Research Article

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Germanium dioxide as agent to control the biofouling diatom *Fragilariopsis oceanica* for the cultivation of *Ulva fenestrata* (Chlorophyta)

https://doi.org/10.1515/bot-2023-0075
Received August 31, 2023; accepted February 6, 2024; published online February 26, 2024

**Abstract:** During the cultivation of *Ulva fenestrata* in a land-based aquaculture system, the colonisation of the water tanks' surfaces and eventually the macroalgal biomass by the biofouling diatom *Fragilariopsis oceanica* compromises the production process. Since germanium dioxide (GeO₂) is an effective growth inhibitor of diatoms, this study aimed to understand how it affects the presence of *F. oceanica* and the photosynthesis and growth of *U. fenestrata* as a primary parameter contribution to the biomass production. A toxicological dose-response experiment showed that the diatom's growth was inhibited at the low GeO₂ concentration of 0.014 mg l⁻¹. In contrast, the photosynthetic performances and growth rates of *U. fenestrata* remained unaffected under a wide GeO₂ concentration range (0.022–2.235 mg l⁻¹) in small and large-scale experiments in 1 l glass beakers and 100 l Plexiglass water tanks, respectively. In the latter, the diatom density in the tanks was reduced by 40 %. The costs arising from the use of GeO₂ can range between €2.35 and €8.35 kg⁻¹ fresh weight of produced *U. fenestrata* biomass under growth conditions resulting in growth rates of 20 and 11.5 % d⁻¹, respectively. GeO₂ is an effective agent to control biofouling diatoms such as *F. oceanica* during the land-based biomass production of *U. fenestrata*.

**Keywords:** *Ulva*; macroalgal aquaculture; diatoms; biofouling; photosynthesis

1 Introduction

Aquaculture is an important part of the world’s food production. In 2019 macroalgal aquaculture accounted for 29 % (34.7 million tons) of the global aquaculture products. While China, Indonesia, South Korea, The Philippines and North Korea together produced 98 % of the macroalgal biomass, Europe only accounted for 0.03 % (Cai et al. 2021). In Norway, macroalgal aquaculture is a relatively young industry sector with a peak production of 336 t of *Saccharina latissima* and *Alaria esculenta* (Fiskeridirektoratet 2023). However, other species such as *Ulva* only play a negligible role despite ambitions to develop aquaculture concepts for them (Roleda et al. 2021; Stévant et al. 2017).

Strains of *Ulva fenestrata* from North Norway are rich in carbohydrates, dietary fibre, proteins and minerals, while being low in total lipids, fatty acids and iodine (Biancarosa et al. 2017; Rautenberger 2022; Roleda et al. 2021). Important vitamins (e.g. C, B₃, B₁₂) and bioactive compounds (e.g. ulvan) could be also present (Holdt and Kraan 2011; MacArtain et al. 2007). Due to these commercially interesting properties, there is a wish to develop a year-round mass production of *U. fenestrata* through land-based aquaculture (Roleda et al. 2021). However, cultivation studies have repeatedly revealed issues with biofouling by the pennate diatom *Fragilariopsis oceanica* (Cleve) Hasle. This diatom is pumped up into the laboratory together with the deep-seawater from the nearby Saltfjord. Insufficient filtration and sterilisation capacities of the high flow-through seawater treatment system allow them to pass through the 5 µm filters. Eventually, *F. oceanica* is flushed into the water tanks and grows rapidly into long and dense filaments. Small brown spots appear first on the *Ulva*’s surfaces, which eventually grow into a dense filaments.

The biofouling by *F. oceanica* not only affects the growth of *U. fenestrata* and reduces the biomass quality, it also increases the production costs. The shading of the macroalgal thalli by the diatom’s dense colonisations on the water tank’s surfaces may reduce light-dependent physiological processes such as photosynthesis, which, eventually, diminishes growth. In addition, there is a competition between the diatoms and *U. fenestrata* for the nutrients dissolved in the seawater.

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Germanium dioxide (GeO₂) showed inconsistent results. While the growth of such as Ulva fenestrata remains unaffected by the treatment of GeO₂, the growth of Fragilariopsis oceanica was expected to be impaired by GeO₂ due to the competition with silicate, growth and photosynthesis of Ulva fenestrata remain unaffected because neither germanium nor silicate are essential for the green macroalgae. The knowledge gained from this study is useful for the contamination control of land-based aquaculture of Ulva.

2 Materials and methods

Ulva fenestrata Postels et Ruprecht was collected from the intertidal in Bodo (67.2759°N, 14.5706°E), Norway, in July 2019 (Hughey et al. 2019). In the laboratory, the collected thalli were cleaned and kept in 600 l water tanks (aeration from the bottom) at 130 ± 5 µmol photon m⁻² s⁻¹ (L36W/954 Lumilux de Lux Daylight, Osram GmbH, Munich, Germany) and 9 ± 1 °C for 7 months prior to the experiments. The deep-seawater (250 m water depth) came from the nearby Saltfjord and was fed into the water tanks with a flow rate of 251 min⁻¹. The nitrate and phosphate concentrations were 9.75–10.26 µM and 0.55–0.91 µM, respectively. The ammonium concentration was low (0.01–0.10 µM).

For the toxicological dose-response experiment with F. oceanica, 21 11 glass beakers (10 % HCl-washed) were filled with 0.81 l of 5 µm-filtered, ESNW-enriched deep-seawater (Berges et al. 2001). NaSiO₃ was omitted because F. oceanica grows in the laboratory without added silicate. Saturated GeO₂ stock solutions were prepared by mixing 1.11735 g of GeO₂ (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) with 0.25 l of sterile-filtered (0.2 µm) seawater, stored at 4 °C in the dark. The mixture was thoroughly homogenised before use (Shea and Chopin 2007). GeO₂ was added to the seawater medium to reach 6 ± 1 mg GeO₂ l⁻¹. Then F. oceanica was allowed to grow at 137 ± 5 µmol photon m⁻² s⁻¹ at 9 ± 1 °C (L36W/954 Lumilux de Lux Daylight; Osram GmbH, Munich, Germany) under aeration from the bottom of the beakers. The seawater was not replenished during the experiment to disturb the growth of the diatoms. The experiment was finished after 22 days when a significant diatom biomass was visible on the beakers’ surfaces. This diatom biomass was carefully removed and filtered through previously heated (105 °C), weighed (at room temperature) and washed (0.5 M NH₄HCO₃) 1.0 µm glass microfibre filters (Number 692; VWR International, Leuven, Belgium). Then the filters were washed twice with 20 ml of 0.5 M NH₄HCO₃ and dried at 105 °C for 24 h. The filters with and without algal biomass were cooled at room temperature in a desiccator. The diatom’s dry weights (DW) were measured using an analytical balance with 0.1 mg precision (VWR International, Leuven, Belgium). The volume-based DW of the diatom biomass in the beakers was calculated as follows:

\[ \text{DW}_{\text{algal biomass}} = (\text{DW}_{\text{algal}} - \text{DW}_{\text{algae}}) \times V^{-1} \]

where \( \text{DW}_{\text{algal}} \) and \( \text{DW}_{\text{algae}} \) are the filters’ dry weights with and without diatom biomass, respectively, and \( V \) is the volume of the seawater medium in the beakers.

Therefore, the presence of F. oceanica needs to be effectively controlled.

Regular cleaning of the water tanks’ surfaces by hand is time-consuming and, thus, an expensive work task. Although artificial seawater does not contain any biofouling organisms, its preparation increases production costs of Ulva fenestrata substantially. A possibly less expensive solution could be the treatment of F. oceanica with germanium dioxide (GeO₂). This cytotoxin inhibits the growth of numerous diatom species by interfering with the silica frustule formation (Lewin 1966). Fragilariopsis oceanica was reported to have silicified frustules consisting of different silification degrees between valves, mantles and bands (Lundholm and Hasle 2010). Although GeO₂ is routinely used to control the contamination of cultures by diatoms, there is still limited information of its effects on green macroalgae such as Ulva. The few studies in which Ulva was treated with GeO₂ showed inconsistent results. While the growth of Ulva fenestrata from Helgoland, Germany, remained unaffected up to 2.2 mg GeO₂ l⁻¹, Ulva australis from Japan was insensitive up to 30 mg GeO₂ l⁻¹ (Markham and Hagmeier 1982; Tatewaki and Mizuno 1979). Therefore, this study was conducted to understand the impact of GeO₂ (1) on the growth of biofouling diatom F. oceanica and (2) on the growth and photosynthesis of Ulva fenestrata as primary physiological processes influencing the macroalgae’s biomass production. Figure 1: Photographs of the water tanks used for the cultivation of Ulva fenestrata in the laboratory while being (A) moderately and (B) strongly colonised by Fragilariopsis oceanica.
For the small-scale cultivation of *U. fenestrata* with GeO$_2$, 12 2 l flasks (10 % HCl-washed) were filled with 1.8 l of 0.45 µm-filtered, ESNW-enriched deep-seawater (no NaSiO$_3$ added), which was aerated from the bottom and changed daily. GeO$_2$ was added to the seawater to treat *U. fenestrata* with three GeO$_2$ concentrations (0.022–2.235 mg l$^{-1}$), while GeO$_2$-free seawater was used as control. Then 12 individuals of *U. fenestrata* were randomly taken from the stock culture and three algal discs (17 mm diameter) were cut from each individual using a stainless steel cork-borer. The three discs from the same individual were randomly assigned to a flask and exposed to 140 ± 5 µmol photons m$^{-2}$ s$^{-1}$ (16 h light:8 h dark, L36W/954 Lumilux de Lux Daylight; Osram GmbH, Munich, Germany) at 9 ± 1 °C for 5 days.

For the large-scale cultivation of *U. fenestrata* with GeO$_2$, nine Plexiglass water tanks were filled with 63 l of deep-seawater and enriched with ESNW medium (no NaSiO$_3$ added). The seawater was aerated from the bottom of the tanks and not replenished during the experiment. GeO$_2$ was added to the seawater to treat *U. fenestrata* with two GeO$_2$ concentrations (0.223 and 2.235 mg l$^{-1}$), while GeO$_2$-free seawater was used as control. The two GeO$_2$ concentrations were used because they are expected to cause the largest effects on the physiology of *U. fenestrata*. Afterwards, individuals of *U. fenestrata* were randomly taken from the stock culture and cut back to a length of 5 cm above the discoid holdfast (or “attachment disc”). Then 3–5 individuals were placed in a water tank to start the experiment with an initial FW of 4–5 g. The macroalgae were cultivated at 140 µmol photons m$^{-2}$ s$^{-1}$ (16 h light:8 h dark, L18W/954 Lumilux de Lux Daylight; Osram GmbH, Munich, Germany) at 9 ± 1 °C for 5 days.

The changes in FW of all macroalgal samples in a flask or a water tank during the experiments were determined gravimetrically. The daily relative growth rates (RGR) were analysed by comparing the initial and final FWs and calculated according to (Lüning 1990).

The photosynthetic performances of *U. fenestrata* were measured on randomly taken samples using PAM-fluorometry (Diving-PAM, Walz GmbH, Effeltrich, Germany) at 10 ± 1 °C at the end of the experiments (Rautenberger et al. 2015). After 5 min of dark adaptation, *U. fenestrata* was exposed to incrementally increasing actinic irradiances ($E_{ac}$) after short saturation pulses (>9000 µmol photons m$^{-2}$ s$^{-1}$, 0.8 s) every 30 s to determine the effective PSII-quantum yields ($F_{v}'/F_{m}' = (F_{m}' - F')/F_{m}'$). Electron transport rates (ETRs) were calculated by multiplying $F_{v}'/F_{m}'$ by $E_{ac}$, the proportion of $E_{ac}$ absorbed by an *U. fenestrata* disc (“thallus absorbance”), and the fraction of absorbed light that was most probably received by PSII (Baker 2008; Figueroa et al. 2009; Lüning and Dring 1985). The maximum ETRs (ETR$_{max}$), the light saturation points of photosynthesis ($E_{s}$), and the initial slopes of light curves (a$_{ETR}$) as photosynthetic parameters were estimated from ETR-E curves, which were fitted after Eilers and Peeters (1988).

The samples for the pigment analyses were taken at the end of the two experiments. All macroalgae in each flask or water tank were frozen after 22 days of cultivation at 137 µmol photons m$^{-2}$ s$^{-1}$ and 9 °C. Diatom biomass (mg m$^{-2}$) in 5 ml of 100 % N,N-dimethylformamide (DMF) in the dark at 4 °C for 72 h. The pigments were analysed spectrophotometrically (UV/Vis UVLine 9400, SCHOTT Instruments GmbH, Germany) from spectra (350–750 nm) with 1.0 nm resolution at 647 and 664 nm (20 °C) with 100 % DMF as reference. Readings at 750 nm were used as correction factors for scattered light. The Chl a, b and total Chl (Chl a + b) contents were calculated after Porra et al. (1989) and normalised on macroalgal FW.

All experiments were designed such that each sample was independent. Means and standard deviations (SD) were calculated from three replicates per GeO$_2$ concentration. The Shapiro-Wilk test and the Levene test were used to test the normal distribution of residuals and the homoscedasticity of the primary data, respectively. When these assumptions were met, one-way ANOVAs were performed to identify statistical differences of means between the treatments with Tukey’s honestly significant difference (HSD) as post hoc test. In the case of heteroscedasticity, Welch-ANOVA were performed with Games-Howel post-hoc tests. The statistical analyses were performed with a 5 % significance level ($P < 0.05$) using JMP 14.0 (SAS Institute Inc., Cary, NC, USA) and R version 4.0 (The R Foundation for Statistical Computing, http://www.R-project.org).

3 Results

3.1 Dose-response relationship in *Fragilariopsis oceanica*

After 22 days of seawater cultivation in 11 glass beakers, brown biofilms of *Fragilariopsis oceanica* became clearly visible on the bottom and walls of the beakers of both the control (0 mg l$^{-1}$) and the lowest GeO$_2$ concentration (0.003 mg l$^{-1}$) with 27.13 mg DW m$^{-2}$ and 24.23 mg DW m$^{-2}$, respectively. In contrast, all GeO$_2$ concentrations ≥0.014 mg l$^{-1}$ had lower diatom biomass (6.56 mg DW m$^{-2}$) than the control and 0.003 mg GeO$_2$ l$^{-1}$ ($P < 0.0001$, 1-way ANOVA; Figure 2).

3.2 Small- and large-scale experiments with *Ulva fenestrata* treated with GeO$_2$

The samples for the pigment analyses were taken at the end of the two experiments. All macroalgae in each flask or water tank were frozen after 22 days of cultivation at 137 µmol photons m$^{-2}$ s$^{-1}$ and 9 °C. Data are means of three replicates per treatment ($n = 3$) and error bars represent standard deviations. Lowercase letters above columns indicate statistically significant differences between the treatments ($P < 0.001$, 1-way ANOVA, Tukey-Kramer HSD post-hoc test).

![Figure 2](image-url)
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However, there was slight decrease in the Chl presence of GeO2 (concentrations tested between 0.022 and 2.235 mg L\(^{-1}\)) (Figure 3). In addition, the contents of Chl \(a\) and Chl \(b\) in \textit{U. fenestrata} remained unaffected by the presence of different GeO2 concentrations in the seawater (Table 1). However, there was slight decrease in the Chl \(a/b\) ratio of \textit{U. fenestrata} from 1.61 \pm 0.08 in the control by 10–15 % in the presence of GeO2 (\(P = 0.0208\), 1-way ANOVA; Table 1).

**Table 1**: Contents of photosynthetic and accessory pigments of \textit{Ulva fenestrata} after 5 days (“small-scale cultivation”) and 14 days (“large-scale cultivation”) of cultivation at 3–4 GeO2 concentrations.

<table>
<thead>
<tr>
<th>GeO2 (mg L(^{-1}))</th>
<th>Chl (a) ((\mu)g mg(^{-1}) FW)</th>
<th>Chl (b) ((\mu)g mg(^{-1}) FW)</th>
<th>Chl (a + b) ((\mu)g mg(^{-1}) FW)</th>
<th>Chl (a/b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small-scale cultivation of \textit{Ulva fenestrata} in glass beakers (after 5 days)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.34 \pm 0.05</td>
<td>0.21 \pm 0.04</td>
<td>0.55 \pm 0.09</td>
<td>1.61 \pm 0.08</td>
</tr>
<tr>
<td>0.02235</td>
<td>0.36 \pm 0.01</td>
<td>0.25 \pm 0.02</td>
<td>0.62 \pm 0.02</td>
<td>1.43 \pm 0.09ab</td>
</tr>
<tr>
<td>0.22347</td>
<td>0.34 \pm 0.02</td>
<td>0.25 \pm 0.01</td>
<td>0.59 \pm 0.03</td>
<td>1.36 \pm 0.01b</td>
</tr>
<tr>
<td>2.23474</td>
<td>0.32 \pm 0.04</td>
<td>0.24 \pm 0.04</td>
<td>0.56 \pm 0.08</td>
<td>1.37 \pm 0.11b</td>
</tr>
<tr>
<td><strong>Large-scale cultivation of \textit{Ulva fenestrata} in Plexiglass water tanks (after 14 days)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.59 \pm 0.08</td>
<td>0.38 \pm 0.03</td>
<td>0.97 \pm 0.10</td>
<td>1.55 \pm 0.09</td>
</tr>
<tr>
<td>0.22347</td>
<td>0.62 \pm 0.14</td>
<td>0.40 \pm 0.08</td>
<td>1.02 \pm 0.23</td>
<td>1.55 \pm 0.06</td>
</tr>
<tr>
<td>2.23474</td>
<td>0.54 \pm 0.13</td>
<td>0.35 \pm 0.08</td>
<td>0.89 \pm 0.22</td>
<td>1.56 \pm 0.01</td>
</tr>
</tbody>
</table>

Fresh weight (FW)-based contents of chlorophyll \(a\) (Chl \(a\)), chlorophyll \(b\) (Chl \(b\)), total chlorophylls (Chl \(a + b\)) and the Chl \(a\)-to-\(b\) ratio (Chl \(a/b\)). Data are means \pm standard deviations of three replicates per treatment (\(n = 3\)). Different lowercase letters behind the data in each column indicate statistically significant differences of the pigments between the GeO2 concentrations (\(P < 0.001\), 1-way ANOVA, Tukey-Kramer HSD post-hoc test).

In the large-scale experiment, the RGR, all three photosynthetic parameters, and the chlorophyll contents, including the Chl \(a/b\) ratio of \textit{U. fenestrata}, were statistically similar between the control and the two tested GeO2 concentrations after 14 days of cultivation (Figure 4 and Table 1). However, the addition of GeO2 to the seawater decreased the density of \textit{F. oceanica} on the wall surfaces of the Plexiglass water tanks by 36–43 % at 0.223–2.235 mg GeO2 L\(^{-1}\) compared to the control (\(P = 0.0077\), 1-way ANOVA; Figure 5).

**Figure 3**: Physiological parameters of the small-scale cultivation of \textit{Ulva fenestrata} with GeO2. (A) Relative growth rates (RGR), (B) photosynthetic electron transport efficiencies (\(\alpha_{ETR}\)), (C) maximum electron transport rates (ETR\(_{max}\)) and (D) light saturation points of photosynthesis (\(E_{s}\)) of \textit{Ulva fenestrata} after small-scale cultivation (5 days) in 1-l glass beakers with four GeO2 concentrations at 140 \(\mu\)mol photons m\(^{-2}\) s\(^{-1}\) and 9°C. Data are means of three replicates per treatment (\(n = 3\)) and error bars represent standard deviations.

### 4 Discussion

In hatcheries and cultivation systems (e.g. land-based aquaculture) of macroalgae, biofouling through unwanted contamination by diatoms can cause serious damage. Therefore, GeO2 is often used to control biofouling diatoms. This study demonstrated that GeO2 controls the growth of \textit{Fragilariopsis oceanica}, while both the growth and the photosynthesis of \textit{Ulva fenestrata} remain unaffected.

#### 4.1 Effects of GeO2 on the growth of \textit{Fragilariopsis oceanica}

GeO2 is widely used as an effective growth inhibitor for diatoms and other silicifying microalgae (e.g. chrysophytes). It competitively inhibits the uptake of Si through Si-transporters (SITs), which eventually interrupts cell wall formation in the algae (Tham astrakoln and Hildebrand 2008).
Toxicological studies have shown species- and strain-specific responses of diatoms to GeO₂. While concentrations of up to 1 mg GeO₂ l⁻¹ inhibited the growth of highly silicified diatom species (e.g. Amphiphora paludosa, Cylindrotheca fusiformis), diatoms with a low degree of silicified cell walls (e.g. Phaeodactylum tricornutum) were insensitive even to 10 GeO₂ mg l⁻¹ (Lewin 1966; Markham and Hagmeier 1982; Tatewaki and Mizuno 1979). By using these results as benchmark for the present study, F. oceanica seems to be highly sensitive to GeO₂ because its growth was inhibited at a low concentration of 0.014 mg GeO₂ l⁻¹. Assuming that Si uptake in F. oceanica is mediated by SITs at low Si concentrations in seawater (<30 µM) as shown for Thalassiosira pseudonana (Thamatrakoln and Hildebrand 2008), a growth inhibition by GeO₂ could have been expected because the seawater Si concentration used was approx. 2.1 µM (Busch et al. 2014). However, if Si was added to the seawater according to formulation of the ESNW medium with a final concentration of 106 µM, a different result would have been observed because diffusive Si uptake predominates at higher Si concentrations (Thamatrakoln and Hildebrand 2008).

Interestingly, the use of GeO₂ in the glass beakers and Plexiglass water tanks showed different effects on F. oceanica. While low GeO₂ concentration inhibited the growth of F. oceanica, the colonisation of the Plexiglass walls by F. oceanica could not be fully prevented even by the use of 2.235 mg GeO₂ l⁻¹. This could be possibly ascribed to the different physical surface properties between glass and Plexiglass. Insoluble extracellular polymeric substances (EPS) secreted by diatoms allow them to adsorb better on hydrophobic surfaces such as Plexiglass than on the hydrophilic surfaces of the glass beakers (Finlay et al. 2013; Holland et al. 2004; Krishnan et al. 2006; reviewed by Thompson and Coates 2017). In addition, EPS-rich biofilms from Roseobacter and Sulfitobacter, which are associated with Ulva, could have enhanced the attachment of F. oceanica on the Plexiglass walls (Bruckner et al. 2011; Buhmann et al. 2016; Spoerner et al. 2012). Thus, the bacterial biofilms could help F. oceanica to overcome the negative effects of GeO₂. Other factors such as the different treatments of the glass beakers (acid-washed) and water tanks (hand washing and sodium hypochlorite) prior to the experiments could
also have contributed to the different outcomes of the two experiments. Nevertheless, since the large-scale experiment showed a considerable reduction in diatom density on the Plexiglass surfaces by 0.223 mg GeO$_2$ l$^{-1}$ (f.c.), the costs for the biomass production of *U. fenestrata* are expected to be lower than without employing any GeO$_2$ at all.

### 4.2 Effects of GeO$_2$ on the growth and photosynthesis of *Ulva fenestrata*

Since there are toxic effects of GeO$_2$ on *F. oceanica*, it is crucial to understand its implications for *U. fenestrata*. This study demonstrated that GeO$_2$ concentrations of up to 2.235 mg l$^{-1}$ had no adverse effect on growth and photosynthesis of *U. fenestrata*. The capture and transfer of light energy through photosynthetic and accessory pigments, as well as the photosynthetic activity, remained unaffected by GeO$_2$ at different cultivation scales (2-l flasks and 70-l water tanks). Consequently, the photosynthetically produced carbon could be allocated to macroalgal growth. Because photosynthesis also interacts with nitrogen metabolism, the constant growth rates imply that nitrogen assimilation remained unaffected by GeO$_2$ even though this was not tested (Huppe and Turpin 1994).

Constant growth and photosynthetic performance suggest that *U. fenestrata* has neither a GeO$_2$-sensitive uptake mechanism nor it is limited by Si, indicating that Si is not essential for the green macroalga. This is in line with a previous study showing that the growth of *U. fenestrata* (studied as *U. lactuca*) is unaffected up to 2.2 mg GeO$_2$ l$^{-1}$ (Markham and Hagmeier 1982). However, this macroalga’s growth was reported to be inhibited at higher concentrations of GeO$_2$ (4.4–8.95 mg GeO$_2$ l$^{-1}$). In contrast, the growth of *Ulva australis* (studied as *Ulva pertusa*) and *Monostroma angicava* was not inhibited up to 30 mg GeO$_2$ l$^{-1}$ (Markham and Hagmeier 1982; Tatewaki and Mizuno 1979). GeO$_2$ seems to be a safe anti-fouling agent for the biomass production of *U. fenestrata* and other green macroalgae. Nevertheless, its effects on the development of the life-cycle stages have yet to be studied because 0.5–1.0 mg GeO$_2$ l$^{-1}$ are often used to prevent diatoms from growing during these stages (Lotze et al. 2000; Marshall et al. 2006; Wang et al. 2012).

When GeO$_2$ is used during the production of *U. fenestrata* for human and animal consumption, knowledge about the bioaccumulation of GeO$_2$ is crucial to understand its safety as a component of human food and animal feed. So far, neither the European Food Safety Authority (EFSA) nor its North American counterparts have any regulations on the food safety of germanium (Ge). To the author’s knowledge, there are no studies available on the bioaccumulation of GeO$_2$ in *U. fenestrata* and other macroalgae. Nevertheless, *U. fenestrata* may accumulate Ge due to the treatment with GeO$_2$ because this has been detected across taxonomic borders: bacteria, cyanobacteria, microalgae and higher plants (Cheong et al. 2009; Chmielowski and Klapcińska 1986; Choi et al. 2013; Djur 2020; Yanagimoto et al. 1983). In the green microalga *Chlorococcum ellipsoideum* (studied as *Chlorella ellipsoidea*), the bioaccumulation of Ge was lowest under optimal pH conditions (6.0–7.4) for growth while it increased 8-fold at a high pH (>9), which inhibited growth (Yanagimoto et al. 1983). Since *U. fenestrata* was cultivated under optimal conditions giving high growth rates, the Ge-content is assumed to be low. Because inorganic Ge-containing compounds, including GeO$_2$, have low toxicity with acute LD$_{50}$ values of 500–5000 mg kg$^{-1}$ (oral application), the assumed low Ge-contents in the tissue of *U. fenestrata* could be considered as food-safe (Gerber and Léonard 1997). Since single oral doses (2000 mg Ge kg$^{-1}$ body weight) of Ge-fortified lettuce did not result in physiological and pathological changes in mice, the bioaccumulated Ge was considered to be safe as food and feed (Kim et al. 2009). However, whether GeO$_2$-treated biomass of *U. fenestrata* is actually a concern for food and feed safety still needs to be determined after we have a better understanding of the bioaccumulation of Ge in *Ulva* and other macroalgae.

### 4.3 Economic considerations of the use of GeO$_2$

For land-based aquaculture of *U. fenestrata*, it was important to understand if the use of a given GeO$_2$ concentration is economically reasonable. Since GeO$_2$ can be currently purchased for an average price of €18.69 g$^{-1}$ from two main laboratory suppliers in Norway, the preparation of 250 ml of saturated GeO$_2$ stock solution costs €20.89. Thus, the use of 5 ml of the GeO$_2$ stock solution for a final concentration of 0.223 mg GeO$_2$ l$^{-1}$ seawater resulting in a 40 %-growth inhibition of *F. oceanica* in a 100-l Plexiglass water tank would cost €0.42. Assuming the average RGR of 11.5 % d$^{-1}$ (this study; over 14 days) with 10 g of initial FW, the cost of adding GeO$_2$ would be €8.35 kg$^{-1}$ FW of *U. fenestrata* produced. However, these costs for GeO$_2$ decline by 70 % to €2.53 kg$^{-1}$ FW of *Ulva* with a higher RGR of 20 % d$^{-1}$, which is not unusual for *Ulva* under optimal growth conditions. Consequently, the 250 ml of GeO$_2$ stock solution costing €20.89 can be used for a production of 2.5 and 8.25 kg FW of *Ulva* under growth conditions resulting in 11.5 and 20 % d$^{-1}$, respectively. However, the costs arising from the use of GeO$_2$ in other laboratories depend on the species-specific toxicological
effects of GeO₂ on predominant diatoms in the cultivation systems used for Ulva or other macroalgal species. While controlling highly silicified diatoms involves lower costs due to the use of lower GeO₂ concentrations, less sensitive species may require higher expenditure for GeO₂. In addition, operational costs are determined by the effects of GeO₂ on the growth and development of the macroalgal species being cultivated. In addition to Ulva, GeO₂ concentrations need to be found for possibly more sensitive red and brown macroalgae that do not cause adverse effects on the macroalgae but keep biofouling diatom densities low.

4.4 Conclusions

GeO₂ is an effective agent for controlling the presence of biofouling diatoms in aquarium systems and hatcheries. It can be used to control the contamination of cultures of U. fenestrata by F. oceanica without compromising the macroalgae’s biomass production. However, studies are needed on the food and feed safety due to the bio-accumulation of Ge when Ulva is produced for human and animal consumption. The concentrations of GeO₂ employed to control biofouling diatoms need to be adjusted to the specific diatom species occurring at an aquaculture facility. In either case, the use of GeO₂ seems to keep the high quality of the produced Ulva biomass, while reducing the workload and production costs.

Acknowledgments: The author wishes to thank Per Magnus Hansen (NIBIO) and Daniel Fonn Aluwini (Norsk fjordsalad AS) for their technical support of the experiments.

Research ethics: Not applicable.

Author contributions: Ralf Rautenberger: conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; resources; software; supervision; validation; visualization; roles/writing – original draft; writing – review & editing. The author has accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The author declares no conflict of interest. The author has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Research funding: The author is grateful to the North Norwegian MABIT programme (“Et næringsrettet FoU program innen marin bioteknologi i Nord-Norge”) for the financial support of this study (grant AF0090).

Data availability: The raw data can be obtained on request from the corresponding author.

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### Bionote

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