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Cultivation of *Melosira nummuloides* cells in the presence of platinum: Preparation of metal-containing frustules

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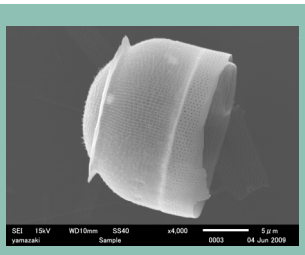
We cultivated *Melosira nummuloides* diatom cells, in the presence of dihydrogen hexachloroplatinate(IV) hexahydrate (DHH) in order to introduce platinum (Pt) into diatom frustules. Diatom cells were successfully grown in a medium containing up to 0.5 mg/mL DHH. Scanning electron microscopy (SEM) images showed that the shape of the formed frustules was not significantly different from that of native frustules. Only doped frustule samples produced a signal specific to Pt on X-ray photoelectron spectroscopy (XPS). Using our method, we successfully produced diatom frustules containing Pt.



A SEM image of a frustule that was obtained after culturing cells in f/2 medium containing Pt. Scale bar, 5 μm.

1 Introduction Diatom frustules are one of the unique biological structures found in nature; these frustules are composed of nanoporous structures present on micron-sized architectures of biosilica [1–5]. Frustules are formed only in natural water and in the presence of sunlight without the need for high temperature or high pressure treatment. Because of their unique structure, frustules find wide industrial application; they are used in water filters, building materials, and chromatography supports [6–9].

As a result of recent progress in nanotechnology, diatom frustules find new industrial applications. Preparation of a metal/carbon replica of frustules is a typical example of combining frustules and nanotechnology [10–14]. Frustules were coated with metals/carbon, and the original frustule was dissolved using chemicals. Another approach involves surface modification of frustules with zeolite or other materials [15–20]. For example, Bao *et al*. used magnesium to anneal frustules and established a single frustule NO_x sensor [20].

Furthermore, 2 research groups have demonstrated the injection of metals into living diatom cells. Townley *et al*. demonstrated the cultivation of *Coscinodiscus wailesii* in a culture medium containing nickel sulfate [21]. When nickel sulfate was added to the culture medium, well-developed frustules containing nickel were obtained. Recently, Jeffryes *et al*. used a similar approach to introduce titanium into *Pinnularia* sp. They added $Ti(OH)_4$ to the culture medium after Si starvation [22]. As compared to the above-mentioned method for the artificial modification of frustules, the cultivation method is more effective for the preservation of frustules because the latter comprises fewer treatment steps. Lengthy experimental steps sometimes tend to destroy frustule structure.

However, thus far, the cultivation method has been described only in 2 reports. It is not known whether this method can be used for producing frustules containing other useful metals. Additional experiments need to be performed to ascertain whether the cultivation method can be used as a standard technique for producing functionalized frustules. In this paper, we demonstrated the cultivation of *Melosira nummuloides* in the presence of dihydrogen hexachloroplatinate(IV) hexahydrate (DHH) in order to introduce platinum (Pt) into frustules.

2 Materials and methods

2.1 Preparation of diatom cells Isolated *Melosira nummuloides* cells were cultured in sea water with f/2 culture medium (G9903; Sigma-Aldrich Inc., St. Louis, MO). The sea water was filtrated through a 0.1-μm membrane filter for sterilization and removal of unknown materials. An aliquot of pre-cultured cells was added to 25 or 100 ml of the culture medium and incubated at 17 °C for 20 d. The maximal brightness of irradiation near the samples was 2000 lux. When dihydrogen hexachloroplatinate(IV) hexahydrate (DHH) was added to the culture medium, the final concentrations of DHH varied from 0 to 4.0 mg/L. Culture media containing 0.04 and 1.5 mg/L Pt was used. After cultivation, diatom frustules were purified according to a method described in previous reports [23, 24]. After purification, the resultant frustules were suspended in pure water.

2.2 Characterization of frustules The purified frustules were characterized by scanning electron microscopy (SEM; JSM-6510; JEOL, Tokyo, Japan) and X-ray photoelectron spectroscopy (XPS; SSX-100; Al kα source; Surface Science Instruments, Finland). For SEM observation, an aliquot of diluted frustule suspension was dropped on a silicon substrate and dried in air. Next, the samples were coated with AuPd for 90 s at 2 mA. For XPS measurements, the frustule suspension was dried overnight at 60 °C in sample tubes.

3 Results and discussion

We selected DHH as a source of Pt because the compound is water soluble and colorless, and thus produces a transparent solution. DHH powder was dissolved in pure water to produce a stock solution, and a suitable amount of DHH was injected into the culture medium at the start of cultivation. Volume of the injected solution was less than 1% of the culture medium in order to avoid changing concentrations of other components of the medium.

First, we examined the frustules formed on cells cultivation in media with different concentrations of DHH. Diatom cells grown in media containing 0.1 and 0.5 mg/L of DHH grew as well as those grown in a medium that did not contain Pt. Figure 1 shows photographs of cells growing in a bottle at 0, 10, and 20 d of incubation in the presence of 0.5 mg/L DHH. Aggregates of diatom cells were easily confirmed by observation. When more than 1.0 mg/L DHH was added to the culture medium, the growth rate was low. In some case, the cells showed no growth. The result suggested that a medium containing

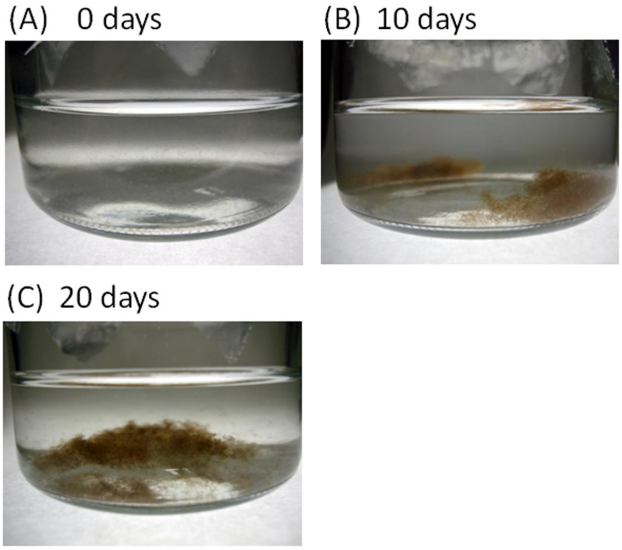


Figure 1 Photographs showing diatom cultivation in the presence of 0.5 mg/L DHH at (A) 0 days, (B) 10 days, and (C) 20 days.

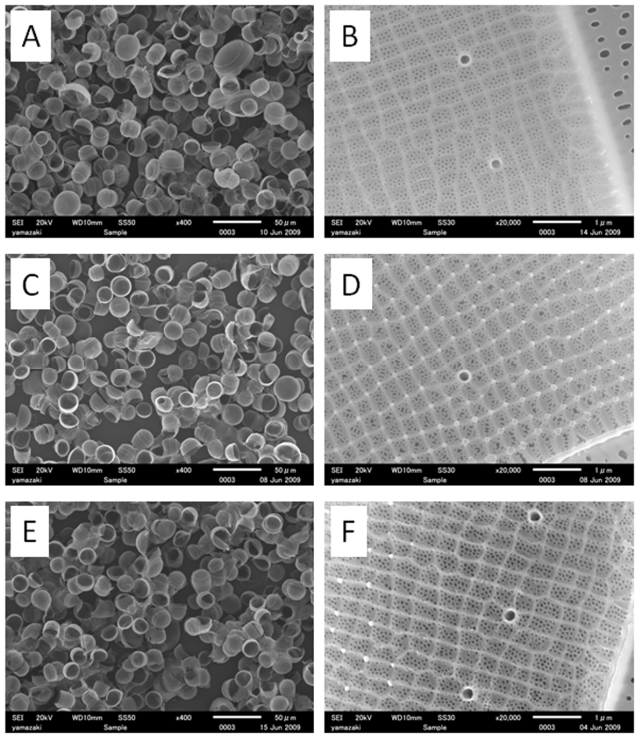


Figure 2 SEM images of purified frustules. (A) and (B) Native frustules. (C) and (D) Frustules cultured in 0.1 mg/L DHH. (E) and (F) Frustules cultured in 0.5 mg/L DHH. Scale bars in (A), (C), and (E) are 50 μm. Scale bars in (B), (D), and (F) are 1 μm.

0.5–1.0 mg/L DHH was suitable for the cultivation of cells.

Although we could not find any previous research about the effect of platinum on diatom cultivation, the effect of platinum on growth rate of *Euglena gracilis* was reported by Barnes and Talbert [25]. They described that growth of the cells was inhibited in the presence of 0.25, 0.50, or 0.75 mg/L of DHH. Comparing with their results, it seems that diatom cells have tolerance for DHH because growth speed was not significantly decreased up to 0.5 mg/L of DHH.

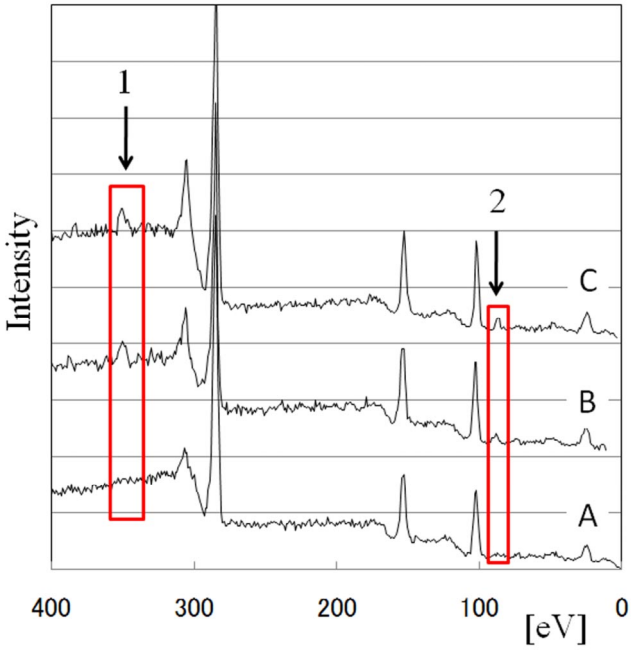


Figure 3 XPS spectra of frustules. (A) Native frustules. (B) Frustules cultured in 0.1 mg/L DHH. (C) Frustules cultured in 1.0 mg/L DHH.

Purified frustules were characterized by observing SEM images. Figure 2 (A, C, and E) shows crane shots of frustules. Even in samples cultured in the presence of 0.5 mg/L DHH, the shape of the frustules could be observed. The result indicated that sufficient amounts of frustules can be produced when cells are cultured in media containing up to 0.5 mg/L DHH. Furthermore, the images proved that the purity of the frustule samples was high because no unknown objects were observed. In all samples, the frustule diameter was about 20 μm.

Figure 2 (B, D, and F) shows a magnified image of the surface structures of frustules. Frustules in all samples were confirmed to have nanoporous structures. In media containing 0, 0.1, and 0.5 mg/L of DHH, nanopores with diameters of 38 (4), 39 (5), and 41 (5) nm, respectively, were obtained. There was no significant difference in the pore sizes of frustules.

In order to confirm the presence of Pt, XPS spectra of dried frustule samples were measured. For samples cultured in the presence of DHH, 2 significant peaks were observed at approximately 88 eV and 361 eV. The peaks were not observed for native frustule samples as shown in Fig. 3A.

It is known that typical Pt reveals 2 specific peaks at 71 eV and 315 eV. In the case of chemical compounds of Pt such as platinum oxides, the peaks should be of a higher value. Although further experiments are required to draw a conclusion, our results suggested that Pt formed some chemical bonds in the frustules.

In conclusion, our experiments indicated that Pt can be injected into frustules at the time of cultivation of living diatom cells. We hope this knowledge will be useful in the development of sensors, electronic devices, and photocatalysts using frustules.

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