Flotation of *Botryococcus braunii* after ionizing irradiation

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Botoryococcus braunii is a green microalga having the ability to produce high amounts of oil, making it suitable for biofuel production. Aiming at the industrial use of this species, several studies, including one on the development of new strains having a high growth rate and cost-effective cultivation methods, are being conducted.[1,2] Another important issue is the harvesting method of *B. braunii* after cultivation. Since this alga contains much oil after cultivation, its specific gravity is low. Thus, the collection of the algae using centrifugation is difficult. Here, we have reported a novel phenomenon of *B. braunii*, in which *B. braunii* floats on the surface of media after the irradiation of ionizing radiation. By applying this phenomenon, an effective method for the collection of the algae can be developed.

The flotation of *B. braunii* was first observed after Ar-ion irradiation. A strain, Hojo, derived from the Hojo-Oh pond in Tsukuba city was cultured and irradiated with Ar ion beams with an LET of 290 keV/μm and a dose range of 25-150 Gy. The flotation was observed 2 h after irradiation. The ratio of floated algae increased in a dose-dependent manner. For example, 11.9% of the algae floated with 25-Gy irradiation, whereas 100% of the algae floated with 100-Gy irradiation. This phenomenon was observed after X-ray irradiation as well. We adopted X-ray irradiation at a dose of 50 Gy for further investigation, in which both floated and sunken algae were observed 2 h after irradiation.

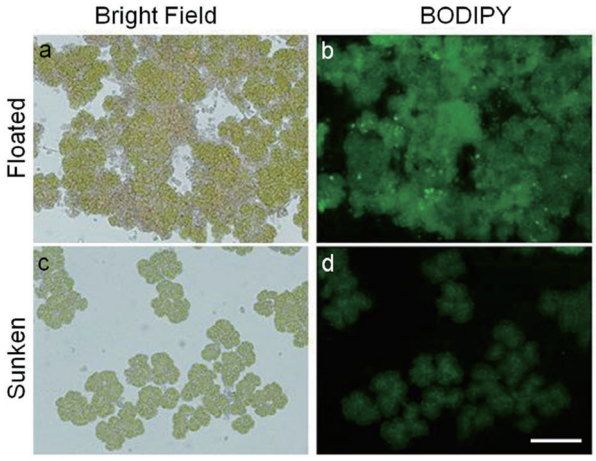

Fig. 1 Microscopic observation of *B. braunii* colonies after X-ray irradiation. Floated and sunken algae were stained with BODIPY and observed by fluorescent microscopy. Scale bar = 50 μm.

The colonies of floated and sunken algae were observed by microscopy after staining with the lipophilic green fluorescent dye, BODIPY (Fig. 1). The colonies of floated algae had large extracellular matrices. These matrices showed higher intensities of BODIPY fluorescence under fluorescent microscopy, indicating that oil was accumulated in the matrices. By contrast, the colonies of sunken cells had less extracellular matrices. This result indicates that floated algae may accumulate more oil than sunken ones.

To investigate the relationship between oil contents and the flotation of the algae, we measured the dry weight and oil weight of both floated algae and sunken algae after irradiation, respectively (Table 1). We also measured the dry weight and oil weight of the algae before the irradiation of X-rays. From these measurements, the oil content in each sample was calculated. Before the irradiation, the oil content was 34.8%. However, 2 h after irradiation, the floated algae showed an oil content of 53.1%. Such an increase of oil content was not observed in the sunken algae. These data indicate that the floated algae accumulate more oil.

By applying this phenomenon, algae having high oil contents can be selectively harvested. Moreover, the sunken algae can be continuously cultured after the removal of the floated algae for the next harvesting. Thus, we suggest that an efficient method of the collection of *B. braunii* can be developed with this approach. To clarify why and how the algae are floated after irradiation, further studies will be needed.

Table 1 Oil contents of irradiated and non-irradiated algae.

	Dry weight (mg)	Oil weight (mg)	Oil content (%)
Before irradiation	71.8	25.0	34.8
Floated algae	30.9	16.4	53.1
Sunken algae	72.5	21.0	29.0

References
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2) P. Cheng et al., Biores. Technol. **138**, 95 (2013).

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