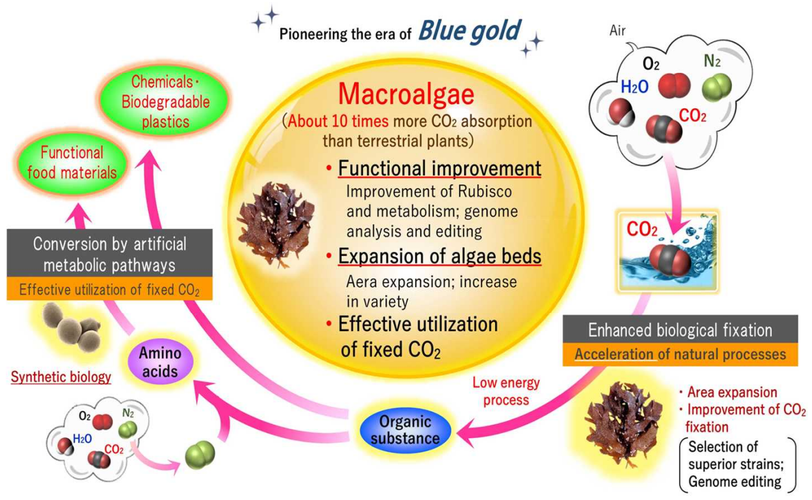
No. A-7-1E

PJ : Redesign of macroalgae for highly efficient CO2 fixation by functional modifications and their product generation

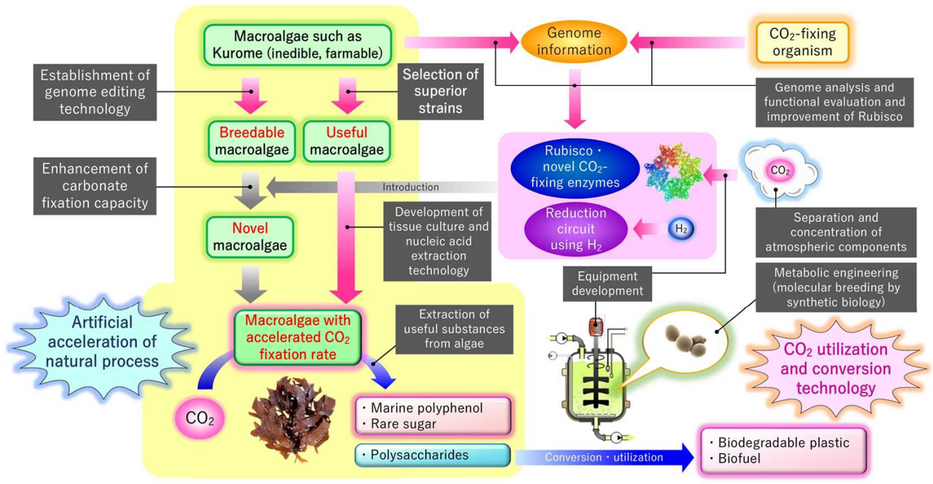
Organization: Kyoto Univ., KIT, Mie Univ., Kansai Chemical Engineering, Green Earth Institute
Contact: Mitsuyoshi Ueda (ueda.mitsuyoshi.7w@kyoto-u.ac.jp)

Nature Positive// Nature Best Solution

Blue Carbon Fixation & Negative Emission



Implementation structure & period(2022−2024)



Basic Technologies

2011-2017
CREST PJ: Development of biological technologies for complete utilization of macroalgae

2021
NEDO-pioneer research PJ: Development of basic technologies for complete utilization of macroalgae

Establishment of breeding technologies of all macroalgae (natural and artificial cultures)

Final targets (2029)

	Starch-Sugar(1G)	Lignocellulose (2G)	Algae (3G)			Algae(3G)
Raw materials	Agriculture products	Forest	Microalgae	Macroalgae		Macroalgae
Productivity (t/ha/y)	11	9	10～20	30		100
CO_2-fixation rate (kg-CO_2/m^2/y)	1.6	0.84	1.5～2.9	3.3	2029	6.0
CO_2fixation ratio	2.3	1	7.6	13		130
Biomass energy production process	simple	conplicated (Removal of lignin)	simple	simple (Key-alginate)		simple
Problems	Competing with food	Using lands	Using lands, Contamination risk, High cost	Enlargement of algae beds		No problem
Production coditions	Sunlight, CO_2	Sunlight, CO_2, Freshwater, Land,Fertilizer, Pesticides	Sunlight, CO_2, Freshwater/ Brackish water, Land	Sunlight, CO_2, Seawater		Sunlight, CO_2, Seawater

Equipment development
- functional improvements & enlargement of algal farms

Collaborations with Offshore Wind Power Projects

Venti-Japan （Akita）& Mitsubishi

（Shunan、Akita、Noshiro Ports）

200 km²

Collaborations of Airports on Sea

54 ha

Kansai-Air Port

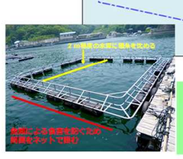
Biomass and Energy Production in Japan

Yokkaichi Port – Carbon Neutral Program

Coupling with Cabon Neutral Port Programs

四日市港・港湾区域面積：約6,600 ha

四日市港CNP形成計画（案）より引用・改変



港湾内への**「海藻養殖ユニット」**の設置による人工的な藻場の造成（2023.11.28-）

「四日市港CNP計画」対象範囲内の年間CO_2排出量

港湾ターミナル内：約0.57万トン
出入船舶・車両：約8.1万トン
港湾ターミナル外：約1687万トン（コンビナート）

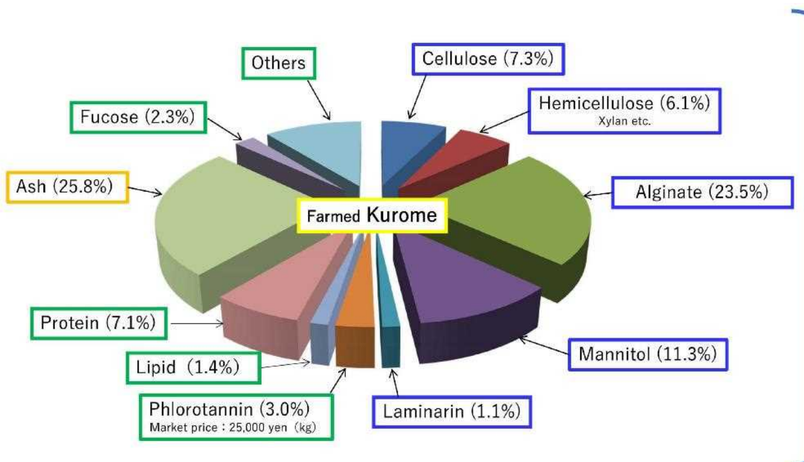
2,200 haの海域（1/3に相当）で海藻養殖を行った場合の試算値

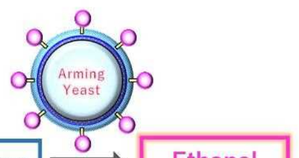
現行の優良選定株養殖技術の導入（210 トン/ha/年）
50万8200 トン-CO_2/年

→ 港湾ターミナル内，出入り船舶・車両の合計CO_2排出量に対して**目標達成は確実！**

MS研究開発事業による海藻養殖の技術革新（420 トン/ha/年）
101万6400 トン-CO_2/年

⑦ Practical application of ethanol fermentation as part of a cascade production process from macroalgae





Optimization of conditions
➡ maximizing ethanol yield

	Percentage in macroalgae	Ethanol obtained from 1 kg (g)
Cellulose	7.3%	480
Hemicellulose	6.1%	270
Alginate	23.5%	520 ※
Mannitol	11.3%	500
Laminarin	1.4%	500
		※：predicted value

(Lab scale)

No. A-7-2E

PJ : Redesign of Macroalgae for Highly Efficient CO_2 Fixation by Functional Modifications and Their Product Generation

Theme: Identification and characterization of CO_2-fixing enzymes to accelerate CO_2 fixation

Organization: Graduate School of Engineering, Kyoto University.
Contact: Haruyuki Atomi (atomi.haruyuki.8r@kyoto-u.ac.jp)

Objective

Our goal is to identify and evaluate the enzymatic properties of CO_2-fixing enzymes from macroalgae. We also aim to isolate autotrophic microorganisms to identify useful enzymes for application.

③-1. Functional evaluation of CO_2-fixing enzymes from macroalgae

Target enzymes

- ○Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)
- ○Phosphoenolpyruvate carboxykinase (PEPCK)
- ○Phosphoenolpyruvate carboxylase (PEPC)

③-2. Screening/characterization of Rubiscos from autotrophic microorganisms

Chemoautotrophic microorganisms are screened by utilizing H_2/O_2 as an energy source and bicarbonate as the sole carbon source.

Summary of progress

③-1. Functional evaluation of CO_2-fixing enzymes from macroalgae

○PEPCK

Kinetic analysis of five purified recombinant PEPCK proteins have been completed and their kinetic parameters were compared with PEPCKs from other organisms.

○PEPC

Five PEPC genes were expressed, and two were obtained as soluble recombinant proteins. Protein purification and enzymatic analyses are ongoing.

○Rubisco

Soluble recombinant proteins were obtained, and Rubisco activity was detected in partially purified proteins.

③-2. Screening/characterization of Rubiscos from autotrophic microorganisms

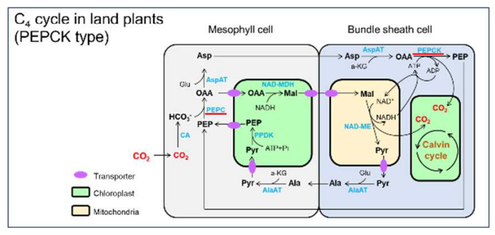
Microorganisms grew under autotrophic condition from 8 samples. Isolation and genome analysis are ongoing.

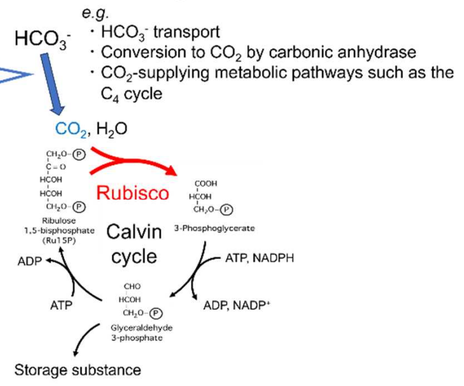
Background

CO_2-concentrating metabolism in plants and algae

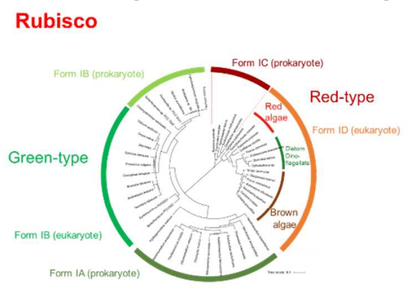
CO_2 concentrating mechanisms (CCMs) supply high concentrations of CO_2 to Rubisco to enhance its CO_2-fixation activity.

Most of the CO_2 exists as HCO_3^- in water, and conversion of HCO_3^- to CO_2 plays an important role

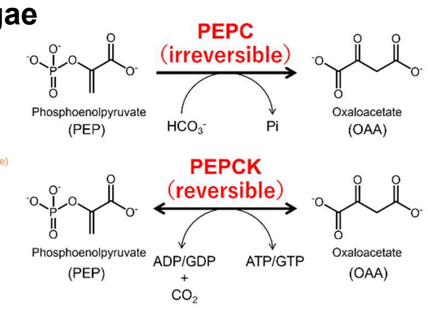




CO_2-fixation genes in macroalgae



Form ID Rubisco is known to have high CO_2 selectivity ($S_{C/O}$), but no purified brown algal Rubisco has been analyzed



- PEPC and PEPCK genes are present on the genomes of brown algae
- PEPCK from extracts of brown algae shows high activity

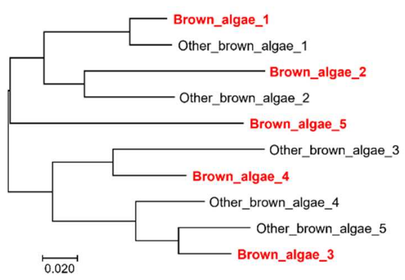
➡ Does a C4 cycle-like CCM exist?

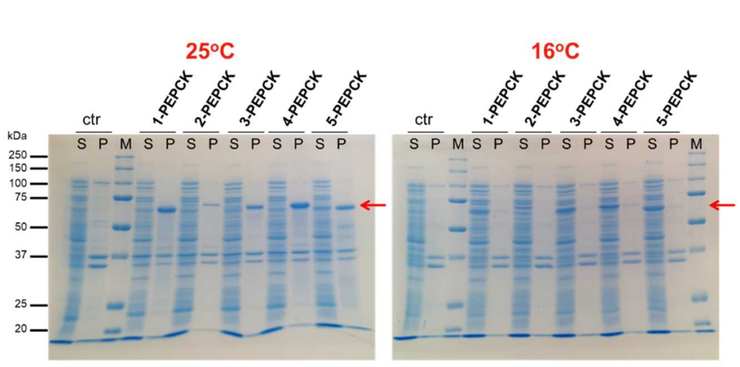
PEPCK

Expression of PEPCK genes from brown algae

Selected brown algae species (based on phylogenetic tree)

Brown_algae_1 (**1-PEPCK**)
Brown_algae_2 (**2-PEPCK**)
Brown_algae_3 (**3-PEPCK**)
Brown_algae_4 (**4-PEPCK**)
Brown_algae_5 (**5-PEPCK**)



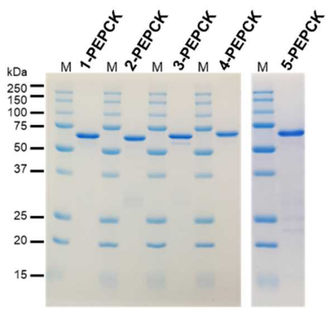


ctr: cells without induction; M: marker; S: supernatant of cell lysate; P: precipitate of cell lysate.

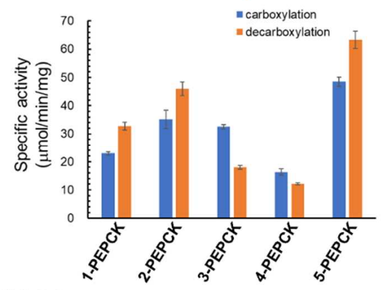
Five recombinant PEPCK proteins were obtained in soluble form by cultivating cells at 16°C.

Purification and specific activity measurement of PEPCKs from brown algae

SDS-PAGE analysis of purified proteins



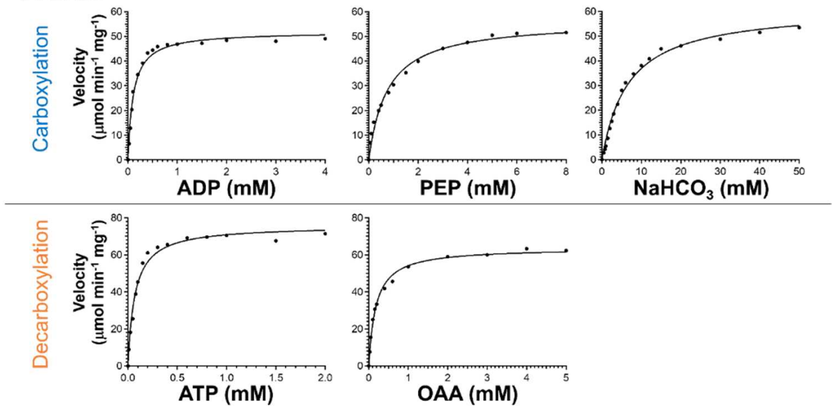
Specific activities of carboxylation and decarboxylation



Substrates:
Carboxylation: 2 mM ADP, 16 mM PEP, 50 mM $NaHCO_3$
Decarboxylation: 2 mM ATP and 4 mM OAA

Kinetic analysis of PEPCKs from brown algae

5-PEPCK



Activity comparison of PEPCKs from various organisms

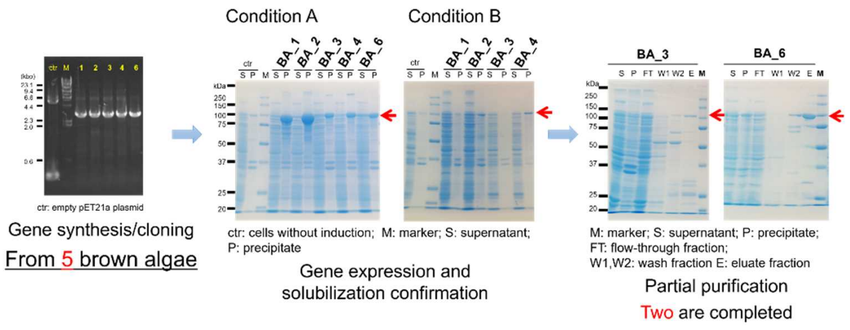
Organism		Carboxylation	Decarboxylation	
		V_{max} or specific activity (mmol min^{-1} $mg\ protein^{-1}$)		
C3 plant, ATP-type	*Arabidopsis thaliana*	**3.2**	**5.4**	
C3 plant, ATP-type	*Oryza sativa* (rice)	**-**	**10**	
C3 plant, ATP-type	*Cucumis sativus* (cucumber)	**48**	**-**	
C4 plant, ATP-type	*Sorghum bicolor* (sorghum)	**-**	**20**	
C4 plant, ATP-type	*Zea mays* (maize)	**-**	**23**	
C4 plant, ATP-type	*Megathyrsus maximum*	**41.6**	**51**	
CAM plant, ATP-type	*Ananas comosus* (pineapple)	**8.1**	**17**	
Green alga, ATP-type	*Chlamydomonas reinhardtii*	**6.0**	**9.6**	
Diatom, ATP-type	*Skeletonema costatum*	**0.037**	**-**	
Diatom, ATP-type	*Phaeodactylum tricornutum*	**0.041**	**-**	
Yeast, ATP-type	*Saccharomyces cerevisiae*	**16.3**	**20**	
Bacterium, ATP-type	*Escherichia coli*	**3**	**26**	
Brown alga, ATP-type	***Ascophyllum nodosum***	**33.03**	**-**	
Brown alga, ATP-type	**Brown_algae_1**	**23.6**	**32.7**	This study
Brown alga, ATP-type	**Brown_algae_2**	**39.2**	**45.9**	
Brown alga, ATP-type	**Brown_algae_3**	**34.3**	**18.0**	
Brown alga, ATP-type	**Brown_algae_4**	**17.8**	**12.2**	
Brown alga, ATP-type	**Brown_algae_5**	**61.6**	**75.8**	
Human, GTP-type	*Homo sapiens*	**43.8**	**39.4**	
Bacterium, GTP-type	*Mycobacterium tuberculosis*	**4.7**	**22.5**	
Archaeon, GTP-type	*Thermococcus kodakarensis*	**76.9**	**44.4**	
Ameba, PP_i-type	*Entamoeba histolytica*	**34**	**-**	

-: Not determined

The PEPCKs from brown algae display high activity.

PEPC and Rubisco

Analysis of PEPCs from brown algae



Gene synthesis/cloning
From 5 brown algae

Gene expression and solubilization confirmation

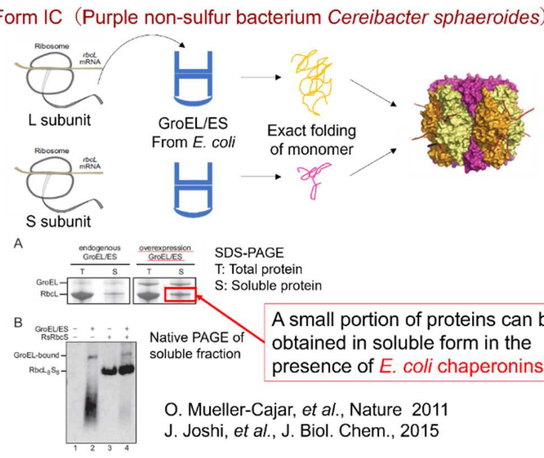
Partial purification
Two are completed

- Further purification of the soluble recombinant proteins
- Evaluation of enzymatic properties of PEPCs

Expression strategy of brown algal Rubisco in *E. coli*

We adopted the strategy for Form IC Rubisco, which displays relatively high identity to brown algal Form ID Rubisco.

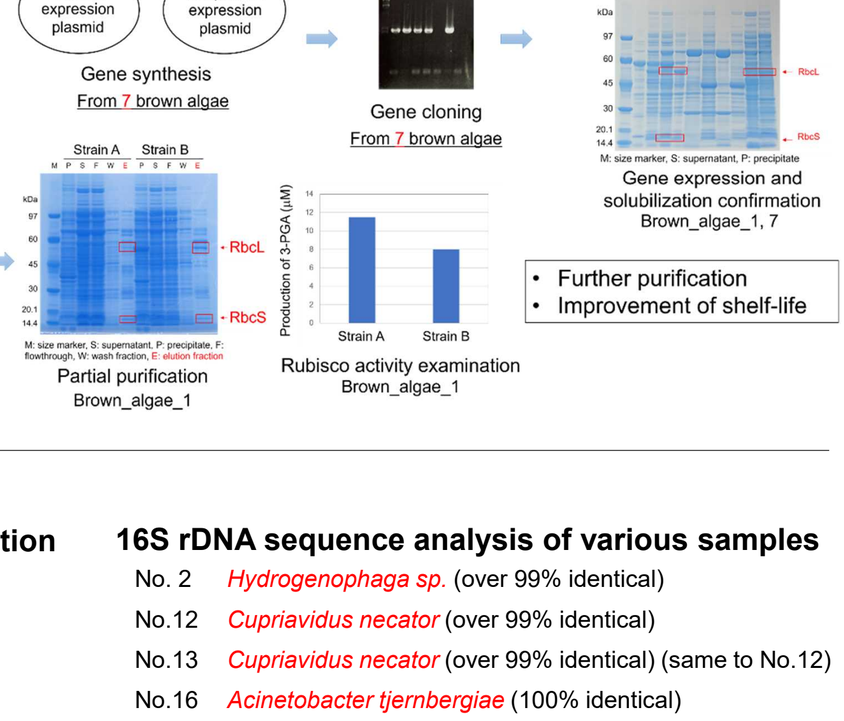
Form IC (Purple non-sulfur bacterium *Cereibacter sphaeroides*)



A small portion of proteins can be obtained in soluble form in the presence of *E. coli* chaperonins.

O. Mueller-Cajar, *et al.*, Nature 2011
J. Joshi, *et al.*, J. Biol. Chem., 2015

Analysis of Rubisco from brown algae

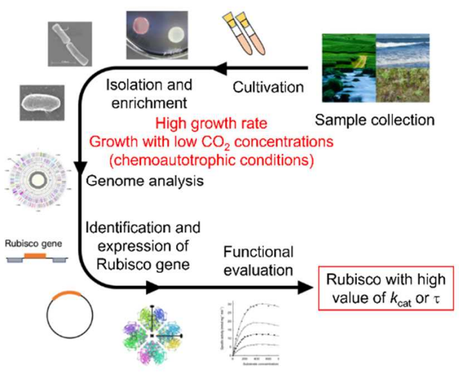


- Further purification
- Improvement of shelf-life

Cultivation of chemoautotrophic microorganisms

Screening/characterization of Rubiscos from autotrophic microorganisms

Screen for chemoautotrophic microorganisms from environmental samples, and identify Rubiscos with superior enzymatic properties.



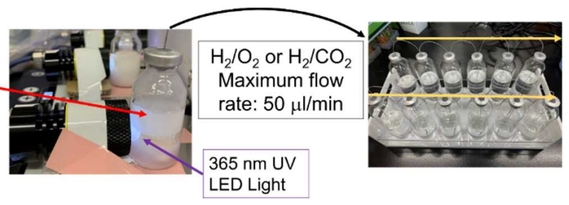
Calvin-Benson-Bassham cycle is used by a large number of aerobic autotrophs

$H_2(aq)+0.5O_2(aq) \leftrightarrow H_2O(l)$

Target microorganisms: utilize the produced ΔG due to the formation of H_2O from H_2 and O_2

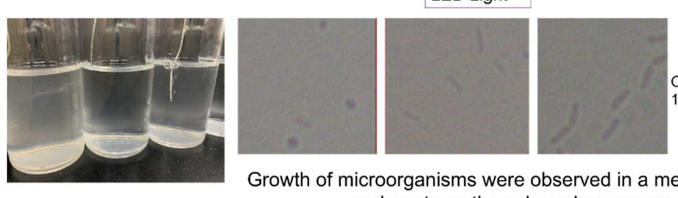
Chemoautotrophic microorganisms that use H_2 oxidation as an energy source

Photocatalyst provided by Laboratory of Prof. Ryu Abe , Kyoto University
($2H_2O \rightarrow 2H_2+O_2$)



H_2/O_2 or H_2/CO_2
Maximum flow rate: 50 μl/min

365 nm UV LED Light



Optical microscope 1000x enlarged photo

Growth of microorganisms were observed in a medium using carbonate as the sole carbon source

16S rDNA sequence analysis of various samples

No. 2 *Hydrogenophaga sp.* (over 99% identical)
No.12 *Cupriavidus necator* (over 99% identical)
No.13 *Cupriavidus necator* (over 99% identical) (same to No.12)
No.16 *Acinetobacter tjernbergiae* (100% identical)
No.17 *Acinetobacter oleivorans* (100% identical)
No.18 *Acinetobacter johnsonii* (over 99% identical)

Samples No. 19 and No. 20 contain several major sequences and further enrichment is necessary.

Growth of CO_2-fixing microorganisms from the above 8 samples was observed

Isolation and genomic analysis are ongoing.

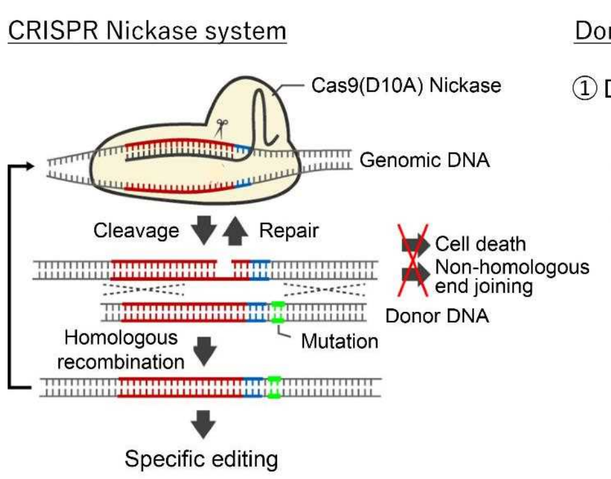
No. A-7-3E

PJ : Redesign of macroalgae for highly efficient CO_2 fixation by functional modifications and their product generation

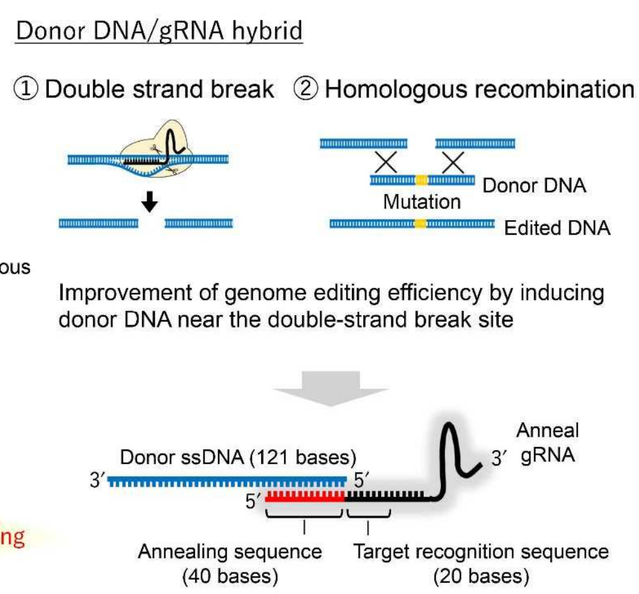
Theme: Establishment of genome editing technology to accelerate macroalgae breeding

Organization: Kyoto Institute of Technology, Kyoto University
Contact: Kouichi Kuroda (kuroda@kit.ac.jp)

① Strategies to improve genome editing efficiency in CRISPR/Cas9 system

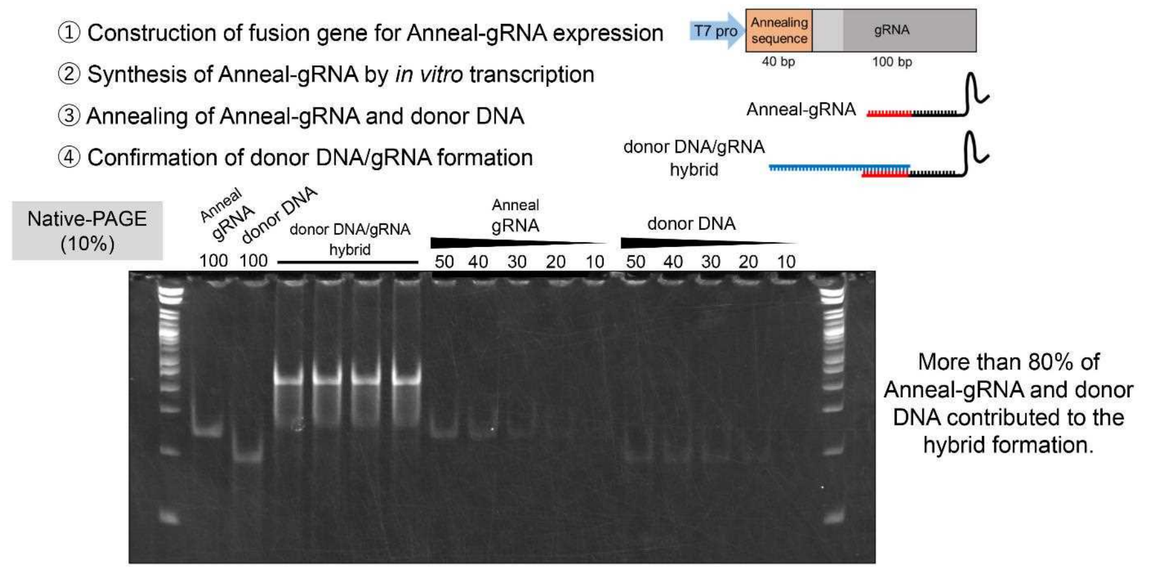


Improvement in accuracy and scope of genome editing



Satomura *et al.*, *Sci Rep*, 2017, 7: 2095

② Preparation of donor DNA/gRNA hybrid

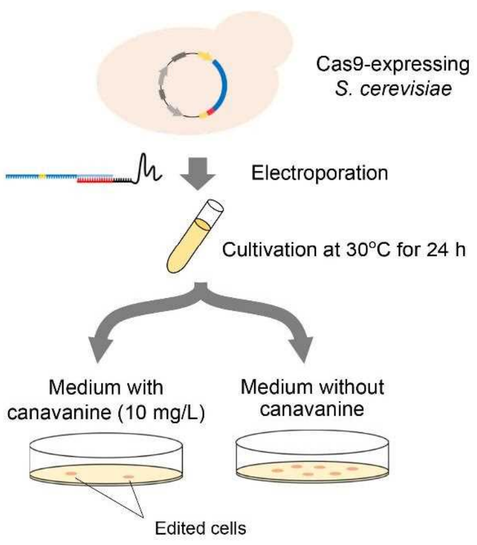


More than 80% of Anneal-gRNA and donor DNA contributed to the hybrid formation.

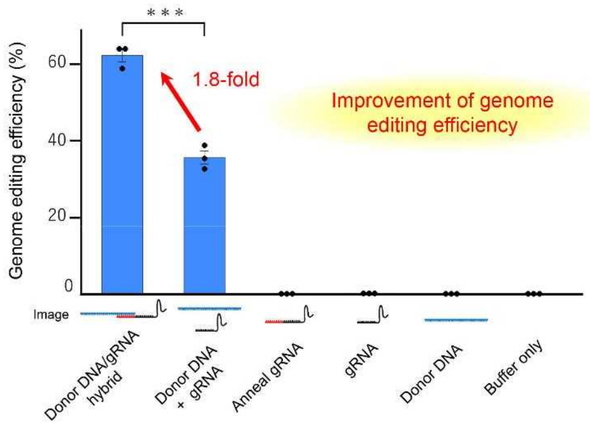
Aiba *et al.*, *Biomolecules*, 2022, 12: 1621

③ Improvement of genome editing efficiency by donor DNA/gRNA hybrid

Insertion of stop codon onto *CAN1* gene



Measurement of genome editing efficiency



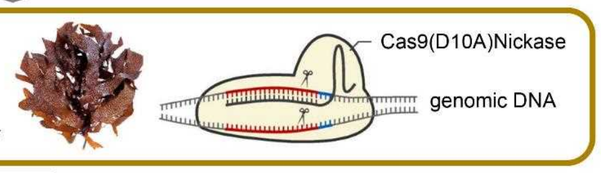
Donor DNA/gRNA hybrid successfully improved the editing efficiency by 1.8-fold compared to the conventional method.

Aiba *et al.*, *Biomolecules*, 2022, 12: 1621

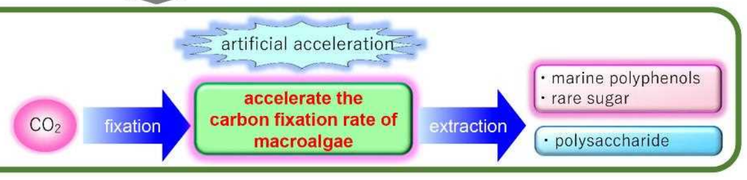
④ Genome editing in algae

***Chlamydomonas*（microalgae）**
- Single-cell green alga
- Model organism in the study of photosynthesis and genetics
- Optimization of experimental conditions

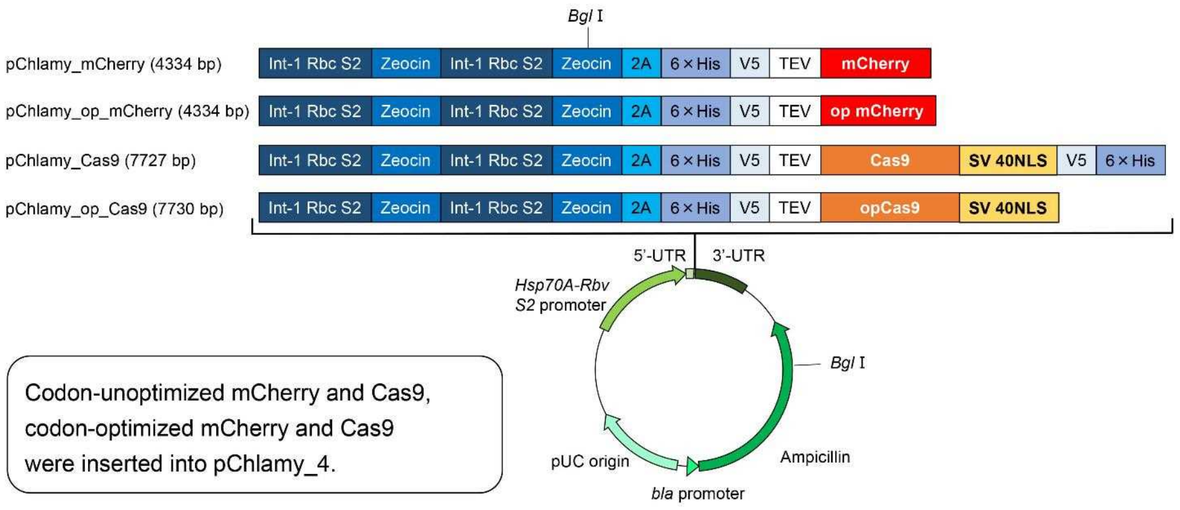
***Ecklonia Kurome*（macroalgae）**
- Brown macroalgae
- Optimization of experimental conditions
- Establishment of genome editing technology



macroalgae
- Functional enhancement (fixation capacity, growth rate)



⑤ Plasmid construction



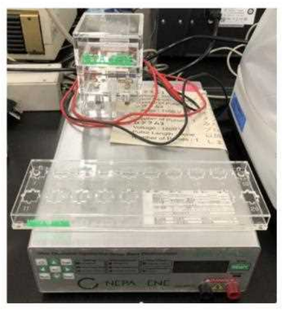
Codon-unoptimized mCherry and Cas9, codon-optimized mCherry and Cas9 were inserted into pChlamy_4.

op: codon optimized
2A: FMDV 2A peptide sequence. Self-cleaving sequence

⑥ Gene transfer into *Chlamydomonas*

- **Gene transfer by electroporation and particle gun**

Expression of fluorescent protein and Cas9 protein
（Optimization of expression vectors and gene transfer methods）
↓
Cas9-fluorescent protein expression, transfer gRNA
↓
Confirmation of gene editing（Evaluation of editing efficiency）

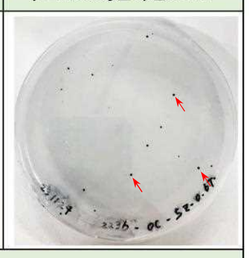


←electroporation

particle gun→

⑦ Particle gun：Result

- *Chlamydomonas* 2236 (5 μg/mL Zeocin, 0.6 μm Tungsten)

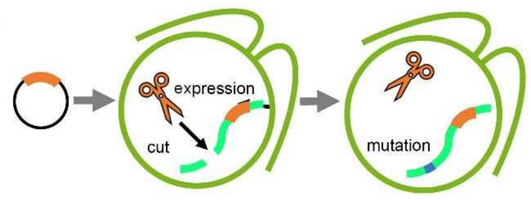
Negative Control	pChlamy_mCherry	pChlamy_op_mCherry	pChlamy_Cas9	pChlamy_op_Cas9
				
number of colonies				
2	47	36	40	21

For Chlamydomonas transformants with pChlamy_mCherry or pChlamy_op_mCherry, mCherry expression will be confirmed by fluorescence microscopy.

⑧ Culture location depending on genome editing content

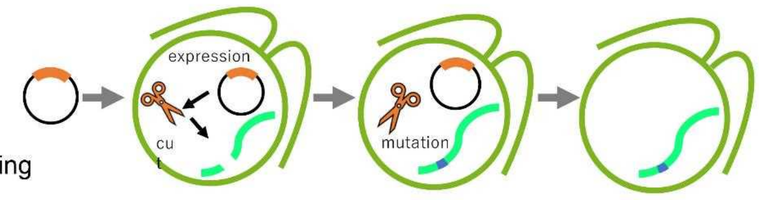
Currently experimental methods

Stable expression system by genome integration
↓
Onshore culture



Ultimate goal

Transient expression system
↓
- Introduction of sequences identified in nature
- Deletion of Cas9 gene after editing
- Sea culture



No. A-7-4E

PJ : Redesign of macroalgae for highly efficient CO2 fixation by functional modifications and their product generation

Theme: Development of fundamental technologies to accelerate breeding, functional enhancement, and full utilization of macroalgae

Organization: Mie University, Graduate School of Bioresources

Contact: Toshiyuki Shibata (shibata@bio.mie-u.ac.jp), Hideo Miyake, Reiji Tanaka, Kousuke Yamamoto, Tetsuya Okuyama

Selection of macroalgae with excellent CO_2 absorption and fixation ability, and development of their tissue culture and seedling production technology

Aims

Laminariaceae

Sargassaceae

In order to drastically increase the amount of **CO_2 absorbed** and **fixed**, it will be select useful macroalgae and developed their seedlings production technology.

Selection of useful macroalgae by measuring ETR value

ETR*	(μmol $m^{-2}s^{-1}$)
Sargassum horneri	7.9
Sargassum micracanthum	6.6
Undaria pinnatifida	**3.0**
Sargassum autumnale	5.7
Sargassum patens	8.3
Sargassum coreanum	6.0
Sargassum muticum	10.9
Sargassum nigrifolium	10.4
Myagropsis myagroides	7.0
Sargassum confusum	11.1
Sargassum yendoi	5.4

ETR (electron transport rate) value indicates the rate at which electrons transfer from the most upstream to the downstream during photosynthesis. → The **ETR** value is thought to be correlated with the **CO_2 absorption** and **fixation ability** of the measurement sample.

The ETR value of the sample was compared with that of *U. pinnatifida*, a macroalgae belonging to the Laminariaceae.
→ Sargassaceae were selected as useful macroalgae.

Preparation of tissue pieces from Sargassaceae and their regeneration by culturing

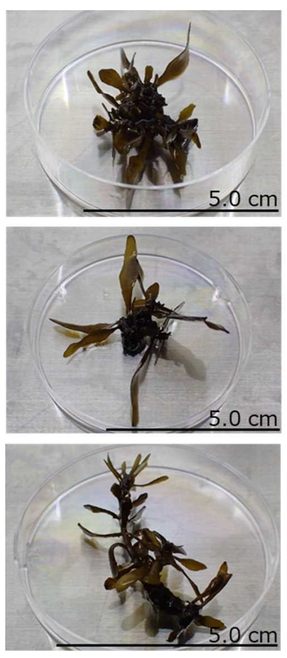
Immerse the algae in a medium containing antibiotics

Preparation of tissue pieces from algal bodies under sterile conditions

Vent cap flask
(PESI medium 30 mL)

Culture conditions

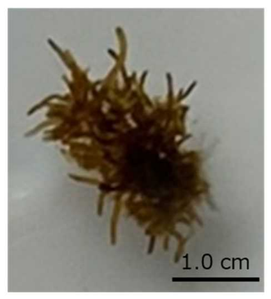
light intensity : 50 μmol/m^2/s
temperature : 15℃
12:12 light-dark cycle
PESI medium (without antibiotics)
Static culture or shaking culture



Achieved regeneration rate of over 50% by optimizing culture conditions
→ It is possible to produce seedlings of Sargassaceae using the tissue culture method.

Collection of fertilized eggs from *S. horneri* and production of their juveniles by culturing in a closed system

***S. horneri* embryos and their germination**

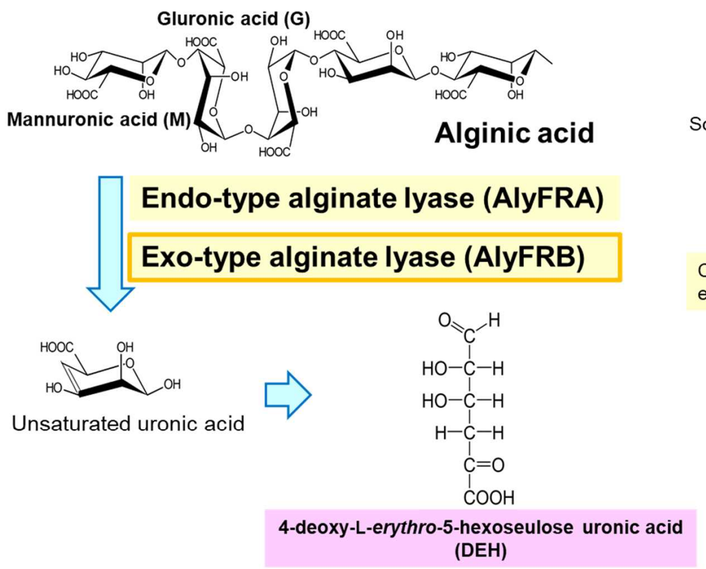


Juveniles of *S. horneri*

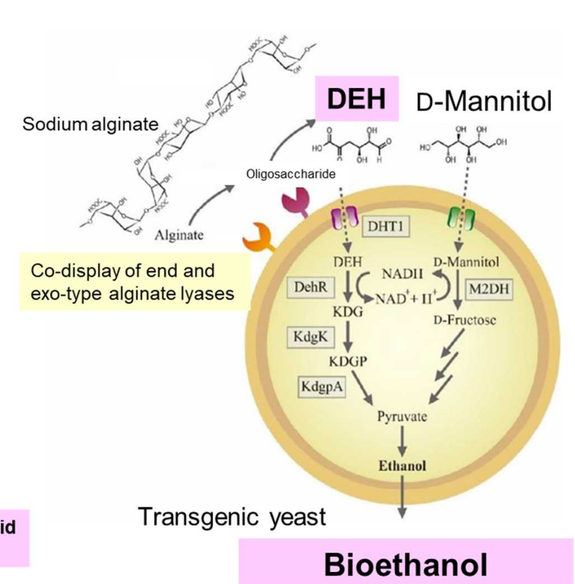
The technology for producing seedlings of *S. horneri* in a closed system has been completed.

Development of a cascade-type material production process combined with microbial pretreatment methods

Previous Studies



WO2017175694 (2021)
Tanaka Y. *et al., Molecules.* 27, 3308, (2022)



Takagi T. *et al.*, *Appl. Microbiol. Biotechnol.*, 101, 6627, (2017)

Problems

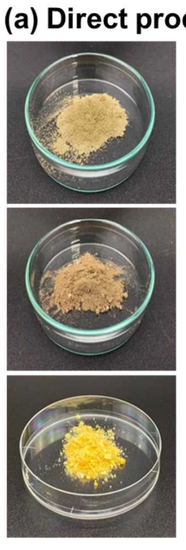
- ✓ Brown seaweeds contain phlorotannins and various carbohydrates.
- ✓ It is preferable to remove phlorotannins because they have the ability to bind to proteins and have antibacterial properties.
- ✓ Alginic acid exists in an insoluble gel state within the algal body.
  → As the concentration of sodium alginate increases in aqueous solution, its viscosity increases, making it difficult to handle in bacterial culture solution and enzyme reaction solution.

Aims

- ✓ Preparation of *E. kurome* powder with polyphenol removed as pretreatment
- ✓ Direct production of DEH from pretreated *E. kurome* powder
- ✓ Selection of optimal brown seaweeds-degrading bacteria for microbial pretreatment

Results

(a) Direct production of DEH



***E. kurome* powder**

Extraction of phlorotannnins

Pretreated *E. kurome*

Enzymatic reaction using AyFRA and AlyFRB

DEH

Conversion of alginic acid contained in *E. kurome* to DEH was achieved with a maximum yield of 63.3%.

(b) Selection of optimal brown seaweeds-degrading bacteria for microbial pretreatment

New marine baciterium

Expresses many polysaccharide-degrading enzymes

- Can degrading algal body
- Cannot utilize alginic acid

Suitable for DEH production

This strain selected as optimal strain for microbial pretreatment method.

Conclusions

- ✓ **It has become possible to directly produce DEH from insoluble alginic acid in brown seaweed.**
- ✓ **Many processes to produce DEH could be reduced. (Acid and alkali are not required.)**
- ✓ **The optimal brown seaweeds-degrading bacterium for microbial pretreatment was obtained.**

No. A-7-5E

PJ : Redesign of Macroalgae for Highly Efficient CO_2 Fixation by Functional Modifications and Their Product Generation

Theme: Investigation on industrial utilization of ethanol fermentation in the cascade production process from macroalgae

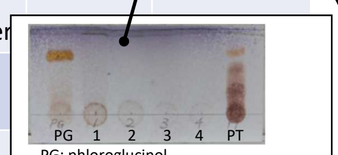
Organization: Green Earth Institute Co., Ltd. Contact: keisuke.yamamoto@gei.co.jp

Applying ethanol fermentation to the cascade process for substance production from brown seaweeds*

*NEDO Feasibility Study Program "Construction of core biotechnologies suitable for complete utilization of seaweeds"

Weight (g) of high value-added chemicals unused for ethanol fermentation

	Seeweed oil	Marine polyphenol
Hexane Extract	0.73	
Water Extract		
Ethanol Extract		Detected on TLC
Ethanol-Washing Water		
Alkaline Extract		
Alkaline-Washing Water		
Enzyme Digest solution		
Total	0.73	
Recovery	0.91%	



PG: phloroglucinol
PT: phlorotannin (provided by Mie Univ.)

Dried Kurome Powder (80g)

→ Hexane Extraction

Hexane Extraction Residue

→ Water Extraction

Water Extraction Residue

→ Ethanol Extraction

Ethanol Extraction Residue

→ Alkaline Extraction

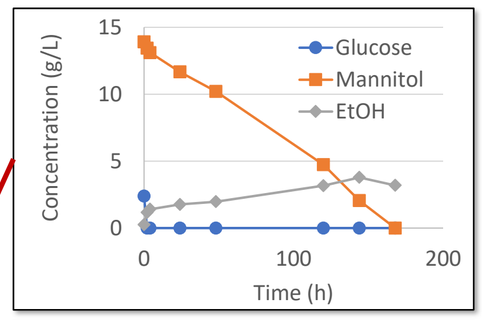
Alkaline Extraction Residue

→ Enzymatic saccharification

Weight (g) of materials used for ethanol fermentation in each fractions

	Mannitol	Laminarin	Alginate	Glucose
Hexane Extract				
Water Extract	13.1	3.14	10.1	
Ethanol Extract				
Ethanol-Washing Water			1.48	
Alkaline Extract			13.4	
Alkaline-Washing Water			0.43	
Enzyme Digest solution				3.13 (2.82)*
Total	13.1	3.14	25.4	2.82*
Recovery	16.4%	3.93%	31.8%	3.53%*

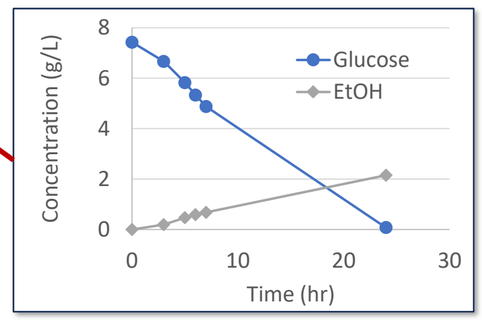
*As cellulose



【Saccharification】
37℃, pH 4.5
Enzyme:CTec3 ca.10％
（to carbon source）
【Fermentation】
35℃, pH 6.0
Strain: AM1
Strain density: OD_{600}= 25

・Vigorous foam formation and precipitation when neutralized
・No ethanol formation by AM1
-> Same observed when reagent grade alginate was used

【Neutralization】
6M $H_2SO_4$was used
【Fermentation】
As above



【Saccharification】
50℃, pH 4.5
Enzyme:CTec3 ca.10％
（to dry weight residue）
【Fermentation】
35℃,
pH not controlled (6.2->4.2)
Strain：Summit/AB Biotek
Strain density:0.5 g/L
(OD_{600}= 1.4)

- Part of Water Extract was subjected to enzymatic saccharification (CTec3/Novozymes) to convert laminarin to glucose. The resultant solution was used for ethanol fermentation by AM1 strain and ethanol formation was confirmed. The result suggested that ca. 4 g of ethanol (ca. 5% to Dried Kurome Powder) would be obtained when all of Water Extract was used.
- Part of Alkaline Extract Residue was subjected to enzymatic saccharification (CTec3/Novozymes). The resultant solution was used for ethanol fermentation by Summit Ethanol Dry Yeast (AB Biotek) and ethanol formation was confirmed. The result suggested that ca. 0.9 g of ethanol (ca. 1% to Dried Kurome Powder) would be obtained when all of Alkaline Extract Residue was used.

Modification of the cascade-type production process using macroalgae with a focus on ethanol production

Dried Kurome Powder

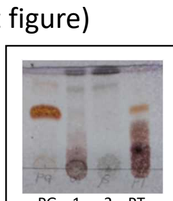
Undisclosed due to preparation of patent application

	Test1	Test2	Test3
Undisclosed due to preparation of patent application			
Maximum EtOH concentration*	7.05 g/L (264h)	5.19 g/L (264 h)	5.15 (192h)
Yield	**8.5%**	**6.2%**	**6.2%**

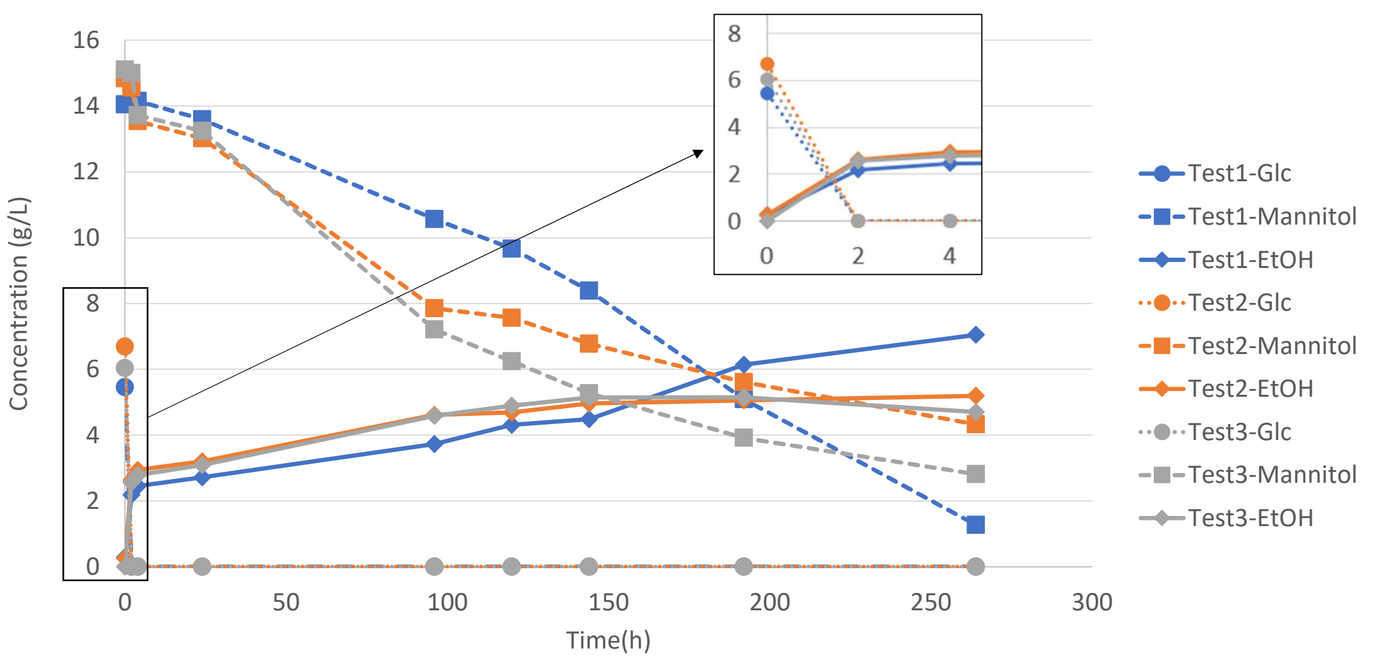
*Quantified by HPLC

→ Ethanol (Right figure)

→ Marine-polyphenols



PG: phloroglucinol
PT: phlorotannin
(provided by Mie Univ.)

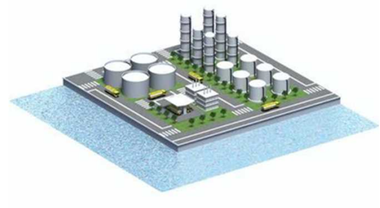


- <u>More than 5% yield (goal of FY 2023) of ethanol production from dried Kurome powder has been achieved by ethanol fermentation using AM1 strain.</u>
- The TLC analysis suggested that Marine polyphenols can be extracted from the fermentation residue.

Issues to be addressed toward industrial application of ethanol production from macroalgae

- Pretreatment method of ocean-fresh macroalgae to be used for ethanol fermentation
- Improvement of the ethanol fermentation conditions (yield, titer, productivity)
- Purification method of ethanol from fermentation broth of macroalgae
- Post-treatmenf of fermentation residue including extraction of high value-added chemicals
- Scaling-up of all the processes (pretreatment, fermentation, purification, waste treatment)
- To make business plan and structure

Example of business images：
Marine Biorefinary Complex on/off the Shore



No.: A-7-6E

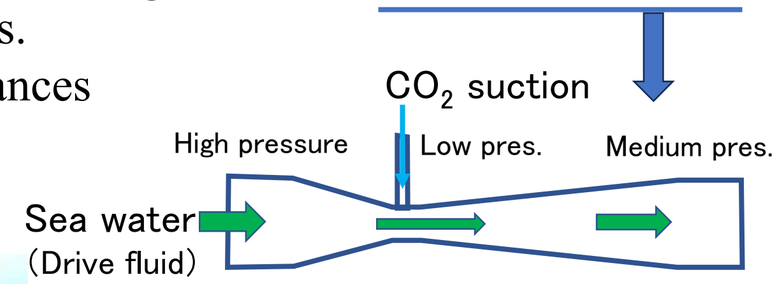
PJ: Redesign of macroalgae for highly efficient CO_2 fixation by functional modifications and their product generation

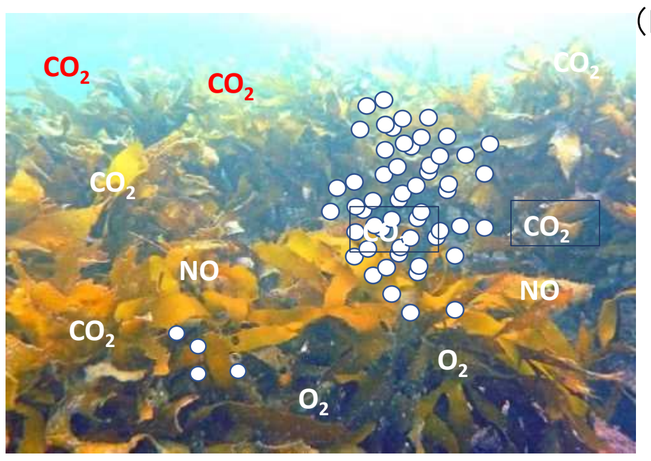
Theme: Development of equipment for accelerating CO_2 fixation of large-scale algae

Organization: Kansai Chemical Engineering Co., Ltd (KCE)
Contact: Hiroshi Ooshima (ooshima@kce.co.jp)

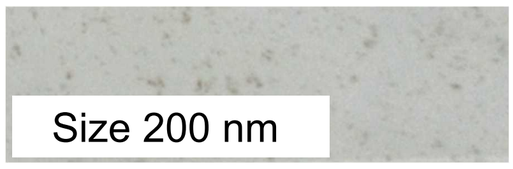
Objectives: (1) To develop a CO_2-fine bubble supply system suitable for accelerating large-scale algae growth in marine algae farms and land-based cultured algae plants.
(2) To improve the circumstances of the marine algae farms.

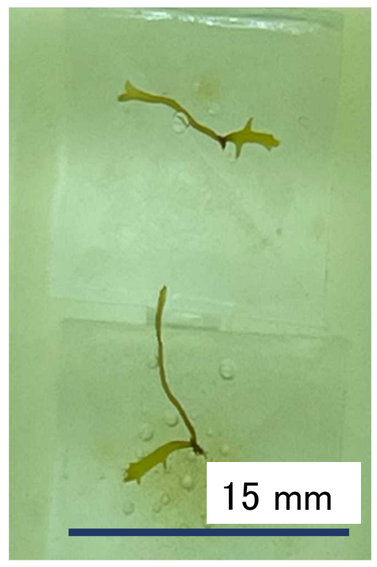
Experiment: Akamoku (academic name: *Sargassum horneri (Turner) C. Agardh*) was selected as a large-scale alga and examined the effect of CO_2-fine bubble supply on the growth of juveniles grown from that juvenile embryo. As a fine-bubble generation mechanism, a venturi tube type was adopted.





Growth of Akamoku





Grow to 10-m size

Juvenile embryo → Juveniles → Growing juveniles

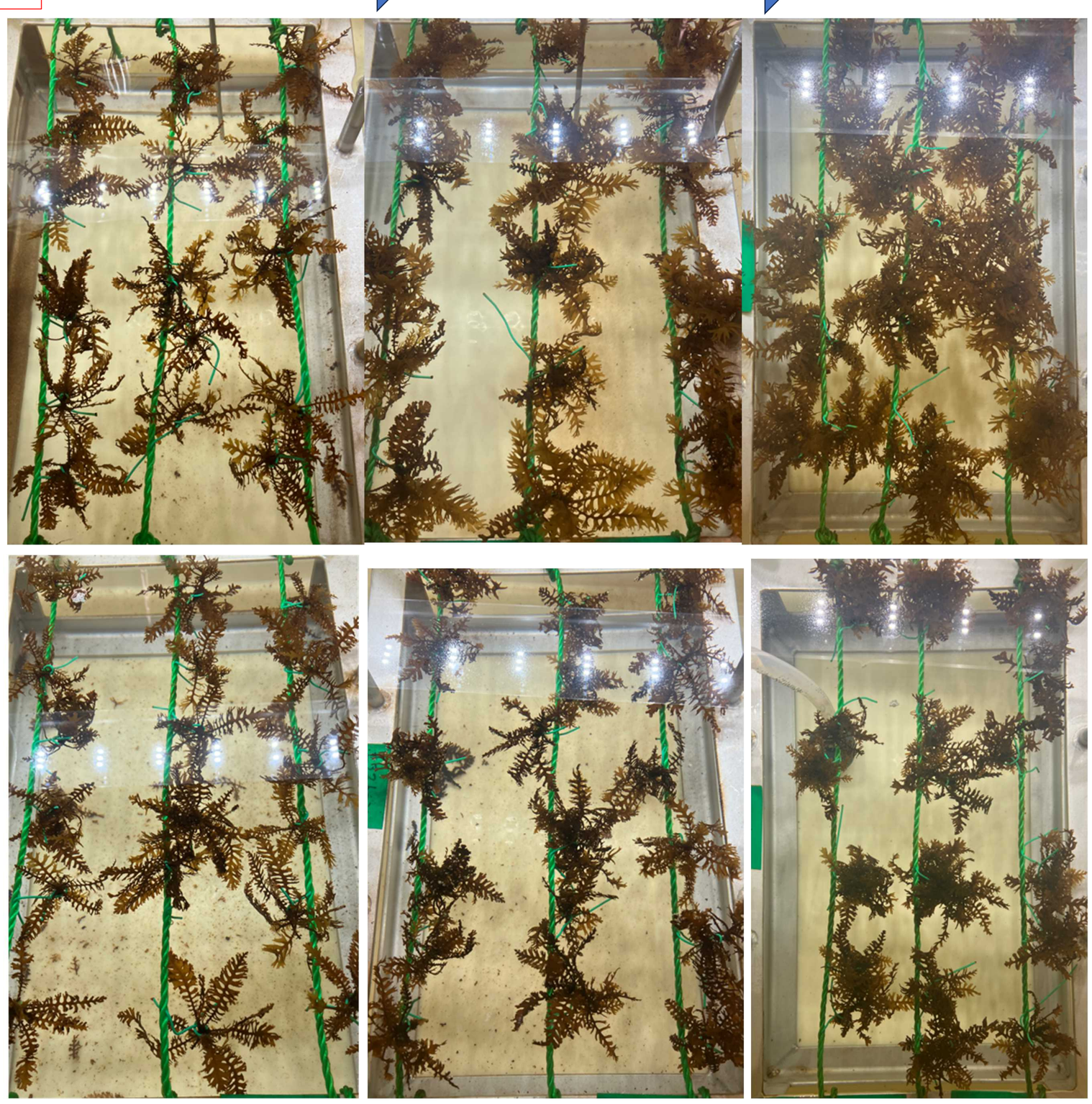
Result (Target 1)

Comparison of the Growth Rate of Juveniles Cultured in a Small Seawater Tank.

Cultivation: 0 d → 15 d → 27 d

Gas supplied: CO_2

Air

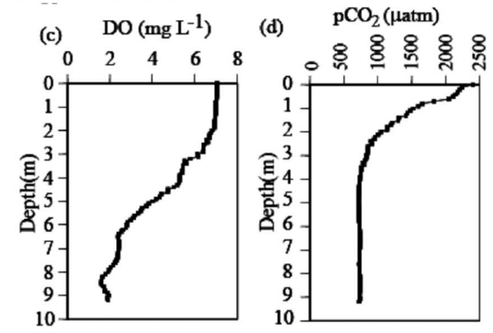


Broth Analyses

Gas supplied	pHav (9:00)	CO_2av, ppm 16:00 (9:00)	O_2av, ppm (9:00)	$HCO_3^-{}_{total}$av, ppm 16:00 (9:00)
Measurement	0 – 48 d	0 - 48 d	19 – 48 d	0 – 48 d
CO_2	(7.49)	13.2* (4.53)**	(4.77)	195.8 (182.4)
Air	(7.93)	(1.41)	(4.79)	(150.4)

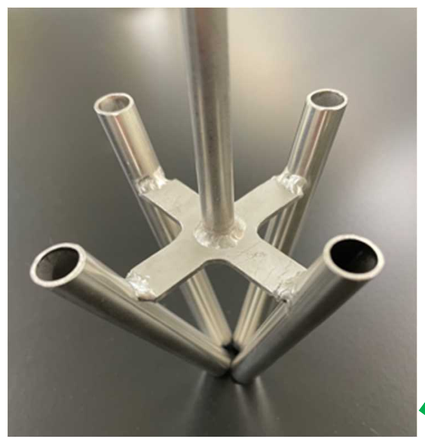
* Maximum/day
** Minimum/day

(Target 2) Improvement of Marine Algae Farm; Vertical Circulation of Seawater



Nishinomia 2014.11.10 (Fijii et al. (2017)

Depth Distribution of pCO_2, DO



WW-mixer^{TR} (KCE)

System Diagram