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Replacement of the Medium for a Natural Phytoplankton Community by Tangential-Flow Filtration, with Special Emphasis on Toxicity Tests

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Short-term photosynthesis toxicity tests with natural phytoplankton communities have received increased attention in recent years, both as an alternative to standardized toxicity tests (Kusk and Nyholm 1991) and as an integrated part of the assessment of the health of aquatic ecosystems (Munawar et al. 1989). The results of a toxicity test with natural phytoplankton are inherently dependent on the actual sensitivity of the algae and on the bioavailability of the contaminants. Both of which can vary significantly with the structure of the phytoplankton community and the chemical composition of the medium (Hongve et al. 1980; Steemann Nielsen and Laursen 1976). If the algae or the medium are replaced before the short-term photosynthesis toxicity test it might be possible to discriminate between the influence of the algae and the medium. This technical note describes and evaluates one approach for carrying out such a replacement.

Since microalgae can adapt to changing conditions within a few minutes (Harris 1986), the time required for the renewal of the medium is critical. It is also critical that the algae do not suffer from mechanical damage, which could bias results if mechanical and chemical stress influence one another. Mechanical stress might affect certain species in particular i.e., those with delicate structures such as flagella. Therefore, conventional laboratory methods such as centrifugation are not readily applicable to natural phytoplankton (Barthel et al. 1989). In biotechnology tangential-flow filtration (TFF) is used to wash sensitive living cells that can not tolerate a centrifugation (Shiloch et al. 1988). In marine biology TFF has been applied in concentration of nano- and picoplankton samples (Barthel et al. 1989; Giovannoni et al. 1990). The advantage of TFF is a water flow along the filter surface (tangential flow) which reduces the contact between the algae and the filter surface.

In this work, the use of TFF in the replacement of the media of natural phytoplankton samples was investigated. The effect of TFF was measured by comparing the photosynthetic rate and the toxic response towards copper before and after the application of TFF.

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MATERIALS AND METHODS

A Millipore Minitan casette system equipped with two filter elements with a pore size of 0.2 µm and a total filter area of 220 cm^2 was applied for tangential flow filtration (TFF) (Millipore Corp). A schematic description of the core of the equipment appears in figure 1; the filter cassette appears in Figure la, and an overview of the entire apparatus is shown in Figure lb.

The pump generates a current of algal suspension from the return reservoir to the filter casette. The pressure of the current is measured by a manometer immediately before the filter casette. The filter casette separates the algal suspension into a tangential flow stream and a filtrate stream. Every particle unable to pass the filter is collected in the return stream. After passage through the filter casette, the filtrate is collected in the filtrate reservoir and the return stream flows into the return reservoir.

With a pumping speed of 0.8 to 1.0 L min^{-1}, algal samples of 300 mL were concentrated to 30 mL in five minutes. During the individual concentrations, the pressure varied between 1.5 and 40 kPa. Thereafter, the newly produced filtrate was added. The concentration and addition of filtrate was repeated two times. This procedure ensures that the observed effects on the algae can only be due to the mechanical stress caused by TFF.

For the natural phytoplankton communities the effects of TFF were assessed by comparison of the photosynthetic activity and toxic response towards copper before and after the application of TFF. The toxic responses were measured by a short-term photosynthesis test. For the cultured algae the cell volume and photosynthetic activity before and after the application of TFF were compared. The algal volume was measured by a Coulter Counter.

The photosynthetic activity was measured as the incorporation of radio-labelled carbon dioxide into acid-stabile carbon (Riemann and Jensen 1991). The incubations were carried out in 20-mL glass scintillation vials, each of which contained 10 mL of algal suspension and 100 µL $NaH^{14}CO_3$ from a stock solution with an activity of 20 µCi ml^{-1} (The International Agency for ^{14}C Determination). The algae were incubated with the ^{14}C-carbonate for two hours. The phytoplankton communities were incubated *in situ* at a depth giving an irradiance corresponding to light-saturated photosynthesis at 320 µ mol $m^{-2} sec^{-1}$. The cultured algae were incubated in a light incubator at the same light intensity as they were grown. Light intensity was measured with a Li-1000 (Li-corr) sensor.

For each phytoplankton community the toxic responses towards copper were determined by a short-term photosynthesis test. Each phytoplankton sample was divided into 20 subsamples. Copper was added according to a geometric concentration series (factor 1.78) to give final concentrations in the range of 0.0316 to 100 µM copper. Five subsamples served as controls without addition of copper. To ensure inhibition of the photosynthesis, a one hour preincubation period was used before the addition of 100 µL $NaH^{14}CO_3$.

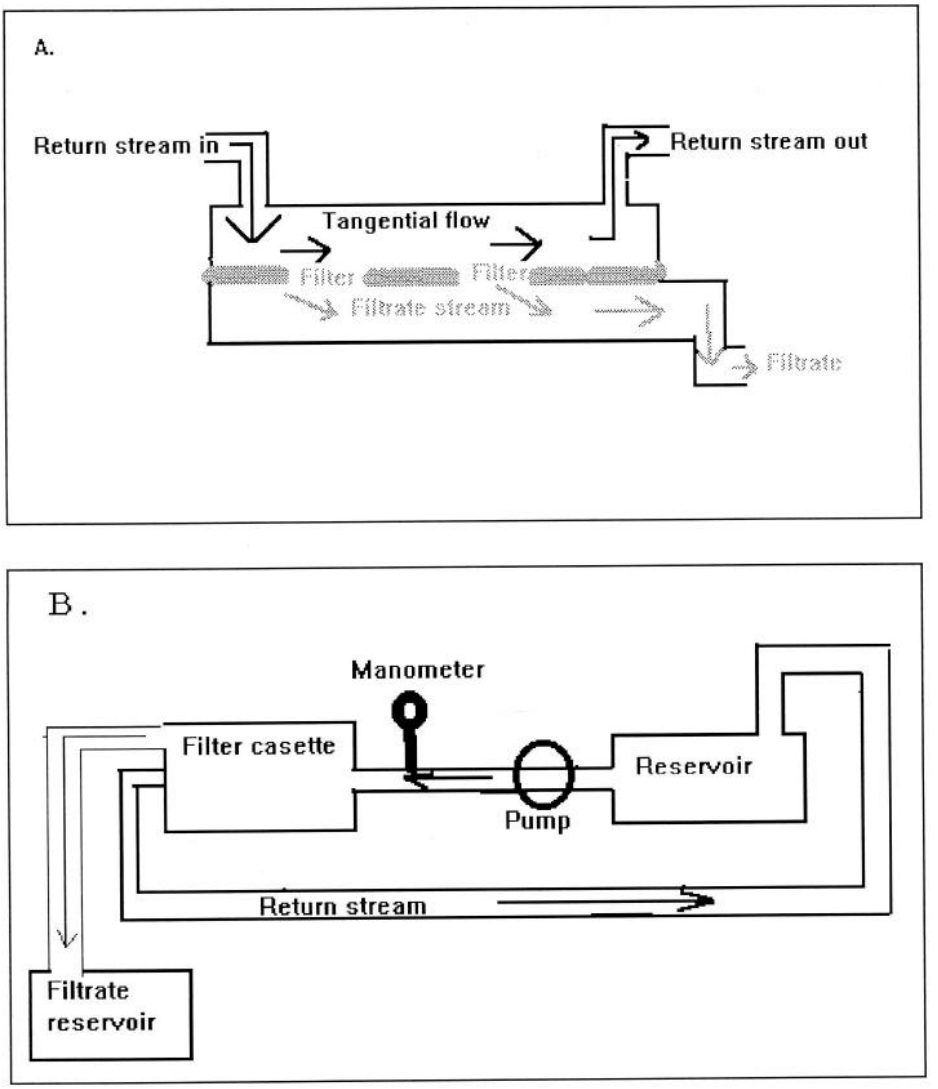

Figure 1. A schematic description of the applied equipment.
(A) the filter casette (B) the entire equipment.

The toxic responses towards copper were expressed as EC50 values (effect concentration). The EC50 values were determined by graphic interpolations on concentration-response curves. To avoid the effects of sample variation and outliers, the concentration-response curves were smoothed using the Lowess procedure (Cleaveland and McGill 1984).

Eight phytoplankton communities were sampled in connection with a marine enclosure experiment. Each enclosure contained 6 m^3 of natural sea water and was attached to a pontoon bridge. To some of the enclosures copper and nutrients were added at the beginning of the enclosure experiment.

The concentrations of copper added to the enclosures were lower than the estimated EC50 values by at least a factor 10. The EC50 values were therefore not corrected for the concentration of copper present in the medium before the beginning of the short-term test. Four phytoplankton communities were sampled in Denmark at Fjord Vellerup, Lake Bure, Lake Esrum, and Lake Frederiksborg. The phytoplankton communities were sampled with a tube as depth-integrated samples of the upper 3.5 m. To avoid grazing by large species of zooplankton, the samples were filtered with a 140-µm net.

Laboratory experiments were carried out with the marine diatom *Skeletonema costatum,* the marine cryptophyceae *Rhodomonas baltica,* and the freshwater green alga *Selenastrum Capricornutum.* Except for a salinity of 1.8%, the marine algae were cultured in accordance with the 1991 guidelines of The International Standard Organization (ISO 1991). The freshwater algae were grown according to the 1989 guidelines of the International Standard Organization (ISO 1989). Immediately before the experiments the algae were inoculated into a new ISO medium, to yield concentrations of 10^4 cells ml^{-1} for *S. costatum* and *R. baltica* and 10^5 cells ml^{-1} for *S. capricornutum*.

RESULTS AND DISCUSSION

For the phytoplankton communities, the application of TFF caused a loss from 1.5 % to 64% (average 37%) of photosynthetic activity (Table 1). For the cultured algae, the loss of photosynthesis varied between 16 and 69%, and the loss of algal volume was between 17 % and 61% (Table 2). For both the photosynthetic activity and the algal volume, the coefficient of variation was 6 to 16%. The loss of photosynthetic activity and algal volume were thus approximately equal, except for *R. baltica,* which showed a minor difference. Our experiments therefore indicate that the photosynthetic activity per algal volume was generally not affected by TFF.

The toxic responses were measured before and after the application of TFF by a short-term photosynthesis test and expressed as EC50 values. Before the application of TFF, the EC50 values varied from 1.5 to 23.4 µM-Cu and after the EC50 values varied from 2.1 to 28.8 µM-Cu (Table 1). The toxic responses towards copper before and after the application of TFF (Figure 2) were significantly correlated ($r=0.86$, $p=0.001$). In addition, a Student's T-test for paired observation showed that the EC values before and after the application of TFF did not differ significantly. The toxic responses of the phytoplankton communities were unaffected by the application of TFF.

The toxic response of an algal towards copper might depend on the integrity of the cell membrane and associated proteins. For instance, the lipid bilayer of the cell membrane protects the inside of the cell against the positively charged copper ion, and the uptake of copper ions often involves binding to transport proteins or diffusion through aqueous pores in the cell membrane (Sicko-Goad and Stoermer 1988; Stauber and Florence 1987). The preservation of the toxic

responses therefore suggests that the cell membrane and associated proteins are not damaged by TFF.

The cell membrane and associated proteins might be among the most vulnerable structures in a filtration process, and the preservation of toxic responses towards copper suggests that the algae are physiologically intact after the application of TFF. This conclusion is further supported by the experiments with the cultured algae, which showed that the loss of photosynthetic activity was due mainly to a loss of algae and not to a decrease of photosynthetic activity per algal volume.

Table 1. Effect of tangential flow filtration on photosynthesis and toxic response (EC50) of all the examined phytoplankton communities.

Phytoplankton community	Loss of Photosynthesis	Toxic response (EC50[μmol-Cu/l])	
		Before	After
Control enclosure 1	30 %	15.1	26.9
Control enclosure 2	28 %	9.3	11.0
Enclosure added 1 μg-Cu/L	41 %	13.5	19.5
Enclosure added 3 μg-Cu/L	35 %	21.9	28.8
Enclosure added 6 μg-Cu/L	31 %	23.4	14.8
Enclosure added 15 μg-Cu/L	28 %	7.9	8.3
Enclosure added N+P	41 %	6.3	8.3
Enclosure added 6 μg-Cu/L +N+P	47 %	1.8	2.1
Lake Bure	36 %	3.0	2.4
Lake Esrum	62 %	1.9	4.3
Lake Frediksborg	15 %	1.5	5.9
Fjord Vellerup	46 %	5.1	4.3

Table 2. Effect of tangential flow filtration on photosynthesis and algal biovolume for cultured species caused by tangential flow filtration.

Alga species	Loss of photosynthesis	Loss of algal volume
R. baltica	67 %	60 %
S. capricornutum	16 %	18 %
S. costatum	31%	32 %

The minor effects of TFF on microalgae are in accordance with the results of previous applications of the technique. For instance, TFF has been used to

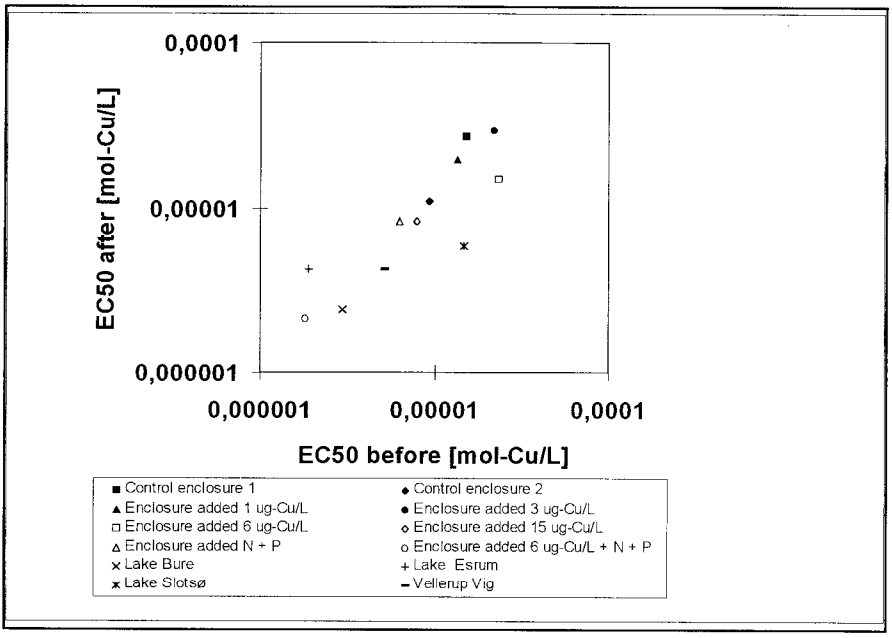

Figure 2. Toxic responses (EC50) for all examined phytoplankton communities before and after the application of tangential flow filtration.

concentrate erythrocytes used in the investigation of membrane proteins (Shiloach et al 1988). In addition, TFF has been used for the harvesting of viable cells of the ciliate *Tetrahymena pyriformis* (Malinowsky 1987). Further more, Barthel et al. (1989) and Giovannoni et al. (1990) have shown through microscopic investigations that nano- and picoplankton were morphologically intact after concentration by TFF.

Our experiment showed that TFF can be used to replace the medium for phytoplankton communities. Furthermore, this concentration technique does not harm the algae, and TFF could therefore be a useful tool with many potential applications in toxicological and physiological investigations.

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