



# The effectiveness of chemical-free water treatment system combining fibre filters, ultrasound, and UV for fish farming on algal control

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**Key words:** algae, Chem-free, ultrasound,  
fibre filters, UV, algae control

## Abstract

**Background and Purpose:** A chemical-free water treatment system consisting of fibre filters, ultrasound, and UV for fish farming was operated in a fishpond to study its effect on the control of algae compared with a non-treated fishpond.

**Materials and Methods:** Phytoplankton and phytobenthos were sampled in both ponds, at the same time Chlorophyll-a, nitrates (NO<sub>3</sub>), ortho-phosphates (PO<sub>4</sub>), pH, water temperature and saturation were monitored.

**Results and Conclusions:** The results showed that ultrasonication caused efficient sedimentation of planktonic algae. The number of determined algal species was lower in the treated pond. The proportion of green algae and Cyanobacteria was higher in the non-treated pond, and diatoms were the predominant group of algae in the treated pond. The species *Oedogonium sp.*, *Mougeotia sp.* and *Spirogyra sp.* were »tolerant« to ultrasonic irradiation. The successful control of algae using the chemical-free water treatment system suggests that the Chem-free water treatment system can be a practical method to control algal bloom in fish farms.

## INTRODUCTION

Aquaculture worldwide has been increasing in production at between 8–10% per annum for the last decade. The potential environmental impacts of aquaculture, e.g. increase of algal population, the dissolved oxygen depletion at the water-sediment interface, organic enrichment of sediments, the effect on bacterial density, biomass, community structure and their possible resistance have been reported in the literature (1, 2). Development of algae is one of the serious problems, which positively affect the fish during the day by O<sub>2</sub> production, while during the night algae consume oxygen and may even reduce the O<sub>2</sub> level below tolerable limits. Another factor is fluctuating pH value due to the diurnal cycles of CO<sub>2</sub> release, causing additional stress to fish. The food conversion ratio in recirculation trout farms is high, e.g. about 64% of P and 53% of N supplied by food are lost. A possible solution to reduce the water pollution by nutrients is diversion of recirculating water into a closed loop treatment system. Treatment of water in which fish are bred is also important due to limited water sources or water saving. Recirculation of water in a closed loop treatment system represents a sustainable method to reduce the environmental impact of aquaculture, especially for small-scale farmers (3, 4)

The CHEM-FREE project (a Co-operative Research Project (CRAFT) funded within the EU 6<sup>th</sup> Framework Programme, Horizontal Research Activities involving SMEs) aimed to develop a chemical free treatment consisting of three well known, water treatment devices: fibre filters, ultrasound and UV-C. The devices are expected to restrain suspended solids as well as dissolved nutrients, to counteract algae growth and care for disinfections. A similar treatment system was tested for four specific applications: swimming pools, reuse of treated wastewater for irrigation of crops, fish farming, and groundwater recharge (5). In this contribution the efficiency of the Chem-free treatment system for fish farming (especially the US device) on algal control is presented. The experiments were carried out at the field site (experiment) in Ajdovščina, Slovenia.

The CHEM-FREE project is in accordance with the Water Framework Directive (2000/60/EC) that aims to achieve sustainable water use, sustainable management and protection of freshwater resources.

## MATERIAL AND METHODS

### Short description of the system operation

The experiment was carried out in two fish ponds (5 m × 9 m × 0.8 m) of which one served as an experimental (Chem-free), and one as a reference (Reference) pond. In each pond 36 carps (*Cyprinus c. carpio* Linnaeus 1758) were added. From the Chem-free pond the water was pumped by a bypass and was treated first by the roughing filter (RF) (1.5m × 1.5m, h=1.1m, 0.5m gravel 4/8mm, 0.3m 8/16mm, 6/22mm 1:1) followed by two batteries of fibre-filters (FF) without pressure, and two UV-C devices (UV) running in parallel. Treated water flowed back to the Chem-free pond. An ultrasound (US- LG SONIC TANK) unit was installed in the Chem-free pond. Both ponds had constant aeration. The Reference pond did not have any treatment. In the case that the water conditions threatened the fish population, groundwater was added. Groundwater was also added in the Chem-free pond occasionally to compensate evaporation losses. The flow rate was approximately 4 m<sup>3</sup>/h. The system started operating at the beginning of May 2007.

### Sampling and identification of algae

Phytoplankton and phytobenthos were sampled every fourteen days from December 2007 until May 2008 in both ponds (12 times in each pond) (6). The samples of phytoplankton were taken with a plankton net, with mesh size 25 µm. For identification we followed standard identification monographs (7, 8, 9–12, 13). The algal taxa were identified directly from living material. The diatoms were examined after preparation according to (14). Light microscope Nikon Eclipse E400 was used to determine the taxa. The relative abundance of algal taxa (Table 1) was estimated by the numbers 1, 3 and 5 (1-single, 3-customary, 5-dominant) (15). We assessed abundance of diatom taxa from permanent slides (14) and

abundance of taxa from other algal groups from living material.

Chlorophyll-*a*, nitrates (NO<sub>3</sub>), orto-phosphates (PO<sub>4</sub>), pH, water temperature and saturation were monitored in both ponds at the same time that phytoplankton and phytobenthos were sampled (6). Chlorophyll-*a* was sampled in the water column (approximately 10 cm below the water surface).

## Multivariate statistical analysis

The relative abundance estimations and the environmental data (Table 2) were analysed by the canonical corresponding analysis (CCA), using the programme CANOCO for Windows 4.5 (16). We did not use any transformation of the environmental data, however after programme CANOCO reads in the environmental variables; it transforms them all to achieve their zero average and unit variance (16).

## RESULTS

### Species composition

Algal species compositions in the Chem-free and the Reference pond are presented in Table 1. In the plankton and benthos of both ponds, we determined altogether 49 different algal species of which 19 belonged to diatoms, 19 to Chlorophyceae, 6 to Cyanophyceae and 5 to Zygnematophyceae. In the Chem-free pond we determined altogether 32 algal species of which 15 species belonged to Bacillariophyceae, 11 to Chlorophyceae, 3 to Cyanophyceae and 3 to Zygnematophyceae. In the Reference pond we determined altogether 40 algal species of which 13 species belonged to Bacillariophyceae, 18 to Chlorophyceae, 6 to Cyanophyceae and 3 to Zygnematophyceae.

The number of determined species was lower in the Chem-free pond in relation to the Reference pond (Table 1). In the phytoplankton of the Chem-free pond we determined only seven different algal taxa, while in the phytoplankton of the Reference pond we determined 26 different algal taxa. In the phytobenthos the number of determined algal taxa in the Chem-free and Reference pond were more or less the same (32, 34, respectively). The difference in determined algal taxa between plankton and benthos of the Chem-free pond amounted to 78% and between plankton and benthos of the Reference pond 35%.

Algal compositions by classes in both ponds are presented in Figure 1. In the Chem-free pond the prevailing group of algae was Bacillariophyceae and in the Reference pond the prevailing groups of algae were Cyanophyceae and Chlorophyceae with numerous planktonic species (e.g. *Actinastrum* sp., *Dictiosphaerium pulchellum*, *Micractinium pusillum*). The number of determined algal taxa from the Zygnematophyceae group were the same in both ponds (3), although only species from the genera *Cosmarium* was present in the Chem-free and in the Reference pond.

TABLE 1

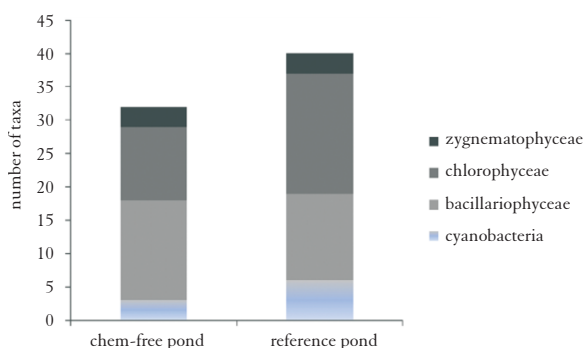
Algal species list in the Chem-free pond and in the Reference pond with acronyms of taxa used in the CCA analysis and with relative abundance of algal taxa (1-single, 3-customary, 5-dominant).

taxa	Chem-free pond		Reference pond	
	plankt.	bent.	plankt.	bent.
<b>CYANOPHYTA</b>				
<b>CYANOBACTERIA</b>				
<i>Aphanocapsa</i> sp.	.	.	1	1
<i>Heteroleibleinia</i> sp.				
<i>Leptolyngbya</i> sp.				
<i>Mycrocystis aeruginosa</i> (Kützing) Kützing	.	.	1	1
<i>Phormidium</i> sp.	.	.	1	1
<i>Pseudoanabaena</i> sp.	.	1	1	1
<b>HETEROKONTOPHYTA</b>				
<b>BACILLARIOPHYCEAE</b>				
<i>Achnanthes lanceolata</i> (Brébisson) Grunow	.	1	.	1
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki				
<i>Achnanthes</i> sp.				
<i>Cocconeis placentula</i> Ehrenberg	.	1	.	1
<i>Cyclotella meneghiniana</i> Kützing	.	.	1	1
<i>Cymbella</i> sp.	.	1	.	.
<i>Fragilaria capucina</i> Desmazières	.	1	.	1
<i>Fragilaria</i> sp.				
<i>Ulnaria ulna</i> (Nitzsch) P. Compère	.	1	.	.
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	.	.	1	.
<i>Gomphonema olivaceum</i> (Hornemann) Brébisson	.	.	1	.
<i>Gomphonema parvulum</i> (Kützing) Kützing				
<i>Navicula halophila</i> (Grunow) Cleve	.	1	.	.
<i>Navicula</i> spp.				
<i>Nitzschia acicularis</i> (Kützing) W.Smith	.	1	.	.
<i>Nitzschia amphibia</i> Grunow				
<i>Nitzschia dissipata</i> (Kützing) Grunow	1	1	.	.
<i>Nitzschia fonticola</i> Grunow	.	.	1	1
<i>Nitzschia palea</i> (Kützing) W.Smith	1	3	1	3
<b>CHLOROPHYTA</b>				
<b>CHLOROPHYCEAE</b>				
<i>Actinastrum</i> sp.	.	.	1	.
<i>Chlamydomonas</i> sp.				
<i>Coelastrum pseudomicoporum</i> Nägeli	.	1	.	1
<i>Dictiosphaerium pulchellum</i> Wood	.	.	5	5
<i>Kirchneriella lunaris</i> (Kirchner) Moebius	.	.	1	1.
<i>Koliela</i> sp.	.	.	1	.
<i>Lagerheimia</i> sp.	.	.	1	.
<i>Micractinium pusillum</i> Fresenius	.	.	1	1
<i>Monoraphidium contortum</i> (Thuret) Komárkova-Legnerová	.	.	.	1
<i>Oedogonium</i> sp.				
<i>Oocystis</i> sp.	1	5	.	1
	.	1	1	1

taxa		Chem-free pond		Reference pond	
		plankt.	bent.	plankt.	bent.
<i>Pediastrum boryanum</i> (Turpin) Meneghini	Ped_bor	1	3	1	3
<i>Scenedesmus abundans</i> (Kirchner) Chodat		.	1	1	1
<i>Scenedesmus obliquus</i> (Turpin) Kützing	Sc_eobl	1	5	1	3
<i>Scenedesmus opoliensis</i> P.Richter		.	.	1	1
<i>Scenedesmus quadricauda</i> (Turpin) Brébisson		.	1	1	1
<i>Scenedesmus</i> sp.		.	1	.	1
<i>Stigeoclonium</i> sp.		.	1	.	1
<i>Ulothrix</i> sp.		.	1	.	.
<b>ZYGNEMATOPHYCEAE</b>					
<i>Closteriopsis acicularis</i> (G. M. Smith) Belch		.	.	.	1
<i>Closterium</i> sp.		.	.	1	.
<i>Cosmarium</i> sp.		.	1	.	1
<i>Mougeotia</i> sp.	Mou_sp.	.	3	.	.
<i>Spirogyra</i> sp.	Spi_sp.	.	5	.	.
<b>No. of taxa</b>		<b>7</b>	<b>32</b>	<b>26</b>	<b>34</b>
			<b>32</b>		<b>40</b>

Dominant species (relative abundance =5) in the Chem-free pond were *Navicula* spp. (benthos), *Chlamydomonas* sp. (benthos), *Oedogonium* sp. (benthos), *Scenedesmus obliquus* (benthos) and *Spirogyra* sp. (benthos). Common species (relative abundance =3) in the Chem-free pond were *Leptolyngbya* sp. (benthos), *Fragilaria* sp. (benthos), *Nitzschia amphibia* (benthos), *Nitzschia palea* (benthos), *Pediastrum boryanum* (benthos) and *Mougeotia* sp. (benthos). In the plankton of the Chem-free pond all determined species were present with relative abundance 1 (single).

Dominant species (relative abundance =5) in the Reference pond were *Leptolyngbya* sp. (benthos), *Chlamydomonas* sp. (benthos) and *Dictiosphaerium pulchellum* (plankton, benthos). Common species (relative abundance=3) in the Reference pond were *Achnanthyidium minutissimum* (benthos), *Nitzschia palea* (benthos), *Pediastrum boryanum* (benthos) and *Scenedesmus obliquus* (benthos).



**Figure 1.** Algal composition by classes in both ponds.

## Chlorophyll-a

Measured values of Chlorophyll-*a* were much higher in the Reference pond compared to the Chem-free pond during the whole experiment (988.5 and 97.5 mg/m<sup>3</sup>, respectively), and especially high values of Chlorophyll-*a* were measured in the Reference pond in May 2008 (1254.9 mg/m<sup>3</sup>).

## Canonical corresponding analysis

CCA analysis was performed on six environmental variables (Table 2) among which three variables (US, saturation and temperature) were chosen by the forward selection method. These three variables statistically significantly ( $P \leq 0.05$ ) explain 33.29% of the variability of algal communities. The environmental variable, which was selected first and was the most explanatory, was the US (14.45%). The saturation explains 12.32% of the variance and the temperature variable 6.52%.

**TABLE 2**

Average values of environmental variables measured in the Chem-free and Reference pond during the experiment.

Variable	Chem free pond	Reference pond
Temperature (°C)	18.6	19.9
US device	1	0
PO <sub>4</sub> (mg/L)	0.67	1.23
Saturation (%)	102	106
NO <sub>3</sub> (mg/L)	0.54	0.34
pH	7.2	7.3

TABLE 3

Summary statistics of the canonical corresponding analysis (CCA).

	Axes				Total inertia
	1	2	3	4	
Eigenvalues	0.543	0.262	0.216	0.175	1.830
Species-environment correlations	0.992	0.906	0.954	0.968	
Cumulative percentage variance					
of species data	29.6	44.0	55.8	65.3	
of species-environment relation	39.6	58.8	74.6	87.3	
Sum of all canonical eigenvalues					1.369

The maximum eigenvalue is the value of the first canonical axis (0.543), which indicates a strong gradient in this direction (Table 3). The first axis statistically significantly explains 29.6% of the variance of the taxa matrix ( $P = 0.002$ ). The eigenvalues of the following canonical axes are lower, which implies a weaker gradient and a smaller percentage of variance explained by an individual axis. The first four axes together explain 65.3% of the total variance of algal data. The correlation coefficients between the first four axes of the taxa matrix and the environmental matrix are larger than 0.9; the correlation coefficient of the first axis amounts to 0.992, while the value of the correlation coefficient of the fourth axis is slightly lower (0.968) (Table 3).

The first CCA axis is in positive correlation with US ( $r = 0.96$ ),  $\text{NO}_3$  ( $r = 0.10$ ), T ( $r = 0.04$ ) and in negative correlation with  $\text{PO}_4$  ( $r = -0.72$ ), pH ( $r = -0.12$ ) and saturation ( $r = -0.11$ ). Only the correlation of the first axis with the US variable is stronger ( $r = 0.96$ ), while the correlations of other environmental variables with the first axis are weaker. The second axis explains less variance than the first and is in weaker correlation with the selected variables. A stronger positive correlation is with saturation ( $r = 0.79$ ). It is in positive correlation also with pH ( $r = 0.60$ ), T ( $r = 0.26$ ),  $\text{NO}_3$  ( $r = 0.16$ ) and US

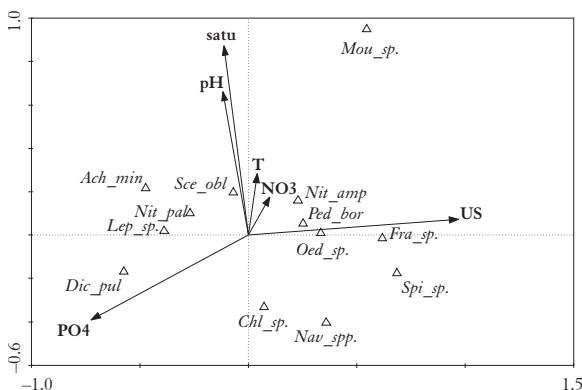
( $r = 0.07$ ). The second axis is in negative correlation with  $\text{PO}_4$  ( $r = -0.35$ ).

On Figure 2 only taxa, which at least once had relative abundance estimation 3 or 5, are shown. The CCA ordination positioned algal taxa typical of higher temperatures, lower  $\text{PO}_4$  values, and »tolerant« to US device (*Mougeotia* sp., *Nitzschia amphibia*, *Pediastrum boryanum*, *Fragilaria* sp. and *Oedogonium* sp.) in the upper right quadrant, whereas taxa more common present at higher pH values, higher saturation are located in the upper left quadrant (*Achnanthydium minutissimum*, *Scenedesmus obliquus*, *Nitzschia palea* and *Leptolyngbya* sp.). Taxa more common at lower pH values, lower saturation and »tolerant« to US are located in the lower right quadrant (*Spirogyra* sp., *Chlamydomonas* sp., *Navicula* spp.), and taxa typical of higher  $\text{PO}_4$  values and »not tolerant« to the US device are positioned in the lower left quadrant (*Dictiosphaerium pulchellum*).

## DISCUSSION

At the beginning of the system operation algal communities in both ponds stabilised after 1.5 month of system operation (17), although in the case of our experiment algal communities in both ponds started to stabilise in seven days from the beginning of the experiment. In the Chem-free pond (operating with US device) the determined number of species (32) was lower compared to the Reference pond (40) (Table 1). In the phytoplankton of the Chem-free pond the determined number of species was much lower (7) in relation to the determined number of species in the phyto-bentos of the Chem-free pond (32), phytoplankton of the Reference pond (26) and phyto-bentos of the Reference pond (34) (Table 1). The average value of Chlorophyll-*a* was much lower in the Chem-free pond (97,5 mg/m<sup>3</sup>) compared to the Reference pond (988,5 mg/m<sup>3</sup>) operating without the US device. In the Reference pond the measured values of Chlorophyll-*a* were lower in relation to the experiment conducted in 2007 (17). Most probably because of the differences in seasons (winter-spring; summer-autumn).

The water in the Chem-free pond was clear with almost no algae present in the water column (all determined species in the phytoplankton had relative abundance 1-single). We assume that the US device caused



**Figure 2.** Results of the Canonical Corresponding Analysis (CCA) carried out in CANOCO shown as biplot environmental variables and algal taxa. Taxa acronyms are listed in Table 1. Satu-saturation,  $\text{PO}_4$ -orto-phosphate,  $\text{NO}_3$ -nitrate. Only taxa which had at least once relative abundance estimation 3 or 5 are shown.

sedimentation of algae in the Chem-free pond (17, 18, 19). In the phytoplankton of the Reference pond the dominant algal species was *Dictiosphaerium pulchellum* (typical planktonic species) as reported also by Krivograd Klemenčič and Griessler Bulc (17), all other determined species in the plankton of the Reference pond were present with relative abundance 1 (single). In the phyto-benthos of the Chem-free pond in the spring of 2008 development of filamentous green algae (*Spirogyra* sp., *Mougeotia* sp., *Oedogonium* sp.) was present (Table 1), indicating that the US device was efficient in the removal of planktonic species but not as efficient in the removal of benthic species (17). According to Krivograd Klemenčič and Griessler Bulc (17) lack of ultrasonication caused major blooms of *Oedogonium* sp. This seems to indicate certain repression of filamentous algal growth (in this case *Oedogonium*) by the presence of ultrasonication. In the Chem-free pond during the experiment filaments of *Oedogonium* sp., *Spirogyra* sp. and *Mougeotia* sp. were short and firm attached to the bottom and the walls of the pond. The majority of the algae determined in the phytoplankton of the Chem-free pond were common benthos species (e.g. *Gomphonema parvulum*, *Nitzschia dissipata*, *N. palea*), which were more or less attached to the filaments of *Oedogonium* or chains of *Fragilaria*. The main effect of ultrasonication on cyanobacteria is sedimentation through the breakdown of gas vesicles (18, 19) in the case that light cannot reach down to the sediment and cyanobacteria are unable to photosynthesise (18, 19). This was not the case in our experiment where both ponds were shallow and algae were just transferred from planktonic to benthos phase – where they were accumulated. Ultrasound treated algae can re-grow if some light is available (19). In the Reference pond we determined potentially toxic cyanobacteria *Microcystis aeruginosa*, which did not occur in the Chem-free pond. The growth of *M. aeruginosa* can be repressed by ultrasonic radiation (20, 21). In the phytoplankton of the Reference pond many planktonic green algae were present, which were not present in the Chem-free pond (e.g. *Dictiosphaerium pulchellum*, *Lagerheimia* sp., *Micractinium pusillum*).

The results (Table 1) showed that species *Oedogonium* sp., *Mougeotia* sp. and *Spirogyra* sp. were not affected by ultrasonic irradiation meaning that they are US »tolerant« species. According to Krivograd Klemenčič and Griessler Bulc (17) *Dictiosphaerium pulchellum*, *Pediastrum boryanum* and *Scenedesmus obliquus* are US »tolerant« species. In the presented experiment *Dictiosphaerium pulchellum* was only present in the Reference pond (dominant in plankton and benthos) and was not a subject of US treatment. *Pediastrum boryanum* and *Scenedesmus obliquus* were present in both ponds; and were more or less equally present in the Chem-free pond with ultrasonication and in the Reference pond without ultrasonication. All determined taxa in this research (Table 1) are typical taxa of Slovenian surface waters (22, 23, 24).

In the Reference pond the proportion of green algae (among which the most common were planktonic species) and Cyanobacteria was higher compared to the

Chem-free pond. In the Chem-free pond diatoms were a predominant group of algae.

Canonical corresponding analysis explained 43.6% of the variability of algal data. CCA was performed on six environmental variables (Table 2) among which three variables statistically significantly ( $P \leq 0.05$ ) explain 33.29% of the variability in algal communities. A variable which has a decisive influence on the distribution of algae is the US, the second variable is saturation and third temperature. According to CCA analysis taxa *Mougeotia* sp., *Nitzschia amphibia*, *Pediastrum boryanum*, *Fragilaria* sp. and *Oedogonium* sp. are typical of higher temperatures, lower  $PO_4$  values, and »tolerant« to US. Taxa *Achnanthydium minutissimum*, *Scenedesmus obliquus*, *Nitzschia palea* and *Leptolyngbya* sp. are more commonly present at higher pH values, higher saturation and taxa *Spirogyra* sp., *Chlamydomonas* sp., and *Navicula* spp. are more common at lower pH values, lower saturation and »tolerant« to US. *Dictiosphaerium pulchellum* is a species typical of higher  $PO_4$  values and »not tolerant« to US.

## CONCLUSIONS

Ultrasonication caused efficient sedimentation of algae in the Chem-free pond, but they were accumulating in the bottom of the pond, where there was enough light for them to photosynthesise. A major problem could be in the late fall when algae start to decompose. For successful treatment of water with the Chem-free system deeper ponds should be introduced, so that sedimented algae at the bottom of the pond would be unable to photosynthesise.

*Acknowledgements:* The work was carried out within the project CHEM-FREE (Development of a chemical-free water treatment system through integrating fibre filters, ultrasound and UV-C; Contract No. COOP-CT-2006-032719; duration: 1.7.2006 – 30.9.2008), a Co-operative Research Project (CRAFT) funded within the Horizontal Research Activities involving SMEs of the EU 6<sup>th</sup> Framework Programme. The authors are grateful for the support.

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