Direct and rapid extraction/concentration technique of useful materials from large-scale micro-algal cultivation

Japan Science and Technology Agency

Advanced Low Carbon Technology Research and Development Program (ALCA)

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Project Name Generation of Diatom Factory through Physiolomics toward a Novel Energy Source

Director Yasuhiro Kashino, Associate Professor, Graduate School of Life Science, University of Hyogo

Objective

Creation of low-carbon society through generation of diatom strains that convert carbon dioxide (CO_2) into useful materials driven by solar energy.

Mission

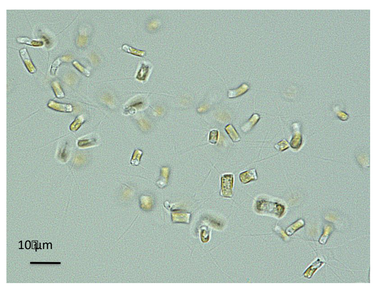
Diatom, one of micro-algae, owes ~25% of annual photosynthetic carbon assimilation on earth, which correspond to that performed by tropical rainforest. It also produce valuable metabolites such as DHA/EPA. In this research work, we are performing molecular breeding of diatom toward the generation of diatom factory that can grow fast by avoiding photoinhibition to efficiently produce valuable metabolite and oil. For this sake, in combination with physiolomics approach to understand cellular physiology based on precise genome, structural and ecological analyses, we are developing gene manipulation system including transformation and genome editing techniques. The large-scale cultivation technique and the following energetically-efficient novel method to collect useful metabolites from cells suspended in the large volume of cultivation medium are also included in the development items. By integrating these elemental technology, we are aiming to establish eco-friendly low-carbon society through photosynthetic conversion of carbon dioxide in the atmosphere or in the gas exhausted from fire plants and waste-water treatment facilities into useful metabolites.

Practical transformation method for the practical micro-algae was established for the first time in the world

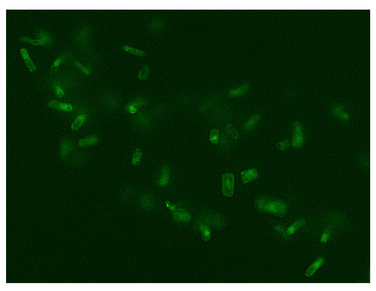
Only 2.5 years was enough for the development of our entirely novel method.

Foreign genes expressed in a marine centric diatom, *Chaetoceros gracilis*, uging a novel high-expressing promotor

~ Expression of fluorescence emitting protein ~



Bright-field image



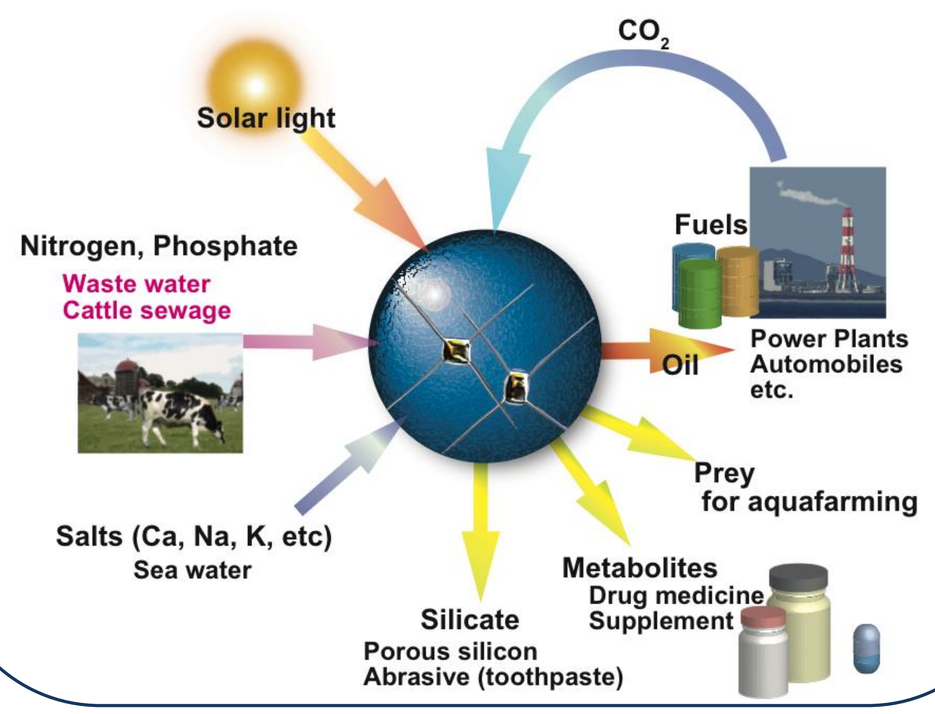
Fluorescence image of transformed diatom cells expressing Azami-Green (mAG)

Patent Pending

Ifuku et al. *Photosynth Res* 123 (2015)

Renewable and sustainable society facilitating diatom (Conversion of carbon dioxide into useful materials including BDF using solar energy)

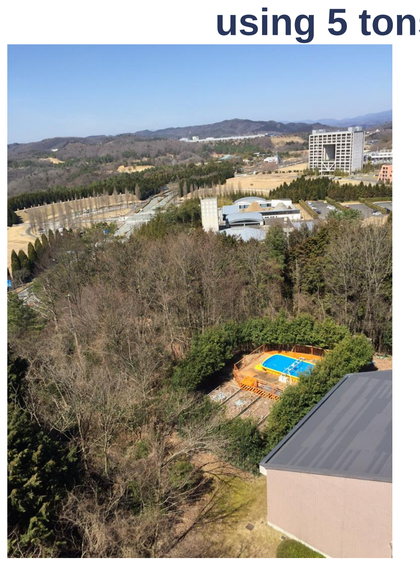
BDF : Bio Diesel Fuel



Strengthening of the physiological function of diatom by molecular engineering
Molecular breeding of diatom toward the production of high-value added metabolites

Large scale cultivation is difficult for most kind of micro-algae in general.

Outdoor cultivation of wild-type diatom using 5 tons of culture medium



15 times within 5 days!

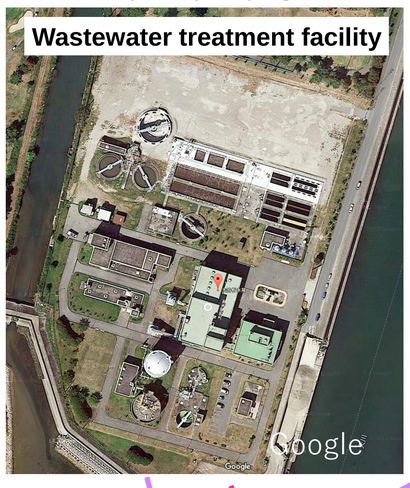
Continuous growth even after typhoon!

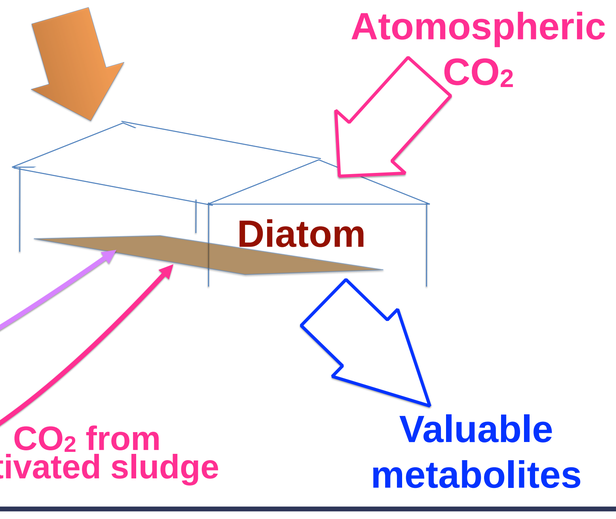
Achievement of favorable growth of shade-adapted diatom under bright solar light

Reducing cultivation cost is important for the practical application!

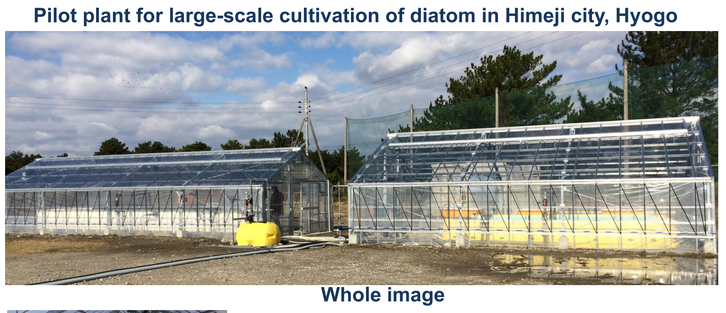
Cultivation in a large-scale pool locating in the wastewater treatment facility

(Himeji City, Hyogo)





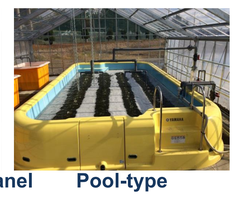
Pilot plant for large-scale cultivation of diatom in Himeji city, Hyogo



Whole image

Raceway-type

Laboratory and control panel



Pool-type

University of Hyogo

kashino@sci.u-hyogo.ac.jp

Yasuhiro Kashino

Graduate School of Life Science
University of Hyogo

<Coworkers> Assoc. Prof. Kazuhiro Ito, Graduate School of Engineering, University of Hyogo
Assis. Prof. Kentaro Ifuku, Graduate School of Biostudies, Kyoto University
Prof. Koji Maeda, Graduate School of Engineering, University of Hyogo

Further problem to be resolved even after the success of large-scale cultivation of micro-algae

Recovering process of valuable metabolites from cells after large-scale cultivation
~ Problems in the existing methods ~

Recovery of micro-algal cells from large volume of culture medium of low cell-density (~0.5% w/w)

Centrifugation
(High energy input and initial investment)
Filtration
(High initial investment; Difficulty in scalability)
Chemical and biological flocculant
(Necessity of waste liquid treatment), etc

↓

Drying of recovered cells

Time consuming air-drying,
or, energy consuming artificial drying

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Breakage of algal cells

Cell breakage with energy consuming ultrasonic or microwave

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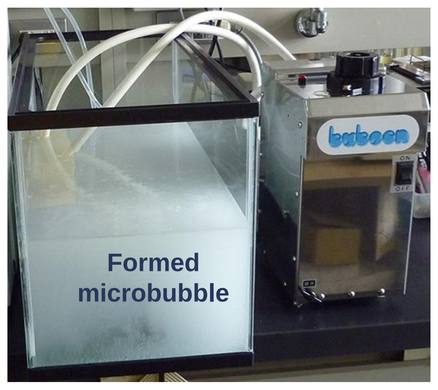
Extraction and purification of valuable metabolites

Extensively high energy input and cost

Extraction and condensation of useful materials w/o harvesting and breaking cells to reduce cost and input energy.

Practical example #1



Microbubble generator

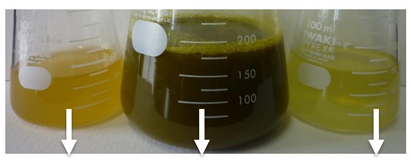
The liquid medium is continuously circulated in the equipment

Simultaneous process of cell-breakage and oil-concentration by microbubble treatment

Before treatment

Abundant foam

After 5 min treatment



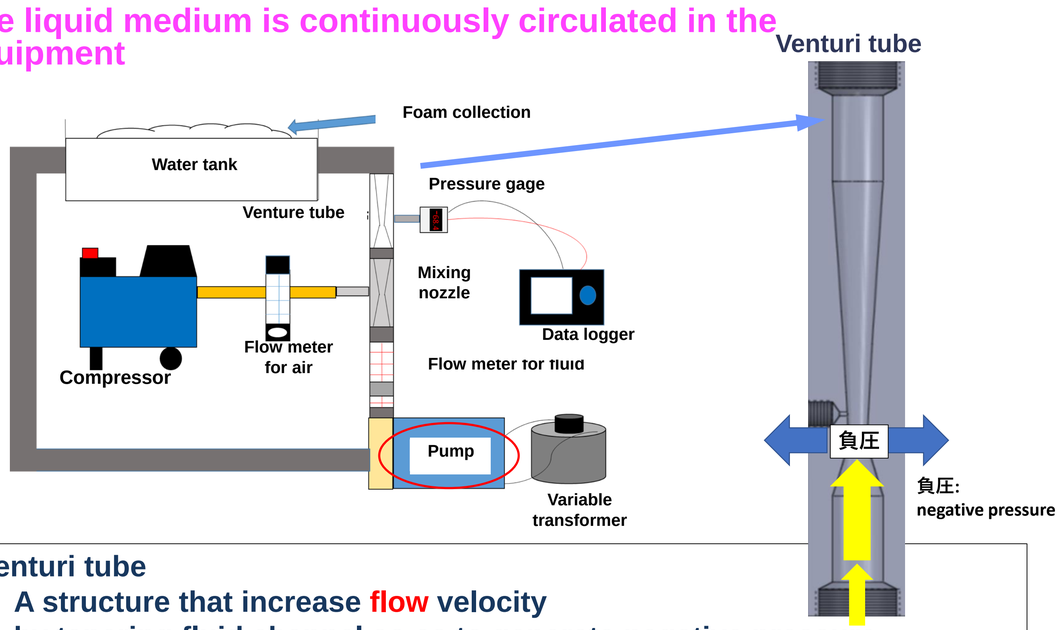
After 30 min treatment

	Before 6.90 L	Foam fraction 0.18 L (2.6%)	After 7.80 L
Triglycerole	100%	65%	18%
Fucoxanthin	100%	57%	16%
Chlorophyll *a*	100%	56%	17%
Chlorophyll *c*	100%	58%	10%

Valuable metabolites are concentrated into the foam upon the cell breakage

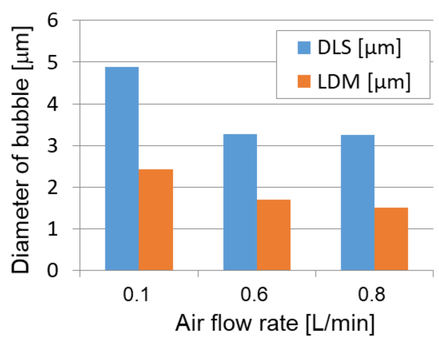
Practical example #2

The liquid medium is continuously circulated in the equipment



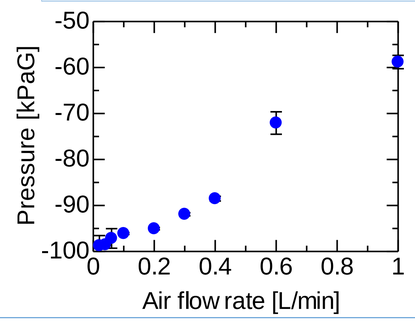
Venturi tube
A structure that increase flow velocity
by tapering fluid channel so as to generate negative pressure

Bubble size determined by Dynamic Light Scattering (DLS) Laser Diffraction Method (LDM)



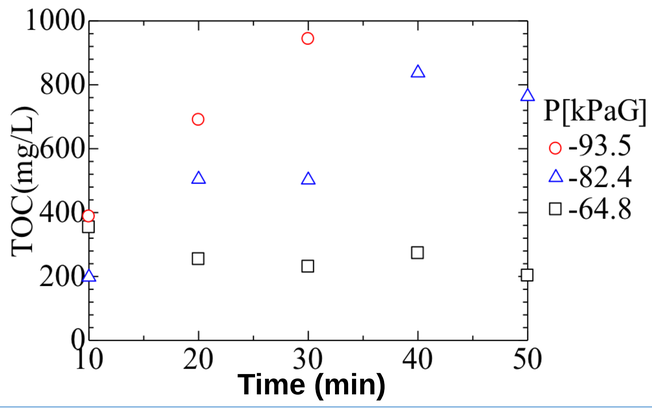
Generation of microbubbles whose diameter is 1.5 – 5.0 μm

Changes in negative pressure by input velocity of air



Negative pressure can be controlled by the air flow rate, e. g.
- 95 kPaG (at 0.1 L/min)
- 70 kPaG (at 0.6 L/min)

Kinetic change of total organic carbon (TOC) in the foam fraction



According to the increase in circulation time, TOC increased.
Longer precessing time leads to progress of cell breakage.

Higher TOC at -95 kPaG than other negative pressure.
Higher negative pressure leads to efficient cell breakage.

[Summary of technique]
Our newly developed technique breaks the cell and concentrate valuable metabolites simultaneously enabling simple and speedy total process.

[Industrial application potency]
It can be reasonably expected that productivity of valuable metabolites including biofuels by micro-algae will be highly improved by extracting and concentrating them from micro-algal cells spread in tremendous volume of cultivation medium in one-step process.

[Items to be resolved]
- ✓ Application to the large-scale cultivation, streamlining efforts
- ✓ Establishment of streamlined recovery system of foam in a large scale
- ✓ Development of purification process for the valuable metabolites concentrated in foam fraction

[Expectation to industrial firms]
- ✓ Collaboration with companies possessing powerful but energy-saving technologies for the generation of microbubble, separation of oil from water, purification of valuable metabolites
- ✓ Collaboration with companies performing production of metabolites using microbes including micro-algae

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NEDO New Energy and Industrial Technology Development Organization

University of Hyogo kashino@sci.u-hyogo.ac.jp