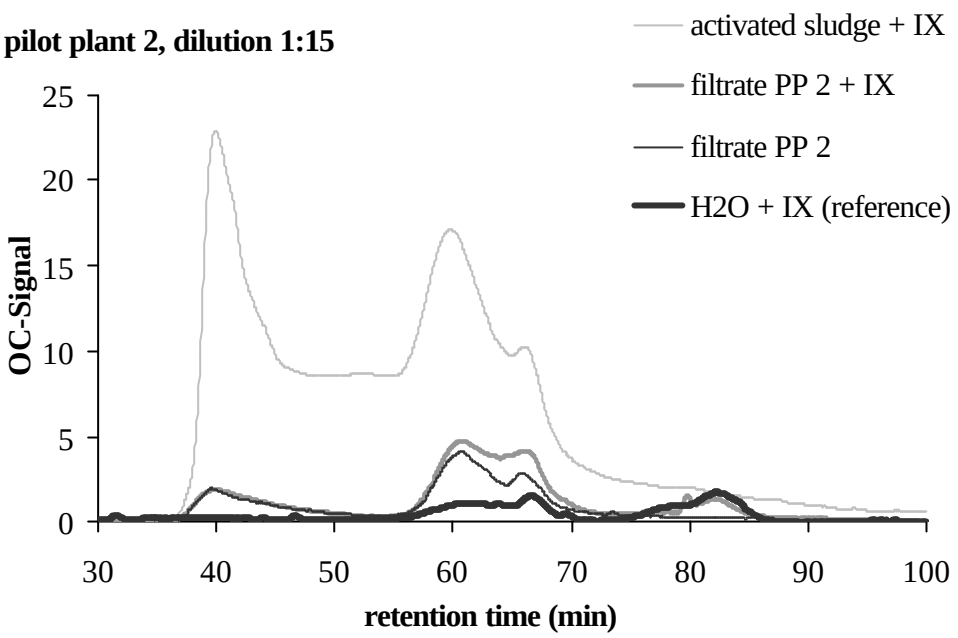

Figure 6.1 Organic carbon chromatograms of EPS extraction experiments (legend from top to bottom corresponds to chromatograms from top to bottom)

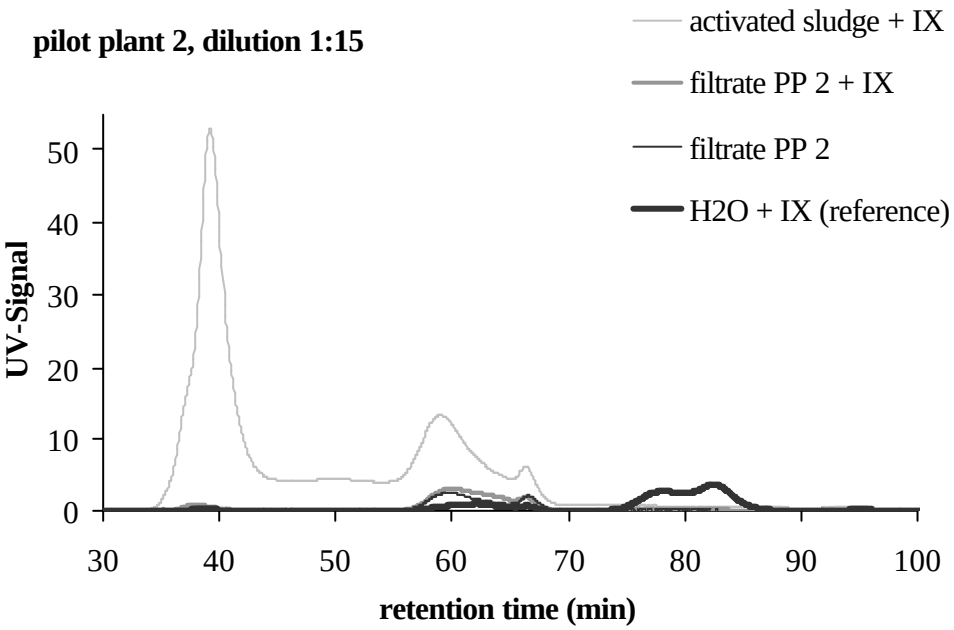

Figure 6.2 UV absorption chromatograms of EPS extraction experiments (legend from top to bottom corresponds to chromatograms from top to bottom)

6.3 Sugar analyses

Furthermore, two samples are given to other institutes for sugar analysis. Investigations in the group of Prof. Kroh (Department for Food Chemistry, Technical University Berlin, Germany) are done with HPLC and thin-film chromatography. A filtrate PP 2 sample is analysed several times after concentration with rotary-evaporation by a factor of 500-1000. Due to the complex matrix of wastewater it is difficult to get clear results with the thin-film chromatography. However, the HPLC shows that most of the polysaccharides in the filtrate PP 2 sample are extremely large molecules as only few single sugars or simple polysaccharides (e.g. disaccharides) are detected. This supports the findings of the LC-OCD that the molecular weight of the polysaccharides is somewhere between 150000 D and > 200000 D (see section 6.1). The highest peak detected by the HPLC of Prof. Kroh is Anhydroglucogen (AHG) which is a sugar typically found in microbiological systems.

The group of Prof. Croué (Laboratory for Water and Environmental Chemistry, University of Poitiers, France) analyses sugars and especially aminosugars using Py-GC-MS (pyrolysis gas chromatography with mass spectrometry). In this case, two samples are sent: i) a filtrate PP 2 sample and ii) the isolated PS peak of the filtrate PP 2 sample. The PS peak is isolated using the LC-OCD by simply collecting the effluent of the UV detector between 33 min and 50 min retention time. Due to the dilution of the PS peak sample by the eluent of the LC-OCD, this sample has to be concentrated by a factor 12 using rotary-evaporation. The filtrate PP 2 sample has been analysed without additional concentration. The filtrate PP 2 sample contains galactosamines and glucosamines while only glucosamines are detected in the PS peak sample. Glucosamines are of biological origin and can be found in chitin, glycolipids, mukopolysaccharides, and glycoproteins. N-acetyl-glucosamin is, for example, part of bacterial cell walls.

Chapter 7 Stirred cell experiments

In addition to the routine samples taken every two weeks, stirred cell tests have been performed with filtrate and permeate samples of the two pilot plants. The aim is to confirm the fouling behaviour found in the pilot plants. For the stirred cell experiments 1-2 L of filtrate / permeate are taken using the same procedure as for the routine samples (see section 3.3). The membrane is the VVLP flat sheet membrane which comes closest to the Memcor hollow fiber membrane installed in the pilot plants, i.e. a hydrophilized polyvinylidene fluoride microfiltration membrane with a pore size of 0.1-0.2 µm. All tests are conducted at a constant pressure of 0.3 bar (see section 3.2). The initial flux J_0 [3] varies between the membrane specimens from 400 L/hm² to 750 L/hm². Hence, the normalized flux J/J_0 is used to compare experimental results.

Figure 7.1 shows the flux decline over the DOC delivered[4] to the membrane surface for the permeate and filtrate of pilot plant 2. It is clear that the filtrate sample has a high fouling potential. After a delivered DOC of 650 mg C/m² the flux has decreased to 20 % of the initial flux whereas the permeate sample has not fouled the membrane at all (J/J_0=1 at 650 mg C/m²). This strong flux decline of the filtrate occurs within the first 15 min of the stirred cell experiment. In the remaining time (total filtration time = 90 min) a further decline of 12 % occurs (final flux = 8 % of J_0) after a total of 1580 mg C/m² are delivered to the membrane surface. In comparison, for the permeate sample nearly 2000 mg C/m² are delivered to the membrane surface within the first 15 min of filtration causing only an overall flux decline of 7 %.

The difference between the filtrate PP 2 sample (taken 14. May 2003) and the permeate PP 2 sample (taken 16. April 2003) is that the permeate sample has passed through the hollow fiber membrane of the MBR pilot plant. Thus, the permeate sample collected during the stirred cell experiment of permeate PP 2 has been filtered twice through the same type of membrane as the feed (permeate PP 2), having already undergone a first filtration in the pilot plant. Since the permeate PP 2 sample does not foul a similar membrane all the foulants must have been retained in the MBR. When comparing the LC-OCD chromatograms it becomes clear that the difference between the two feed waters is the PS peak which is only present in the feed of sample “filtrate PP 2” and not in the feed of sample “permeate PP 2” (Figure 7.2). The corresponding permeate samples as collected during the stirred cell experiments give important information on the foulants. Permeate samples collected during the stirred cell experiments will be referred to as “perm” samples to avoid any confusion with the permeate of the MBR pilot plants (= permeate PP 1 or permeate PP 2). For the test performed with permeate PP 2, the chromatograms of feed and

[3] J_0 is the flux measured after 1 min of sample filtration [L/h m²]

[4] delivered DOC [mg C/m²] = DOC_{feed} [mg C/L] * permeate volume [L] / membrane surface area [m²]

perm mimic each other with the only difference being that the perm chromatogram is slightly smaller in all areas than the feed chromatogram (Figure 7.2 right). Hence, no specific compound is preferentially retained by the membrane. Instead, the overall organic carbon load is responsible for the slight flux decline exhibited during the stirred cell test, i.e. humic substances, organic acids, and low molecular weight substances.

Although these substances are much smaller than the membrane pore size of 0.1 –0.2 µm, it is normal that small amounts are rejected by the membrane. This is due to the fact that the nominal pore size is never a 100 % cut-off as it is assumed for an ideal membrane. In fact, the correlation between the permeation coefficient and the molecular weight/ size is a continuous function [Eberle et al 1979].

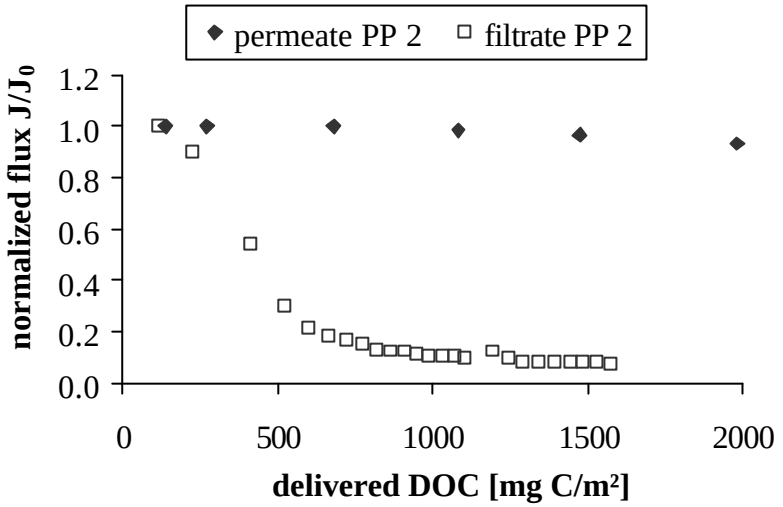

Figure 7.1 Flux decline of permeate and filtrate from pilot plant 2 in stirred cell tests with hydrophilized PVDF microfiltration membranes

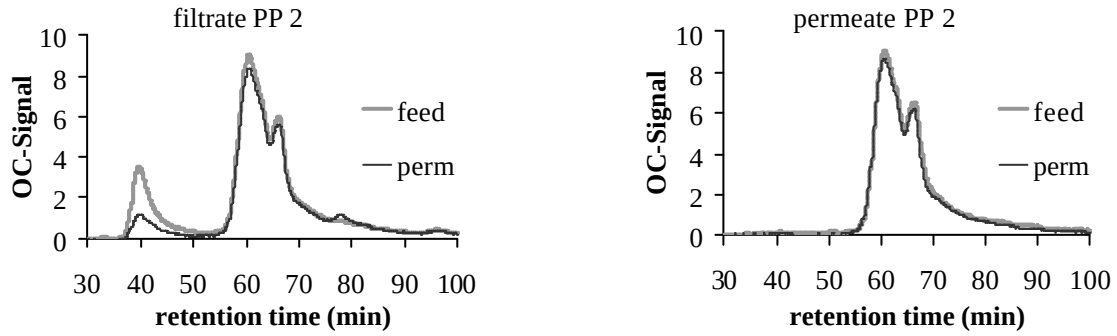

Figure 7.2 LC-OCD chromatograms for stirred cell test with filtrate PP 2 (left) and permeate PP 2 (right); "feed" means the corresponding sample used for the filtration test while "perm" is the permeate collected during the stirred cell test

The feed and perm chromatograms for the stirred cell test performed with filtrate PP 2 are depicted in Figure 7.2 (left). In this case, a clear difference between the feed and perm chromatograms is found in the PS peak around 40 min retention time. The humic substances and acid peaks are only diminished to a small extent as seen in the pilot plants too. In contrast to the pilot plants behaviour, the PS peak is not completely retained by the membrane in the stirred cell test. One third of the initial PS concentration goes through the flat sheet membrane (VVLP) and is found in the perm sample. Thus, from the large macromolecules (organic colloids, polysaccharides, and proteins), 0.8 mg C/L of the initial 2.4 mg C/L are truly dissolved and smaller than the membrane pore size of 0.1-0.2 µm.

Nevertheless, the PS peak is completely removed by the MBR pilot plants. Because the filtrate and permeate samples from the pilot plants are always taken in the middle of a filtration cycle (7 min after the last backwash), these samples might not be the correct comparison for the stirred cell experiments with clean unfouled membranes. In order to check this, permeate samples are collected from pilot plant 2 during a filtration cycle (10 s, 30 s, 60 s, 2 min, 3 min, 10 min, and 12 min after the last backwash on 4. Nov. 2003) and each sample is analysed with the LC-OCD. However, none of the samples exhibits a PS peak and no evolution trend of the TOC (as measured in the by-pass peak) over the filtration time is found. A second sampling campaign of a subsequent filtration cycle (sampling date: 4. Nov. 2003) gives the same results suggesting that the permeate quality does not depend on the filtration time. Unfortunately, a similar sampling campaign could not be done directly after a chemical cleaning of the membrane in the pilot plants which would have come closer to the state of a clean unfouled membrane than after a backwash.

This means that the stirred cell experiments do not accurately replicate the fouling situation in membrane bioreactors for most of the time (possible exception would be directly after a chemical cleaning). Nevertheless, stirred cell experiments do give a qualitative indication of the fouling potential and behaviour. The difference between the stirred cell tests and the pilot plants is the presence of suspended biology, i.e. the activated sludge, in the latter. This means that the activated sludge plays an important role in the rejection characteristics of the membranes since a paper filtered sample from the membrane reactor of the pilot plants (=filtrate) is being used as feed water for the stirred cell tests. Possible explanations for the complete rejection of the PS peak, including any truly dissolved compounds with a molecular size smaller than the pore size of the membrane, are:

- the formation of a layer of activated sludge flocs on top of the membrane surface acting as an additional filter entrapping any dissolved large macromolecules and/or
- the formation of a fouling layer including activated sludge flocs and large colloidal macromolecules forming a secondary membrane and thereby, reducing the nominal pore size of the microfiltration hollow fiber membrane.

With regards to the fouling rate of the pilot plant membranes, the amount of organic carbon present in the PS peak plays a crucial role (see section 5.2). Stirred cell experiments with filtrate PP 1, filtrate PP 2, and filtrate CAS (conventional activated sludge, see section 3.3)

indicate that the specific characteristics of the compounds eluting in the PS peak play an important role in the fouling behaviour, too. Figure 7.3 depicts the flux decline curves of the three aforementioned samples. While filtrate PP 1 and filtrate PP 2 show similar fouling behaviour, the filtrate CAS sample exhibits a lower flux decline. However, all three samples have similar TOC concentrations and nearly identical organic carbon concentrations of the PS peak (see Table 7.1). The sludge retention time of all three systems is around 15 days at the time of sampling. Besides the operational difference, i.e. conventional activated sludge versus membrane bio-reactor, the water temperature in the conventional WWTP is lower at the time of sampling (filtrate CAS sampled 16.4.03) than in the membrane bio-reactor pilot plants (sampled 19.8.03). A lower temperature should induce higher fouling as more EPS is produced by the microorganisms [Barker & Stuckey 1999]. Besides differences in operational conditions as the cause for the different characteristics of the compounds eluting in the PS peak, the activated sludge microbiology differs somewhat between conventional systems and MBRs [Rosenberger 2003].

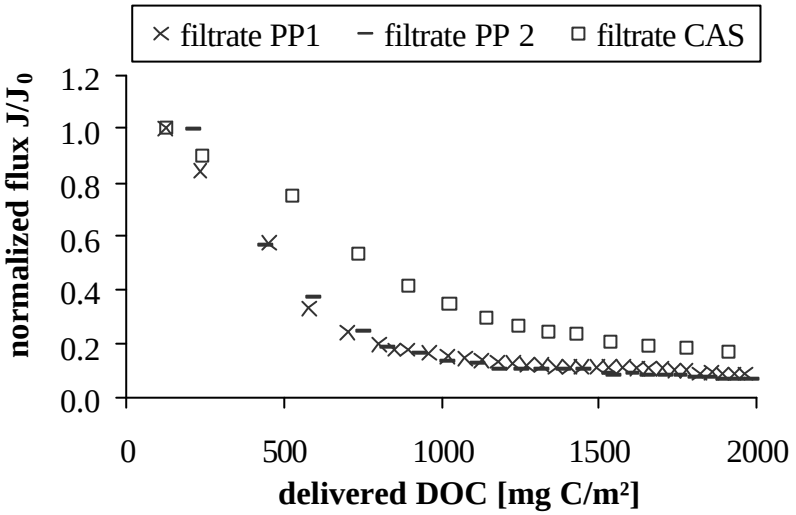

Figure 7.3 Flux decline curves for stirred cell experiments with filtrate PP 1, filtrate PP 2, and filtrate CAS

Table 7.1 Organic carbon concentrations of the bulk sample and PS peak for filtrate PP 1, filtrate PP 2, and filtrate CAS

	TOC [mg C/L]	OC of PS peak [mg C/L]
Filtrate PP 1, 19.8.03	14.6	1.4
Filtrate PP 2, 19.8.03	16.3	1.3
Filtrate CAS, 16.4.03	15.6	1.4

Additional stirred cell experiments are carried out with filtrate PP 1 using either a microfiltration membrane (MF, VVLP) or an ultrafiltration membrane (UF, YM100). The results of these are depicted in Figure 7.4 as normalized flux versus delivered DOC. As can be seen, both membranes are fouled to the same extent after 90 min filtration (the initial flux has declined to less than 10 %). However, there seems to be a difference in the fouling

behaviour with the microfiltration membrane fouling more slowly than the ultrafiltration membrane: 187 mg C/m² delivered DOC cause 40 % flux decline with the ultrafiltration membrane while 521 mg C/m² are necessary for the same flux decline with the microfiltration membrane.

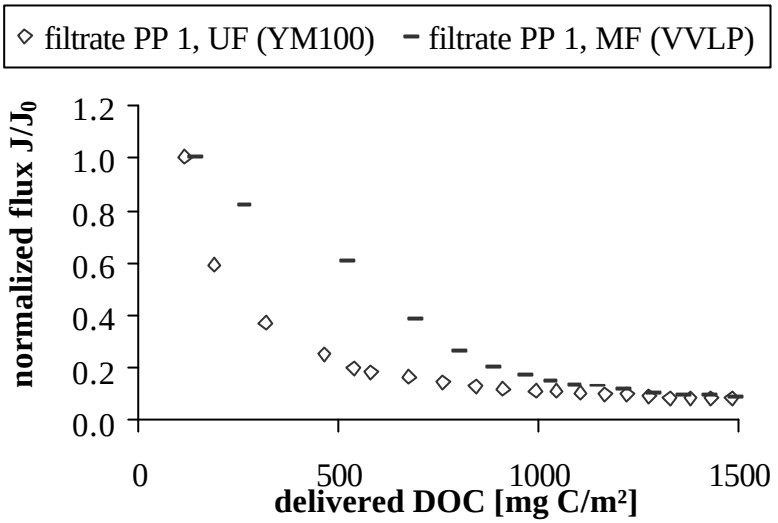

Figure 7.4 Flux decline curves of stirred cell tests for filtrate PP 1 with a microfiltration membrane (VVLP) and an ultrafiltration membrane (YM100)

A linearization of the curves according to Kilduff et al [2002] is possible. Linear plots of flux (J) versus filtration volume (V), t/V versus t (time), and t/V versus V indicate pore blockage, pore constriction, and cake formation, respectively, as dominat fouling mechanism. Figure 7.5 gives the graph for t/V versus V. The data of the ultrafiltration stirred cell experiment can be linearized using the t/V versus V plot and thus, the dominant fouling mechanism here is cake formation. The results with the microfiltration membrane, on the other hand, are not linearized in Figure 7.5 nor using one of the other two plots (data not shown). This means that either two or more fouling mechanisms are relevant, e.g. pore blockage and cake formation, or back-transport due to the stirring during the experiment is more important with the microfiltration membrane than with the ultrafiltration membrane, convoluting the data.

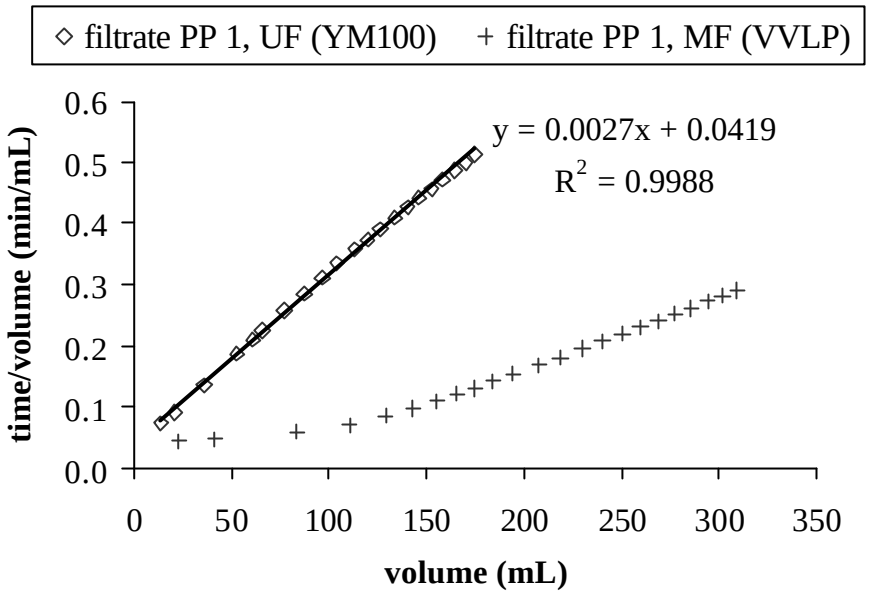

Figure 7.5 Identification of fouling mechanism for filtrate PP 1 with a microfiltration membrane (VVLP) and an ultrafiltration membrane (YM100); a linear correlation between time per volume and volume indicates cake formation as is the case for the UF membrane here (equation and R^2 value given in graph)

Chapter 8 Conclusions and outlook

8.1 Conclusions

The development of the sampling procedure and sample pretreatment has shown that it is very important to sample on the same weekday and at the same time in order to minimise daily and weekly variations. Paper filtration is a standard protocol to separate the biomass from the aqeous phase in activated sludge samples and has been chosen over centrifugation of the samples due to the possibility of on-site filtration and therefore, faster handling of the samples.

Reproducibility of all analyses lies within 10 % error including the sampling routine. Samples should always be analysed as soon as possible. In the case of the pilot plants this means within two weeks if the samples are stored under refrigeration.

The comparison between LC-OCD and photometric polysaccharide measurements reveals a good correlation although, a factor of two is found between the two methods. This is due to an overestimation of the polysaccharides by the LC-OCD as the PS peak includes colloids. On the other hand, the photometric method underestimates the amount of polysaccharides as it is calibrated with glucose. This means that only polysaccharides consisting of glucose monomers are measured quantitatively while the method is less sensitive for polysaccharides which have fructose or other sugars as monomers.

The survey of the seasonal variation in the two pilot plants indicates colloids and large macromolecules such as polysaccharides as most important foulants. No differences in the fouling behaviour due to the different process configurations, i.e. pre-denitrification versus post-denitrification, are detected. Instead, the amount of extracellular polymeric substances (EPS) produced by the microorganisms depends on the stress situation of the bacteria (low dissolved oxygen concentrations in PP 2 at end of operation resulted in high PS; sludge loss in PP 1 on 07/12/02 with long recovery time of nearly 4 months), the wastewater temperature (decreasing PS with increasing temperature), and perhaps the sludge retention time (SRT; tendency: PS of 15d < PS of 8d).

The polysaccharides analysed with both methods are most likely part of the EPS as demonstrated by a comparison of the LC-OCD chromatograms between extracted EPS and a filtrate PP 2 sample. Furthermore, sugar analyses by research groups of Prof. Kroh and Prof. Croué suggested a biological origin of the polysaccharides (main peaks are anhydroglucogen for the HPLC analyses by Prof. Kroh and glucosamines for the Py-GC-MS analyses by Prof. Croué).

Finally, stirred cell experiments confirmed the high fouling potential of the filtrate samples from both pilot plants and that this is primarily due to the PS peak (permeate samples of

the pilot plant do not foul the membranes). One third of the PS peak, however, is truly dissolved and passes the microfiltration membrane (VVLP) in the stirred cell tests while the whole peak is retained in the pilot plants. This difference between pilot plants and stirred cell experiments is attributed to the biomass present in the pilot plants and the formation of a cake layer during filtration. Besides the amount of polysaccharides present in the feed of the stirred cell tests, their characteristics play an important role for the fouling behaviour (different fouling behaviour of filtrate PP1 and filtrate PP 2 samples versus filtrate CAS samples).

A comparison of the fouling behaviour of filtrate PP 1 on an ultrafiltration membrane revealed different fouling mechanisms: the main fouling mechanism on the ultrafiltration membrane is cake formation whereas no single fouling mechanism can be determined for the microfiltration membrane.

8.2 Research recommendations

This research clearly shows the importance of polysaccharides as produced by the microorganims in activated sludge systems to the fouling behaviour of membrane bio-reactors. A better understanding of the parameters influencing EPS production is needed. A cooperation with microbiologist, for example, could be helpful in determining the biocoenosis and its behaviour under varying operating conditions. However, it would be important to only vary one operational parameter at a time, e.g. temperature or sludge retention time, while all other parameters are held constant at standardized conditions.

Furthermore, investigations on the character of the PS peak (in the OC response chromatograms), the inorganic colloids (in the UV response chromatograms), and hydrophobic compounds (not accessible by the chromatographic method) are recommended. For this purpose an SEC column with larger pores such as HW-65S or HW-75S would be necessary. At the same time thoughts should be given to appropriate calibration standards. Another interesting question is the adsorption capacity of polysaccharides and hydrophobic compounds on micro- and ultrafiltration membranes as well as in the SEC columns.

References

Barker, D. and Stuckey, D. (1999): A review of soluble microbial products (SMP) in wastewater treatment systems, Wat. Res., Vol. 33, No. 14, pp. 3063-3082.

Dubois, M.; Gilles, K.; Hamilton, J.; Rebers, P.; and Smith, F. (1956): Colorimetric method for determination of sugars and related substances, Analytical Chemistry, Vol. 28, No. 3, pp. 350-356.

Frolund, B.; Palmgren, R.; Keiding, K. and Nielsen, P. (1996): Extraction of extracellular polymers from activated sludge using a cation exchange resin, Wat. Res., Vol. 30, pp. 1749-1758.

Gnirss, R. (2004): personal communications.

Gnirss, R.; Lesjean, B.; Buisson, H.; Adam, Chr.; Kraume, M. (2003a): Enhanced biological phosphorus (EBPR) in membrane bioreactors (MBR), Proceedings of the AWWA Membrane Technology Conference, 2.-5. März 2003, Atlanta, USA.

Gnirss, R.; Lesjean, B.; and Buisson, H. (2003b): Biologische Phosphorentfernung mit einer nachgeschalteten Denitrifikation im Membranbelebungsverfahren, Tagungsband zur 5. Aachener Tagung Siedlungswasserwirtschaft und Verfahrenstechnik, A17, 30.9.-1.10. 2003, Aachen, Germany.

GROM Analytik + HPLC GmbH (2003): Catalogue, bulk materials, gel permeation, p. 141, www.grom.de/products/framprod1.htm.

Huber, S. and Frimmel, F. (1991): Flow injection analysis of organic and inorganic carbon in the low ppb range, Analytical Chemistry, Vol. 63, pp. 2122-2130.

Huber, S. and Frimmel, F. (1996): Gelchromatorgraphie mit Kohlenstoffdetektion (LC-OCD): Ein rasches und aussagekräftiges Verfahren zur Charakterisierung hydrophiler organischer Wasserinhaltsstoffe, Vom Wasser, Vol. 86, pp. 277-290.

Kilduff, J.; Mattaraj, S.; Sensibaugh, J.; Pieracci, J.; Yuan, Y.; and Belfort, G. (2002): Modeling flux decline during nanofiltration of NOM with poly(arylsulfone) membranes modified using UV-assisted graft polymerization, Environ. Eng. Sci., Vol. 19, No. 6, pp. 477-495

Lowry, O.; Rosebrough, N.; Farr, A.; and Randall, R. (1951): Protein measurement with the folin phenol reagent, Journal of Biological Chemistry, Vol. 193, No. 1, pp. 265-275.

Rosenberger, S. (2003): Charakterisierung von belebtem Schlamm in Membranbelebungsreaktoren zur Abwasserreinigung, Fortschritt-Berichte VDI, Reihe 3, Nr. 769, VDI Verlag, Düsseldorf, Germany.

Rosenberger, S.; Lesjean, B.; Bonhomme, A.; Laabs, C.; Jekel, M.; and Gnirss, R. (2003): Zusammenhang zwischen Membranfouling und gelösten Substanzen in Membranbelebungsreaktoren, Tagungsband zur 5. Aachener Tagung Siedlungswasserwirtschaft und Verfahrenstechnik, A24, 30.9.-1.10. 2003, Aachen, Germany.

Späth, R. (1998): Rolle der extrazellulären polymeren Substanzen in Biofilm un belebtem Schlamm bei der Sorption von Schadstoffen, Dissertation am Lehrstuhl für Wassergüte- und Abfallwirtschaft an der Technischen Universität München, Germany.

Appendix A Turbidity and organic carbon results

Table A.1 Turbidity and organic carbon values of routine samples for pilot plant 1

	permeate PP 1				filtrate PP 1			
sampling date	TOC mg C/L	PS mg C/L	HS+acids mg C/L	turbidity NTU	TOC mg C/L	PS mg C/L	HS+acids mg C/L	turbidity NTU
28.08.2002	14.1	0.08	9.98		17.5**	0.5	10.86	
30.09.2002	13.7	0.3	11.5		21.3	4.4	13.3	
11.12.2002					24.7	9.2	11.4	2
23.12.2002					24.7	12.7	12.3	5.7
08.01.2003					23.1	10.4	12.6	9.4
22.01.2003					22.8	7.8	12	7.2
06.02.2003					19.1	6.4	10.7	5.8
19.02.2003	14.6	0.02	11.5	0.2	22.8	8.4	12.5	7.7
05.03.2003	13.3	0.07	9.9	0.17	21.2	7.8	11.3	9
19.03.2003	12.3	0.17	10.2	0.18	22.5	7.4	11.8	7.1
02.04.2003		0	9.3	0.2	23.6	9	12	8
16.04.2003	13.5	0	10.2	0.13	22	7	12	3.1
30.04.2003	13.1	0.3*	10.2	0.18	22	4.9	11.1	4
14.05.2003	12.9	0	9.5	0.4	16.3	3.9	10.4	3.7
28.05.2003	12.7	0	10.2	0.3	17.3	3.6	11.3	3.8
11.06.2003	13.4	0	9.9	0.3	17	2.7	11.4	5.3
25.06.2003	11.7	0	8.5	0.2	15.5	3.6	9.6	4.7
09.07.2003	11.4	0	9.7	0.6	15.5	2.3	10.2	2.7
22.07.2003	12	0	10.7	0.3	15.9	2.1	11.1	5
05.08.2003	13.3	0	9.8	1.1	13.8	1	9.9	4.8
19.08.2003	13.9	0	10.5	0.2	15.5	1.5	11.7	4.4
02.09.2003	9.4	0	8.1	0.3	11.8	1.7	9.5	7
16.09.2003	11.8	0	9.6	0.25	15.8	2.8	10.9	7.6
23.09.2003	12.4	0	9.6	0.3	15.4	1.8	10.7	1.5
30.09.2003	10.6	0	8.3	0.33	11.7	1.1	8.4	2
07.10.2003	11.3	0	8		13.4	1.4	8.8	2.2
15.10.2003	12.5	0	9.4	0.43	15.5	1.3	10.1	2.9
28.10.2003	12.2	0	9.2	0.8	13.9	1.6	9.5	4.2
11.11.2003	12.2	0	9.4	0.3	19.6	3.8	11.3	13
25.11.2003	12.2	0	9.5	0.5	17.5	3	11.7	13

* peak could be an artefact as it is very narrow

** filtrate sample from AE4 reactor

Table A.2 Turbidity and organic carbon values of routine samples for pilot plant 2

	permeate PP 2				filtrate PP 2			
Sampling date	TOC mg C/L	PS mg C/L	HS+acids mg C/L	turbidity NTU	TOC mg C/L	PS mg C/L	HS+acids mg C/L	turbidity NTU
28/08/2002	16.1	0.01	10.5		20.1	1.1	12.47	
30/09/2002	13.3	0.08	10.4		19.2	3.7	12	
19/11/2002	12.5	0	10.8	0.13	16.3	5.7	11.9	7.5
29/11/2002*					20.1	5.3	12.4	5.1
11/12/2002					17.2	2	10.1	7.7
23/12/2002					16.4	4.7	11.8	2.6
08/01/2003					17.4	5.2	11.8	5.8
22/01/2003					18.1	5.1	12.1	7.7
06/02/2003					16.5	4.7	10.2	4.2
19/02/2003	33.4	0		24.4	24.5	7	14.3	11.6
05/03/2003	12.0	0	9.4	0.2	19.2	5.8	10.6	6.5
19/03/2003	13.3	0.04	10.5	0.5	20.7	6.1	11.7	9.6
02/04/2003	12.9	0	9.7	0.2	20.5	7	10.8	7.6
16/04/2003	13.2	0	10.0	0.2	22.8	7.7	11.7	3.9
30/04/2003	13.7	0	9.7	0.2	19.1	5.2	10.6	5.6
14/05/2003	11.5	0	8.7	0.2	14.8	2.8	9.7	5.4
11/06/2003	12.3	0	9.7	0.3	15.5	1.8	10.8	6
25/06/2003	11.2	0	8.0	0.3	13.7	1.7	9.1	3.6
09/07/2003	12.3	0	9.4	0.5	16	2.1	10.6	4.3
22/07/2003	11.9	0	9.9	0.3	15.6	1.9	10.6	6.7
05/08/2003	12.6	0	9.7	0.3	15.1	1.9	10.1	4.4
19/08/2003	15.5	0	10.2	0.3	18.2	1.5	10.9	3.3
01/09/2003	9.4	0	7.8	0.4	11.1	1.6	8.7	5
02/09/2003	10.1	0	8.3	0.4	11.7	2	9.3	8.1
16/09/2003	11.2	0	9.3	0.2	13.2	1	9.8	4.9
23/09/2003	11.9	0	9.3	0.4	17.2	3.1	11.4	4.5
30/09/2003	11.2	0	9.0	0.5	15.8	3.6	9.7	5
07/10/2003	12.9	0.06	9.6		18.9	4.6	10.8	7.1
15/10/2003	16.2	0.09	11.2	0.4	26.7	7.1	12.9	12.3
28/10/2003	12.9	0	9.0	0.4	16.1	5.4	10.2	9.9
11/11/2003	11.2	0.04	8.9	0.3	18.5	3.9	10.9	6.4
25/11/2003	12.8	0	9.4	0.4	19	4.1	11.7	24.6

* average of samples 1-6

Appendix B LC-OCD chromatograms of routine samples

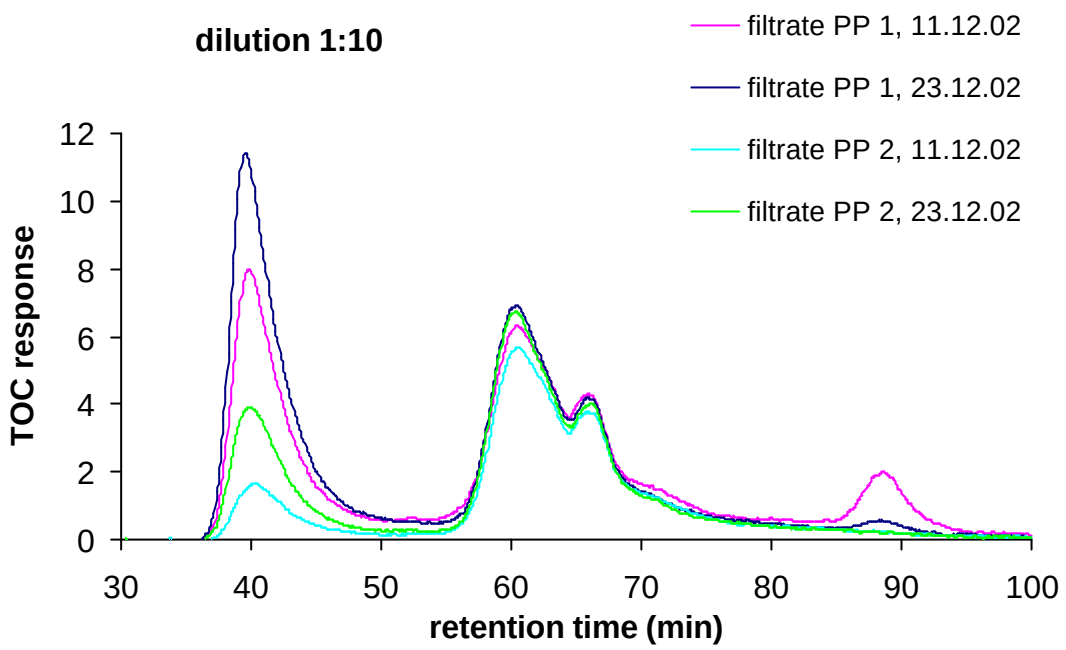

Figure B.1 Chromatograms for organic carbon response for filtrate samples of both pilot plants taken on 11th and 23rd December 2002

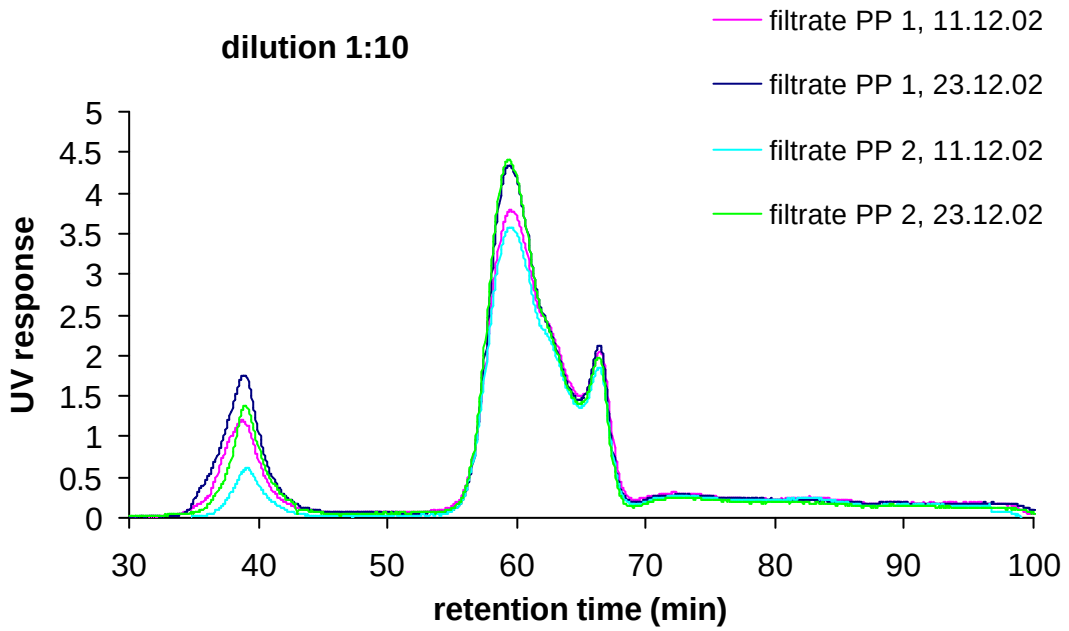

Figure B.2 Chromatograms for UV response for filtrate samples of both pilot plants taken on 11th and 23rd December 2002

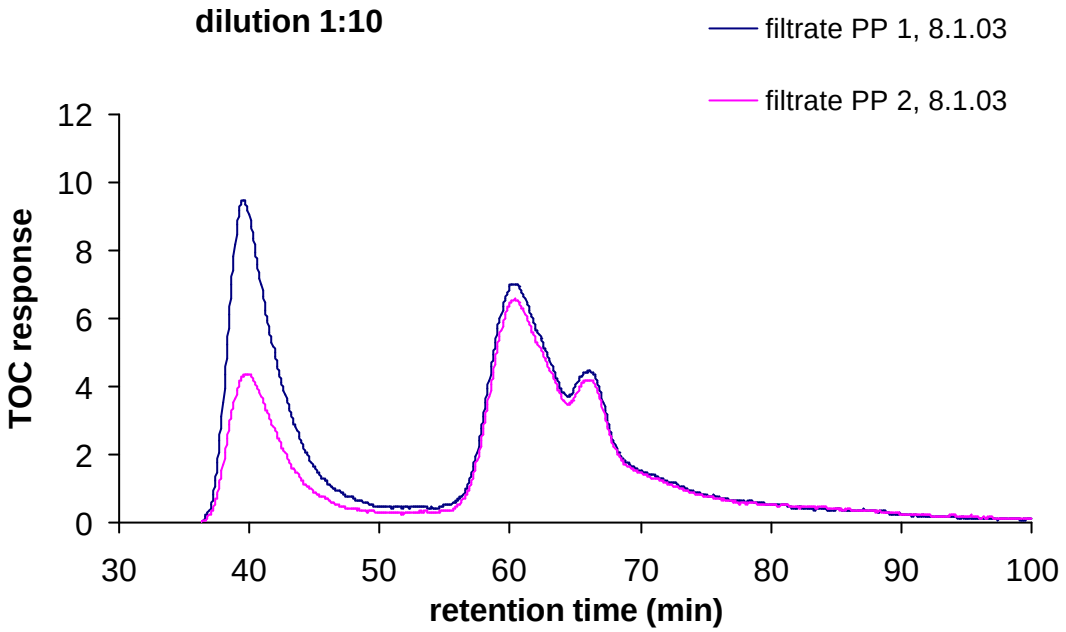

Figure B.3 Chromatograms for organic carbon response for filtrate samples of both pilot plants taken on 8th January 2003

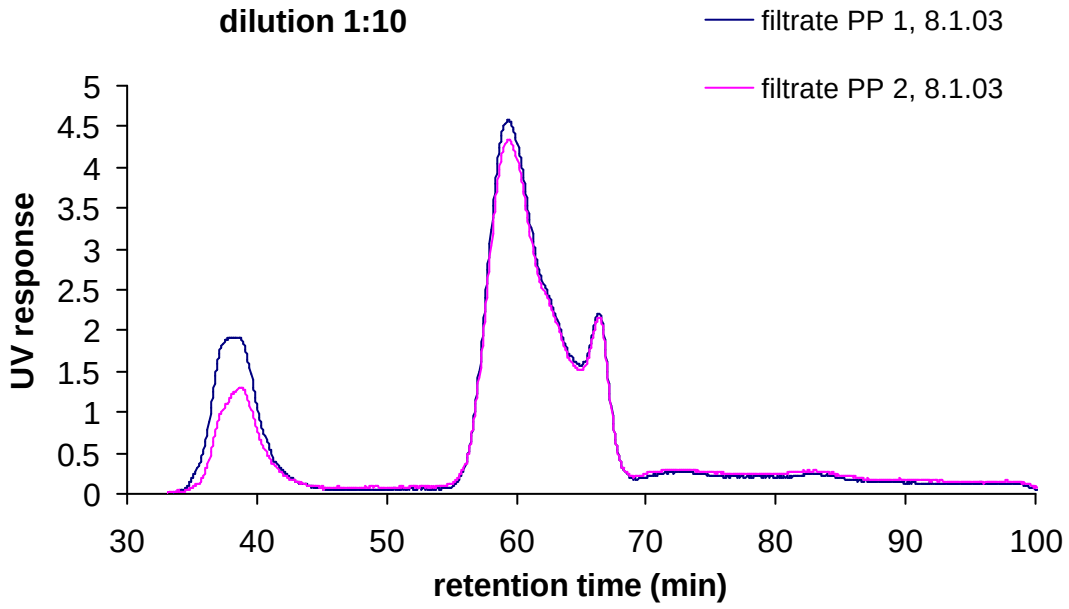

Figure B.4 Chromatograms for UV response for filtrate samples of both pilot plants taken on 8th January 2003

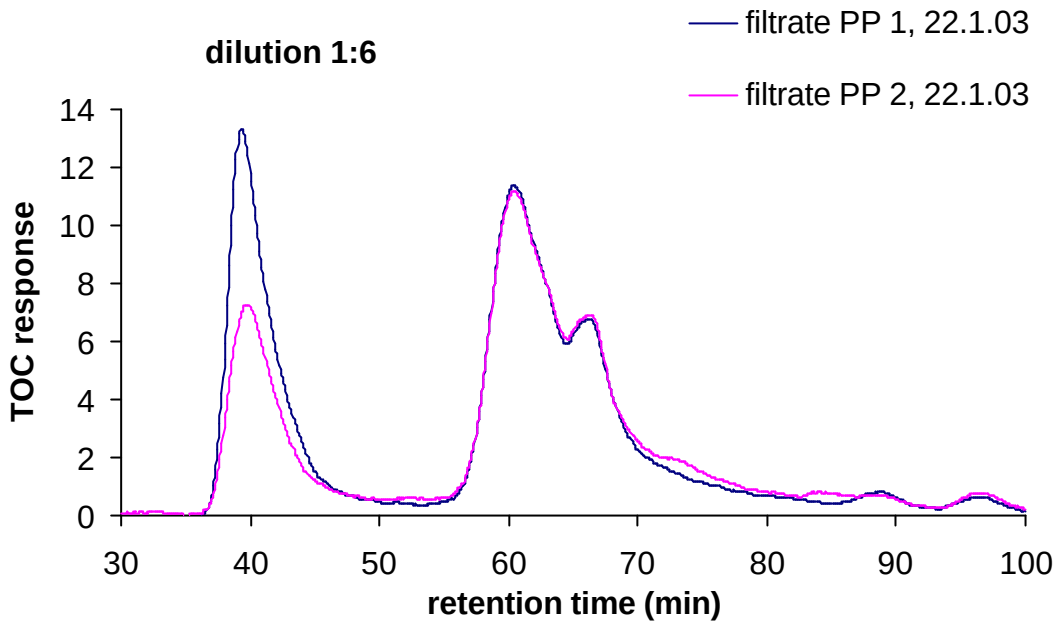

Figure B.5 Chromatograms for organic carbon response for filtrate samples of both pilot plants taken on 22nd January 2003

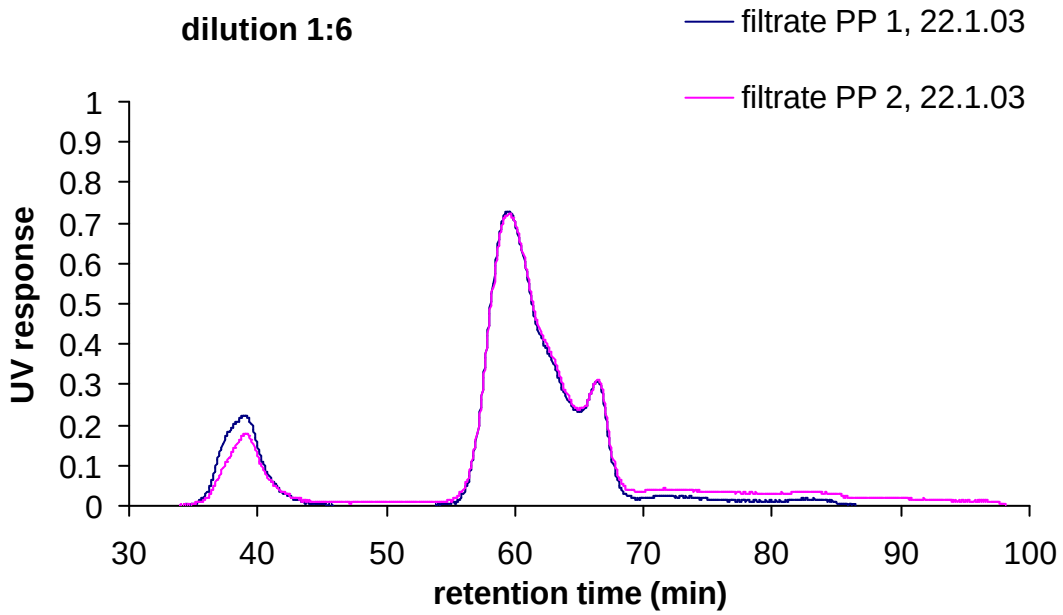

Figure B.6 Chromatograms for UV response for filtrate samples of both pilot plants taken on 22nd January 2003

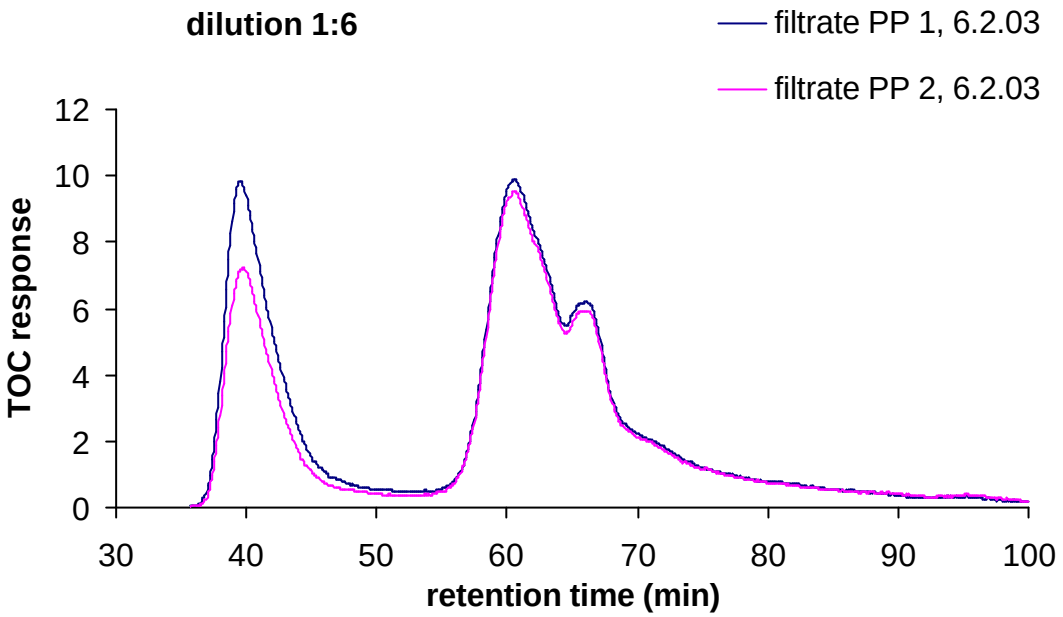

Figure B.7 Chromatograms for organic carbon response for filtrate samples of both pilot plants taken on 6th February 2003

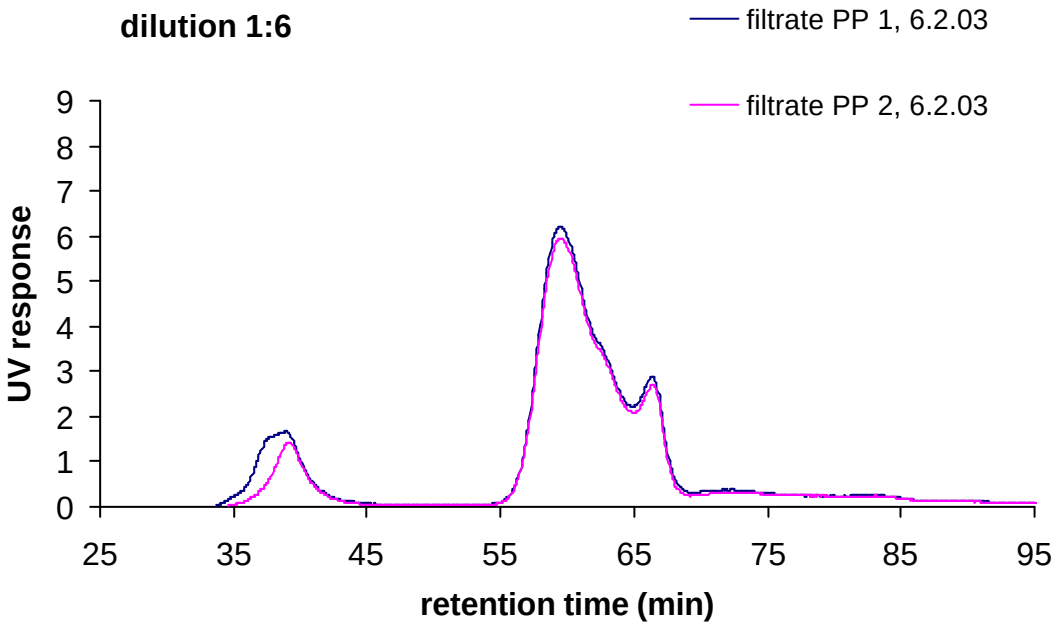

Figure B.8 Chromatograms for UV response for filtrate samples of both pilot plants taken on 6th February 2003

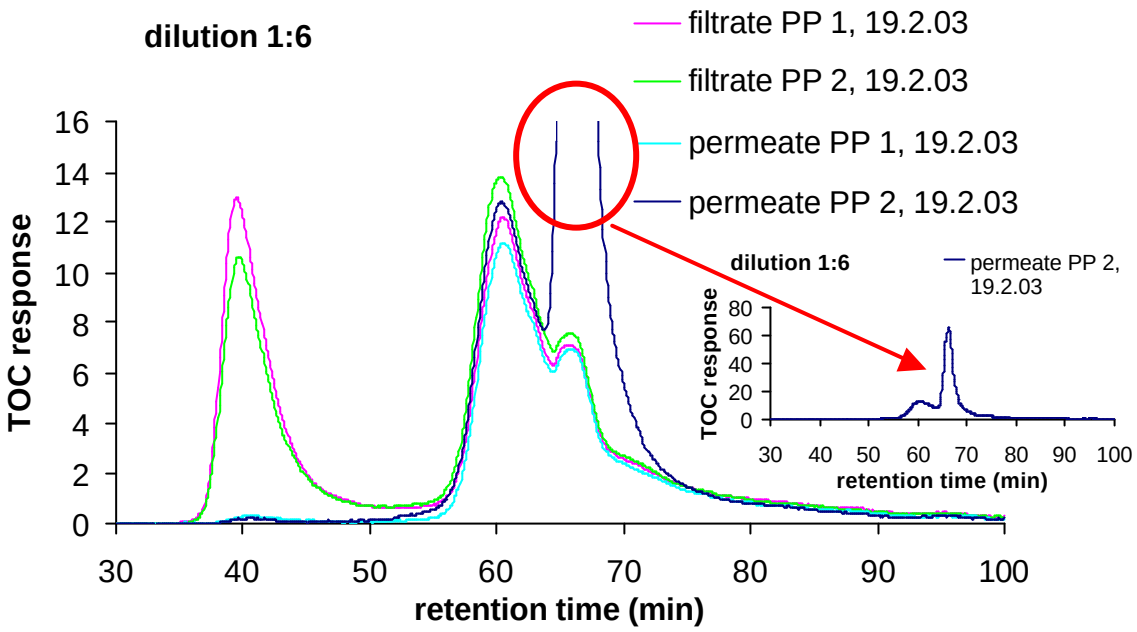

Figure B.9 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 19th February 2003

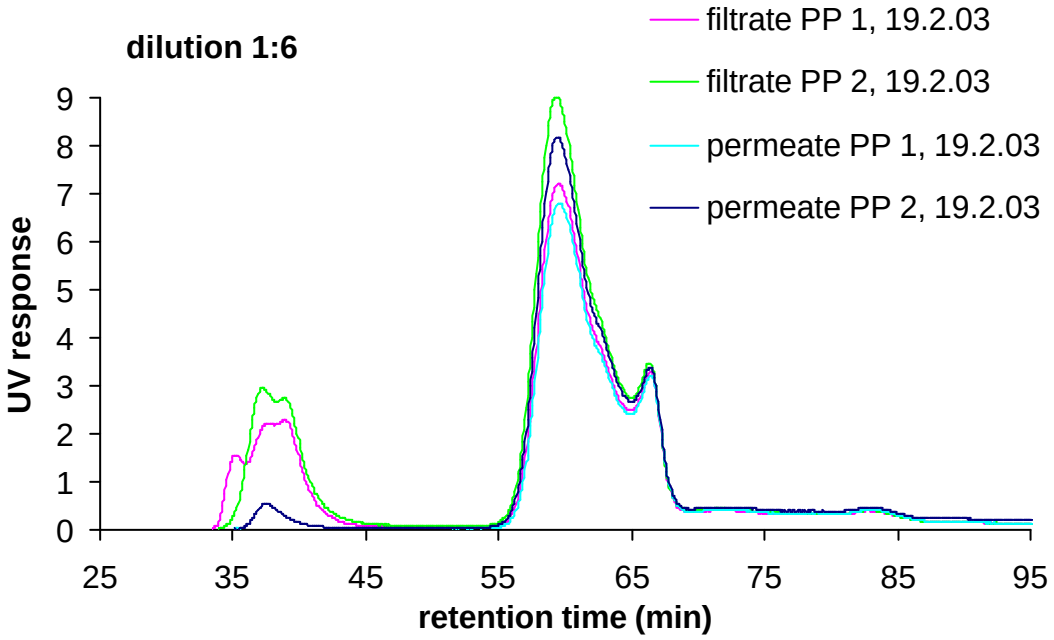

Figure B.10 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 19th February 2003

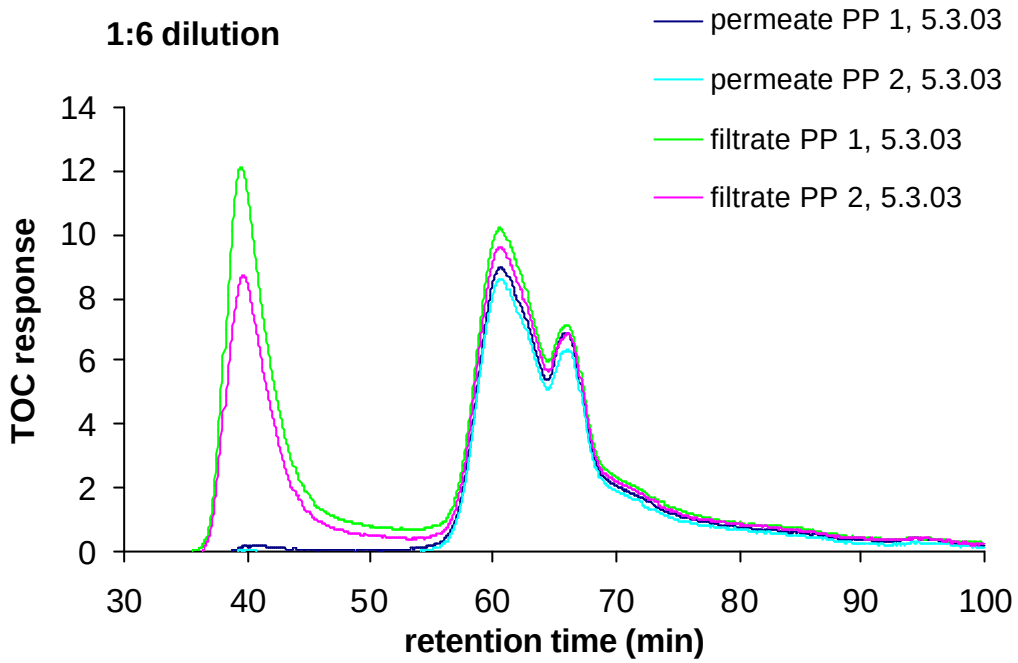

Figure B.11 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 5th March 2003

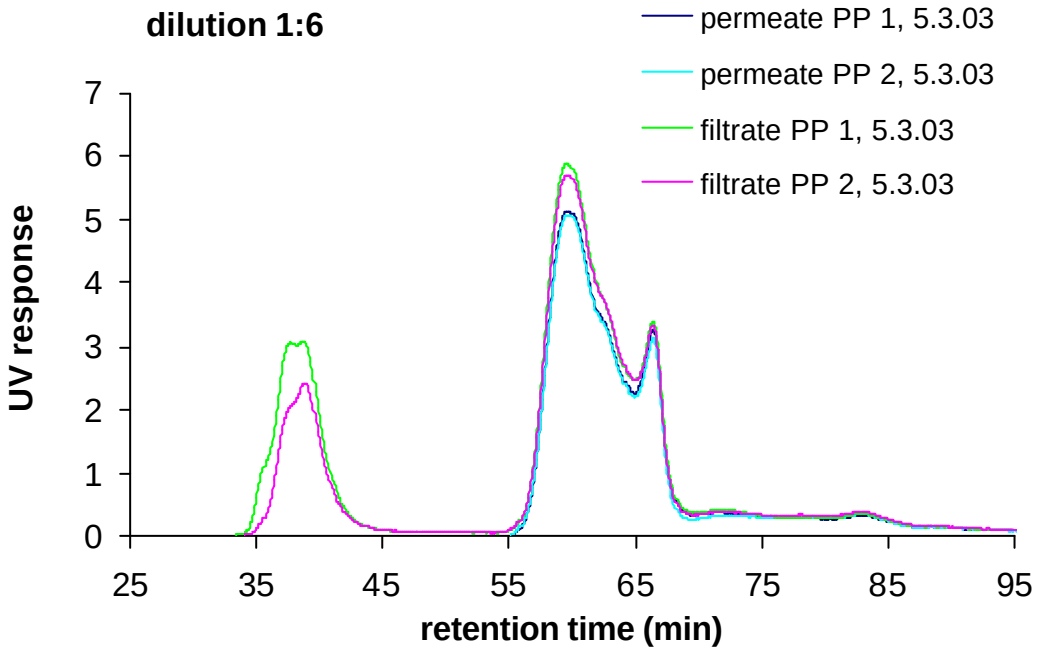

Figure B.12 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 5th March 2003

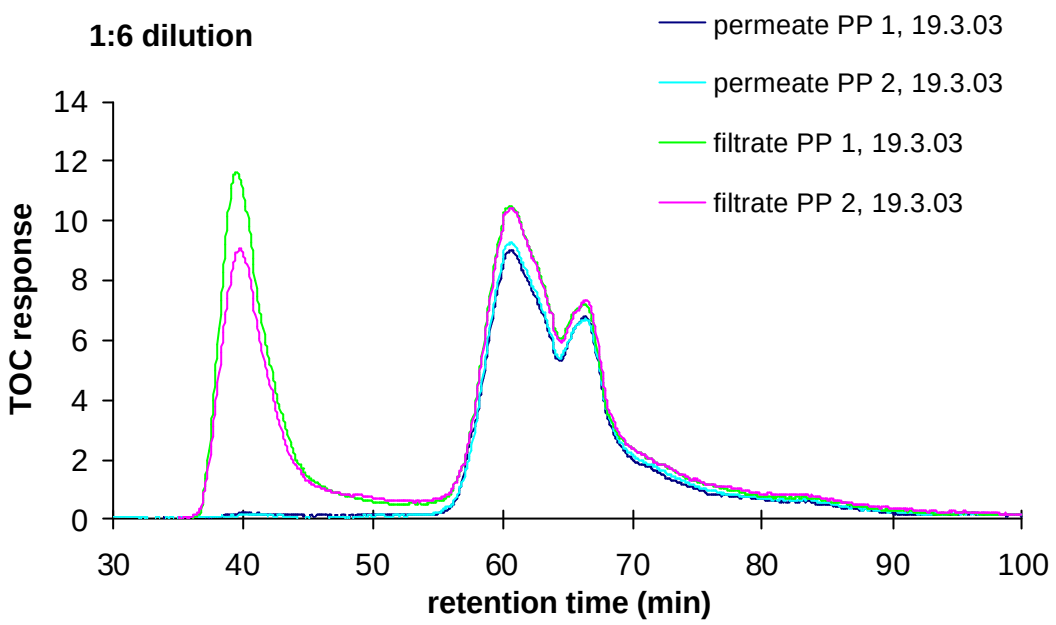

Figure B.13 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 19th March 2003

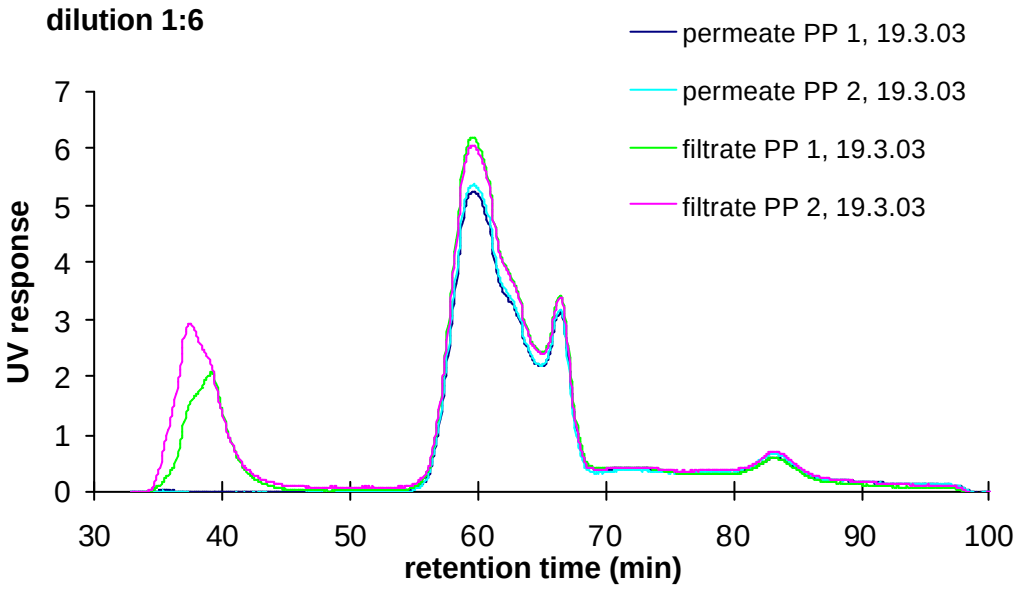

Figure B.14 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 19th March 2003

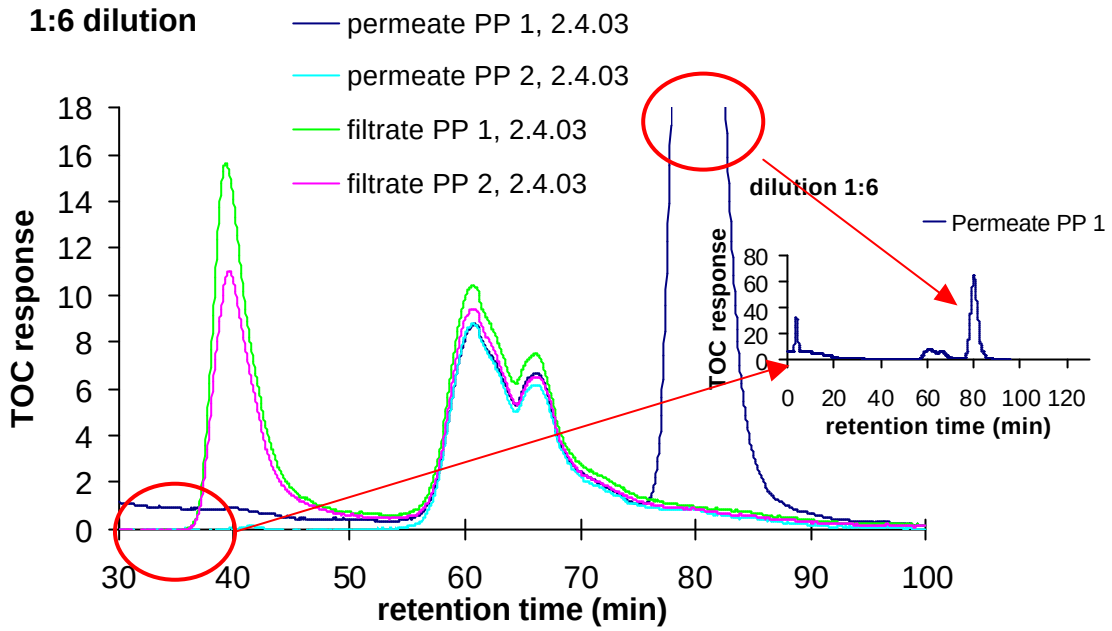

Figure B.15 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 2nd April 2003

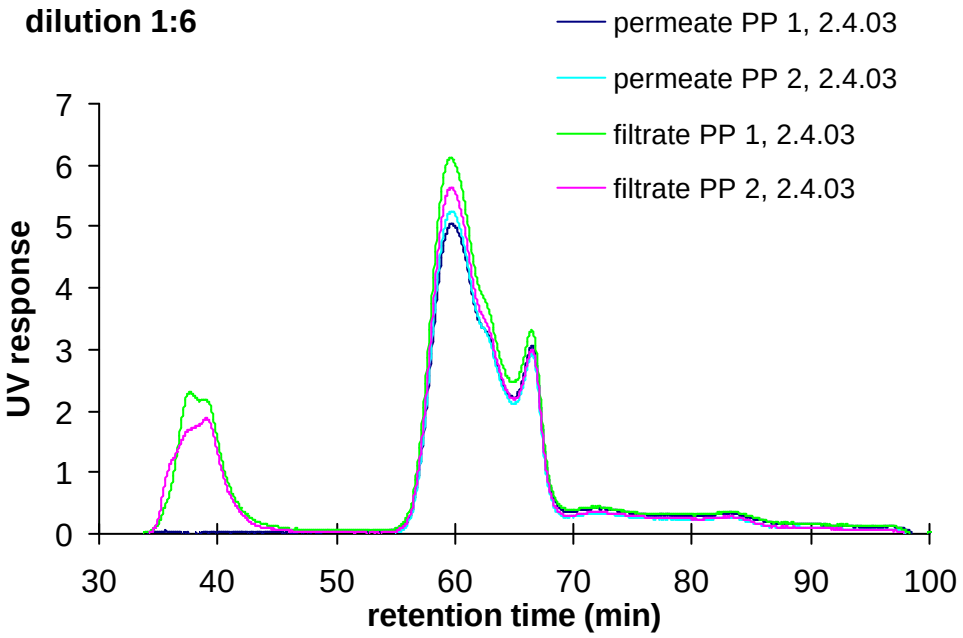

Figure B.16 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 2nd April 2003

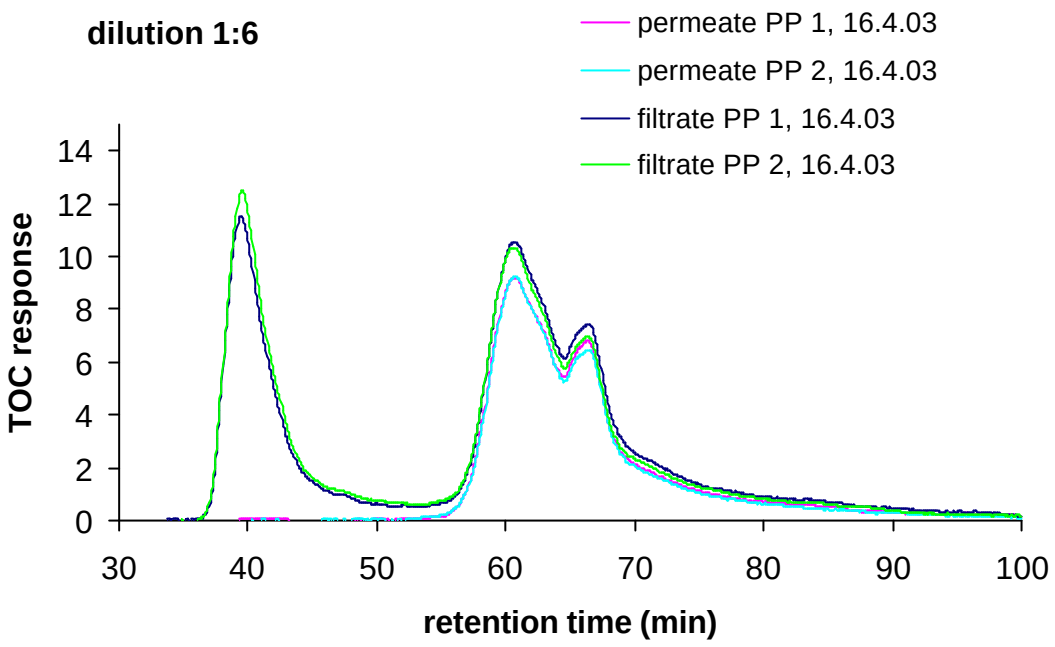

Figure B.17 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 16th April 2003

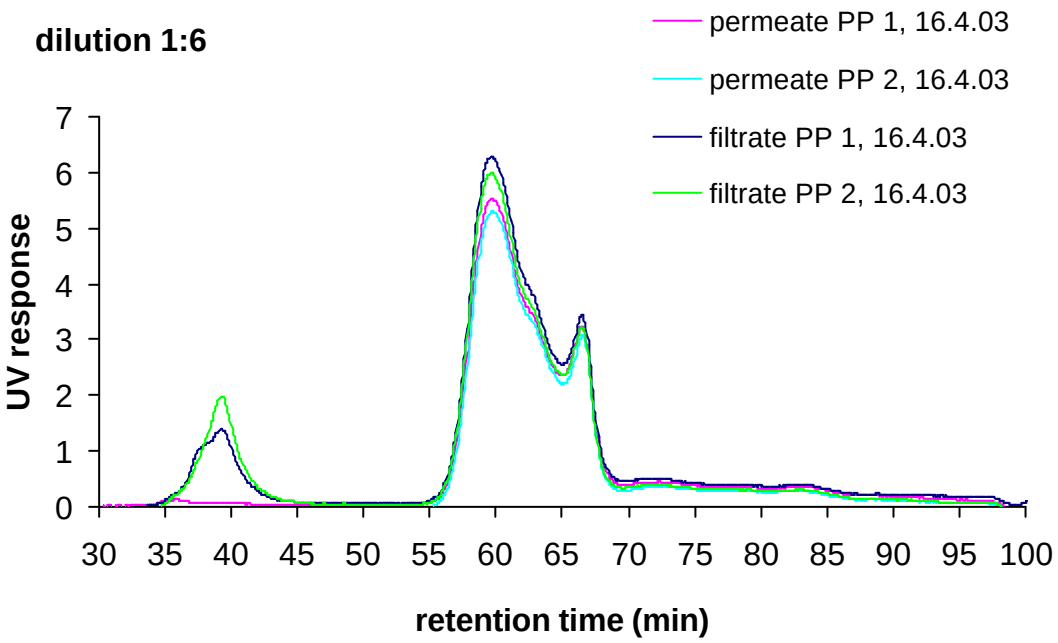

Figure B.18 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 16th April 2003

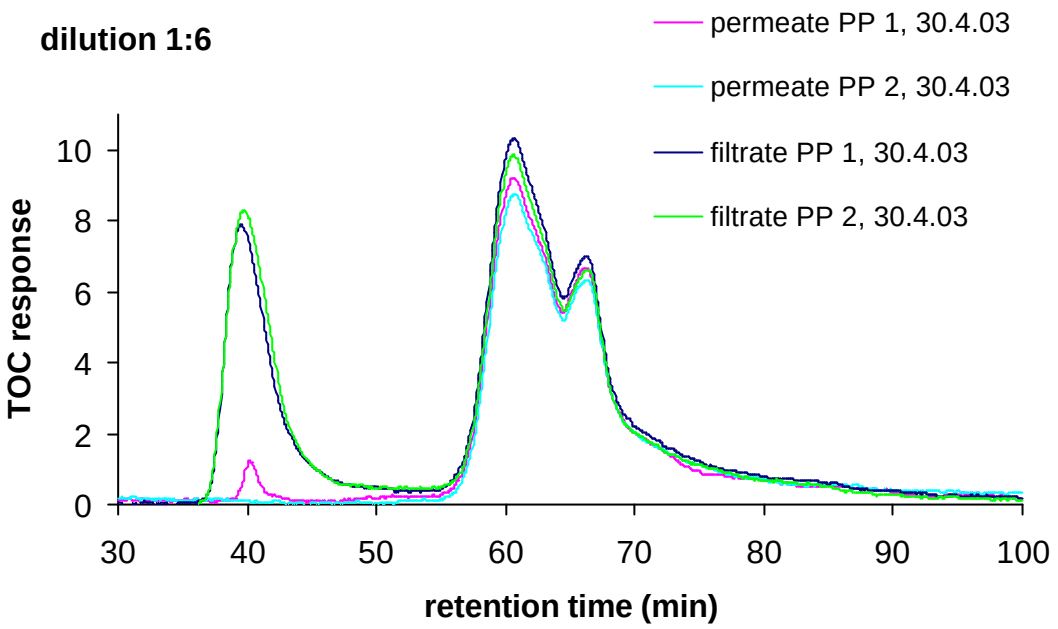

Figure B.19 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 30th April 2003

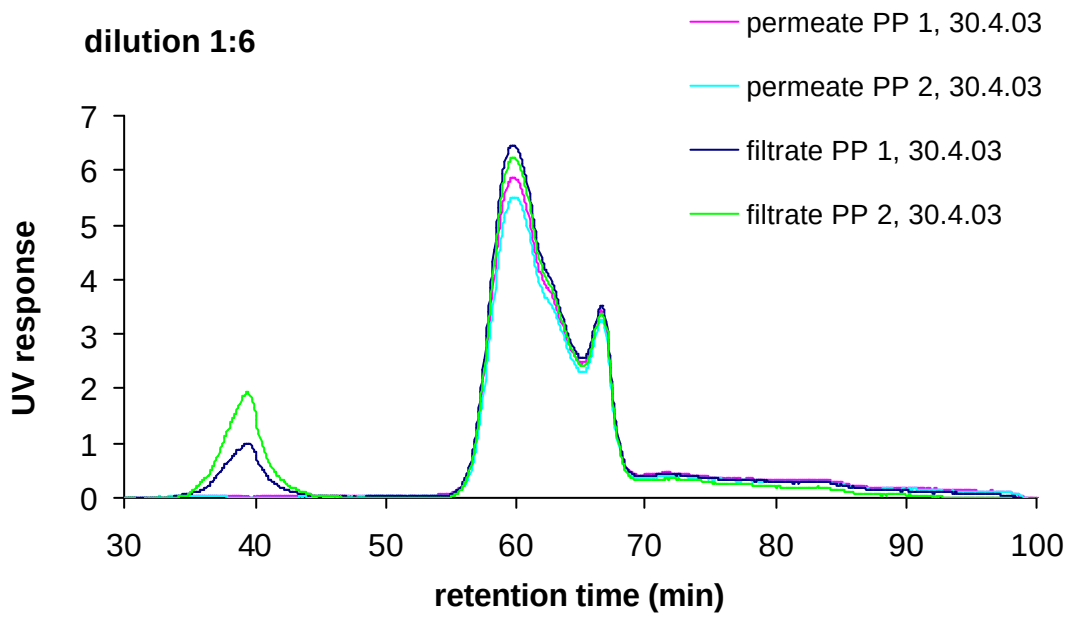

Figure B.20 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 30th April 2003

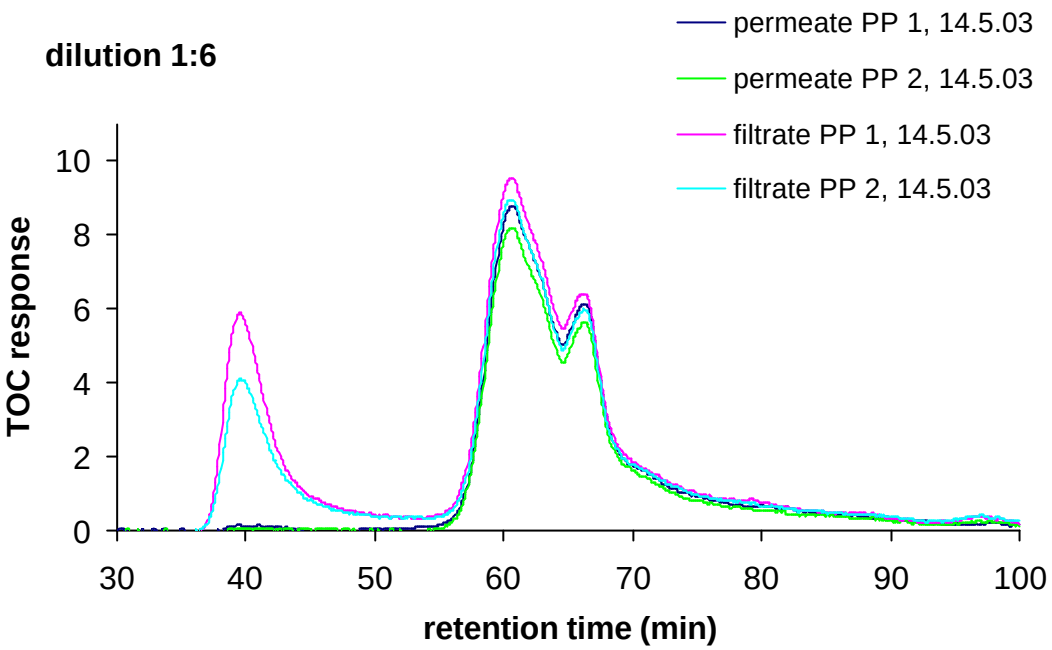

Figure B.21 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 14th May 2003

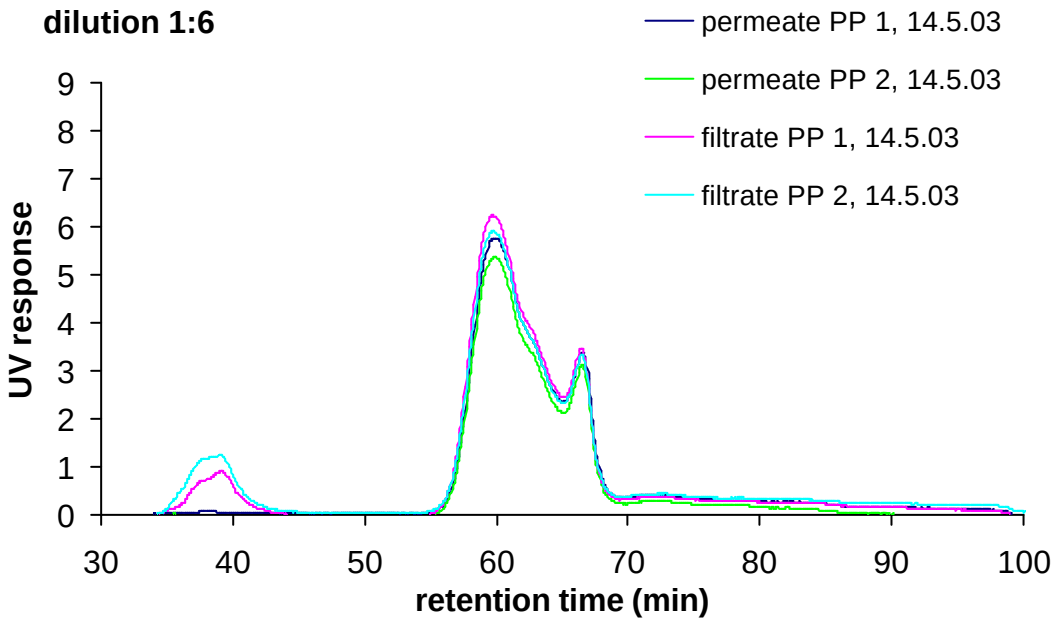

Figure B.22 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 14th May 2003

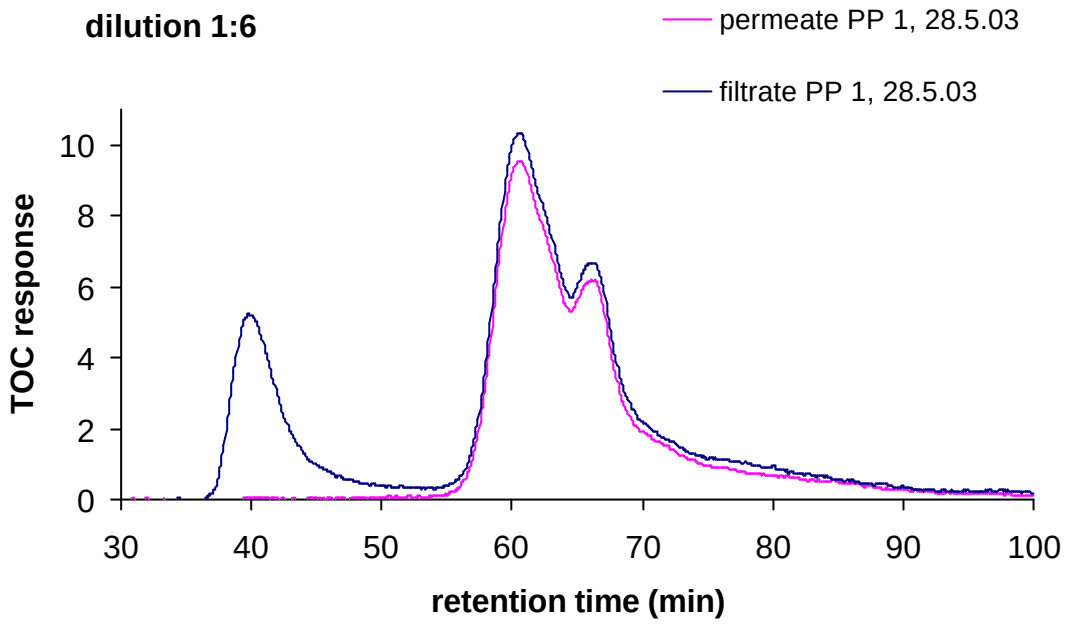

Figure B.23 Chromatograms for organic carbon response for filtrate and permeate samples PP 1 taken on 28th May 2003

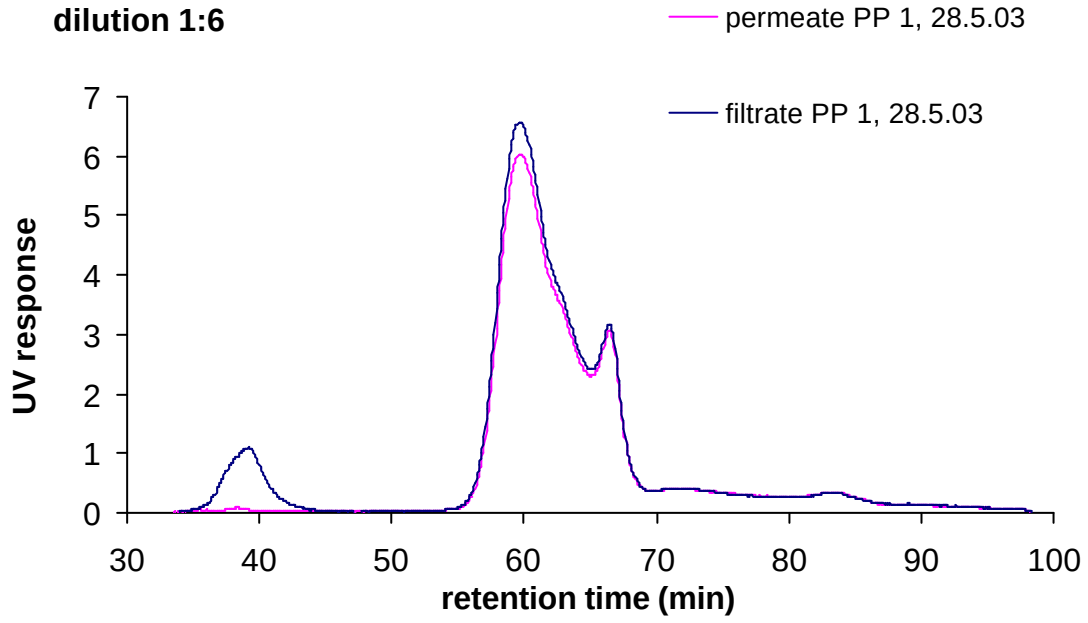

Figure B.24 Chromatograms for UV response for filtrate and permeate samples PP 1 taken on 28th May 2003

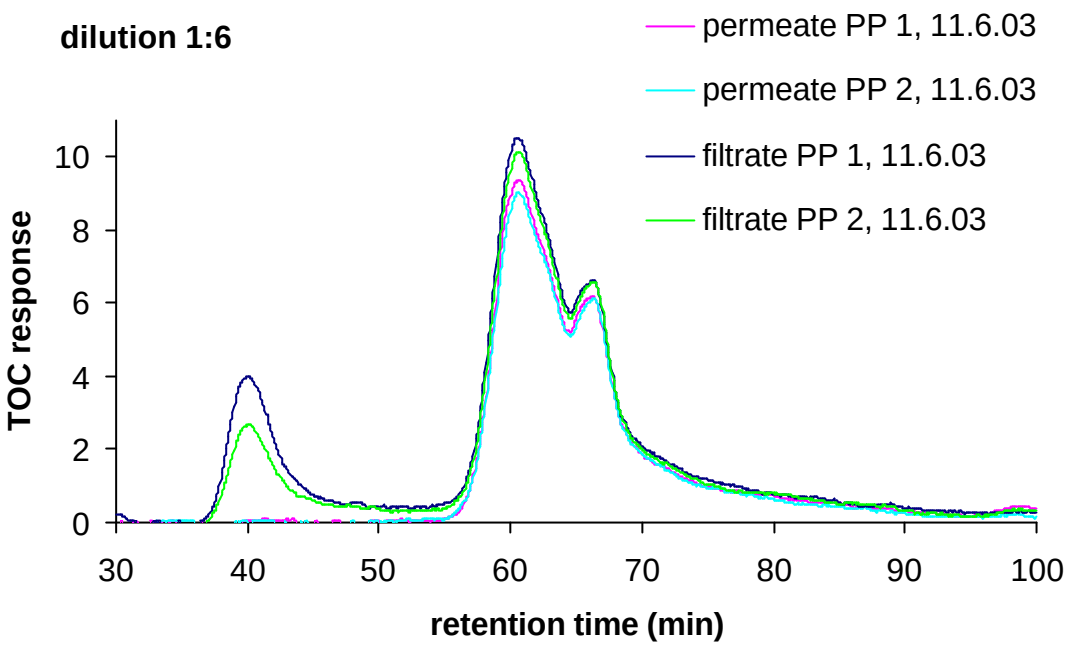

Figure B.25 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 11th June 2003

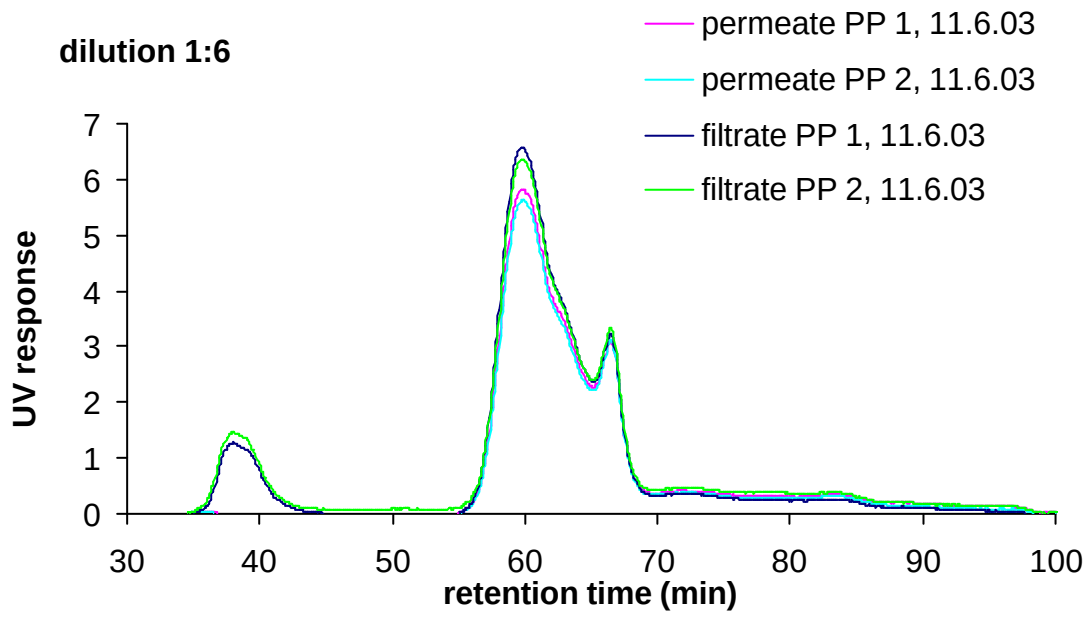

Figure B.26 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 11th June 2003

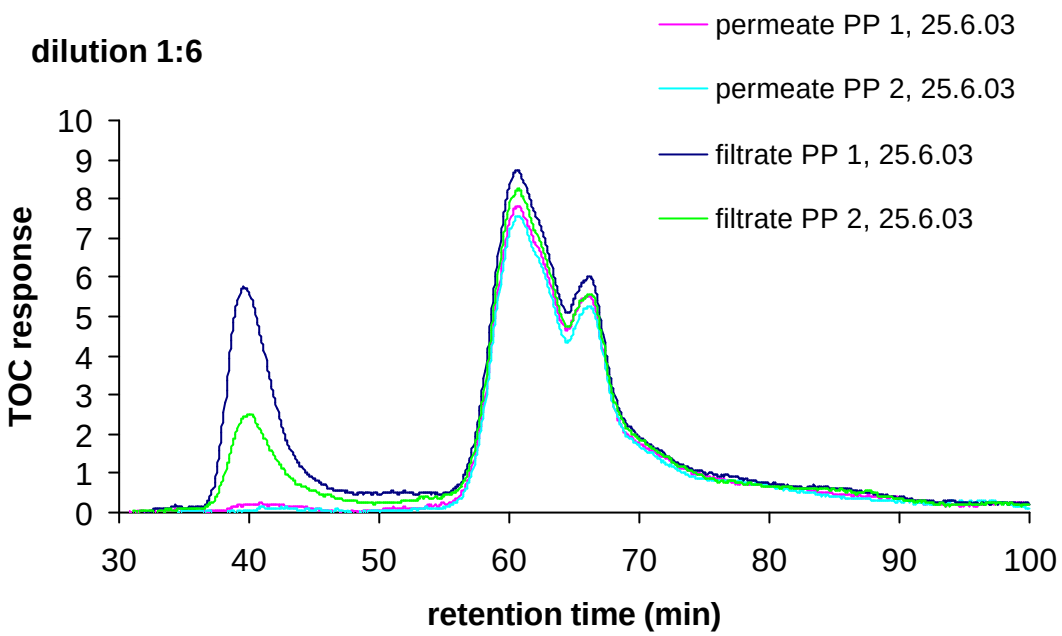

Figure B.27 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 25th June 2003

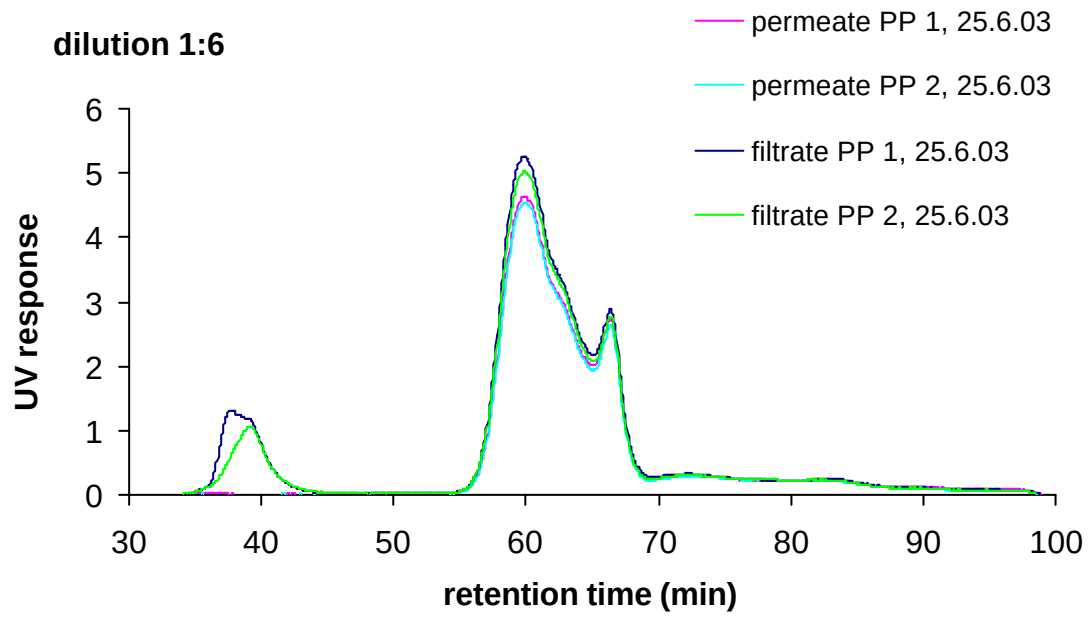

Figure B.28 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 25th June 2003

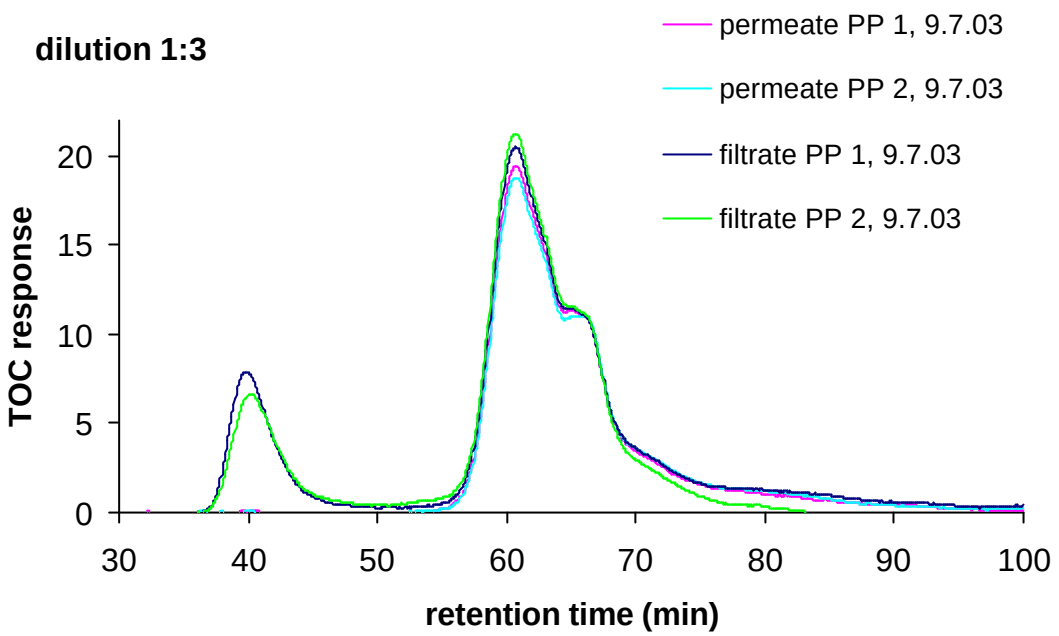

Figure B.29 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 9th July 2003

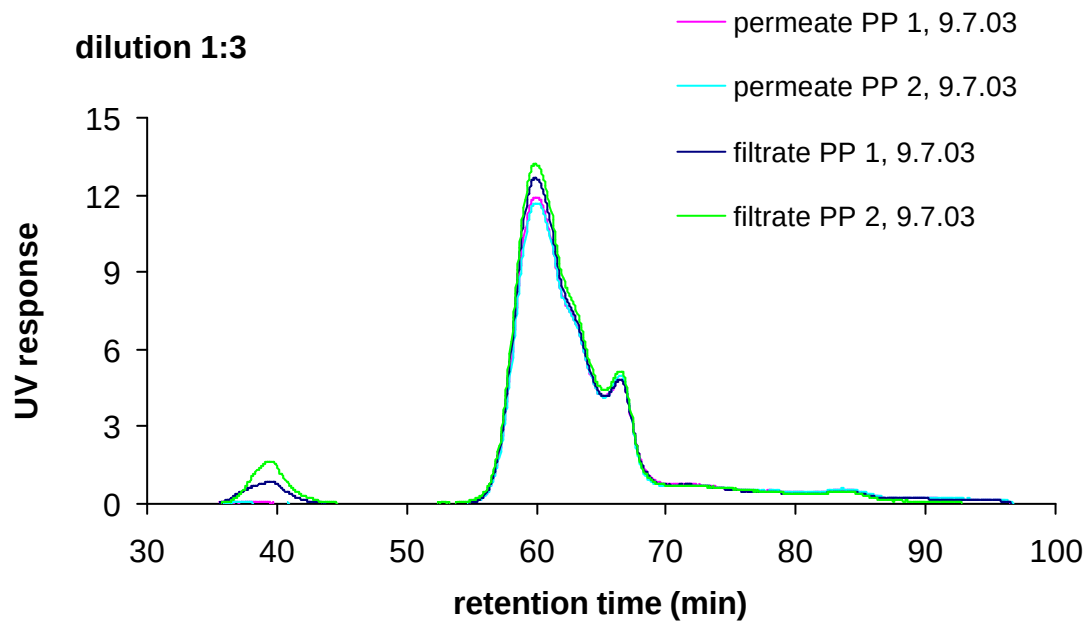

Figure B.30 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 9th July 2003

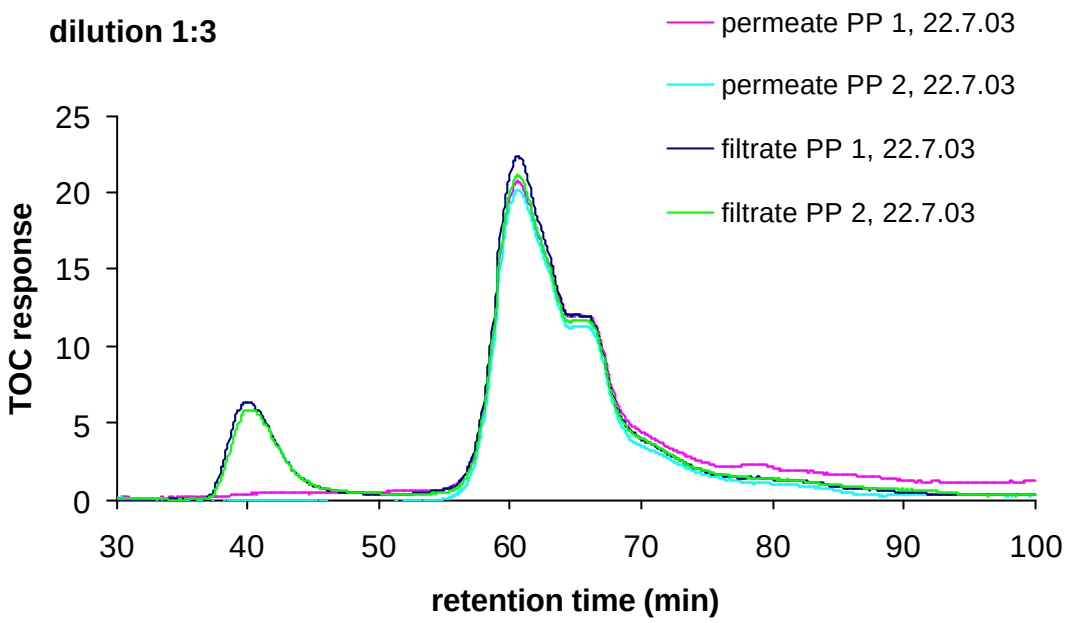

Figure B.31 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 22nd July 2003

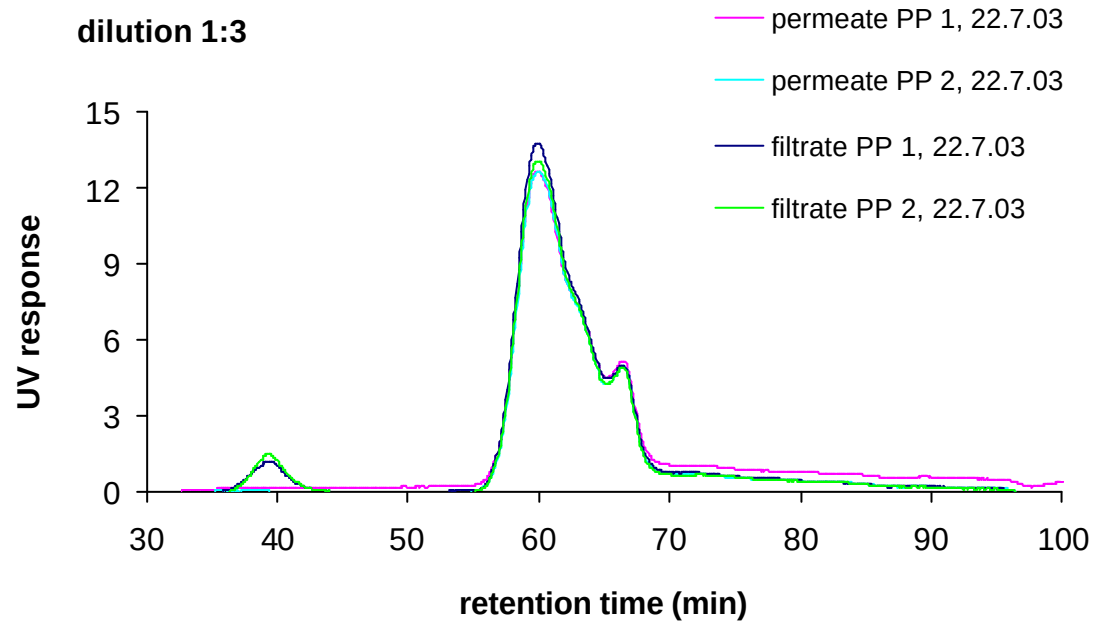

Figure B.32 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 22nd July 2003

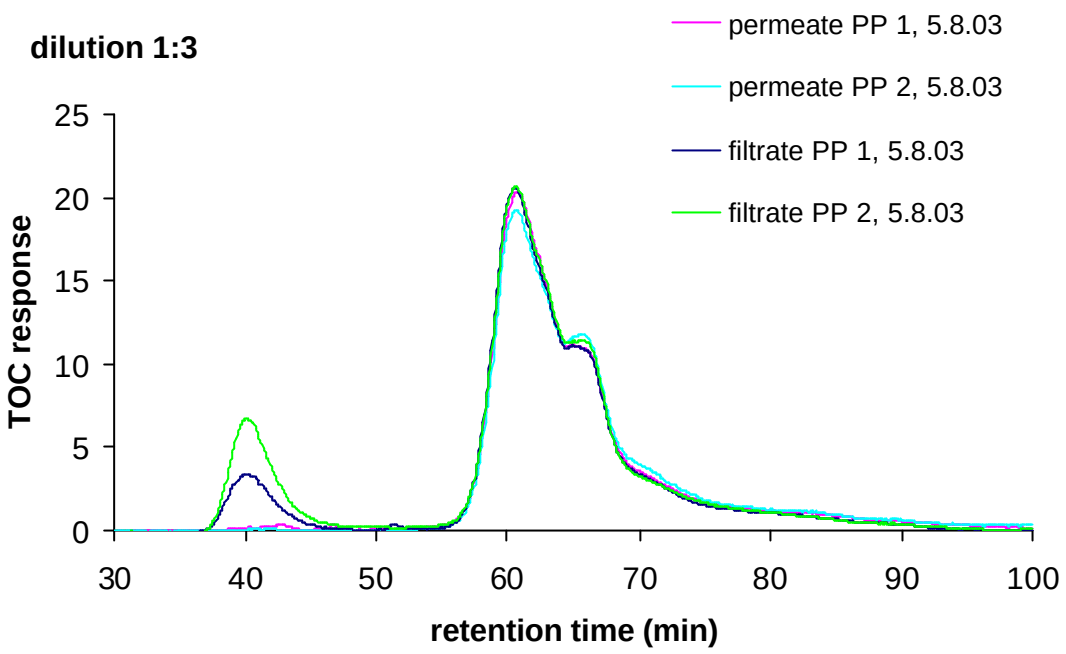

Figure B.33 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 5th August 2003

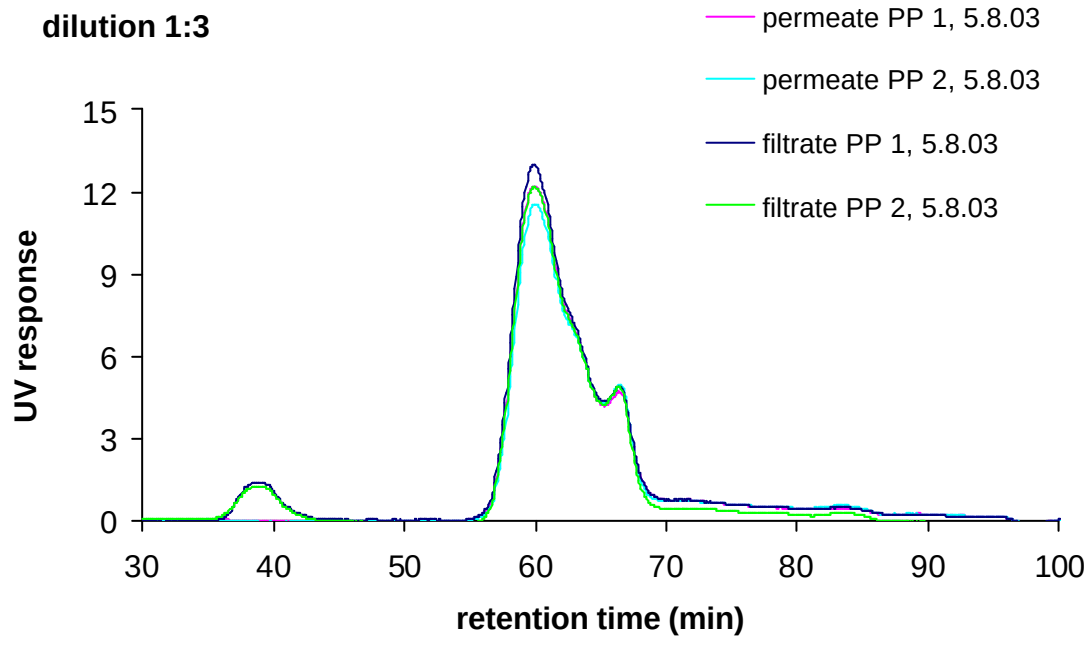

Figure B.34 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 5th August 2003

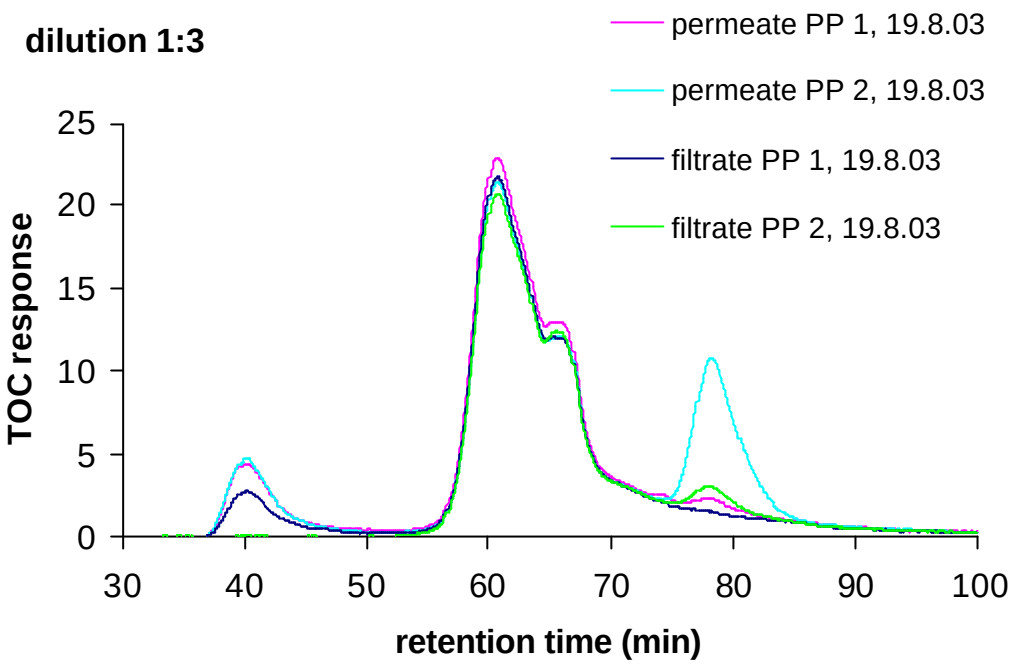

Figure B.35 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 19th August 2003

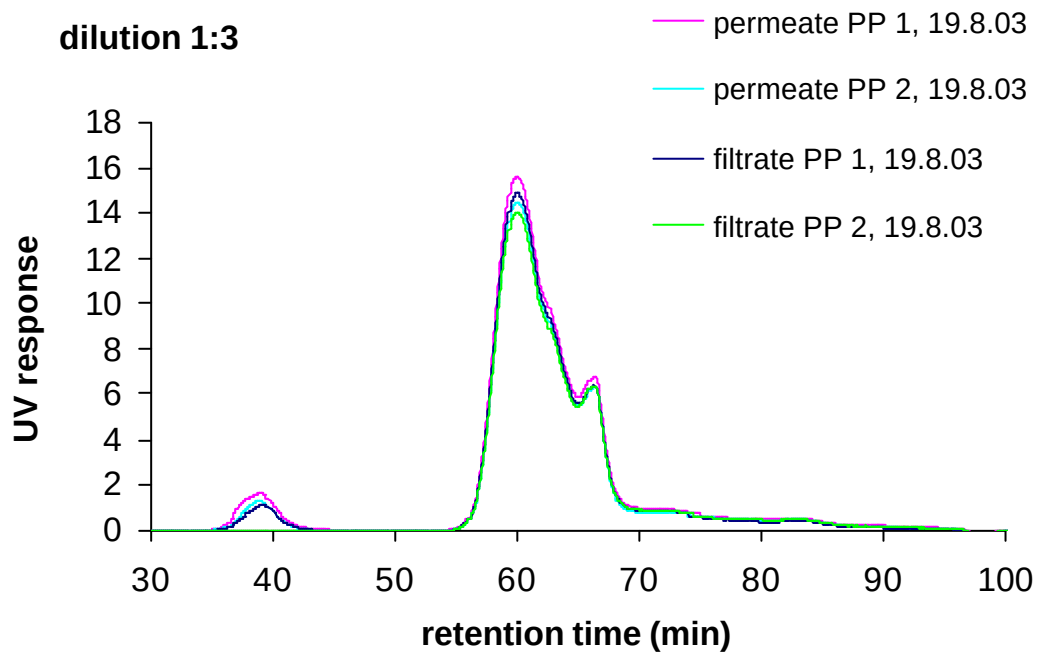

Figure B.36 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 19th August 2003

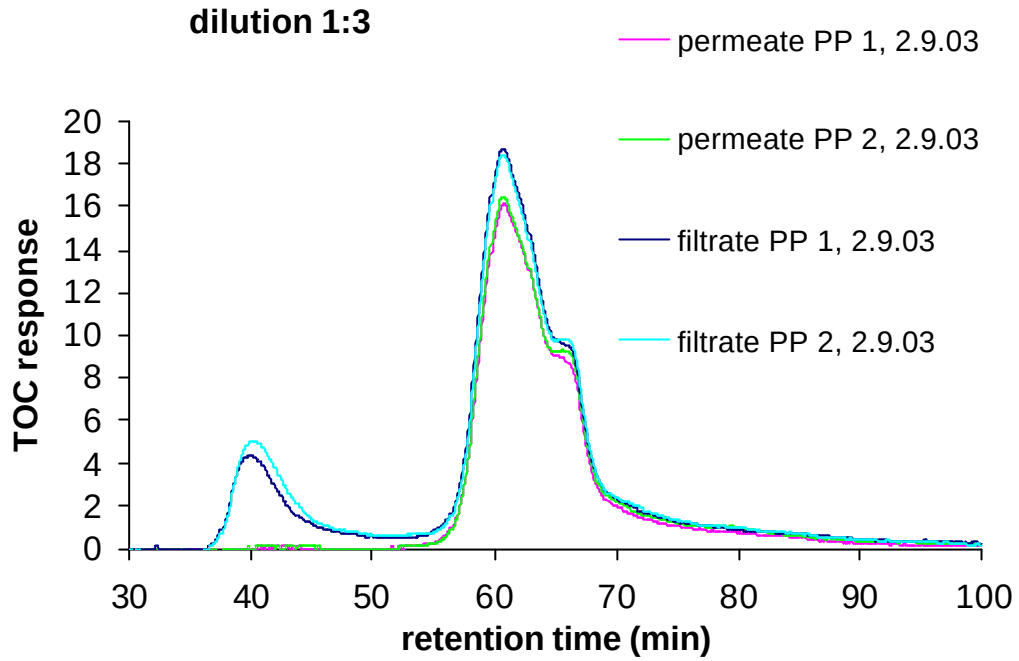

Figure B.37 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 2nd September 2003

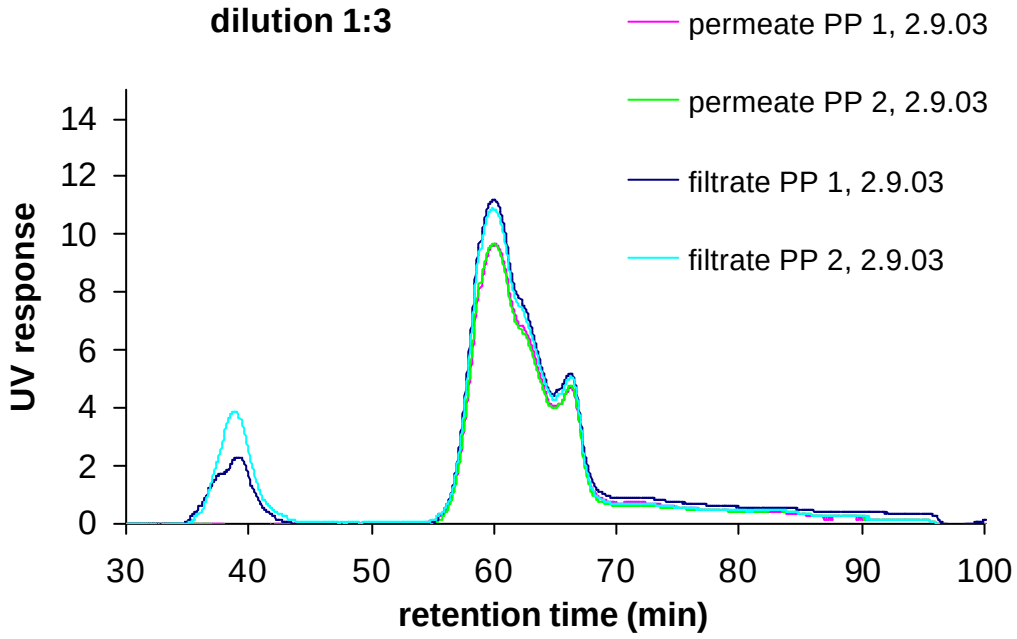

Figure B.38 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 2nd September 2003

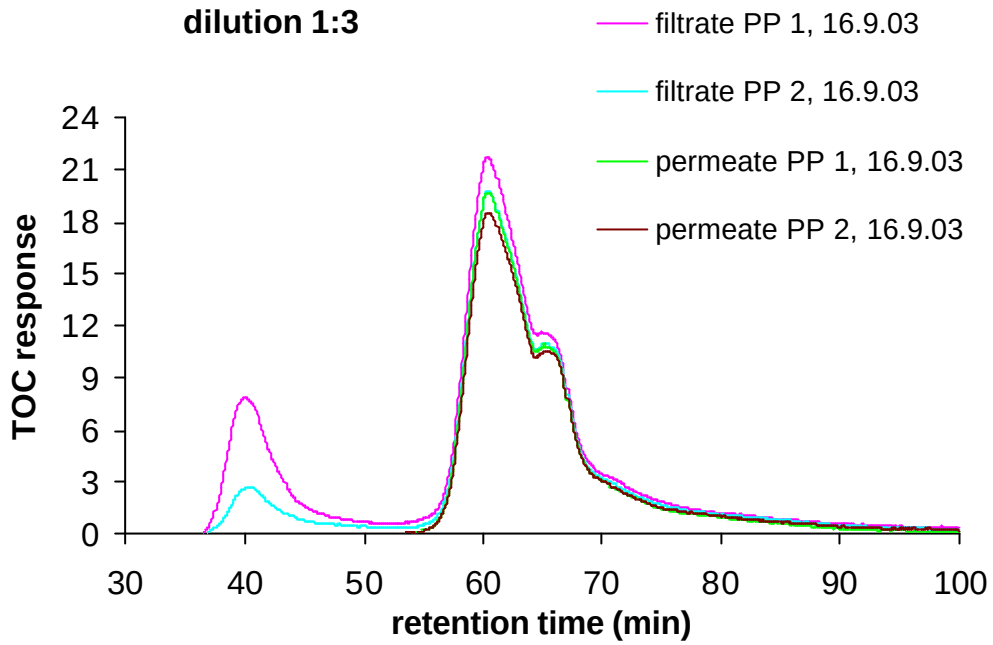

Figure B.39 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 16th September 2003

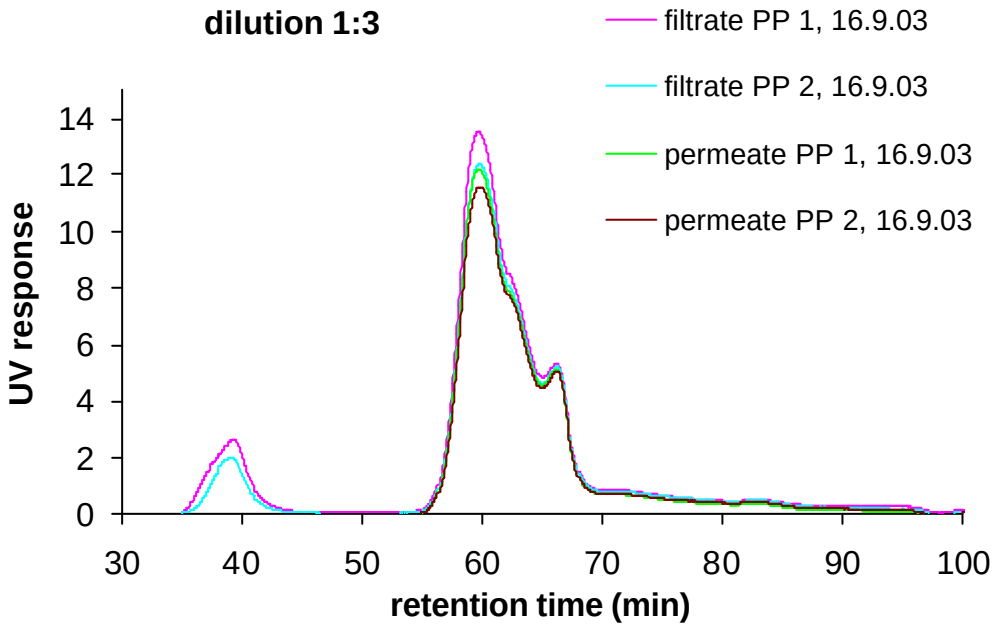

Figure B.40 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 16th September 2003

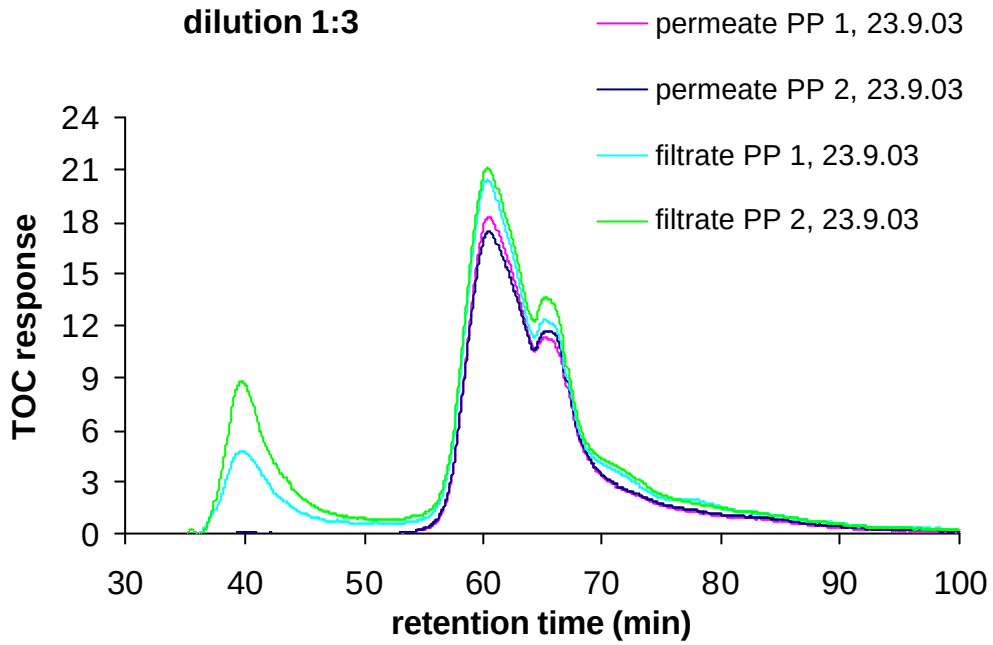

Figure B.41 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 23rd September 2003

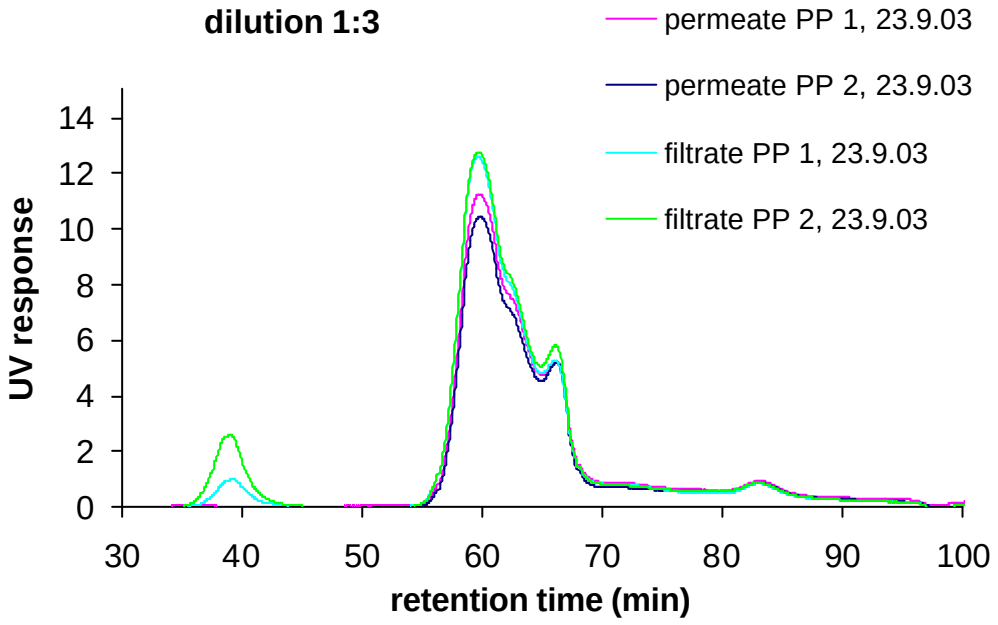

Figure B.42 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 23rd September 2003

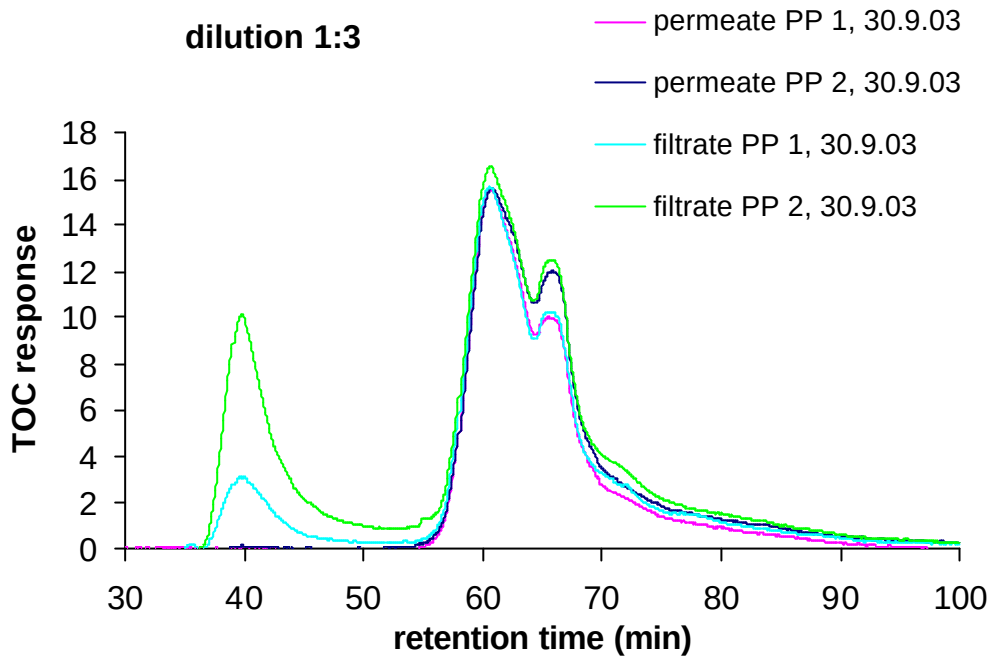

Figure B.43 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 30th September 2003

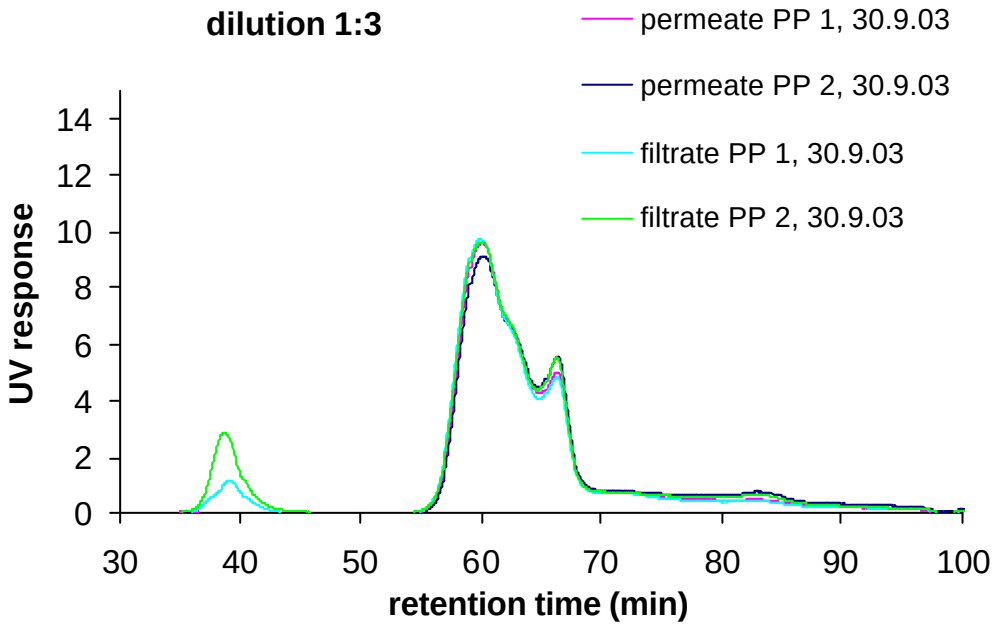

Figure B.44 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 30th September 2003

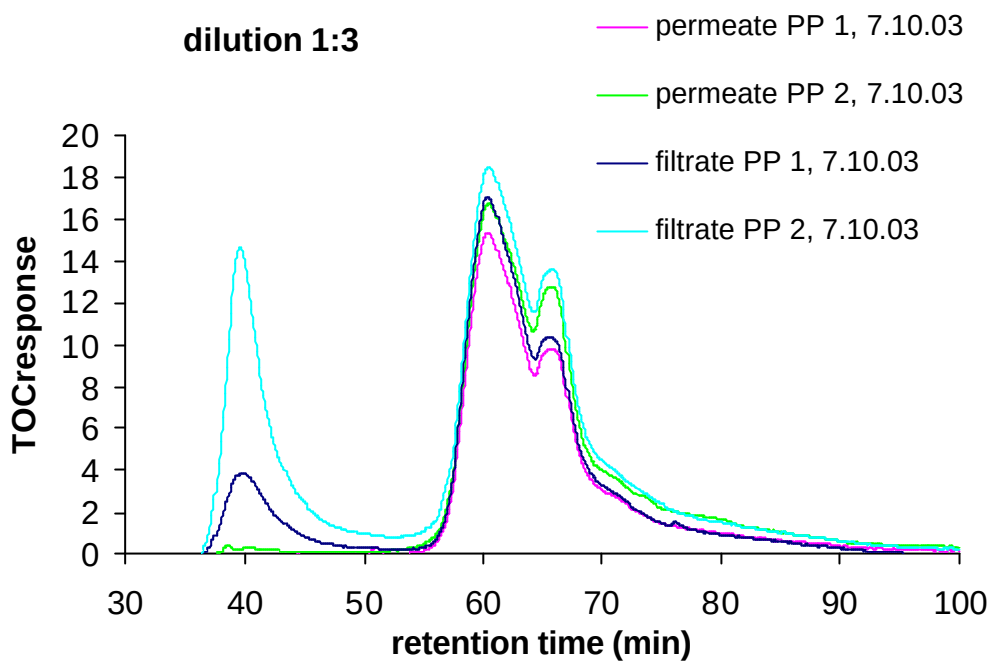

Figure B.45 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 7th October 2003

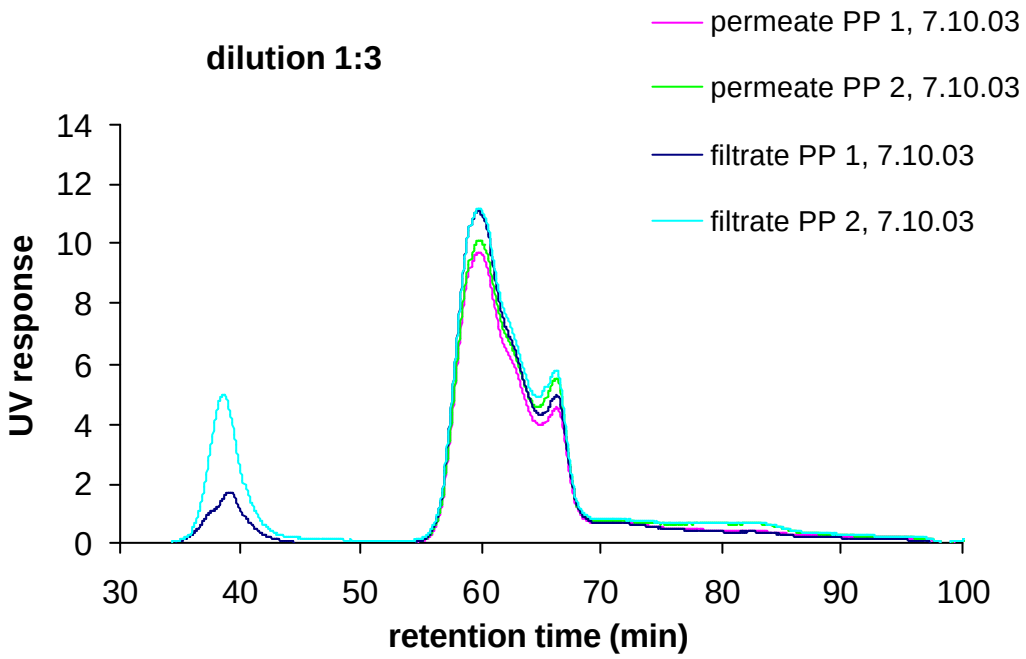

Figure B.46 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 7th October 2003

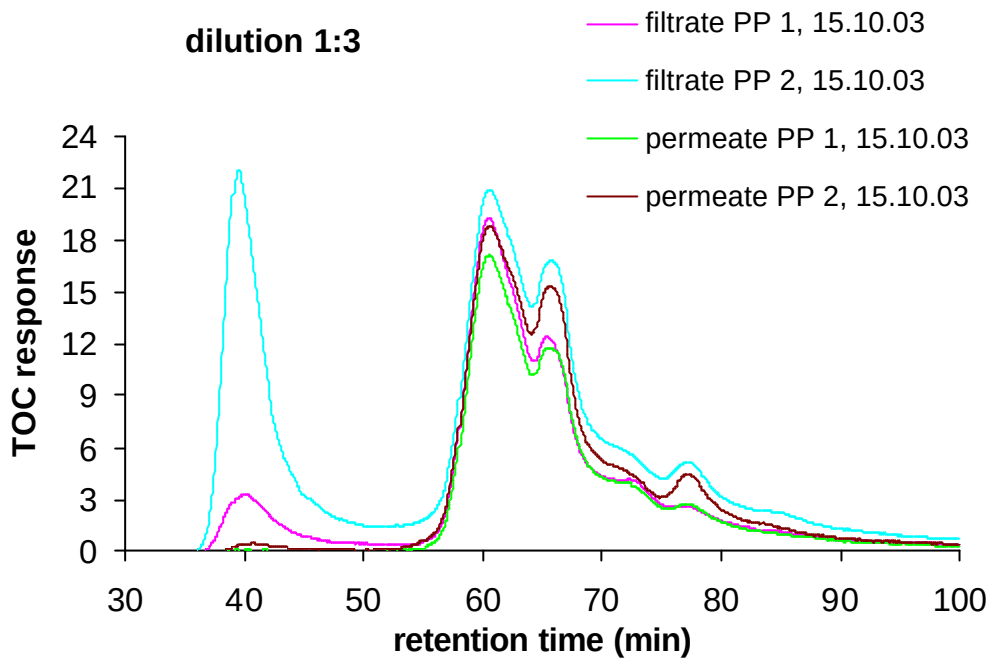

Figure B.47 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 15th October 2003

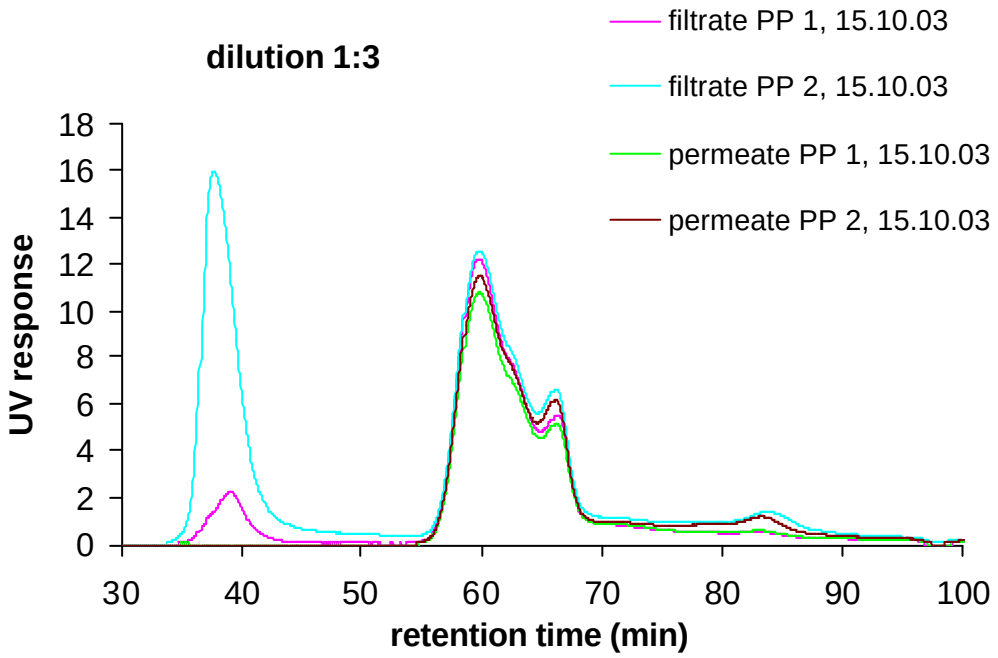

Figure B.48 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 15th October 2003

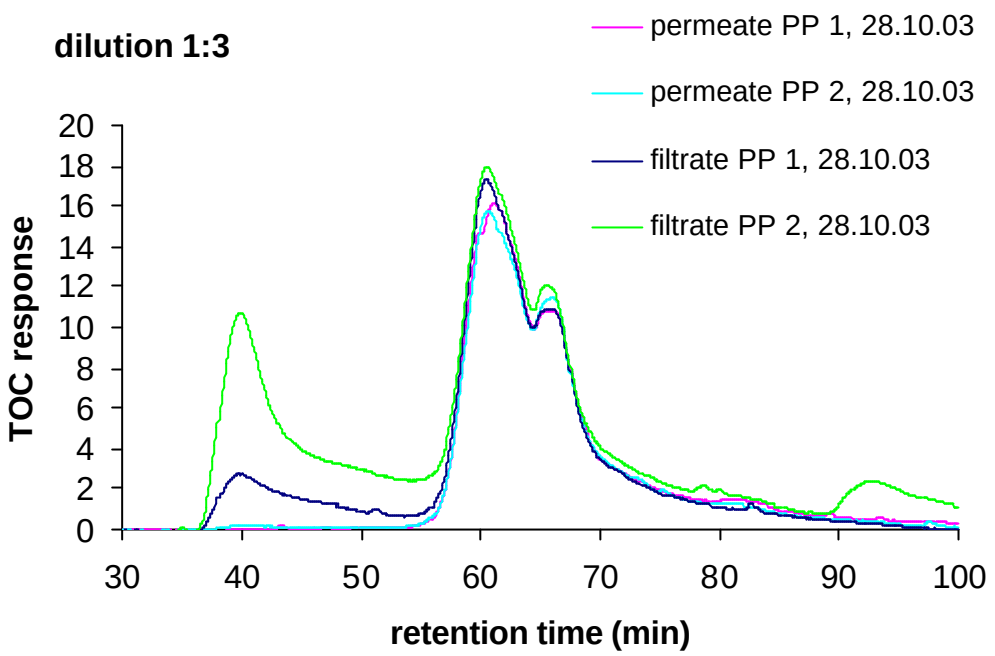

Figure B.49 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 28th October 2003

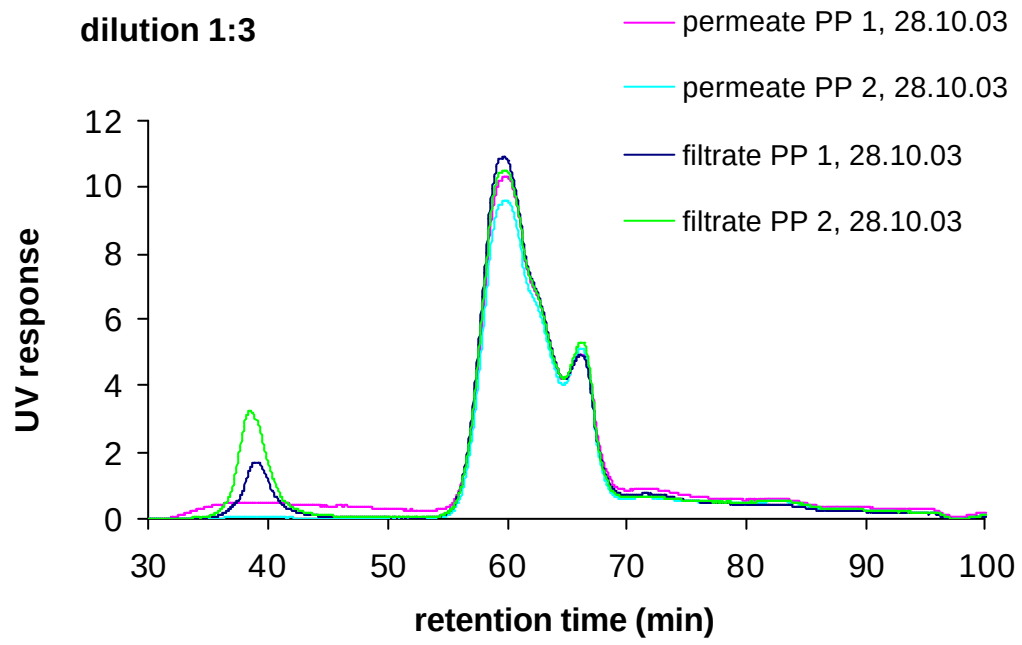

Figure B.50 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 28th October 2003

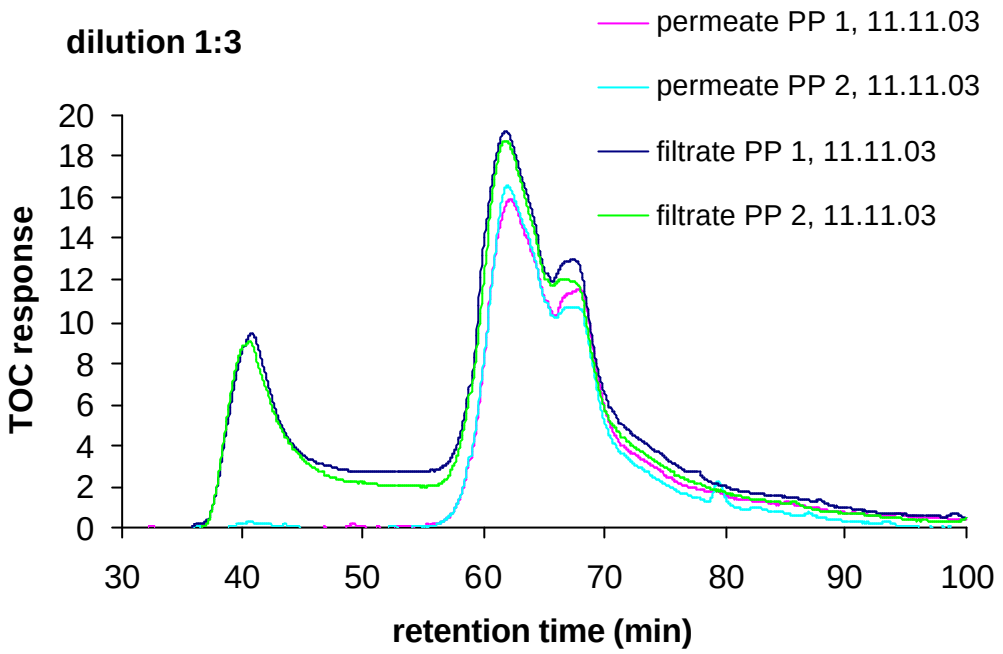

Figure B.51 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 11th November 2003

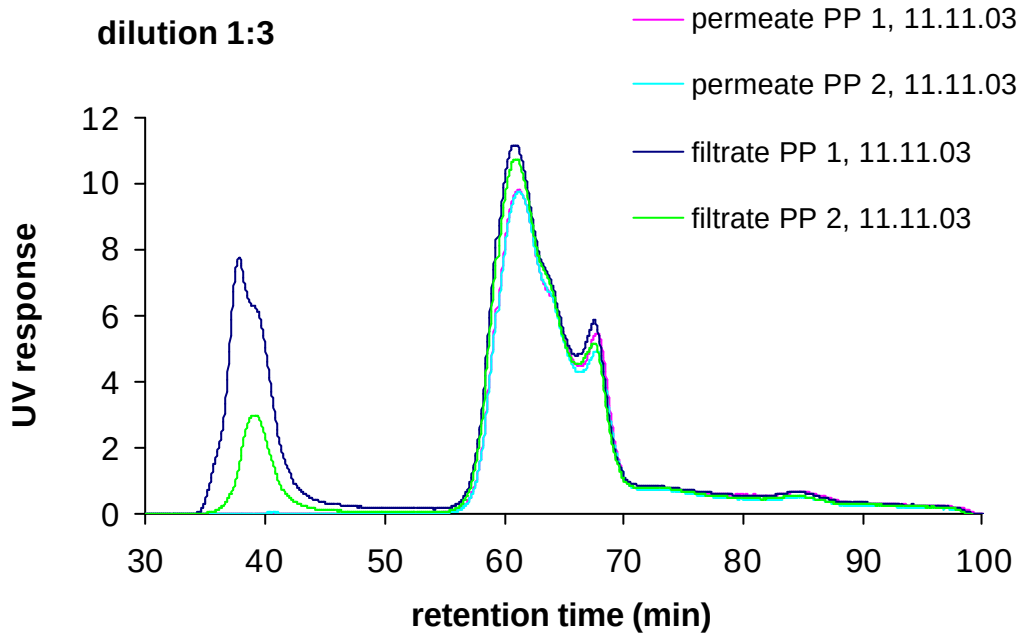

Figure B.52 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 11th November 2003

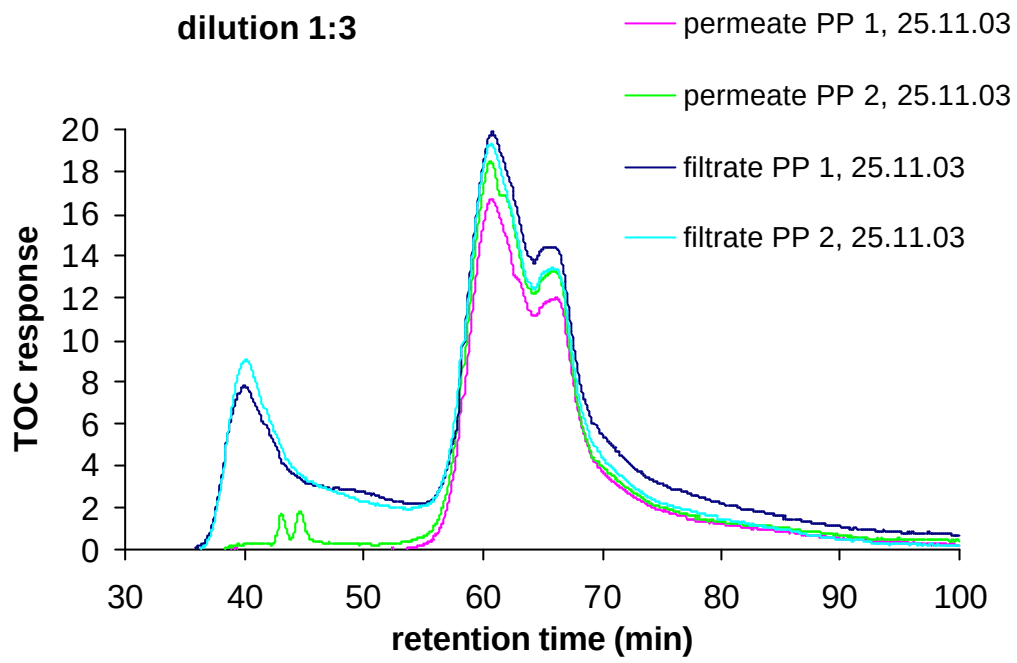

Figure B.53 Chromatograms for organic carbon response for filtrate and permeate samples of both pilot plants taken on 25th November 2003

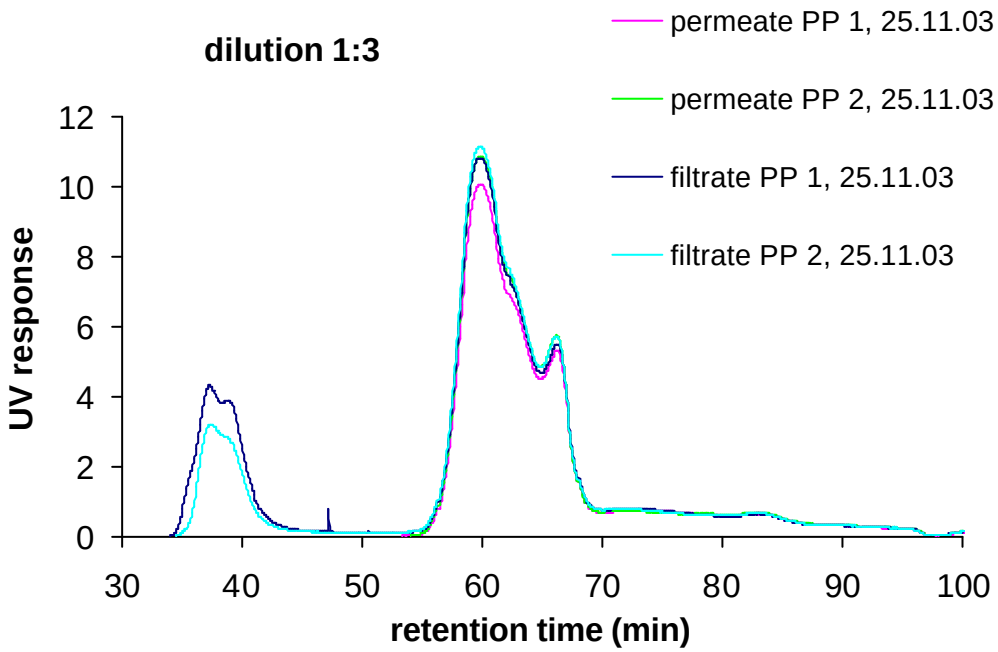

Figure B.54 Chromatograms for UV response for filtrate and permeate samples of both pilot plants taken on 25th November 2003