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Characterization of Intracellular Iodine Accumulation by Iodine-Tolerant Microalgae

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Abstract

High iodine concentrations are thought to be toxic to algal growth. However, in this study, among tested 10 algae, 6 species (*Bellerochea* sp., *Chlamydomonas reinhardtii*, *Dunaliella tertiolecta*, *Isochrysis galbana*, *Skeletonema costatum*, *Tetraselmis* sp.) exhibited high iodine tolerance and 2 species showed positive growth responses (*Emiliania huxleyi*, *Gephyrocapsa oceanica*). On the other hand, growth of *Chlorella kessleri* and *Chaetoceros sociale* were strongly inhibited. Interestingly, the effect of iodine differed among algal species and between the molecular forms of iodine, namely iodide (I^-) or iodate (IO_3^-) ions. Neutron activation analysis indicates that *E. huxleyi* intracellularly accumulated more iodine when grown in KIO_3 than when grown in KI. Such high intracellular iodine accumulation may stimulate the growth. Moreover, *E. huxleyi* accumulated ca.10 times more iodine than that found in seawater, suggesting that microalgae can be used in iodine-related industries for extracting iodine from seawater and iodine-containing wastewater.

Keywords: growth stimulation; growth inhibition; haptophyte; iodine; iodine accumulation

1. Introduction

Iodine is an abundant, biophilic, and redox-sensitive trace element in the ocean [1]. It plays an important physiological role as an essential element in higher animals. In particular, the thyroid hormone had long been known as the only iodine-containing substance whose physiological function was

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understood. Therefore, physiological studies on iodine atoms mainly focus on organisms that possess thyroid glands, i.e., higher animals such as humans [2].

Consequently, the role of iodine is far less studied in organisms without the thyroid glands than in organisms that possess them. In higher plants, iodine is not an essential element for growth but is considered to have an inhibitory effect by causing Akagare disease, which exhibits red spots on leaves as well as death in farm crops [3]. Growth inhibition by iodine is also reported in the green microalga *Chlorella vulgaris* [4]. From these observations, iodine was thought to be toxic to both land plants and freshwater algae, although information supporting this notion is quite limited.

Some physiological studies on iodine in macroalgae have been performed, because brown and red algae are known to accumulate high concentrations of iodine [5]. In particular, extremely high iodine concentrations are present in the brown alga *Laminaria digitata* with a maximum close to 5% of its dry weight [6]. This shows that brown algae have a high ability to accumulate iodine at concentrations as low as 0.3–0.5 μM in seawater [6] and utilize iodine as an antioxidant [7]. Furthermore, Hsiao [8] reports that the brown alga *Petalonia fascia* requires iodine for growth, morphogenesis, and reproduction. Iodine is also required for the growth of the brown alga *Ectocarpus fasciculatus* [9] and the red alga *Polysiphonia urceolata* [10]. On the other hand, growth inhibition by high iodine concentrations is reported in the brown alga *E. fasciculatus* [9] and in the red alga *Goniotrichum elegans* [10]. Brown algae are also known to transform iodate to iodide and release stored iodine as iodide, volatile iodocarbons, and molecular iodine [11, 12].

Some data from oceanological, geoscientific, and biological studies suggest a relationship between iodine and microalgae. First, diatoms produce thyroid hormones or at least hormone-like substances which play an essential role in the development of adult-stage sea urchins [13]. Second, microalgae participate in the iodate (IO_3^-)-to-iodide (I^-) speciation and emit iodine as monoiodomethane into the marine environment [1, 14, 15, 16, 17]. Third, microalgae are thought to play an important role in the geochemical functioning of the global iodine cycle [1, 18]. These reports strongly suggest that microalgae clearly exhibit a different response to iodine compared to higher plants and that their physiology is closely associated with their iodine usage. In this context, basic physiological research is required. This study examines the effects of high concentrations of iodine, iodate, and iodide levels on the growth of 10 algae species from 4 classes of eukaryotes. Iodine-accumulating microalgae were observed to exhibit iodine tolerance. The biological significance of iodine in algae and their possible role in the geochemical cycle of iodine is also discussed.

2. Materials and methods

2.1. Material

Strains of the marine haptophycean algae *Emiliania huxleyi* (Lohmann) Hay et Mohler (NIES 837) and of *Gephyrocapsa oceanica* Kamptner (NIES 838) and *Isochrysis galbana* Parke (UTEX LB 2307) were obtained from Drs. I. Inouye (University of Tsukuba, Japan) and M. Okazaki (Tokyo Gakugei University, Japan), respectively. Two strains of freshwater chlorophycean algae, *Chlamydomonas reinhardtii* Dangeard (strain 137c mt^+) and *Chlorella kessleri* Fott et Nováková [K&H] (strain 11h), were obtained from Dr. Y. Tsubo (Kobe University, Japan) through Dr. H. Takeda (Niigata University, Japan) and from the algal culture collections of Göttingen University (Germany) by Dr. S. Miyachi (University of Tokyo), respectively. The marine chlorophycean alga *Dunaliella tertiolecta* Butcher (strain CS-175) was obtained from the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Australia) by Dr. A. Goyal (Michigan State University). The marine prasinophycean alga *Tetraselmis* sp. was obtained from Dr. S. Tunkijjanukij (Kasetsart University, Thailand). The marine diatoms

Chaetoceros sociale Lauder (NIES 377) and *Skeletonema costatum* (Grev.) Cleve (NIES 353) were obtained from the National Institute for Environmental Studies (NIES, Japan). *Bellerochea* sp. was kindly provided by Dr. N. Okamoto (University of Tsukuba).

For the stock cultures, the haptophycean algae were grown in natural seawater medium enriched with Erd-Schreiber medium (ESM) solution containing 10 nM sodium selenite in the place of soil extracts. For experimentation, artificial seawater (Marine Art SF-1; Tomita Pharmaceutical Co., Ltd., Tokushima, Japan) enriched with ESM solution containing 10 nM sodium selenite was used [19]. The green algae *C. reinhardtii C. kessleri,* and *D. tertiolecta* were grown in High Salt minimum medium (HSM) [20], MC medium [21], and defined medium [22], respectively, on 1.5% (w/v) agar slants for the stock cultures and in suspension culture for experiments. The prasinophycean alga *Tetraselmis* sp. was grown in Marine Art SF-1 enriched with Daigo's IMK medium (Wako, Osaka, Japan), and the diatoms were grown in f/2 medium [23] enriched with $Na_2SiO_3 \cdot 9H_2O$ at 10 $mg \cdot L^{-1}$ [24]. All stock cultures were maintained under low light intensity (24 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20°C. Growth tests were subsequently performed in an L-shaped tube (Taitec, Tokyo, Japan) under continuous high light intensity (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20°C; the tubes were hand shaken daily for experiments in Figure 1 and 2. For the experiments in Figure 3, *E. huxleyi* was also cultured in a flat, oblong glass vessel (inner height, 25 cm; inner width, 10 cm; inner thickness, 2.5 cm) containing 0.5 L of the medium at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with continuous bubbling of air containing approximately 0.04% CO_2 at a flow rate of 100 $mL \cdot min^{-1}$ as described by Iwamoto and Shiraiwa [25]. Strains and culture conditions are summarized in Table 1.

Table 1. Algae and growth media used in this study.

Species	Medium for stock culture	Medium for experimentation	Strain condition
Haptophyceae			
Emiliania huxleyi (NIES 837)	NS-ESM	MA-ESM	unialgal
Gephyrocapsa oceanica (NIES 838)	NS-ESM	MA-ESM	unialgal
Isochrysis galbana (UTEX LB 2307)	NS-ESM	MA-ESM	unialgal
Chlorophyceae			
Chlamydomonas reinhardtii (strain 137c mt+)	HSM	HSM	axenic
Chlorella kessleri (strain 11h)	MC medium	MC medium	axenic
Dunaliella tertiolecta (strain CS-175)	defined	defined	axenic
Prasinophyceae			
Tetraselmis sp.	MA-IMK	MA-IMK	unialgal
Diatoms			
Chaetoceros sociale (NIES 377)	f/2	f/2	axenic
Skeletonema costatum (NIES 353)	f/2	f/2	axenic
Bellerochea sp.	f/2	f/2	unialgal

NS, natural seawater; ESM, Erd-Schreiber medium; MA, artificial seawater Marine Art SF-1; IMK, Daigo's IMK medium. MA and NS contain 0.42 μM iodide and natural iodine levels, respectively. No iodine was present in HSM, MC, or defined media.

2.2 Analysis

2.2.1 Growth assay

Changes in the cell concentrations during cultivation were monitored by measuring the optical density using a spectrophotometer (Spectronic 20A or UV-2200; Shimadzu, Kyoto, Japan) at 750 nm (expressed as OD_{750}); diatoms were measured at 680 nm (expressed as OD_{680}) to enhance the measurable quantity. To monitor growth in large-scale *E. huxleyi* cultures for neutron activation analysis, cell number was determined under a microscope using a Thoma's hemocytometer; chlorophyll concentration was calculated as described by Jeffrey and Humphrey [26].

2.2.2 Neutron activation analysis

Iodine was quantified by neutron activation analysis at the following phases of cell growth: at the start of culturing, early linear phase, late linear phase (where the effect of iodide on growth began to appear), stationary phase in the control and iodide-supplemented cultures, and stationary phase in iodate-supplemented culture. Samples for neutron activation analysis were prepared as follows: algal cells with a density factor of 50 (OD_{750} × suspension volume [mL]) were harvested by centrifugation (1200 × *g* for 10 min) using a swing rotor after the addition of 0.01% polyoxyethylene (20) sorbitan monolaurate to prevent the cells from adhering to the tubes [27]. The pellet was washed twice with 300 mM sucrose and dried on a membrane filter (47 mm, 12.0 μm, Nuclepore Polycarbonate; Whatman, Brentford, UK) using a lyophilizer (Lyphlock 6; Labconco, Kansas City, MO, USA). The dried sample was sealed in a double polyethylene bag with an approximately 2 mg 0.1% Au/Al alloy (IRMM-530) flux-monitor wire and irradiated using the JRR-4 research reactor at the Japan Atomic Energy Research Institute (JAERI, Tokai, Japan) for 20 s. The γ-ray spectrum around 443 keV from ^{128}I was counted for 500 s [28]. The iodine concentration was determined by a calibration curve obtained by the signals of standard IK solution (Wako, Osaka, Japan). Cell volumes of *E. huxleyi* were calculated on the basis of packed cell volume determination as reported by Sekino and Shiraiwa [29].

3. Results and Discussion

3.1. Effect of high iodine on the algal growth

Fig. 1 shows the effects of iodine—1 mM iodide (I^-) or iodate (IO_3^-)—on the growth of 10 algal species from 4 classes of microalgae. The concentration of iodine corresponds to a level around 2000–3000 times greater than that in natural seawater, which is reported to range from ca. 0.3–0.5 μM in open sea [30, 31, 32]. The results indicate that 1 mM KIO_3 has various effects on growth depending on the algal species. In the coccolithophorids *E. huxleyi* and *G. oceanica*, growth increased by around 20% at the late linear and stationary growth phases (Fig. 1A, B). On the other hand, the growth of the diatom *C. sociale* was severely inhibited by iodate. The growth of other algae species was not affected by the addition of KIO_3, namely the green algae *C. reinhardtii* and *D. tertiolecta*, the prasinophycean alga *Tetraselmis* sp., the diatoms *Bellerochea* sp. and *S. costatum,* and the haptophycean alga *I. galbana* (Fig. 1C–H). When 1 mM KI was added, the growth of the diatom *C. sociale* and the green alga *C. kessleri* was suppressed by 20–30% (Fig. 1J, I). However, 1 mM KI had no effect on other algal species tested. The observed growth stimulation and inhibition were considered to be caused specifically by iodine and not potassium, because each iodine-enriched medium used in this study contained the same concentration of

potassium. In addition, the growth of *E. huxleyi* was stimulated by 1 mM $NaIO_3$ but not by 1 mM NaI (data not shown).

Fig. 2 shows the dose responses of *C. sociale*, *C. kessleri*, and *E. huxleyi* to iodide and iodate. In *E. huxleyi*, KIO_3 stimulated growth between 0.1 and 20 mM (Fig. 2A); KI did not stimulate growth even at 20 mM and actually inhibited it slightly (by less than 10%) (Figure 2B). The growth of the diatom *C. sociale* was inhibited by KIO_3 concentrations above 0.1 mM; its growth was suppressed by approximately 90% at 1 mM (Fig. 2A), although 1 mM KI resulted in only 15% inhibition (Fig. 2B). The growth of

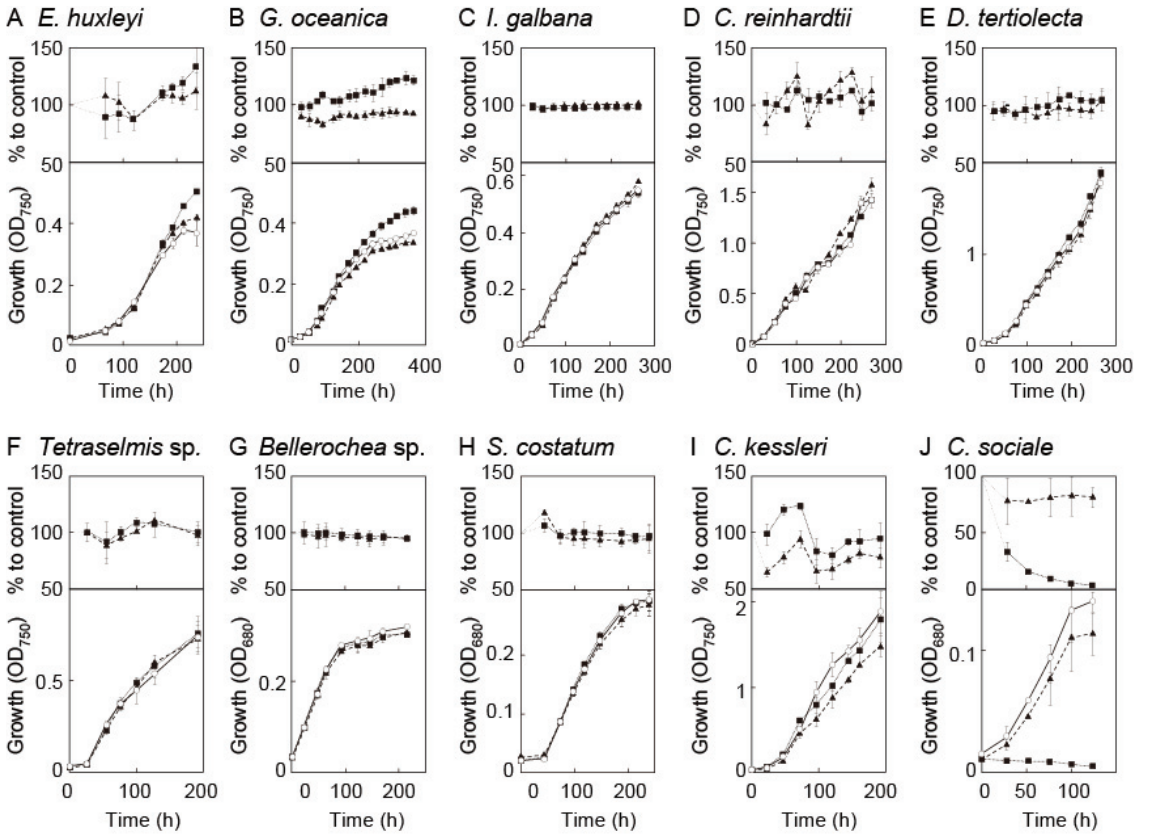

Fig. 1. Effect of iodine on the growth of various microalgae: *E. huxleyi* (A), *G. oceanica* (B), *I. galbana* (C), *C. reinhardtii* (D), *D. tertiolecta* (E), *Tetraselmis* sp. (F), *Bellerochea* sp. (G), *S. costatum* (H), *C. kessleri* (I), and *C. sociale* (J). Lower panel, growth curve expressed by changing OD_{750} or OD_{680}. Upper panel, ratios of OD values of iodine-treated to non-treated cultures. Open circles with solid line, control; closed triangles with dashed line, +1 mM KI; closed squares with dotted line, +1 mM KIO_3. Typical growth curves are shown here, but all experiments were repeated more than twice with duplicate samples.

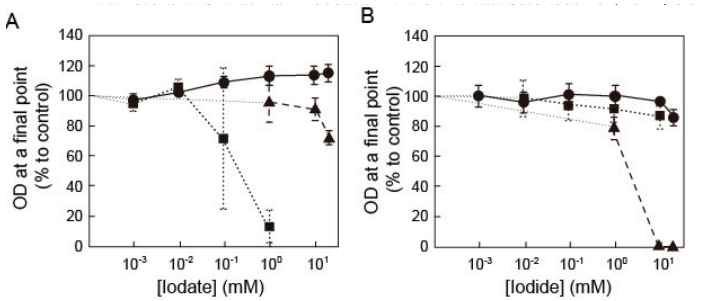

Fig. 2. Effect of various concentrations of iodide (A) and iodate (B) on the growth of *C. sociale* (squares), *E. huxleyi* (circles), and *C. kessleri* (triangles).

C. kessleri was completely inhibited in 10 mM KI (Figure 2A) and by 30% in 20 mM KIO_3 (Fig. 2B).

These results demonstrate that high iodine concentrations affect algal growth, including iodine ion-dependent inhibition and stimulation. The inhibitory effects of iodine on growth are well documented in photosynthetic organisms such as *Chlorella* [4]. Akagare disease is a well-known example of iodine-induced plant growth inhibition. This disease was first reported in Japan affecting rice plants grown on reclaimed land. It has been shown that type III Akagare disease (one of 3 types) is due to the inhibition of photosynthesis by high concentrations of iodide (I^-) above 1 ppm, which corresponds to 8 μM. In macroalgae, iodine-induced growth inhibition is reported in the brown algae *E. fasciculatus* [9] and *G. elegans* [10] above iodine concentrations of 64 and 0.4 μM, respectively. Regarding iodine toxicity, Greenfield [4] reports that short-term treatment with 10 mM iodide harms photosynthesis by inhibiting CO_2 fixation in the freshwater green alga, *Chlorella vulgaris*. In the present study, this result was confirmed in that the growth of *C. kessleri* was completely inhibited in the presence of 10 mM KI (Fig. 2B). In addition to iodide, iodate-induced growth inhibition was observed in the diatom, *C. sociale* (Fig. 1J). Interestingly, the growth of *C. sociale* was not affected by 1 mM KI but was strongly suppressed by 1 mM KIO_3 (Fig. 2A). The difference in the sensitivity to different species of iodine may be due to different transport and accumulation activities, because algae are known to take in only certain chemical forms of iodine [1, 33]. Iodate is actively absorbed by microalgae as is the case in the diatoms *Thalassiosira oceanica* and *S. costatum*, the haptophycean alga *E. huxleyi,* and the green alga *D. tertiolecta*, although these mechanisms are poorly understood [1]. In the case of brown algae, iodide is taken up after it is converted to HIO by haloperoxidase; it subsequently crosses the plasmalemma, resulting in a facilitated diffusion mechanism [33].

This study shows that all algae tested in this study except *C. sociale* can grow in the presence of≧1 mM iodine, either in the form of iodide or iodate (Figs. 1, 2). The results indicate that some microalgae possess high tolerance to iodine; this is distinctive, because disease is caused by 8 μM iodine in rice plants. The results also raise the question of whether such iodine tolerance mechanisms are due to an inability to transport iodine, low iodine accumulation activity, or other mechanisms. The intracellular concentration of iodine in *E. huxleyi* grown in the presence of 1 mM KI and KIO_3 was determined using neutron activation analysis during a complete growth cycle (Fig. 3). The concentration increased as the cell stage progressed. At the stationary phase, cultures supplemented with iodate and iodide, and controls had concentrations of 0.53, 0.14, and 4.4 μM, respectively (Fig. 3B, phase d). Because an appreciable amount of iodine accumulated in the cells, this suggests that the photosynthesis and metabolism of these cells are not disturbed by iodine as is the case in *Laminaria* species.

The stimulation of growth by high iodate concentrations was observed in the coccolithophorids *E. huxleyi* and *G. oceanica* for the first time in the present study (Fig. 1). The neutron activation analysis shows that *E. huxleyi* accumulates much more iodine in iodate-supplemented culture than in an iodide-supplemented one, especially at the stationary phase in which cell growth was stimulated (Fig. 3). This

result indicates that iodine plays an important physiological role in these algae. Iodine possibly functions as an antioxidant, which was also recently suggested by Küpper et al. [7] in kelp cells.

3.2. Iodine accumulation by microalgae

Neutron activation analysis revealed that the internal iodine concentration in *E. huxleyi* cells was 4.4 μM, which is 10 times that in the sea water (Fig. 3). Since the coccolithophorid *E. huxleyi* functions as a biological pump that transports carbon and other materials from the sea surface to sediment, the bio-concentration of iodine by marine photosynthetic-calcifying organisms may greatly contribute to global iodine circulation. In the ocean, approximately 70% of iodine is found in oceanic sediments, whereas only 0.8% is present in seawater [34]. The biological pump by the coccolithophorids is thought to contribute to the concentration of iodine in underground brine-storing natural gases over geological time [18]. The results in the present study suggest that marine microalgae such as coccolithophorids act as carriers for iodine immigration and play a significant role in the global iodine cycle.

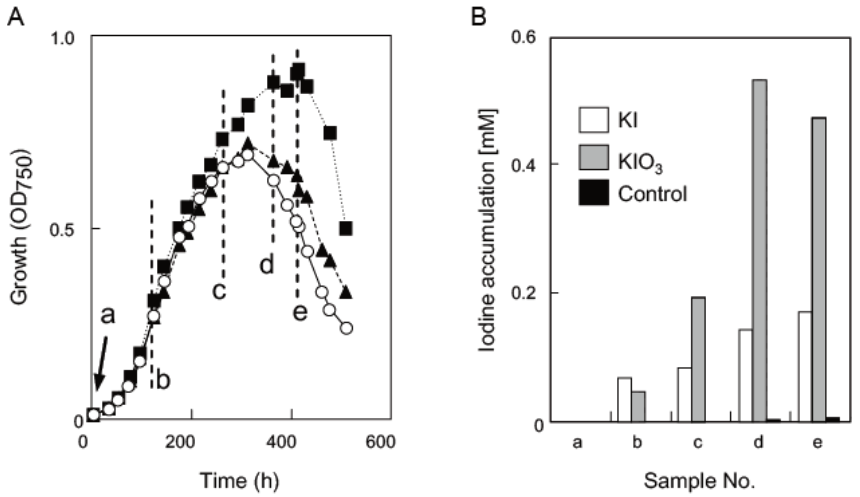

Fig. 3. Intracellular accumulation of iodine represented by the bio-concentration of iodine in *E. huxleyi* cells. A, growth curves: open circles with solid line, control; closed triangles with dashed line, +1 mM KI; closed squares with dotted line, +1 mM KIO_3. Cells were grown in flat oblong glass vessels under air bubbling. B, intracellular iodine concentrations: black bars, control; white bars, +1 mM KI; gray bars, +1 mM KIO_3. "a" to "e" indicate the time for harvesting (A) and the iodine assay using neutron activation analysis (B).

3.3. Industrial application

This study revealed that some algae had high iodine tolerance while other algae were sensitive to iodine. This finding may be applied to prevent outdoor mass culture of algae being contaminated with unwanted species, when the target species is iodine tolerant. For instance, the coccolithophorids *E. huxleyi,* and *G. oceanic* are iodine resistant algae. These algae are known to synthesize large amounts of long-chain alkenones and alkyl alkenoates, and are useful candidates for the production of renewable liquid and gaseous fuels through pyrolysis [35, 36]. An iodine supplement in the growth medium could contribute to a unialgal mass culture.

The results of this study also reveal the ability of microalgae to accumulate iodine. This may demonstrate the potential of using microalgae to accumulate and recover iodine from seawater and iodine-containing industrial effluent and, hence, contribute to the development of a novel source of iodine.

4. Conclusion

This study demonstrates that many algae exhibit high iodine tolerance and/or positive growth responses. Furthermore, the effects of iodine differ among algal species and between the molecular forms of iodine. In addition, more iodine was accumulated in *E. huxleyi* cells grown in KIO_3 than in those grown in KI. Such high intracellular iodine accumulation may have stimulated their growth. Furthermore, *E. huxleyi* accumulates ca. 10 times more iodine than that found in seawater. These results suggest that microalgae could be used in iodine-related industries for extracting iodine from seawater and iodine-containing wastewater.

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