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SEASONAL MICROALGAE VARIATION IN A SUBARCTIC WASTEWATER STABILIZATION POND USING CHEMICAL PRECIPITATION

Säsongsvariation av mikroalger i en norrländsk fällningsdamm

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Abstract

Surface water microalgae samples were collected during the ice-free period in a small subarctic wastewater stabilization pond system, complemented with chemical precipitation during the winter period (fellingsdam), and serving 310 persons. In the primary pond microalgae dominance alternated between the Cryptophyte *Cryptomonas* and green algae (Chlorophyta). In the second and third pond the general pattern was that *Cryptomonas* dominated during summer but was replaced by green algae in autumn and the following spring. Estimations of the microalgae part of the effluent COD and phosphorus showed that microalgae dominated these parameters for only 3–4 weeks of 12 evaluated. This does not support the reasoning behind the European Union directive of the use of filtered samples for effluent BOD, COD and SS from stabilization ponds, in contrast to other wastewater treatment methods. The reasons behind the EU's procedure for ponds are based on the assumption that stabilization ponds convert "sewage BOD" to "algal BOD". The results of this study suggest that further investigations of the microalgae function in subarctic wastewater stabilization ponds and fellingsdams should be conducted, before implementing the EC directive into Swedish law, or into similar laws in other countries with subarctic regions.

Key words – phytoplankton, sewage, oxidation pond, lagoon, fellingsdam, cold climate, EC directive.

Sammanfattning

I en jämtländsk fällningsdamm, med belastningen 310 p.e., samlades mikroalg-prover in från 10–20 cm djup under den isfria perioden. Biomassan och förekommande algtyper undersöktes därefter. I primärdammen växlade dominansen mellan släktet *Cryptomonas* (Cryptophyta) och olika grönalger (Chlorophyta). I den andra och tredje dammen var det generella mönstret att *Cryptomonas* dominerade under sommaren men ersattes av grönalger under vår och höst. Uppskattningar av algernas andel av utgående COD och fosfor visade att algerna dominerade dessa parametrar bara under 3–4 veckor av 12 undersökta. Resultaten är inte i överensstämmelse med de antaganden som finns bakom EG-direktivet (91/271/EEC) om att använda filtrerade prover i biodammar för utgående BOD, COD och SS. Detta till skillnad från andra reningsmetoder där ofiltrerade prover används. Orsaken till denna särbehandling baseras på antagandet att biodammar omvandlar »avlopps-BOD» till »alg-BOD». Denna undersökning visar att mikroalgers roll i subarktiska bio- och fällningsdammar bör undersökas mer, innan det aktuella EG-direktivet implementeras i svensk lag, eller motsvarande lagar i andra länder med subarktiska regioner.

Introduction

Microalgae are important for the functioning of facultative and maturation wastewater stabilization ponds. Their ability to produce oxygen by photosynthesis is vital to the ecology of the water environment. In a symbiotic relationship between algae and bacteria, algae are needed to supply oxygen to aerobic, heterotrophic bacteria, which in turn mineralize the organic wastes into nutrients used by the algae (Tchobanoglous and Burton 1991). Though the oxygenation function of microalgae is often the only one emphasized, Mara and Pearson (1986) and others point out that many of the microalgae present in ponds can also act like bacteria, growing heterotrophically by mineralizing organic energy sources. Pearson et al. (1987) showed that for a wastewater stabilization pond strain of *Chlamydomonas*, the dark utilization (without photosynthesis) of acetate was about 30 % of the utilization by bacteria. In the presence of light this utilization increased to 70 % compared to bacteria. The corresponding figures for the population of *Euglena* were less than 1 % in darkness and only 2–3 % in light.

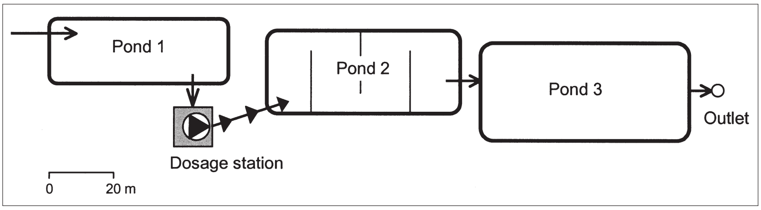

Figure 1. *The fellingsdam wastewater treatment plant at Orrviken.* (From Hanæus et al. unpublished). Ponds 1–3 are in the following figures and tables labeled D1–D3.

Palmer (1980) suggested that examination of the pond effluent for its algal flora may be a useful tool for operators of sewage stabilization ponds. Genera suggested as indicators in sewage oxidation ponds were *Chlamydomonas*, *Chlorella*, and *Scenedesmus*. If the effluent contains principally *Chlorella*, the pond is assumed to be working at or over its capacity; if it contains a mixed flora, the pond can handle a heavier load (Palmer 1980).

Objective

The objective of this study was to investigate the presence of microalgae in a full scale subarctic wastewater stabilization pond system over the productive season.

The investigated plant, situated in the mid Sweden region, was of the fellingsdam type, using chemical precipitation as a complement to the pond function. However, during the investigated period the ponds were mainly operated without chemical precipitation.

Method

The investigated plant and sampling periods

This investigation was performed at the wastewater treatment plant of Orrviken 17 km south of Östersund in the mid Sweden region. The treatment plant is of fellingsdam type in which wastewater stabilization pond technology is combined with chemical precipitation (in-pond precipitation, Hanæus 1991, Hanæus et al. unpublished). A principal overview of the pond system is given in figure 1 (the ponds are from here called D1 to D3). An experiment of seasonal operation of the fellingsdams was performed during the summer period 2001 and 2002, in which the chemical precipitation was interrupted from 5 July–20 September 2001 and from 20 May–14 September 2002 (Hanæus et al. unpublished). The microalgae sampling in this investigation started during the second period of interruption, and was performed from 3 July to 29 October 2002, and from 5 May to 29 June 2003. The ponds were ice covered from 18 October 2002 to the last week of April 2003. The differences in investigation period for D1, D2 and D3 are due to selection of samples for evaluation.

The characteristics of the Orrviken treatment plant is shown in table 1.

The temperature and photosynthetically active radiation (PAR) in Orrviken from May 2002 to June 2003 is shown in figure 2. For comparison 1 µE/m^2*s (PAR) equals 1 µmol/m^2*s (PAR), which may be converted to W/m^2 (PAR) with a factor of 1/4.6 when the light source is daylight (LI-COR, inc. 1979).

The total and phosphate phosphorus (Tot-P and PO_4-P) and organic material (as COD_{Cr}) of the influent and effluent were measured by Hanæus et al. (unpublished) and are shown in figure 5 to 8 for the period June to August/September 2002. The conversion of organic material from COD_{Cr} to TOC (mg C/l) was calculated with a conversion factor derived from Crites and

Table 1. *Characterization of the Orrviken treatment plant* (from Hanæus et al. unpublished).

Persons connected:	310 p.e. (person equivalents)
Average flowrate (1997–2001):	240 m^3/d
Number of ponds:	3
Total pond volume:	8,300 m^3
Precipitant:	AVR (8 % Al, 0.8 % Fe)
Average influent organic material (1995–99):	128 mg/l as COD_{Cr}
Average influent total phosphorus, Tot-P (1995–99):	3.22 mg P/l
Average effluent organic material (1995–99):	29 mg/l as COD_{Cr}
Average effluent total phosphorus, Tot-P (1995–99):	0.16 mg P/l

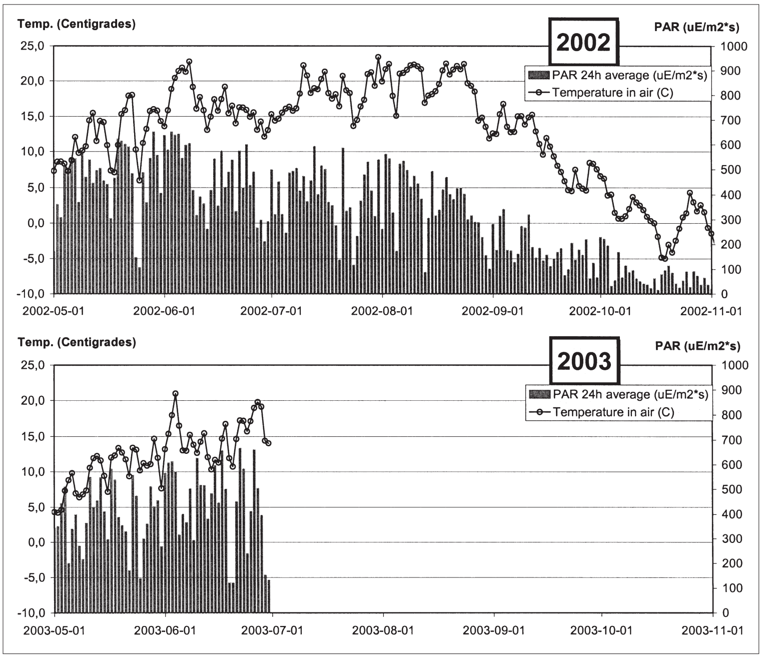

Figure 2. *Temperature and photosynthetically active radiation (PAR) in Orrviken 2002–2003.*

Tchoubanoglous (1998), where COD/TOC is theoretically 2.7, if the organic compound is $C_5H_7NO_2$. Hanæus et al. (unpublished) also measured influent ammonia (NH_4-N) which in 2002 varied between 10.5 mg/l and 24.0 mg/l. The mean flow rates were approximately 135 m^3/d in 2002 and 115 m^3/d in 2003, i.e. two dry years compared to the average.

During the period 20 May to 16 June 2002, the second pond (D2) was desludged, and therefore out of operation. During this period the influent wastewater was pumped from the dosage building directly to the third pond. The first microalgae samples were collected almost 3 weeks after D2 was in operation again.

Sampling and analysis

Microalgae samples were collected close to the outlets of the three ponds approximately 1.5 m from the wall, with a 1 litre beaker attached to a stick and submerged upside down to the sample depth of 10–20 cm below the surface, where it was turned and filled. The samples were conserved with Lugols solution (Tikkanen & Willen, 1992). Samples stored longer than a year were re-conserved.

Algal populations were described and quantified using a Fuchs-Rosentahl cell counting chamber (depth 0.200 mm). The volume of the microalgae was estimated with recommended volume formulas from Tikkanen & Willen (1992) och Olrik et al (1998). The density of the microalgae was assumed to be 1 g/ml.

For the estimation of nutrient content in the microalgae the standard approximation of microalgae composition, $C_{106}H_{181}O_{45}N_{16}P$ (Oswald 1988 a), was used and the following conversion factors from Olrik et al. (1998): Cyanophytes C=Bx0.22, dinoflagellates C=Bx0.13, Diatoms C=Bx0.11, Chlorophytes C=Bx0.16, Other C=Bx0.11, where C is phytoplankton carbon in µg/l, and B is phytoplankton wet biomass in µg/l.

Temperature and PAR were measured every 10 seconds, and saved in a data logger as 10 minute average values. In figure 2 the data has been further aggregated to 24 hour averages.

Algae classification

In this paper a botanical classification of the microalgae from Tikkanen and Willén (1992) is used. However, in the wastewater treatment context a practical classification based on significance to sanitary scientists and engineers is sometimes used rather than the alga's evolutionary relationship as botanists would normally classify them (Palmer and Ingram 1955 in Palmer 1980). In such a classification the algae are divided into pigmented flagellates, blue-green algae, green algae, and diatoms. Only a few wastewater related forms of brown, red, and yellow-green algae do not fit into these four groups. It must be remembered that there is no evident choice of what type of classification to use for microalgae. Curtis (1983) summarized this discussion by pointing out that the species definition works well for animals, but not as

Table 2. *Conversion table for algae present in this investigation, from the botanical classification by Tikkanen&Willén (1992) to the wastewater engineering classification according to Palmer (1980).*

Species, Genera, etc.	Abbrev. used in table 3.	Classification – Tikkanen&Willén (1992)	Classification – Palmer (1980)
Achroonema leutum	Achro	Cyanophyceae – Oscillatoriales	blue-green
Chlamydomonas spp.	Chla	Chlorophyceae – Volvocales	flagellate
Chlorella spp.	Chlo	Chlorophyceae – Chlorococcales	green
Chrysochromulina parva	Chrys	Chrysophyceae – Pedinellales	flagellate
Coelastrum microporum	Coel	Chlorophyceae – Chlorococcales	green
Cryptomonas spp.	Crypt	Cryptophyceae – Cryptomonadales	flagellate
Dacthylosphaerium sp.	Dact	Chlorophyceae – Chlorococcales	green
Euglena sp.	Eugl	Euglenophyta – Euglenales	flagellate
Fragilaria virescens	Frag	Diatomophyceae – Pennales	diatom
Golenkinia radiata	Gole	Chlorophyceae – Chlorococcales	green
Gymnodinium lantzschii	Gymn	Dinophyceae – Peridinales	flagellate
Koliella spp.	Kol	Chlorophyceae – Ulotrichales	green
Micractinium pusillum	Micr	Chlorophyceae – Chlorococcales	green
Peridinales *sp.*	Peri	Dinophyceae – Peridinales	flagellate
Planktothotrix sp.	Plank	Cyanophyceae – Oscillatoriales	blue-green
Scenedesmus spp.	Scene	Chlorophyceae – Chlorococcales	green
Selenastrum capricornutum	Selen	Chlorophyceae – Chlorococcales	green
Stichococcus sp.	Stich	Chlorophyceae – Ulotrichales	green

well for all types of plants and microorganisms, and microbiologists are "more apt to consider species as a category of convenience, existing rather in the human mind than in the natural world". Graham and Wilcox (2000) exemplify this by presenting three major species concept approaches (the biological, the morphological, and the phylogenetic), and at the same time point out that an increasing number of algal studies are employing molecular methods to define algal species. However this is expensive and not possible for e.g. fossils (Graham and Wilcox 2000). The relation between the Tikkanen and Willén (1992) and the Palmer (1980) classification is given in table 2, where also results from specific samples are given with genera or species resolution (table 3).

Results

Algal composition

In the primary pond D1 during 2002 (figure 3) there was a dominance of Cryptophytes (*Cryptomonas*) in July and late August, interrupted by a total dominance of green algae (Chlorophytes, mainly *Chlorella* followed by *Chlamydomonas*) in August, and another *Chlorella* bloom in September. During spring and early summer 2003 (figure 4) the green algae *Chlamydomonas* dominated, accompanied by *Chlorella.* Other types with some significance in D1 during the sampling period 2002–03 were the Dinoflagellates *Gymnodinium lantzschii* and another Peridinales type (possibly *Massartia vorticella*), an Euglenophyte (*Euglena sp.*), and the green algae *Scenedesmus sp.*

In pond D2 during 2002 (figure 3) the dominance of Cryptophytes (*Cryptomonas*) lasted longer than in D1, including not only July but also August. In September *Cryptomonas* were slowly replaced by Chlorophytes (at first *Micractinium*, then mainly *Chlamydomonas* and to a lesser degree *Chlorella*). The samples from 2003 (figure 4) in D2 were dominated in May by Chlorophytes (mainly *Chlamydomonas* followed by *Chlorella*), and in June dominated by Cryptophytes (*Cryptomonas*). Other types present with some significance were the Dinoflagellate *Gymnodinium lantzschii*, an Euglenophyte (*Euglena sp.*), and the Haptophyte *Chrysochromulina parva.*

Pond D3 during 2002 (figure 3) followed the same pattern as D2 with a dominance of Cryptophytes (*Cryptomonas*) in July and August, replaced by Chlorophytes during September (at first *Chlorella*, then mainly *Chlamydomonas* and to a lesser degree *Chlorella*, *Koliella* and *Coelastrum*, the two latter in the end of September as common as *Chlamydomonas*). In mid October the Chlorophytes peaked (with a *Coelastrum* dominance accompanied by *Koliella* and *Chlamydomonas*). After the ice cover formation (the 18 October) Cryptophytes (*Cryptomonas*) increased again. The samples from 2003 (figure 4) in D3 was dominated in May by green algae (*Chlamydomonas* and *Chlorella*), in early June by Cryptophytes (*Cryptomonas*), and in late June again by green algae (*Coelastrum*). In early June there was also a significance of Cyanophytes (*Planktothrix*). Other types

Table 3. *Genera and species found in this investigation.* Abbreviations are explained in table 2.

Date	Taxonomic group	D1 µg/l	D2 µg/l	D3 µg/l
2002				
3 July	Crypt	440,000	–	–
4 July	Chla			2,400
	Chlo			400
	Crypt	750,000[+]	780,000	132,000
	Gymn	3,500		
9 July	Chlo			50
	Chrys		50	
	Crypt	230,000	305,000	142,000
	Gymn	p	800	
22 July	Chla			3,900
	Crypt	200,000[+]	816,000	670,000
	Gymn			7,000
	Scene	950[+]		
25 July	Chlo	30	–	–
	Chrys	30	–	–
	Crypt	69,000	–	–
30 July	Chlo	5,250	35	
	Chrys		140	
	Crypt		364,000	175,000
	Frag			250
	Gymn			1,400
	Scene	150		
	Stich	15		
5 Aug	Chla	750		
	Chlo	28,500	300	
	Chrys	60	30	50
	Crypt		93,000	97,000
6 Aug	Chlo	–	600	50
	Crypt	–	113,000	279,000
	Scene	–		200
7 Aug	Chla	5,700		700
	Chlo	31,600	650	20
	Chrys	50		
	Crypt		710,000	241,000
	Gymn		1,400	
	Scene	200		
	Stich	5		
8 Aug	Chlo	–	930	
	Chrys	–		60
	Crypt	–	1,049,000	701,000
	Gymn	–	2,800	8,500
9 Aug	Chlo	–	200	10
	Chrys	–	100	30
	Crypt	–	379,000	783,000
	Gymn	–		14,000
	Scene	–		100
10 Aug	Chla	–	1,300	
	Chlo	–	800	
	Crypt	–	95,000	1,029,000
	Gymn	–		12,700
12 Aug	Chlo	24,600	100	
	Crypy		356,000	508,200
	Frag			300
	Gymn		2,800	4,200
	Scene		200	
	Stich	25		
21 Aug	Chlo	1,000	100	
	Chrys	60	100	1,000
	Crypt	60,000	34,400	119,500
	Stich	10		
26 Aug	Chla	8,800	650	
	Chlo	20		
	Chrys	20	300	
	Crypt	140,000	117,000	18,400
2 Sept	Chlo	–	–	500
	Chrys	–	–	50
	Crypt	–	–	10,900
	Frag	–	–	250
5 Sept	Chla	–		100
	Crypt	–	5,100	5,600
9 Sept	Chlo	–	–	300
	Coel	–	–	300
	Crypt	–	–	16,900
18 Sept	Chla	–		2,000
	Chlo	–		500
	Coel	–		300
	Crypt	–	11,200	8,600
	Eugl	–	1,600	
	Kol	–		300
	Micr	–	7,300	
26 Sept	Chla	–	–	9,000
	Chlo	–	–	200
	Coel	–	–	13,800
	Crypt	–	–	7,900
	Kol	–	–	9,300
7 Oct	Chla	–	77,500	7,000
	Coel	–		37,400
	Crypt	–	2,000	3,300
	Eugl	–	800	
	Gymn	–		400
	Kol	–	50	8,800
	Micr	–	4,800	
14 Oct	Chla	–	–	13,500
	Coel	–	–	12,900
	Crypt	–	–	3,000
	Dact	–	–	150
	Eugl	–	–	3,300
	Kol	–	–	6,700
21 Oct	Chla	–	–	12,400
	Chlo	–	–	200
	Coel	–	–	9,100
	Crypt	–	–	44,500
	Kol	–	–	5,500
29 Oct	Chla	–	–	1,400
	Coel	–	–	1,400
	Crypt	–	–	9,100
	Kol	–	–	1,000
2003				
5 May	Chla	900	200	7,600
	Chlo	900		
	Gymn			200
	Micr		150	
	Peri	1,000		
22 May	Achro			p
	Chla	138,000	20,500	350
	Chlo	7,400	4,300	2,000
	Chrys	p	900	
	Crypt	1,600		100
	Scene			p
	Selen		10	30
	Stich			p
10 June	Chla	297,000		
	Chlo	7,600		
	Chrys	250		
	Coel			9,200
	Crypt		36,300	28,100
	Plankt			2,700
27 June	Chla	45,500	650	
	Chlo	25,900		
	Coel			131,400
	Crypt	8,200	20,700	3,300
	Gole	p		
	Gymn	200		
	Selen	p		
	Chrys	p		

[+] Due to aggregation of the algae this figure is a low estimate. p = present, but not within the counted field. – = no sample.

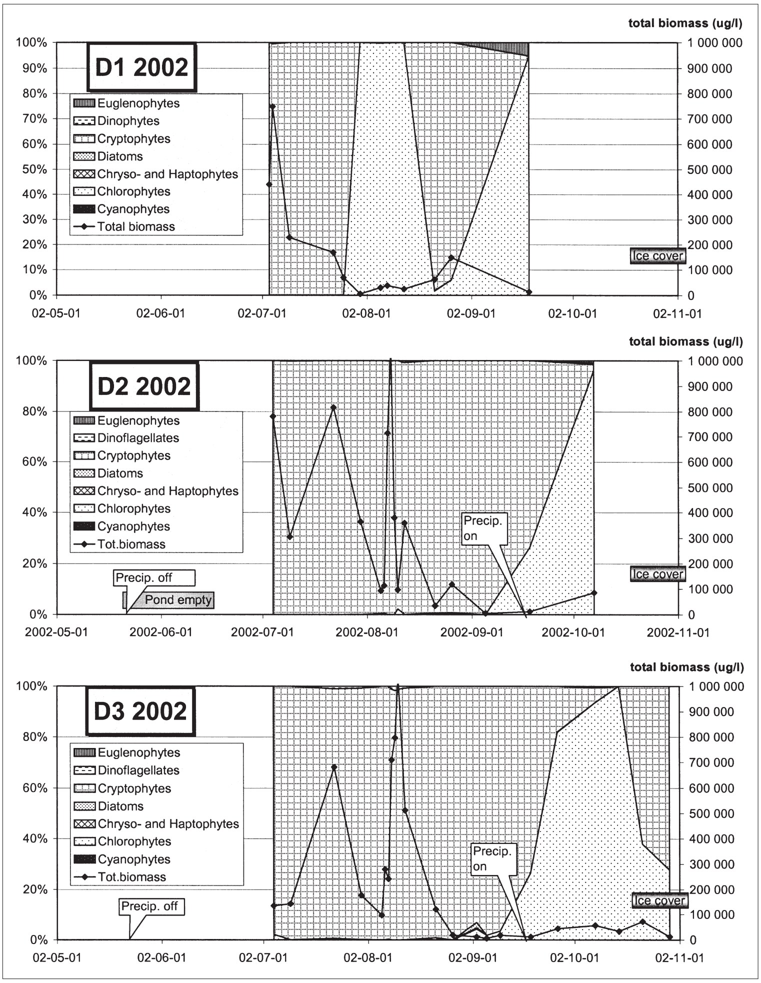

Figure 3. *Algal distribution in wastewater ponds D1, D2, and D3, year 2002.*

present with some significance in D3 were the Dinoflagellate *Gymnodinium lantzschii*, and the Haptophyte *Chrysochromulina parva.*

The abundance of microalgae was highest during July and the first half of August 2002, with peaks up above 500,000 µg/l, and lower during late August and September (figure 3). In D3, samples collected in October showed a low biomass. However, the values were higher than in September. In spring and early summer 2003 the biomass was fairly high in D1 but low in D2 and D3, though rising in the last collected sample in June in D3 (figure 4).

Comparison of algal biomass with influent and effluent data

In the primary pond D1 the estimated phosphorus content in the microalgae was very low compared to the total phosphorus (Tot-P) in the raw wastewater (figure 5).

In the sampling point for microalgae in D3, close to the effluent outlet, figure 6 shows that during some periods microalgae was the reason for a significant part of the total phosphorus leaving the treatment system. In early July the microalgae contributed to about one third of total phosphorus content in the treated wastewater. At one sampling occasion in late July there was a microalgal peak that did not coincide with the wastewater sampling program, and thus was not identified in the effluent total phosphorus. That also occurred in early August. When sampling of both microalgae and wastewater was performed on the same day it could be seen that microalgae may contain about two thirds of the total phosphorus content in the wastewater. From late August the microalgal phosphorus had a very small contribution to the high total phosphorus concentration existing at that time in the effluent.

For organic matter the same pattern as for phosphorus was found. In the pond D1 the estimated algae car-

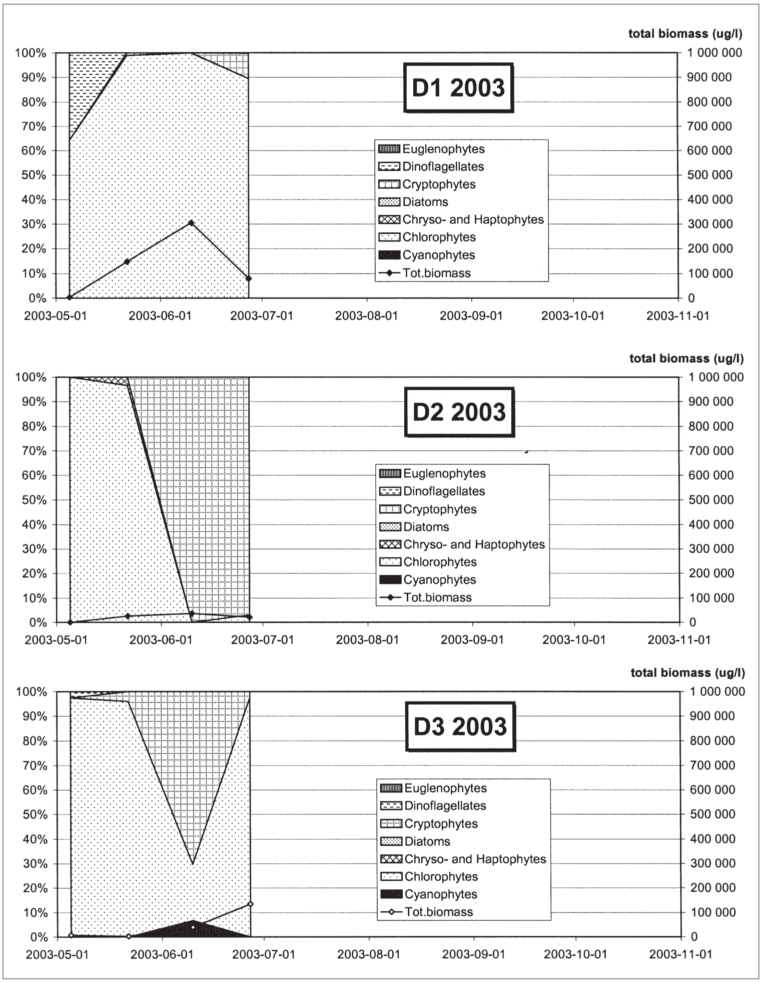

Figure 4. *Algal distribution in wastewater ponds D1, D2, and*

bon was generally low compared to the total carbon in the wastewater estimated from the concentration of COD_{Cr} (figure 7). In the pond D3 it was shown that algal biomass may be the main cause of organic material in the effluent during algal bloom. However, the two microalgal peaks were not registered in the effluent sampling schedule of organic matter content.

Discussion

Species and genera development

Palmer (1980) refers to a large survey of sewage pond microalgae in the USA, covering 74 ponds in 18 states: 125 types of microalgae were recorded, approximately 50 % green algae (excluding *Chlamydomonas*, see table 2), 25 percent pigmented flagellates (including *Cryptomonas*, *Chlamydomonas*, and *Euglena*, see table 2), 15 % blue-green algae (Cyanophyta), and 10 % diatoms. The Orrviken fellingsdam in this investigation was also dominated by pigmented flagellates (*Cryptomonas* and *Chlamydomonas*) and green algae (*Chlorella* and *Coelastrum*). Blue-greens and diatoms, however, were rare. Holmgren (1983) made an observation of the generally low competitiveness of blue-green algae (Cyanophyta) in subarctic regions, suggesting this might be caused by blue-greens being more sensitive to photoinhibition due to relatively low temperatures in combination with abundance of light during the summer period. However, this suggestion has not been confirmed by extensive studies of current literature (Falk et al. 1996). Palmer (1980) also produced a ranking list of the 25 most common sewage pond algae. Many of these were common also in Orrviken: *Chlorella* (ranked by Palmer, 1980, as most common), *Chlamydomonas* (number five), and *Cryptomonas* (number 18). Types with a high ranking by Palmer (1980), but present in the Orrviken fellingsdams with a low abundance were:

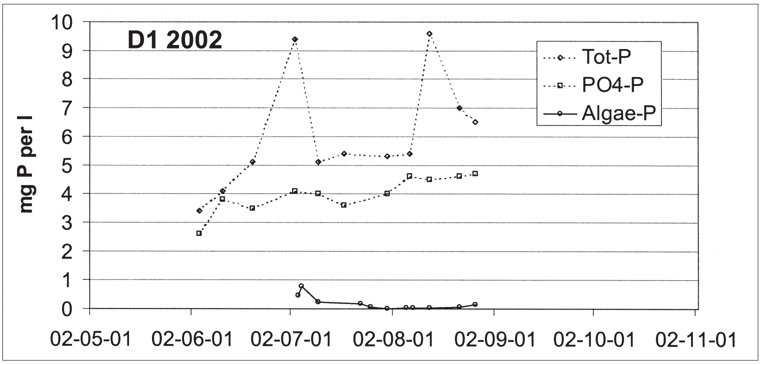

Figure 5. *Estimated microalgal phosphorus contribution to total phosphorus content in the wastewater in D1 2002.*

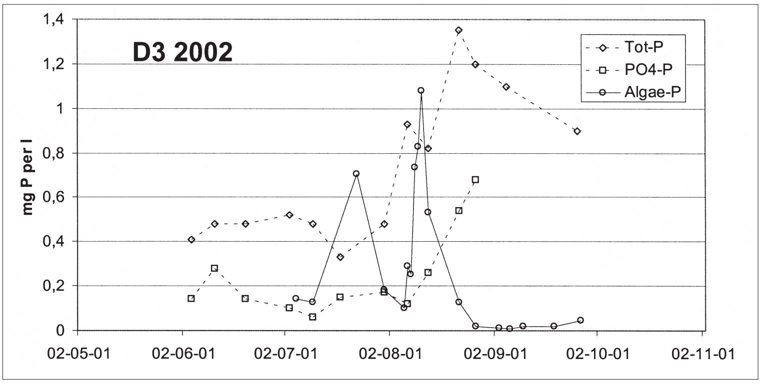

Figure 6. *Estimated microalgal phosphorus contribution to total phosphorus content in the wastewater in D3 2002.* Note the different scale on the Y-axis compared to figure 5.

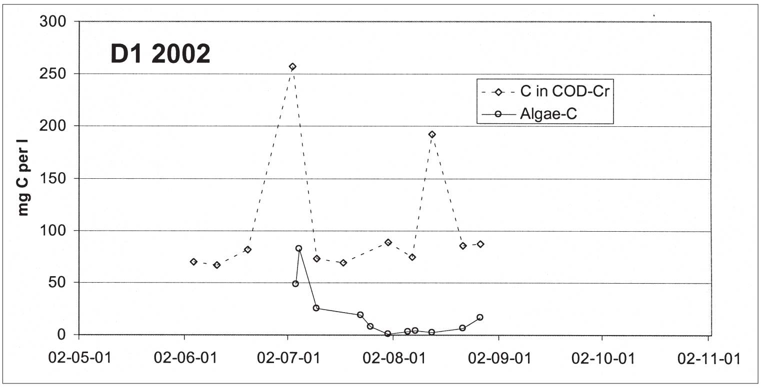

Figure 7. *Estimated organic matter content in the algae compared to estimated total organic content in the wastewater in D1 2002.*

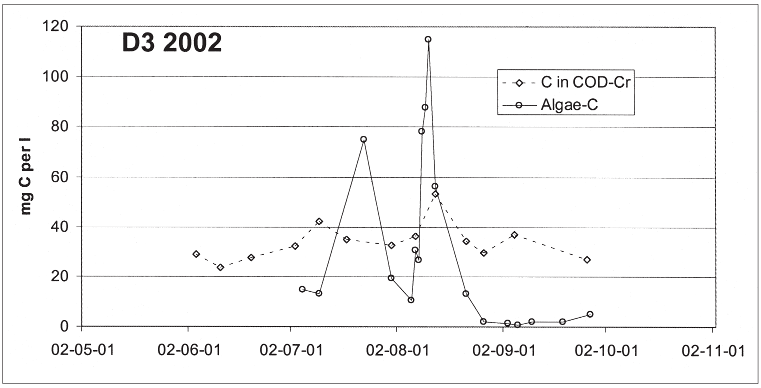

Figure 8. *Estimated organic matter content in the algae compared to estimated total organic content in the wastewater in D3 2002.* Note the different scale on the Y-axis compared to figure 7.

Scenedesmus (ranked number three), *Euglena* (number four), *Micractinium* (number seven), and *Golenkinia* (number eight).

In pond D1 *Chlorella* played a more important role than in the following ponds, which was not surprising since *Chlorella* is known to often dominate under conditions of abundant nutrients. *Chlorella* is also known to be more tolerant to ammonia inhibition than many other species (Pearson et al. 1987), which may explain the replacement in pond D1 in late July of *Cryptomonas* with mainly *Chlorella*. Ammonia concentrations measured by Hanæus et al. (unpublished) in 2002 at Orrviken were, however, lower than what is suggested to severely inhibit algal growth (Pearson et al. 1987).

The development over the season in pond D2 and D3 suggests a pattern of summer dominance by *Cryptomonas*, and spring and autumn dominance by green algae. The only exception to this pattern is the last early summer sample in D3 2003, where the green algae *Coelastrum* takes dominance. The increase in D3 of *Cryptomonas* in late October 2002 was during ice development, when the ice was still transparent and yet not covered with snow. It has been suggested that motility is an advantage in stabilization ponds by moving up towards the surface shading out non-motile species below (Mara and Pearson 1986). This may be an important factor in non-turbulent water under ice, and here the non-motile *Coelastrum* decreased and motile *Cryptomonas* and *Chlamydomonas* increased. This may also be exemplified in pond D2 where *Cryptomonas* decreased in early September 2002, and instead a non-motile green algae (*Micractinium*) could increase and probably dominate for a short period, just to again be replaced by motile flagellates (*Cryptomonas* and *Euglena*) in the mid September sample.

Algal biomass

The algal standing crop in efficiently operating facultative ponds is frequently in the range of 1000–3000 µg chlorophyll *a* per litre of pond water, according to Mara and Pearson (1986). Assuming a chlorophyll *a* to biomass ratio of 0.2 % wet weight (Jørgensen et al. 1991), this corresponds to 500,000–1,500,000 µg algae biomass per litre. Those levels were reached during the summer in the ponds, but more often in ponds D2 and D3 than in the primary pond D1. This observation suggests some inhibiting process occurring in D1, possibly ammonia. From mid August and in the spring period, however, the microalgae biomasses were considerably lower in all ponds. In warmer climate, for comparison, and for another type of wastewater ponds (the raceway or HRAP type) Oswald (1988 a) suggested a minimum of 125 Langleys/day, which corresponds to approximately 270 uE/m^2*s of photosynthetically active radiation (PAR), to be a lower limit for the use of this type of algae intensive wastewater treatment. The daily average of PAR insolation in Orrviken decreased below that level more often in early September (figure 2), which may be the reason for lower microalgae biomass during September and October. Oswald (1988 b) suggested 10°C as a lower temperature limit. In Orrviken the air temperature was above 10°C until mid September 2002, and from early May 2003. However, in a subarctic fertilizing experiment by Holmgren (1984), relevant in this context for the high nutrient levels in the fertilized lakes, temperature did not correlate at all with the biomass.

In D2 and D3 there was a *Cryptomonas* peak in early August 2002 sharper than other periods. This peak was identified due to more frequent sampling, which suggests this to be the general pattern also for the strongly fluctuating periods before and after the sharp peak, i.e. for the period early July to mid August. Samples collected during this period might represent random positions in similar peaks. From mid August the risk of this was lower, since all samples had considerably lower biomass.

The samples were collected from 10–20 cm below the pond surface for practical reasons. Pearson et al. (1987) concluded in their investigation of a facultative pond in Portugal that most of the photosynthetic activity occurred in the top 20 cm layer, even though the maximum algal concentrations occurred between 30 and 40 cm below the surface. This probably means that there is an overestimation of flagellated motile algae able to move up to the surface layer more effectively than non-motile species in our investigation. Consequently, periods when non-motile genera dominated may be underestimated from a biomass point of view, since the highest densities may have been present 10–20 cm deeper than where the samples were taken. This is illustrated in pond D1 30 July 2002 where non-motile green algae dominated in the sample, but the total biomass was lower than in adjacent samples.

The estimations of the microalgal part of total phosphorus and carbon in pond D1 during 2002 (figure 5 and figure 7) show as expected that the contribution was not very high. The influent concentration of phosphorus was high and carbon was expected to be most present as undegraded dead organic matter and bacteria. The estimations in pond D3 during 2002 (figure 6 and figure 8) show another pattern. During some periods microalgae phosphorus was the dominating part of the total phosphorus. Phosphorus in microalgae may of course not exceed total phosphorus as is shown in figure 6, and this may be due to phosphorus content in the microalgae diverging from the one used in the calculations, or that the total phosphorus measures were real effluent values while the microalgae were collected in the pond possibly shallower than the effluent outlet. More likely may be that the sampling dates of microalgae and wastewater effluent often were not the same, and other microalgae peaks as the one visible around 22 July and 5–12 August may have occurred. The pattern is the same for carbon in figure 8.

The D3 results emphasize the fact that microalgae may contribute significantly to the effluent phosphorus and organic matter. There is debate about whether discharge of microalgal phosphorus and organic material is as hazardous as discharge of other types of phosphorus and organic material. Mara and Pearson (1998) characterize facultative ponds as converting "sewage BOD" to "algal BOD", and that microalgae in the stabilization pond effluents "readily disperse and are consumed by zooplankton in receiving waters, so they have little chance to exert their oxygen demand, and during daylight hours they of course *produce* oxygen". Mara and Pearson (1998) also point out that this view has been accepted in the EU: "In the member States of the European Union WSP effluents have to meet the same BOD and COD requirements as other effluents (not above 25 and 125 mg/l) but with one very important difference: *filtered* samples are used to determine the BOD and COD, which are therefore the residual non-algal values (Council of the European Communities, 1991), although of course filtration removes non-algal solids as well – but in WSP effluent the algae comprise most (>80 %) of the suspended solids. Furthermore in the EU, pond effluents can contain up to 150 mg SS per litre, whereas effluent from other treatment processes can contain only 35 mg SS/l. This recognises the distinctions between algal and sewage BOD (and COD) and algal and sewage SS." Mara and Pearson (1998) urge other countries not within the EU to take into account the inherent difference between algal and sewage BOD, COD and SS, and allow filtered samples to replace unfiltered BOD.

Though recognizing the important difference between algal COD and sewage COD, the results from this investigation suggest a precautionary implementation in subarctic climates. The D3 results do not fully support the basis for the special use of filtered samples in wastewater stabilization ponds effluent, namely that microalgae dominate the effluent COD. In this investigation microalgae only dominated the effluent COD for a period of 3–4 weeks of 12 weeks investigated (figure 8). However, to achieve more reliable results the microalgae should be examined in the actual effluent samples and not in the pond close to the outlet as in this case, since the position of the outlet might have impact.

The low microalgae contribution to the organic matter in September may partly be explained by high wastewater flow in the second half of September causing shorter retention times, and not only by decreasing light and temperature as suggested above. A longer retention time might have resulted in higher algal production. Another consideration on the regional scale is that in some subarctic areas a trend of decreasing nutrient status (oligotrophication) from an already low level is present in the recipients, rather than too much nutrients causing eutrophication (Stockner et al. 2000). Discharge of nutrients in the form of microalgae may then be an interesting nutrient management option.

Pond performance and algae as process indicators

Pond D1 was totally dominated by *Chlorella* between 30 July and 12 August, which indicate full capacity or overload according to Palmer (1980). As mentioned the *Chlorella* biomass at this time may have been underestimated due to the use of a too shallow sampling depth. In pond D2 there was as expected no *Chlorella* signs of overload. The same situation existed in pond D3, with a possible exception for a small *Chlorella* appearance in early September 2002, which also may be underestimated because of shallow sampling. However, still the flagellate *Cryptomonas* dominated this period. The dominance by *Cryptomonas* in ponds D2 and D3 during the summer period represents the clear-"brownish tint"-type of the four main types of conditions in facultative or maturation ponds suggested by Palmer (1980), and further described below (under Future research).

An impact on the microalgae from the interruption of the chemical precipitation may be observed by the dominance shift from *Cryptomonas* to green algae in both D2 and D3 in connection to the restart of dosing precipitant at September 15. However, it is more likely that the shift was not connected to the precipitant dosage since one would expect the microalgae biomass to decrease when phosphorus was precipitated to a higher extent, which was not the case. On the contrary, microalgae biomass increased during late September-early October. In D2 this observation is, however, supported by only two samples. In 2003 the higher microalgae biomass in D1 than D2 and D3 may be interpreted as a result of inhibition of microalgae in D2 and D3 due to the phosphorus precipitation, as the chemical is added into D2.

Conclusions

The investigated subarctic seasonal stabilization pond/fellingsdam treatment plant was dominated by the genera *Cryptomonas* during the summer period in the second and third pond. In autumn green algae dominated for a period but were in October under a transparent ice layer once again replaced to large extent by *Cryptomonas*. During spring conditions green algae dominated. During the summer period the primary pond was also investigated and *Cryptomonas* and green algae alternated dominance.

Approximately for three to four weeks of the twelve summer and early autumn weeks of the investigation, microalgae were dominant in the effluent. This does not support the resoning behind the European Union recommendation of using filtered samples for effluent BOD, COD and SS, which is that the effluent consists of mainly microalgal biomass. A further investigation of this contradiction is suggested together with a suggestion of developing regional guidelines for the use of microalgae information in management of stabilization ponds and fellingsdams.

Future research

The above discussed differences between sewage and algal BOD and COD in the effluent, and its consequences for subarctic wastewater pond management and legislation is an important area for future research.

The use of microalgae as process indicators in wastewater plant operation is another interesting area. Palmer (1980) introduced a general guideline table for further development and local adaptation, where seven types of conditions for wastewater stabilization ponds are identified, three for primary ponds and four for secondary and subsequent ponds. The conditions are characterized by observation of colour, odour and other easily made observations together with identification of dominating microalgae, which are then correlated to mid afternoon pH, dissolved oxygen, inlet and outlet BOD, and total nitrogen reduction. Of course these factors all vary in actual ponds, but Palmer's table is a basic guideline.

The regional level might be the appropriate level to further develop these type of guidelines to be useful for more efficient wastewater stabilization pond management. In such an investigation regional knowledge should be established of the relation between easily accessible surface samples, and more extensive full water column samples from different sections of the ponds.

References

Council of the European Communities, 1991. Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC). *Official Journal of the European Communities*, L135/40 (30 May). (also available at http://europa.eu.int/comm/environment/water/water-urbanwaste/amendment.html)

Crites, R., and G. Tchobangolous, 1998. *Small and decentralized wastewater management systems*. McGraw-Hill.

Curtis, H., 1983. *Biology*. Worth Publishers, New York.

Falk, S., Maxwell, D.P., Laudenbach, D.E., Huner, N.P.A., 1996. Photosynthetic adjustment to temperature. In Baker NR (ed.) Photosynthesis and the Environment, pp. 367–385, Kluwer Academic Publishers, The Netherlands. ISBN 0-7923-4316-6

Graham, L. E., and L. W. Wilcox, 2000. *Algae*. Prentice-Hall, Inc, Upper Saddle River, NJ., USA

Hanæus, J, 1991. Wastewater treatment by chemical precipitation in ponds. *Doctoral thesis 1991:95D, Luleå University of Technology*, Luleå, Sweden.

Holmgren, S., 1983. Phytoplankton biomass and algal composition in natural, fertilized and polluted subarctic lakes. *Acta Universitatis Upsaliensis 674, Faculty of Science*. Uppsala Universitet, Uppsala

Holmgren, S., 1984. Experimental lake fertilizations in the Kuokkel area, northern Sweden. Phytoplankton biomass and algal composition in natural and fertilized subarctic lakes. *Int. Revue ges. Hydrobiol* 69: 781–817.

Jørgensen, S. E., S. N. Nielsen, and L. A. Jørgensen, 1991. *Handbook of Ecological Parameters and Ecotoxicology*. Elsevier, Amsterdam.

LI-COR, inc., 1979. *Radiation measurement*. Lincoln, Nebraska, USA

Mara, D. D., and H. Pearson, 1986. Artificial freshwater environment: Waste stabilization ponds. Pages 177–206 in H.-J. Rehm and G. Reed, eds. *Biotechnology, Vol. 8*. Verlagsgesellschaft, Weinheim.

Mara, D., and H. Pearson, 1998. *Design manual for Waste stabilization ponds in Mediterranean Countries*. Lagoon Technology International Ltd., Leeds, England.

Olrik, K., P. Blomqvist, P. Brettum, G. Cronberg, and P. Eloranta, 1998. *Methods for Quantitative Assessment of Phytoplankton in Freshwaters, part 1*. Naturvårdsverket, rapport 4860, Stockholm.

Oswald, W. J., 1988a. Large-scale algal culture systems (engineering aspects). Pages 357–394 in M. A. Borowitzka and L. J. Borowitzka, eds. *Micro-algal Biotechnology*. Cambridge University Press, Cambridge.

Oswald, W. J., 1988b. The role of microalgae in liquid waste treatment and reclamation. Pages 255–282 in C. A. Lembi and J. R. Waaland, eds. *Algae and human affairs*. Cambridge University Press, Cambridge.

Palmer, C. M., 1980. *Algae and Water Pollution*. Castle House Publications Ltd.

Palmer, C. M., and W. M. Ingram, 1955. Suggested classification of algae and protzoa in sanitary science. *Sewage and Indust. Wastes* 27: 1183–1188.

Pearson, H., D.D. Mara, S.W. Mills, and D.J. Smallman, 1987. Factors determining algal populations in waste stabilization ponds and the influence on pond performance. *Water Science and Technology* 19: 131–140.

Stockner, J. G., E. Rydin, and P. Hyenstrand, 2000. Cultural Oligotrophication: Causes and Consequences for Fisheries Resources. *Fisheries* 25(5): 7–14.

Tchobanoglous, G., and F. L. Burton, 1991. *Wastewater engineering. Treatment, disposal and reuse*. McGraw-Hill, Inc.

Tikkanen, T., and T. Willén, 1992. *(Phytoplankton flora; in Swedish) Växtplanktonflora*. Naturvårdsverket, Stockholm.