
Assessment of diatom growth using fluorescence imaging

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Abstract. Monitoring the growth of diatom microalgae requires sensitive, fast, and easy-to-use methods for selecting the most productive strains for further research and application. Here we propose a novel, rapid and non-destructive approach for monitoring the viability and growth of diatom colonies using the IVIS fluorescence imaging system. The fast-growing *Achnanthes sibiricum* diatoms were cultivated in favorable laboratory conditions in a specially designed bioincubator for 100 days. The results showed that the diatoms reached the end of the exponential growth phase after 56 days of culture, after which there was a slight decrease in fluorescence intensity. This method relies on using chlorophyll *a* fluorescence without any sample preparation and has proved to be successful for monitoring algae growth in an incubator during long-term batch cultivation. This result paves the way towards controlled diatom cultivation aimed at collecting diatom-derived bioactive compounds for various purposes.

1. Introduction

Diatoms, unicellular photosynthetic microalgae, which provide up to 20% of the oxygen on planet Earth and account for about 40% of marine primary productivity [1], are among the most influential biological groups from an ecological point of view. One of the essential features that distinguish them from other organisms is their hierarchically structured, highly porous cell wall - the frustule formed by amorphous silicon dioxide. Due to their wide distribution, low cost, structure, ease of modification, good mechanical

and optical properties, and biocompatibility, diatom shells are widely used for various purposes [2,3]. In addition, they contain a significant concentration of chromophores such as chlorophylls *a*, *c*, and the carotenoid fucoxanthin [4] and can be easily cultivated in laboratory conditions, given the optimal temperature, light, salinity, and nutrients. Bioactive substances obtained from properly grown diatoms can be used for pharmaceutical and biomedical purposes. Therefore, it is necessary to use highly efficient methods to track their growth not only in laboratory conditions but also in natural environments to facilitate the harvesting of diatom cultures.

In this study, we proposed a novel approach for monitoring the growth of diatoms *Achnanthes sibiricum* (*A. sibiricum*) in a specially assembled incubator under optimal conditions for 100 days using the IVIS SpectrumCT In vivo Imaging fluorescence tomography system. To the best of our knowledge, this non-invasive, easy-to-use monitoring method, based on the chlorophyll fluorescence, has not previously been used for such purposes. We believe that this method is up-and-coming for further applications in aquaculture and bioreactor practice as it expands the possibilities of the cultivation of diatoms and their observation without affecting the integrity of the cells.

2. Materials and methods

2.1. Cultivation of diatom algae

A. sibiricum strain 256, isolated from the coastal bottom of Lake Baikal, was cultivated in the DM medium [5] in Eppendorf cell culture flasks "T-25" (Helicon, Moscow) for 100 days. Their growth was maintained under optimal conditions ($12 \pm 1^\circ\text{C}$, 12:12 day-night cycle) in the reactor. The algae reactor was assembled around 2 main elements: time controller (Feron TM41) with low-flickering PWM lamp brightness regulator (10 kHz operating frequency) and thermal controller (STL0052) with 2 DS18B20-IP67 probes. In the thermal regulation circuit, the purified water from the growth chamber is sucked by an impeller pump into a water block attached to the 80W Peltier element's cold side. The hot side of the Peltier element is cooled by a maintenance-free liquid cooling system. Continuous temperature monitoring in the growing chamber possesses feedback to the water chilling system. The growth chamber represents foamed polyethylene-insulated glass tank. Flasks with algal cultures are located on the frame. A maintenance rack, mounted around, contains slots for inlet-outlet hoses and thermal probes. The top part of the chamber is covered with a thick acrylic thermal screen. A lighting system is located on the top of the thermal screen: two LED strips on the aluminum frame with LEDs that correspond to the bands of maximum light absorption by chlorophyll. A schematic representation of the diatom algae incubator is shown in Figure 1.

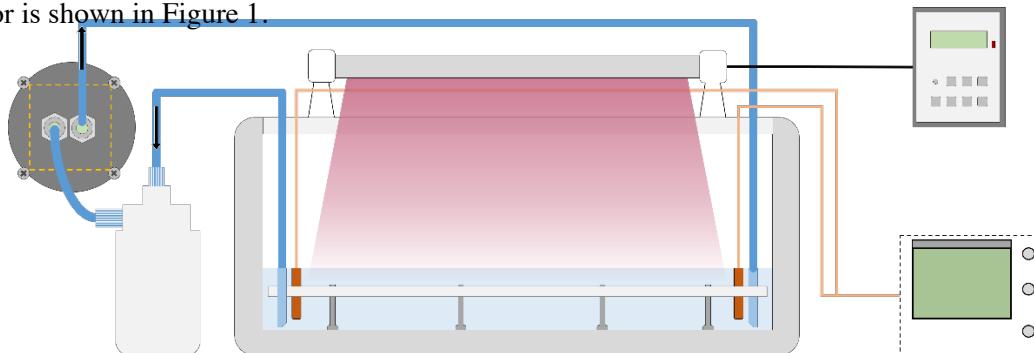


Figure 1. A simple scheme of the algae reactor

2.2. Scanning electron microscopy

The morphology of precleaned diatom frustules was investigated using scanning electron microscopy (Quattro S, Thermo Fisher Scientific) at an accelerating voltage of 3 kV. Acid treatment was performed as described before [5] to remove organic components from diatom shells.

2.3. Fluorescence measurements with IVIS SpectrumCT In vivo Imaging system

The growth of diatom cells was monitored over 100 days using IVIS CT Spectrum In Vivo Imaging System (Xenogen Corp., CA, USA). The fluorescence signal from flasks directly transferred from the reactor to the imaging chamber was obtained with a 465/680 nm excitation/emission filter and analyzed using Living Image software 4.7.3. All the fluorescence images were acquired with 3 s exposure.

2.4. Fluorescence spectroscopy

Fluorescence spectroscopy measurements of diatom suspensions were carried out in a 96-well plate with the Infinite M Nano+ dual-mode microplate reader (Tecan Trading AG, Switzerland). The excitation wavelength was 465 nm, while the emission was observed in the region 620–750 nm.

3. Results and discussion

Fluorescence visualization of flasks containing diatoms by IVIS imaging system is shown in Figure 2a. According to Figure 2a, a strong fluorescence signal originating from chlorophyll *a* upon excitation at 465 nm was successfully obtained. Total radiant efficiency increases with increasing incubation time up to 56 days, after which we observed the slight decrease (Figure 2b), indicating the end of the exponential growth or declining relative growth phase, followed by a relatively stable period where the cell division is slowed down due to the lack of resources necessary for the development. During the 100 days of culturing, we didn't encounter the substantial decline of fluorescence or the final death phase when nutrients are depleted to a level incapable of sustaining growth. The results are in line with a particular path of microalgae growth [6].

A. sibiricum is monoraphid pennate diatom species with bilateral symmetry, whose raphe is present only on one valve of the frustule, as shown in Figure 2c. The length of the cell wall is in the range 7.5–10.5 μm , while the width ranges from 1.7 μm to 2.7 μm .

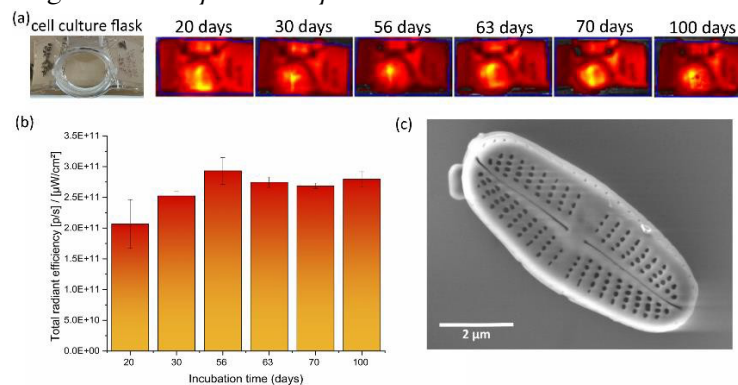


Figure 2. (a) A top view of cell culture flask containing diatoms (left) and IVIS fluorescence images, (b) A total radiant efficiency of *A. sibiricum* diatom cells during the cultivation period, (c) SEM image of diatom frustule.

The results of fluorescence measurements were also compared with fluorescence spectra collected during 100 days of incubation. Figure 3a shows the fluorescence intensity spectra of *A. sibiricum* cells, obtained by excitation at 465 nm. An emission band with a maximum at about 680–682 nm is attributed to chlorophyll *a*. Fluorescence intensity was normalized to one by dividing the intensity of all points by the maximum intensity at a wavelength of 680 nm to compare the obtained results with the normalized total radiant efficiency depending on the sampling days. According to the obtained spectra, the maximum values of fluorescence intensity were achieved after 30 days of cultivation. The total radiant efficiency reaches the maximum after 56 days in the incubator (Figure 3b).

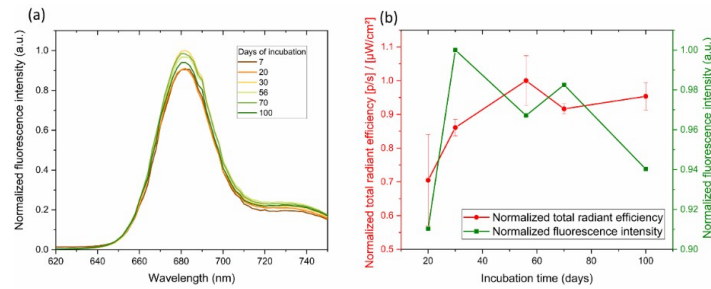


Figure 3. (a) Fluorescence spectra of *A. sibiricum* cells, (b) Comparison of normalized total radiant efficiency and normalized fluorescence intensity at 680 nm depending on the incubation time

It should be noted that for spectroscopic measurements, we extracted the culture, which introduces certain errors that necessarily appear when such a factor as the intensity of shaking the medium when taking an aliquot interferes. On the other hand, using the IVIS system, we measured the total amount of chlorophyll in the volume without taking aliquots and disrupting cell integration. In our previous work [7], we performed the cell counting the first four days of cultivation and observed the lag phase and the onset of the exponential growth of the culture. However, small species of diatoms such as *A. sibiricum* begin to form clusters a few days after seeding, which makes the counting almost impossible with more prolonged cultivation, so we continued to monitor their growth using the IVIS setup, choosing long enough time intervals between measurements to observe differences in fluorescence signals.

4. Conclusions

In this study, we monitored the growth of *A. sibiricum* freshwater diatoms cultivated in the incubator for 100 days via the IVIS fluorescence imaging technique. Owing to the presence of chlorophyll, this method allowed us to quickly assess the viability and growth of diatoms during long-term batch cultivation. The successful demonstration of the IVIS system for the visualization and monitoring of diatom cultures promises further advances in diatom culture and allows experiments to be carried out without compromising cell integrity, which is of paramount importance for aquaculture monitoring.

Acknowledgments

The IVIS fluorescence imaging was performed using the equipment of the "Bioimaging and Spectroscopy" Core Facility of the Skolkovo Institute of Science and Technology, while the SEM images were acquired using the equipment of the Advanced Imaging Core Facility. The cell culture of *A. sibiricum* strain 256 was provided from the culture collection of the cell ultrastructure department of the Limnological Institute of the Siberian Branch of the Russian Academy of Sciences (Irkutsk).

References

- [1] Kale A, Karthick B. The diatoms. *Resonance*. 2015 **20**(10) 919–30
- [2] Mishra M, Arukha AP, Bashir T, Yadav D, Prasad GBKS. All new faces of diatoms: Potential source of nanomaterials and beyond. *Front Microbiol*. 2017 **8**1–8
- [3] Sprynskyy M, Pomastowski P, Hornowska M, Król A, Rafińska K, Buszewski B. Naturally organic functionalized 3D biosilica from diatom microalgae. *Mater Des*. 2017
- [4] Kuczynska P, Jemiola-Rzeminska M, Strzalka K. Photosynthetic pigments in diatoms. *Mar Drugs*. 2015 **13**(9) 5847–81
- [5] Bedoshvili Y, Gneusheva K, Popova M, Morozov A, Likhoshway Y. Anomalies in the valve morphogenesis of the centric diatom alga *Aulacoseira islandica* caused by microtubule inhibitors. *Biol Open*. 2018 **7**(8) 1–10
- [6] Farag I. Resources Conservation in Microalgae Biodiesel Production. *Int J Eng Tech Res*. 2013 (1) 2321–0869
- [7] Cvjetinovic J, Bedoshvili Y, Nozdriukhin D, Efimova O, Salimon A, Volokitina N, et al. In situ fluorescence/photoacoustic monitoring of diatom algae. *Proc. SPIE 11641*, 116410G 2021