Influence of ozonated cyanobacteria on bacterial growth in rapid sand filters

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ABSTRACT

Ozone treatment of the raw water results in strong bacterial growth in the rapid sand filters during the winter months at Zurich's lake-water treatment plants. The source of the assimilable organic carbon (AOC) needed for this growth is not known. Evidence is provided that AOC is generated by ozone treatment of cyanobacteria present in the raw water which are disrupted during ozonation. Firstly, bacterial growth correlates with the presence of cyanobacteria at the intake during the winter months and secondly, the quantification of specific phytoplankton pigments, as a parameter for intact biomass, suggests that cells of cyanobacteria are readily disrupted by ozone treatment whereas other phytoplankton cells, such as diatoms, are more resistant to ozone. It is proposed that the released cellular content of phytoplankton provides the AOC source for bacterial growth.

Key words | AOC, cyanobacteria, ozone, pigments, rapid sand filters

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INTRODUCTION

The use of surface waters for the production of drinking water requires several treatment steps. In lake-water plants, pre-oxidation with chlorine, chlorine dioxide or ozone is often used as a first disinfection step (US EPA, 1989) as well as to oxidize the dissolved organic carbon (DOC) compounds (Hoigné, 1998). The reaction between chlorine and organic matter forms trihalomethanes (THM) (Rook, 1974), and is the reason why this oxidation agent has been replaced by ozone in many surface water plants (Geering, 1987). This is also the case in the Lengg lake-water treatment plant, one of Lake Zurich's drinking water plants, where ozone treatment has been used as the pre-oxidation step since 1988. However, the oxidation of DOC in water results in the formation of assimilable organic carbon (AOC) compounds which, typically, are more easily assimilated by bacteria (van der Kooij *et al.*, 1989). Oxidative treatment of water containing DOC often results in bacterial growth after the treatment (van der Kooij, 1992). The degree of this growth is measured by the increase in heterotrophic plate counts (HPC). Moreover, comparisons between different oxidation agents showed that ozone is a significantly more potent disinfectant than chlorine but causes more extensive bacterial growth (e.g. Jäggi, 1986; Marhaba *et al.*, 2000). In the Lengg plant long-term studies showed that ozone treatment of raw water results in an increase of bacterial growth in the rapid sand filters but only during the winter months (Zimmermann & Cao, 1997). However, there are no seasonal differences in the DOC content of the raw water and, hence, the basis for the strong bacterial growth specifically during the winter period needs to be explained. Moreover, no such growth has been observed in other Lake Zurich plants which use a mixture of chlorine and chlorine dioxide instead of ozone as the oxidation agent (Zimmermann & Cao, 1997).

The aim of this study was to show that this bacterial growth in the rapid sand filters correlates with the presence of specific phytoplankton species at the lake intake. In addition the influence of the various treatment steps, especially the ozonation, were to be studied. With an understanding of the underlying mechanisms it was expected to show that this effect has no impact on the drinking water quality.

MATERIALS AND METHODS

To control water quality through the different treatment steps in the Lengg lake-water plant, weekly samples are analysed for heterotrophic plate count (HPC) and monthly samples for determination of phytoplankton content and biomass in addition to other chemical parameters. HPC is determined according to the *Schweizerisches Lebensmittelbuch* (Anonymous, 1985) using PC-agar (Fa. Becton Dickinson Code 43116338) at 20°C for 72 h.

For the analysis of phytoplankton, the samples were immediately fixed with Lugol's solution. Phytoplankton genera and species were differentiated and counted by means of an inverted microscope (Utermöhl, 1958). The biomass was calculated by multiplying the counts of the different phytoplankton species by their respective bio-volumes. Since the distinction between living and dead cells under the microscope is often difficult, all cells that were not obviously damaged, i.e. morphologically intact, were counted. This procedure is routinely used for analysing raw water and treated water samples since morphologically intact cells still represent distinct particles that have to be eliminated by filtration. However, careful inspection of raw water samples showed that up to 60% of the diatoms were found to be empty shells, whereas most of the cyanobacterial filaments looked as though they consisted of intact cells. After pre-ozonation, most of the diatom cells were devoid of pigments but retained their characteristic shape. During routine counts, such cells were therefore counted.

For this study, a method to measure the content of intracellular pigments was developed by the laboratory of Zurich Water Supply. The pigment concentrations are a measure of the intactness of phytoplankton cells and the relative abundance of cells of specific phytoplankton groups. Three litres of water were filtered through Whatman GF/F filters and immediately stored at −20°C. The filters were homogenized in 10 ml of 90% acetone and filtered through Whatman GF/F filters. The filtrate was concentrated under nitrogen gas to about 5 ml. Chlorophyll a, b and c, and up to 15 carotenoids were separated by high performance liquid chromatography (HPLC) at a flux rate of 1 ml/min and identified and quantified by analysis of their spectra between 300 and 600 nm. The HPLC consisted of a Series 1050 system (Agilent Technologies) with a DAD detector (440, 450, 475, 538 nm) and a quaternary pump. The stationary phase used was a Nucleosil column (100 C_{18}, 5 μm, 250 × 4.6 mm, Bischoff Chromatography), and for the mobile phase a gradient from water, methanol, acetonitril and ethylacetate was used (Table 1). The injection-volume was 100–500 μl depending on the pigment concentration.

Turbidity was measured online and for this study the mean daily turbidity values on the dates for HPC analysis samples were used. Assimilable organic carbon (AOC) content was determined by the method as described in

Table 1 | Gradient timetable of the HPLC method for determination of different algal pigments

Time (min)	Water	Ethylacetate	Acetonitril	Methanol
0	10	0	2.0	88.0
3	10	0	3.6	86.4
4	5	0	3.8	91.2
11	5	0	3.8	91.2
13	5	0	30.4	64.6
14	0	0	80.0	20.0
17	0	0	85.0	15.0
18	0	0	90.0	10.0
20	0	11	82.0	7.0
25	0	13	72.0	15.0
28	0	17	45.0	38.0
36	0	15	22.5	62.5
38	0	10	22.5	67.5
40	5	5	22.5	67.5
46	10	0	2.0	88.0

Hambsch *et al.* (1992). This microbiological test is based on turbidity (12° forward scattering) as a parameter for the increase of biomass after sterile filtration of the water samples and inoculation with autochthonous bacteria. The automated turbidity measurements produce a continuous growth curve. The growth experiment is stopped when the stationary phase is reached. The AOC of the sample is calculated as acetate-C equivalents by comparing its turbidity increase with the turbidity increase of an acetate dilution series. This AOC analysis covers only dissolved organics in the water (filtration with 0.25 μm membrane filters).

RESULTS AND DISCUSSION

Comparison of HPC values in the rapid sand filter effluents

The city of Zurich produces about 70% of its drinking water from lake water which is extracted at a depth of 30 m. Figure 1 shows the treatment processes used at the Lengg lake-water plant. The ozone dosage is adjusted continuously in a range of 1.1 mg/l ± 25%, throughout all seasons, in order to maintain a constant ozone residual of 0.4 mg/l after a contact time of 11 min. This achieves a more-or-less constant ozone exposure (ct-value) in the pre-ozonation regardless of seasonal variations in temperature, pH, turbidity, etc.

During winter the heterotrophic plate count (HPC) values in the effluent of the rapid sand filters are considerably higher than those in plants using other oxidation agents (e.g. the Moos plant; Table 2). This has been previously reported (Zimmermann & Cao, 1997) in the Lengg plant as well as in other plants using pre-ozonation. Figure 2 shows that the high HPC values in the Lengg plant have only been observed since ozone was introduced as the pre-oxidation step in 1988, suggesting that the higher HPC values are due to the ozone treatment. Moreover, the higher values in the Lengg plant show seasonal variations, whereby the highest values are consistently observed during the winter months (Figure 2).

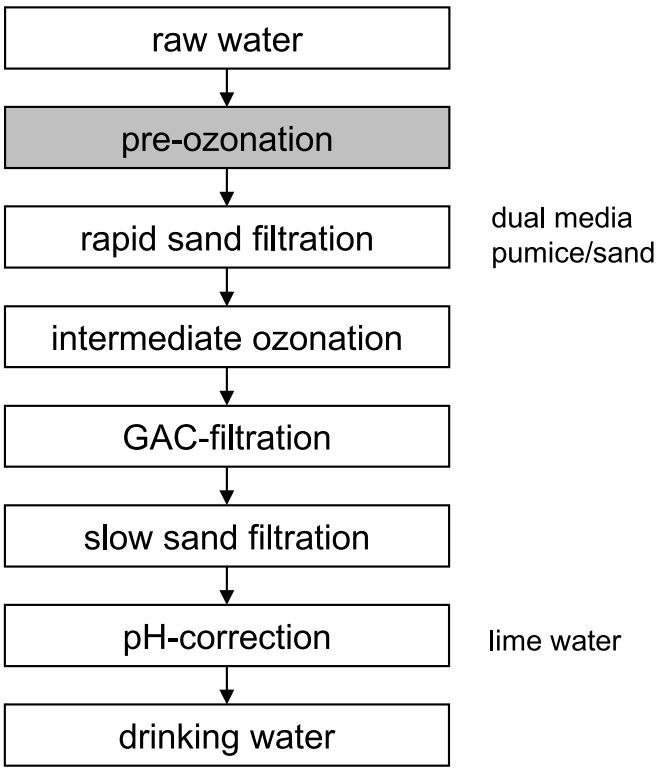

Figure 1 | Unit treatment processes used at the Lengg lake-water plant, Zurich Water Supply.

Evaluation of possible reasons for high HPC values in the rapid sand filter effluent

Several factors can lead to high HPC values. Firstly, changes in the operational conditions of the plant (e.g. filtration rate, backwash cycles of the filters or the residence time of the water between the various treatment processes which may cause such effects) can be excluded as the plant works all the year under the same operational parameters. Secondly, another obvious reason for the changes in HPC values in the Lengg plant could be changing DOC levels in the raw water. However, this explanation can be excluded since the DOC levels as measured by Zurich Water Supply have been more-or-less constant over the past 20 years, lying in the range between 1.0 and 1.3 mg/l with no seasonal variation. A third reason might be changes in the number of bacteria in the raw water. However, this explanation can also be excluded since there is no evidence of seasonal variations in HPC values

Table 2 | Dependence of bacterial growth in the effluents of the rapid sand filters on pre-oxidation agent in different lake-water plants (from Zimmermann & Cao 1997)

Lake Zurich treatment plant	Pre-oxidation	Cyanobacteria	Strong bacterial growth during winter
Lengg	O_3	+	+
Küsnacht	O_3	+	+
Meilen	O_3	+	+
Oberland	O_3	+	+
Männedorf	Cl_2	+	–
Rüschlikon	Cl_2	+	–
Moos	Cl_2/ClO_2	+	–

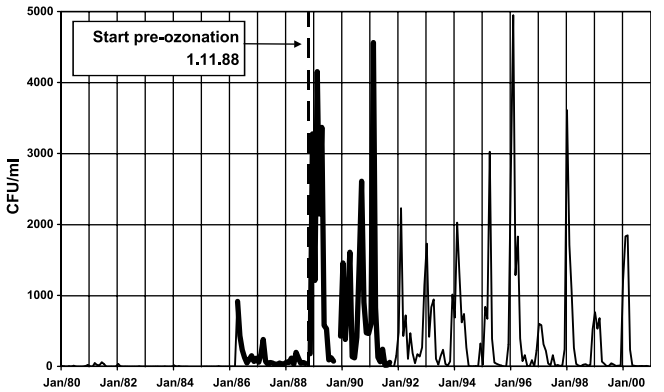

Figure 2 | Heterotrophic plate count (HPC) in the effluent of the rapid sand filters of the Lengg lake-water plant (January 1980–December 2000).

in the raw water samples (Zurich Water Supply, unpublished data). Finally, during the winter months, turbidity of the raw water seems to be correlated with HPC values in the effluents of the rapid sand filters (Figure 3). However, high raw water turbidity values are also observed during the summer months without corresponding high HPC values. Thus, there is no general correlation between turbidity and HPC values in the rapid filters. The high turbidity values in winter are probably mainly caused by large amounts of phytoplankton (mostly cyanobacteria) at the intake. As shown below, the HPC values correlate well

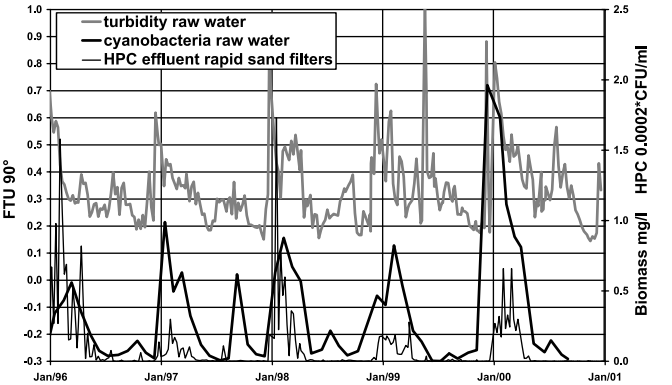

Figure 3 | Time series of turbidity, cyanobacteria biomass and heterotrophic plate count (HPC) of the Lengg lake-water plant (January 1996–December 2000).

with the presence of cyanobacteria at the intake (Figure 3). This correlation leads us to propose that the cellular contents that are released after ozone-mediated disruption of cyanobacteria cells provide the AOC source for bacterial growth in the rapid sand filters (Zimmermann & Cao, 1997).

Study to confirm the hypothesis

Description of the study

To test the hypothesis that cellular material derived from disrupted phytoplankton cells provides the nutrients for bacterial growth, AOC, intracellular pigments and biomass in the raw water as well as in samples from the different treatment processes of the Lengg plant were measured. These measurements were performed on five different dates and in addition to the routine examinations of the water. Two of the analyses were done during periods with minimal bacterial growth (25 August 1999 and 10 July 2000) and three analyses were done during periods with high bacterial growth (13 December 1999, 24 January 2000 and 14 February 2000).

AOC analysis

Table 3 shows the results of the AOC analysis. The AOC values in the raw water are relatively low in both seasons,

Table 3 | HPC (CFU/ml) and AOC values (μg/l) after the different treatment processes at the Lengg lake-water plant

Sampling date	Raw water		After pre-ozonation		Rapid sand filtration		Intermediate ozonation		GAC		Slow sand filtration	
	HPC	AOC	HPC	AOC	HPC	AOC	HPC	AOC	HPC	AOC	HPC	AOC
Summer 25.08.99	60	4	0	65	30	22	0	45	1	13	0	11
Winter 13.12.99	65	23	0	241	55	32	0	72	1	35	0	24
Winter 24.01.00	42	7	0	128	870	25	0	49	1	26	2	20
Winter 14.02.00	380	9	0	179	1200	23	0	66	0	20	1	14
Summer 10.07.00	440	14	0	88	2	18	2	35	2	15	1	8
Mean												
Summer	250	9	0	77	16	20	1	40	1	14	0	10
Winter	160	13	0	183	710	27	0	62	1	27	1	19

HPC—CFU/ml; AOC—μg/l.

generally below 20 μg/l. After pre-ozonation, the AOC-content always shows a significant increase compared with that of the raw water content. However, AOC values exceeding 100 μg/l after pre-ozonation are only observed during the winter months. It is also during this period that high HPC values in the effluent of the rapid sand filters are observed (Figure 2). These observations strongly suggest that high bacterial growth requires AOC values above 100 μg/l, as measured with the applied method, and that there is only very poor bacterial growth below this AOC concentration. The AOC content in samples from the subsequent treatment steps lies clearly below the value of 100 μg/l (Table 3), and this explains why there is no substantial increase in HPC counts observed in samples taken from these treatment steps.

Biomass and pigment analysis of phytoplankton

To test the degree of phytoplankton cells disrupted by the ozone treatment, both biomass and the pigment content in the different samples were measured. The rationale for analysing the pigment content (mainly chlorophylls and carotenoids) is that earlier studies showed that different phytoplankton groups in Lake Zurich each contain characteristic group-specific carotenoids (Cao *et al.*, 1999). Hence, these specific carotenoids can be used as indicators to identify the corresponding groups. Importantly, these pigments are bound intracellularly and they are quickly released if the cells are disrupted. The content of carotenoids can therefore be used as a measure of the intactness of phytoplankton cells. In particular, the reduction of the intracellular pigment content after ozonation can be taken as a parameter to estimate whether organic material has been released after ozone treatment.

Before analysing the effects of ozonation on phytoplankton cells, their pigment content and total biomass were determined in the raw water samples at the lake intake. These results are summarized in Table 4. In winter the raw water samples contain about three times more biomass and significantly higher amounts of total pigment than in summer. These seasonal differences are most likely due to the stratification of the lake during the summer months when the majority of the phytoplankton is present in the epilimnion, while at the intake (30 m below the surface) only sedimenting phytoplankton is found. During the summer months the predominant group at the intake

Table 4 | Total pigments and biomass in the raw water of the Lengg lake-water plant

Sampling date	Total pigment (μg/l)	Total biomass (mg/l)
Summer 25.08.99	0.527	0.397
Winter 13.12.99	16.440	2.242
Winter 24.01.00	5.041	2.052
Winter 14.02.00	4.524	1.375
Summer 10.07.00	2.796	0.857
Mean		
Summer	1.662	0.627
Winter	8.668	1.889

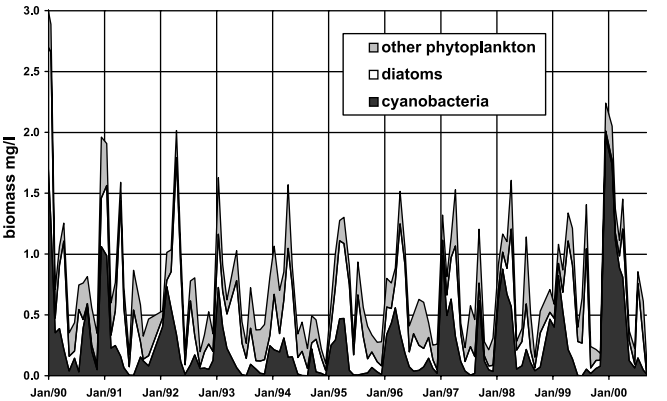

Figure 4 | Raw water at Lengg: time series of composition of phytoplankton biomass (January 1990–December 2000).

are diatoms. The careful microscopic analysis of the August 1999 samples suggests that the majority of the cells (>60%) are empty shells (see Materials and Methods). Consistent with this, the ratio of diatom-specific pigments to diatom biomass in the summer samples is comparatively low compared with the winter samples (Table 5). These observations suggest that the majority of the diatom cells present at the intake have lost their pigments as well as other cellular contents and therefore do not represent intact biomass.

In contrast, in winter, the lake is mixed down to a depth below 30 m. Cyanobacteria, predominantly *Planktothrix rubescens*, are the most abundant group in the lake during this period and they represent the bulk of the total biomass at the intake (Figure 4). These cells are

Table 5 | Specific carotenoids and biomass of diatoms and cyanobacteria in the raw water and after pre-ozonation at the Lengg lake-water plant (detection limit of carotenoids: 0.005 μg/l)

		Pigments (specific carotenoids μg/l)				Biomass (mg/l)			
		Diatoms (fucoxanthin)		Cyanobacteria (myxoxanthophyll+zeaxanthin)		Diatoms		Cyanobacteria	
Sampling dates		Raw water	After pre-ozonation	Raw water	After pre-ozonation	Raw water	After pre-ozonation	Raw water	After pre-ozonation
Summer	25.08.99	0.075	<0.005	<0.005	<0.005	0.111	0.089	0.025	0.021
Winter	13.12.99	0.077	0.030	0.270	<0.005	0.050	0.041	1.963	2.274
Winter	24.01.00	0.060	0.014	0.218	<0.005	0.037	0.033	1.734	1.517
Winter	14.02.00	0.077	0.018	0.221	<0.005	0.057	0.058	1.117	0.988
Summer	10.07.00	0.425	0.022	0.078	<0.005	0.610	0.656	0.149	0.111
Mean	Summer	0.250	0.011	0.039	<0.005	0.361	0.373	0.087	0.066
	Winter	0.071	0.021	0.236	<0.005	0.048	0.044	1.605	1.593

morphologically intact, contain pigment, and therefore most likely constitute intact biomass. To summarize, there is a major distinction between the winter and summer raw water samples. In the winter samples a substantial amount of intact phytoplankton cells are present at the intake due to mixing of the lake, whereas the summer samples contain primarily sedimenting cells. Long-term studies by the Zurich Water Supply in the lake support this assumption (the ratio of diatom-specific pigments to diatom biomass at 30 m depth is 0.69×10^{-3} during the summer months and 1.05×10^{-3} during the winter period).

The extent to which ozone disrupts cells of different phytoplankton groups was investigated. The routine biomass determination by cell counts does not show a clear difference. The biomass of both cyanobacteria and diatoms appears essentially unaltered after ozonation. As mentioned above, the reason for this is that we do not differentiate between intact cells and cell skeletons during routine biomass determination. However, after pre-ozonation, cyanobacteria-specific pigments such as myxoxanthophyll and zeaxanthin, are no longer detectable (Table 5). This indicates that ozonation disrupts most of the cyanobacteria cells and results in the release of pigments and other cellular components. In contrast, diatom-specific pigments such as fucoxanthin are still detected after pre-ozonation (Table 5). This implies either that a fraction of diatom cells remains intact after ozonation or that ozone treatment results only in a partial release of organic material from these cells. A likely explanation for the comparatively less efficient disruption of diatom cells by ozone is that they are more resistant to destruction due to their shells. These results thus show that cyanobacteria are more easily disrupted by ozone treatment compared with diatom cells. Their released cellular components then constitute the nutrients (AOC) that are required for bacterial growth in the rapid sand filter.

Correlation between HPC values and cyanobacteria biomass

Both cyanobacteria biomass and pigment content (myxoxanthophyll and zeaxanthin) in the raw water samples correlate well with the AOC levels after pre-ozonation (Figure 5). Consistent with this, it is found that the HPC values in the rapid filter samples show no correlation with the total biomass but they do specifically correlate with the biomass of cyanobacteria at the intake (Figure 6). These observations further support the interpretation that AOC stems primarily from the ozonation of cyanobacteria cells.

During periods of high bacterial growth, bacteria populations grown from rapid sand filter samples are morphologically distinct from those isolated during periods with little growth. Previous studies showed that the composition of a bacteria population could change depending on the products present after ozonation (van

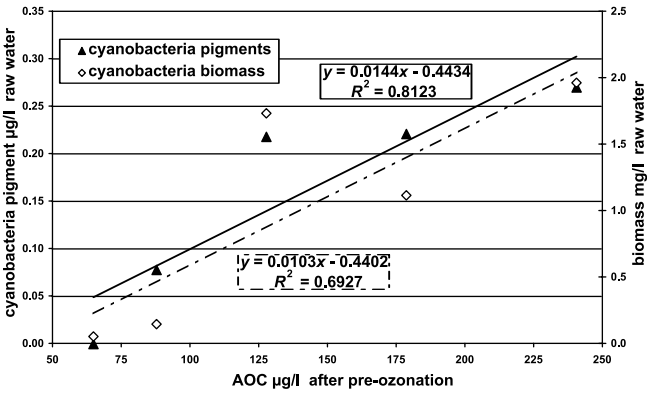

Figure 5 | Correlation of cyanobacteria pigments and biomass in the raw water to AOC after pre-ozonation.

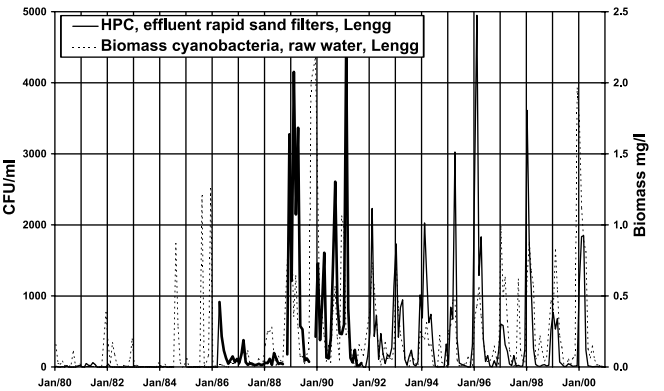

Figure 6 | Time series of heterotrophic plate count (HPC) in the effluent of the rapid sand filters and biomass of cyanobacteria in the raw water, Lengg lake-water plant (January 1980–December 2000).

der Kooij, 1992; Kang *et al.*, 1997). An understanding of the dynamics of these bacteria populations will require the isolation and identification of the bacteria species present in the rapid sand filter.

CONCLUSIONS

This study provides evidence that in water treatment plants which use ozone for pre-oxidation, ozone-mediated disruption of certain phytoplankton groups present in lake water creates a source of AOC that can cause substantial bacterial growth during subsequent treatment. The results also suggest that not all phytoplankton cells are disrupted with the same efficiency. Cyanobacteria are more readily disrupted than cells of diatoms.

In the Lengg lake-water plant, increased bacterial growth is observed in the rapid sand filters, the first step after pre-ozonation. After final purification through a slow sand filter AOC levels again lie below a critical threshold. This demonstrates the importance of biofilters after ozone treatment to prevent bacterial regrowth in the network (Klein & Forster, 1999). Finally, this study also shows that long-term monitoring of phytoplankton populations in raw water sources is an important aspect of quality control since these populations can significantly affect the process of drinking water production.

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