



Carbon dioxide mitigation by photosynthetic microorganisms and application of their biomass

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Outline

- **Introduction**
- **Material and methods**
- **Results and discussion**
- **Conclusions**

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Bioconversion of CO₂ and biomass processes

- **Methanogenic and acetogenic bacteria**
- **Photosynthesis of microorganism**
 - *Cyanobacteria*
 - *Microalgae*
 - *Macroalgae*
- **Enzyme-Catalyzed Reaction**
- **Biomimetic reactions**

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Mass transfer between carbon dioxide and water

- Other than the light limitation of photosynthesis, carbon dioxide mass transfer was a key factor in cultivating photosynthetic microorganisms due to the low mass transfer coefficient between carbon dioxide and water (Eckert et al., 1967).
- Gas volumetric mass transfer coefficients for some reagents with carbon dioxide using 38.1 mm Intalox saddles have been studied (Eckert et al., 1967).

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Mass transfer coefficients

Gas	Reagent	Product		$K_G a$
		Hydrate	Ion	
CO ₂	H ₂ O	H ₂ CO ₃	H ⁺ CO ₃ ⁻²	0.072
CO ₂	H ₂ O · KOH	K ₂ CO ₃	K ⁺ CO ₃ ⁻²	3.10
CO ₂	H ₂ O · NaOH	Na ₂ CO ₃	Na ⁺ CO ₃ ⁻²	2.25
CO ₂	H ₂ O · MEA	MEA · CO ₃	MEA ⁺² CO ₃ ⁻²	2.50

Mass transfer coefficients (KGa) for some reagents with carbon dioxide using 1½-inch Intalox saddles (Eckert et al., 1967)

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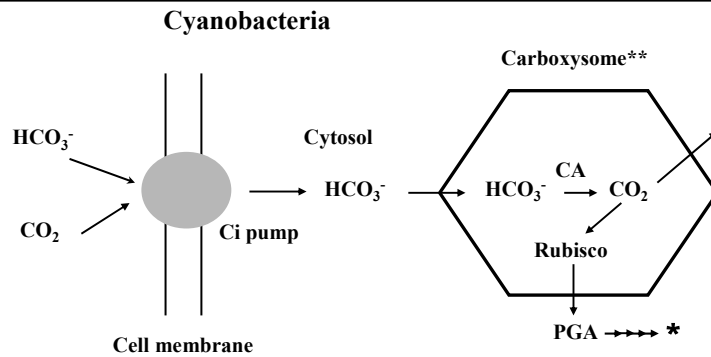
Integrated system of chemistry and biology

- **Reagent cost?**
 - *Regeneration*
- **Biological limitation?**
 - *Affinity for carbon under alkaline conditions*
- **Monod type thinking?**
 - *What carbon concentration is enough?*

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Mass transfer for photosynthesis (1/2)



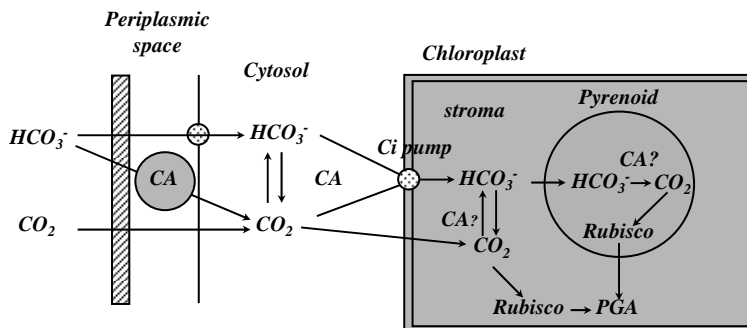
[Badger and Price, 1994]

- CA: Carbonic anhydrase
- Rubisco: Ribulose biphosphate carboxylase-oxygenase
- PGA: 3-phosphoglyceric acid
- * : Carbohydrates, Lipids etc.
- **Carboxysomes are bacterial microcompartments that contain enzymes involved in carbon fixation



Mass transfer for photosynthesis (2/2)

Microalgae



[Badger and Price, 1994]

- CA: Carbonic anhydrase
- Rubisco: Ribulose biphosphate carboxylase-oxygenase
- PGA: 3-phosphoglyceric acid



DIC (dissolved inorganic carbon) pump

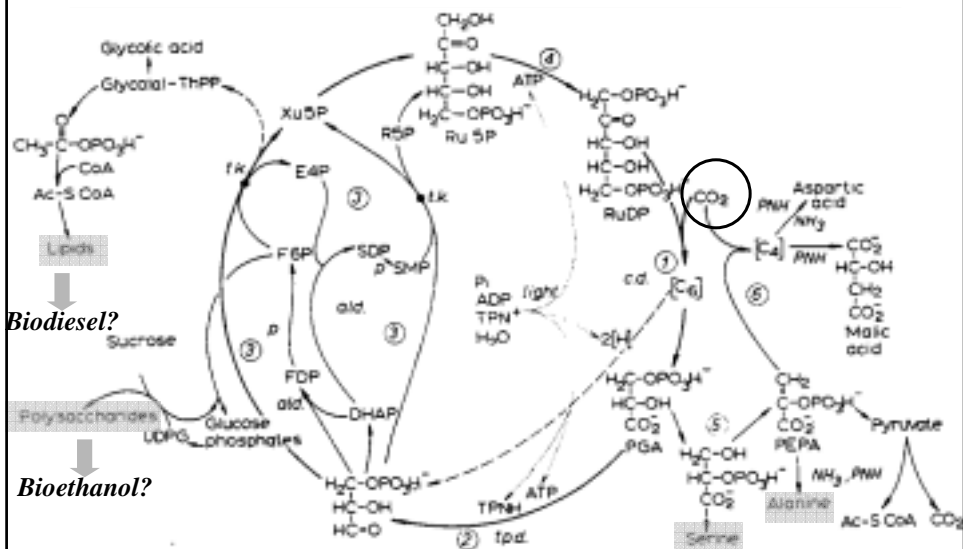
【Borowitzka, 1987】

- $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$
 - $\text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$
 - $\text{HCO}_3^- \rightleftharpoons \text{CO}_2$ (inside) + OH^- (outside)
(photosynthetic DIC pumps)
 - CO_2 (photosynthetic CO_2 reduction) \rightarrow $(\text{CHO})_n$ (inside) \rightarrow Biomass production
Bio-fuel?
- Regeneration!**
Enough?

*DIC means dissolved inorganic carbon



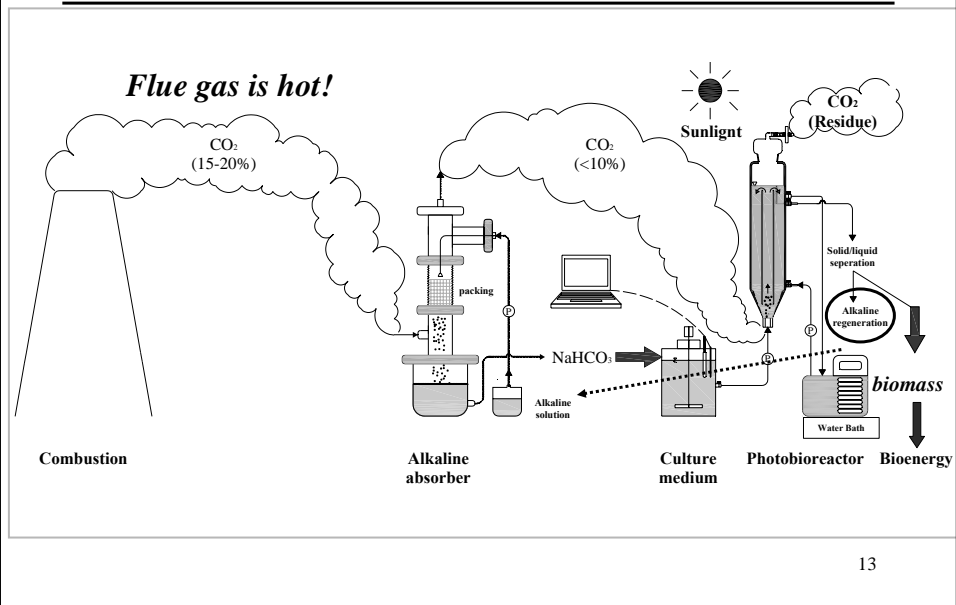
Calvin Cycle



The photosynthetic carbon cycle and its relation to quantum conversion and to succeeding biosynthesis (Calvin, 1961)



The idea of CO₂ fixation and biomass production

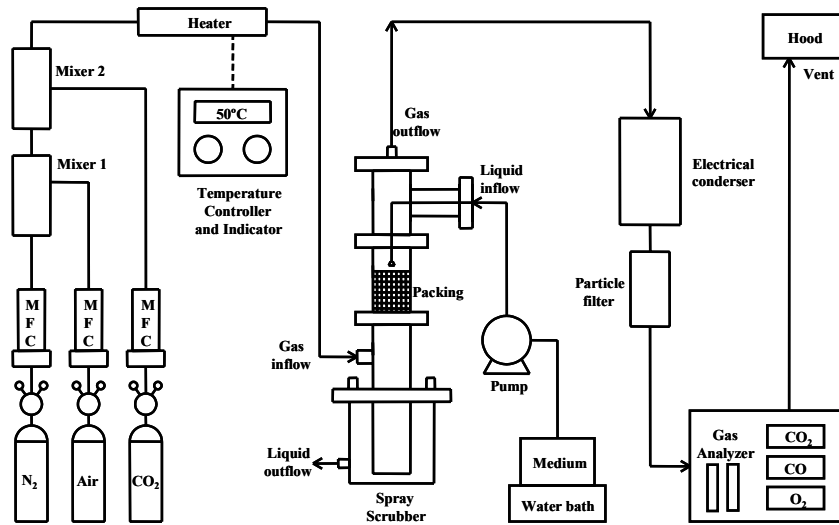


Integrated system and bio-energy

- We have developed a system integrated alkaline absorption with microbial photosynthesis to remove CO₂ from flue gases and a hot spring cyanobacterium with high DIC affinity under alkaline and high temperature conditions has been isolated.
- Cellular components (in accordance with bio-energy production) have been studied in various conditions in a either batch or continuous cultivation.



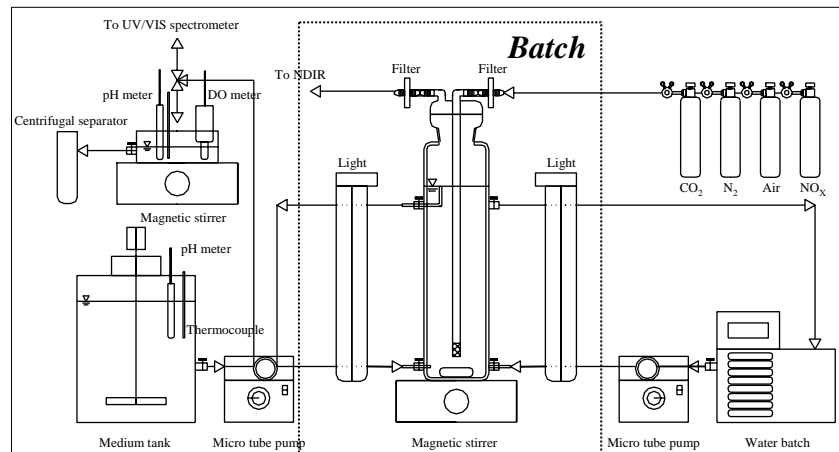
Packed tower



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Bio-reactor



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Cultivation conditions (1/2)

- **The growth conditions of Microorganisms**

- *Isolated species with the high alkaline affinity from Chin-Lun (CL) hot spring*
- *Identify the isolated species using 16S rRNA and/or other genes with standard procedure*
- *7,000 or 10,000 lux was maintained with 24 hr a day for hot spring species*
- *The temperature was controlled at 50 °C*

- **Growth medium**

- *Modified Fitzgerald medium (Takeuchi, et al., 1992) for HSC*
- *The changes with the concentrations of CO_3^{2-} , SO_4^{2-} , NO_3^- , PO_4^{3-} in the medium were determined by IC*

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Cultivation conditions (2/2)

- **Biomass analysis**

- *Three macro-components (lipids, proteins, and carbohydrates) were determined by wet chemistry method*
- *Metal contents were determined by ICP*
- *The contents of C, H, N, S, and O were determined by EA*

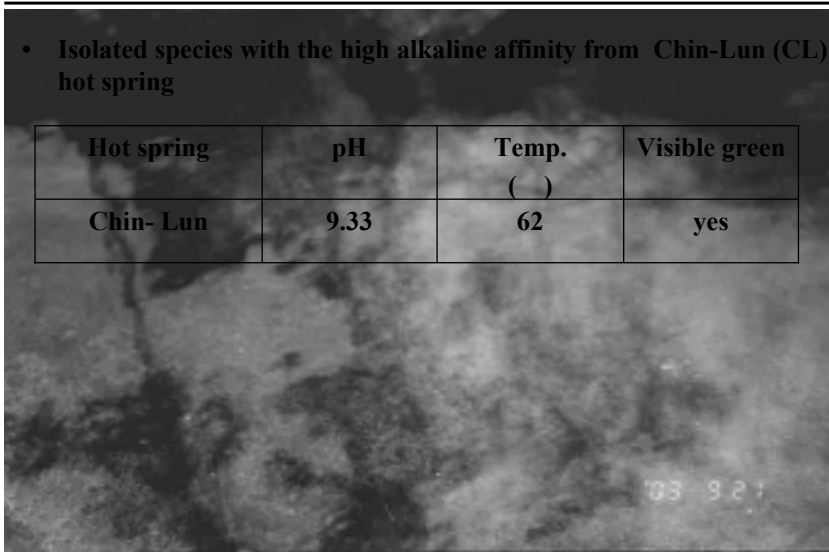
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Source

- Isolated species with the high alkaline affinity from Chin-Lun (CL) hot spring

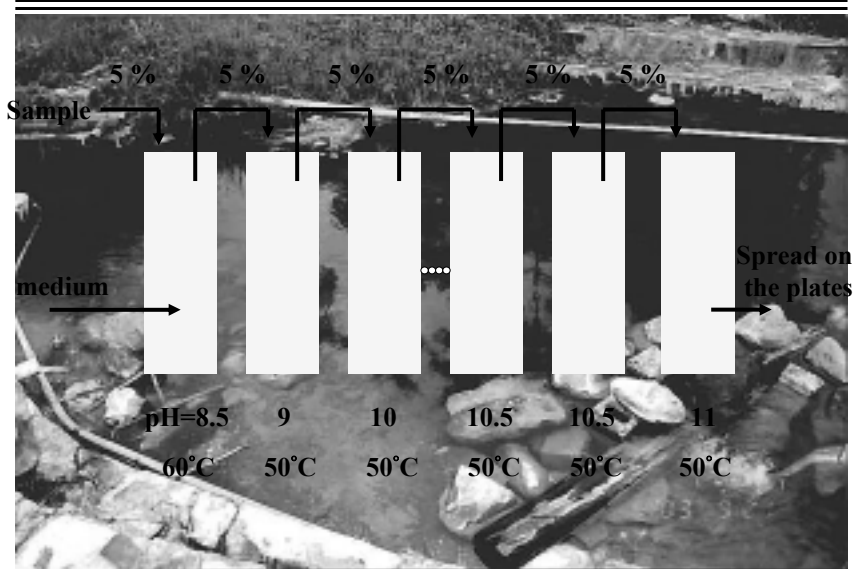
Hot spring	pH	Temp. ()	Visible green
Chin- Lun	9.33	62	yes



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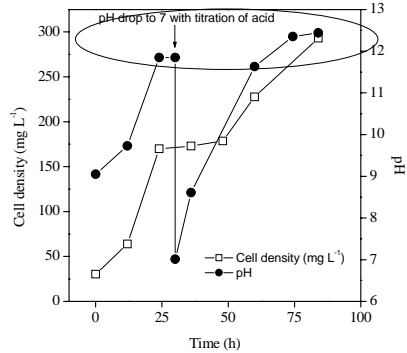
Isolation procedure



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The upper pHs physiological limitation



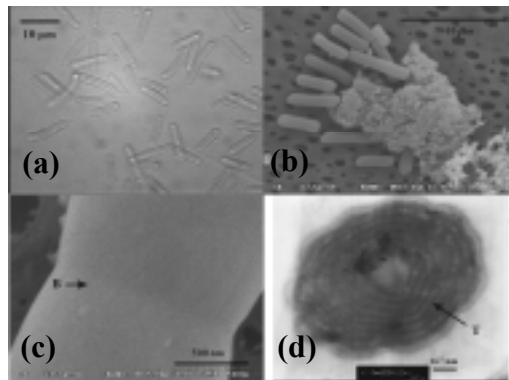
The upper pHs physiological limitations of HSC under batch cultivation.

This species can regenerate alkaline in the medium well (over pH 12).

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Morphology

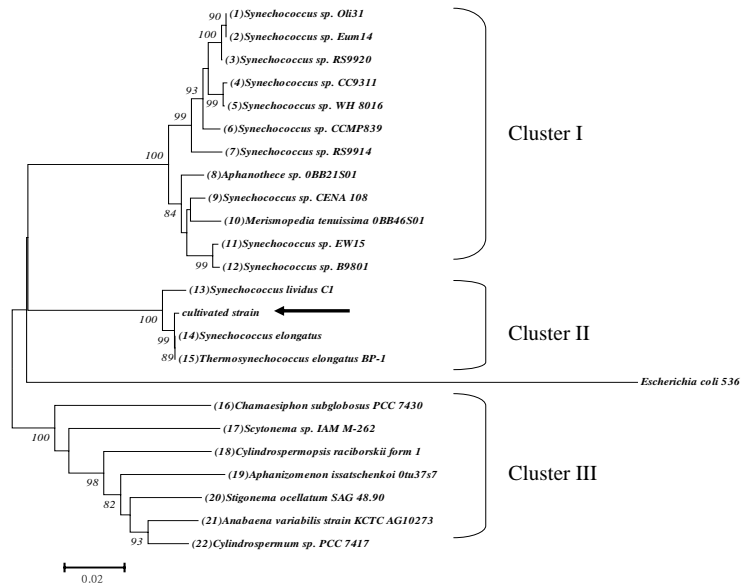


- The photo-images of cultivated strain, (a) light microscopy image; (b), (c) SEM images, B: the section of binary transverse fission; (d) TEM image, T: thylakoids.

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Phylogenetic analysis



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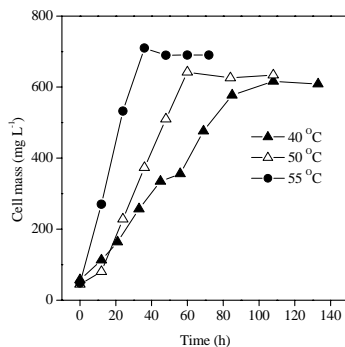
Phylogenetic analysis and nomenclature

- Distance tree was based on partial 16S rRNA sequence analysis of the cultivated strain named as *Thermosynechococcus* sp. CL-1 (TCL-1) and other complete and partial cyanobacterial sequences published in the NCBI database. *E. coli* (also published in the NCBI database) was used for rooting the tree. Numbers at nodes indicated the numbers of difference from which the cluster descending from the node was found in the 500 bootstrap trees. Cluster I and Cluster II: unicellular and colonial forms lacking specialized cells or reproduction; Cluster III: others (16: exopore-producing; 17-19, 21-22: heterocyst and akinete-producing; 20: true-branched)

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The effect of temperature



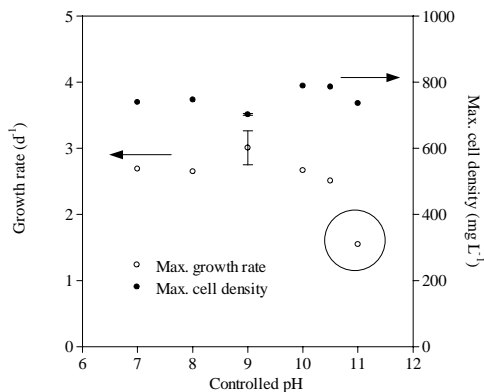
- Growth curves at the constant DIC concentration 28.3 mM at different temperatures (pH: 9.5; Light intensity: 10 klx.)
- At 40, 50, and 55 °C, the carbon uptake rate was 2.9, 5.9, and 9.9 mg L⁻¹ h⁻¹, respectively.

Over 55 °C

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The effect of pH



The growth rate and cell density of TCL-1 cultivated in alkaline media by adding Na₂CO₃ to let the carbon concentration be 34 mM and controlling pH values from 7 to 11 by adding 0.1N HCl (pH variations were within ±0.1 for all cases), growth conditions: 7,000 Lux, 50°C over 3 d.

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Mass recovery

The mass recovery comparisons between the consumption of carbonate and nitrate from the media and the contents of carbon and nitrogen from biomass and amount of other important elements for HSC (biomass cultivation conditions: 7,000 Lux, 50°C over 3 d).

pH		Carbon (C)			Nitrogen (N)			P	S	H	O	Cl*
		a	b	r	a	b	r	b	b	a	a	a
10.0	Percentage (%)	41.4	43.0	96.1	6.5	6.1	105.6	0.3	0.6	6.1	38.8	133.2
10.5	Percentage (%)	39.8	39.9	99.7	5.1	5.9	87.0	0.5	0.7	6.8	39.2	237.2
11.0	Percentage (%)	43.1	45.6	94.5	8.4	8.4	99.5	0.3	0.7	6.5	33.5	154.4

a: the contents calculated from the elemental analysis of biomass

b: the contents calculated from the nutrients (C and N) consumption from the medium, for example, b for carbon =

$$\frac{(DIC_{initial} - DIC_{final})_{medium} \times V_{medium}}{W_{bio-accumulation}}$$

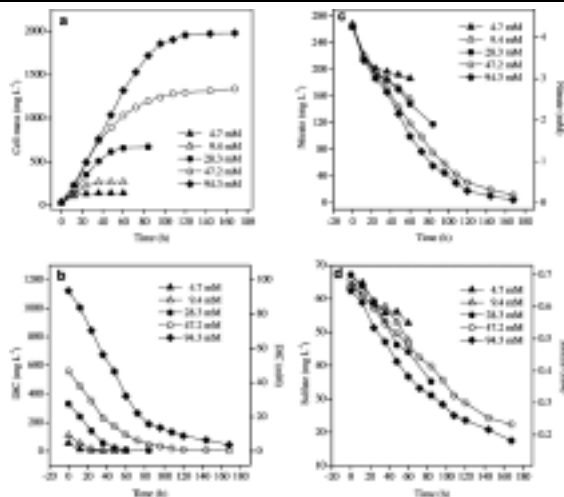
r: the percentages of a/b

P: phosphorous; S: sulfur; H: hydrogen; O: oxygen; Cl: chlorine

*: the unit is ppm



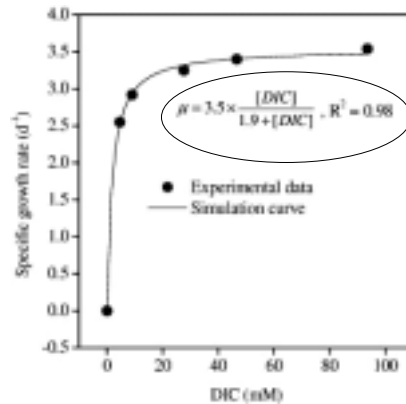
The effect of DIC conc. (1/2)



- Growth curves of TCL-1 and concentrations in the medium under different DIC concentrations. (a) Growth curves. (b) DIC concentration in the medium. (c) Nitrate concentrations in the medium. (d) Sulfate concentrations in the medium (Light intensity: 10 klx; pH: 9.5; temperature: 50 °C.)

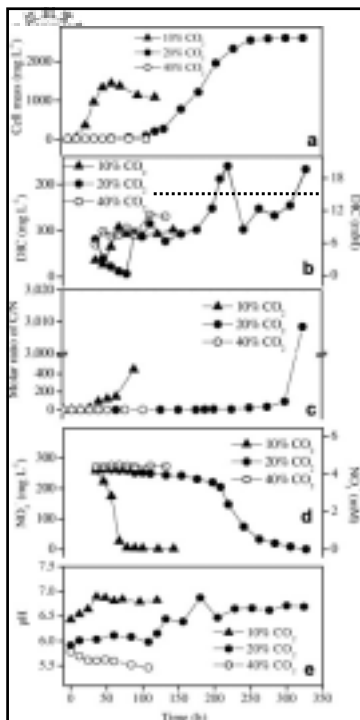


The effect of DIC conc. (2/2)



- A Monod model simulation

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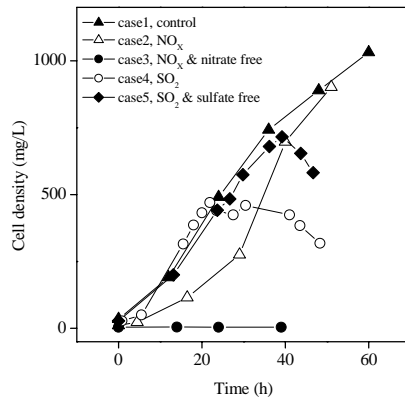
The effect of P_{CO_2}

- (a) Growth curves. (b) DIC concentrations. (c) Molar ratio of C/N. (d) Nitrate concentrations and (e) pH variations of TCL-1 with CO_2 bubbling of different CO_2 concentration. (Light intensity: 10 klx; temperature: 50 °C; gas flow rate: 1 L min^{-1} ; O_2 : 6%.)

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The effect of P_{NO_X} and P_{SO_2}

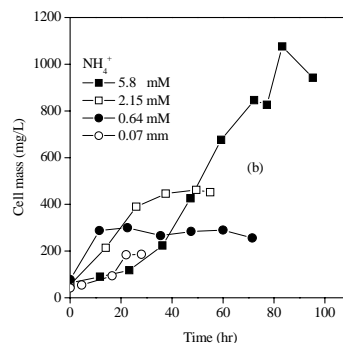
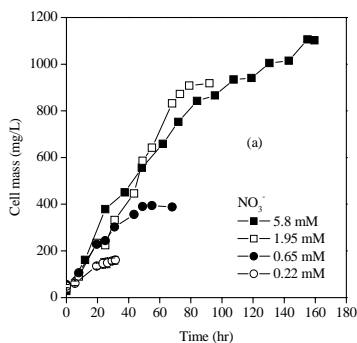


- NO_X : 250 ppm; SO_2 : 500 ppm; CO_2 : 15%; O_2 : 6%
- Control: 5.8 mM DIN; no NO_X and SO_2 inflow

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The effect of DIN conc.



(Light intensity: 10 klx; pH: 9.5; temperature: 50 °C; DIC: 47.2 mM)

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Cellular components under various P_{CO_2}

CO_2 (%)	μ (d^{-1})	C (%)	H (%)	O (%)	N (%)	Total (%)	Carbohydrate (%)	Lipid (%)	Protein (%)
10	2.7	42.7±0.0	7.4±0.0	42.1±0.0	2.8±0.0	95.0	60.6±2.4	13.8±3.0	16.9±1.6
20	1.0	41.8±0.1	7.1±0.0	46.5±0.0	2.4±0.0	97.8	ND	ND	ND

- The carbohydrate content reached 61%

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Cellular components under various DIC conc.

Table 1

Growth rates of TCL-1 and its composition under various DIC concentrations.

DIC (mM)	μ (d^{-1})	C (%)	H (%)	O (%)	N (%)	Total (%)	Carbohydrate (%)	Lipid (%)	Protein (%)
4.7	2.6	48.6±0.0	6.5±0.0	41.4±0.1	8.1±0.0	94.6	2.1±1.3	18.7±1.5	90.1±1.8
8.4	2.9	48.9±0.1	6.3±0.0	41.8±0.1	7.8±0.0	94.8	8.6±1.0	28.5±2.5	32.1±5.7
28.3	3.3	49.1±0.1	6.5±0.1	41.8±0.5	7.9±0.0	95.5	35.4±4.1	18.7±8.6	19.0±2.3
42.2	3.4	44.7±0.0	7.5±0.0	40.2±0.0	7.2±0.0	98.6	37.1±1.0	18.3±8.6	19.5±1.6
94.3	3.5	40.9±0.1	6.2±0.0	50.1±0.0	3.4±0.1	98.6	32.4±3.8	13.6±8.9	12.9±2.8

Cultivation at pH 9.5 and 58 °C.

- Carbohydrate content of TCL-1 increases significantly from 2 to 33% as the DIC concentration increases from 4.7 to 94.3 mM

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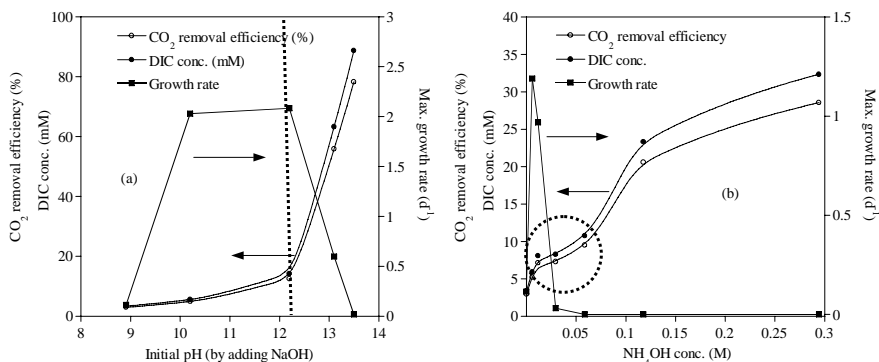
Cellular components under various DIN conc.

	DIN (mM)	Cellular components content (%)		
		Lipid	Carbohydrate	Protein
[NO ₃ ⁻]	5.8	23.0	45.8 ± 5.1	12.3
	0.7	12.3	61.1 ± 5.8	4.5
[NH ₄ ⁺]	5.8	15.0	53.8 ± 1.3	16.7 ± 1.0
	0.7	10.7	43.8 ± 2.8	13.4 ± 1.8

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Cultivation under various absorption reagents

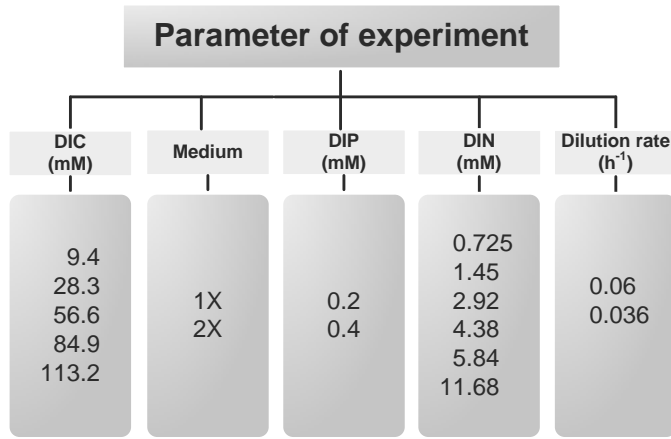


The CO₂ removal efficiencies with various absorbents concentrations in a packed tower and the growth rates as well as cell densities of TCL-1 cultivated with the absorbed solutions, (a) NaOH (b) NH₄OH, growth conditions: 7,000 Lux, 50°C over 7 d. Max. growth rate = Max. (k) = Max. $(2.303 \times \log(N_{t2}/N_{t1})/(t_2-t_1))$; k: growth rate (d⁻¹), N_{t1}: the cell density at time t₁, N_{t2}: the cell density at time t₂.

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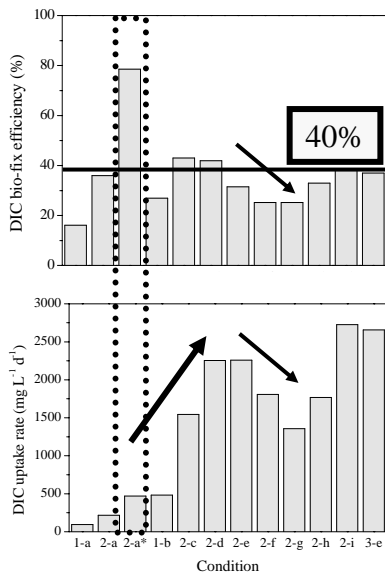
Continuous cultivation



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DIC bio-fixation & DIC uptake rate



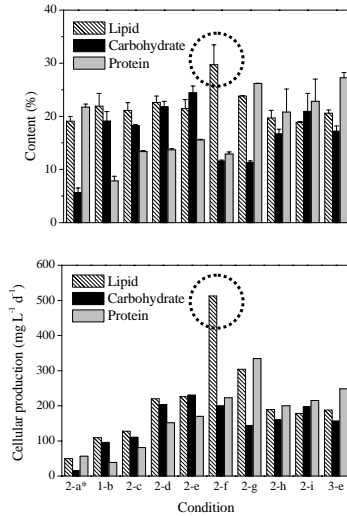
Condition	DIC (mM)	DIN (mM)	DIP (mM)	C (mM)
1-a	9.4	5.8	0.2	1.5
2-a	9.4			3.4
2-a*	9.4			7.4
1-b	28.3			7.6
2-c	56.6			24.4
2-d	84.9			35.6
2-e	113.2	35.6		
2-f	113.2	11.6	0.4	28.5
2-g	84.9	11.6	0.4	21.4
2-h	84.9	5.8	0.4	27.9
2-I	113.2	5.8	0.4	43.0
3-e	113.2	5.8	0.2	41.9

$$\text{DIC bio-fix efficiency} = \frac{\Delta C}{\text{DIC}}$$

$$\text{DIC uptake rate} = \Delta C \times 44 \times 1.44$$



Cellular components production

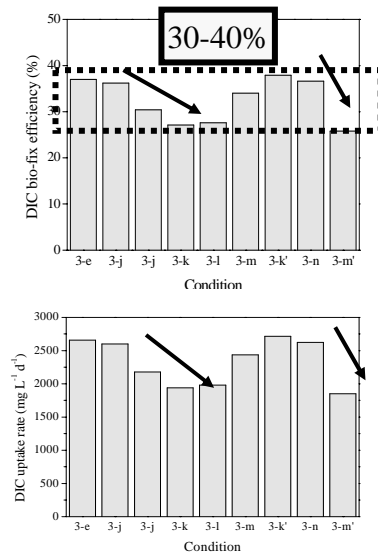


Condition	DIC (mM)	DIN (mM)	DIP (mM)
2-a*	9.4	5.8	0.2
1-b	28.3		
2-c	56.6		
2-d	84.9		
2-e	113.2		
2-f	113.2	11.6	0.4
2-g	84.9	11.6	0.4
2-h	84.9	5.8	0.4
2-I	113.2	5.8	0.4
3-e	113.2	5.8	0.2

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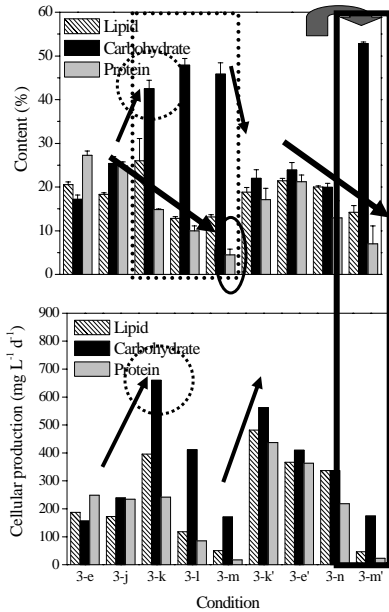
DIC bio-fixation & DIC uptake rate



Condition	DIN (mM)	C (mM)
3-e	5.8	41.9
3-j	4.4	41.0
3-k	2.9	34.4
3-l	1.45	30.6
3-m	0.725	31.3
3-k'	2.9	38.5
3-e'	5.8	42.9
3-n	11.6	41.4
3-m'	0.725	29.2

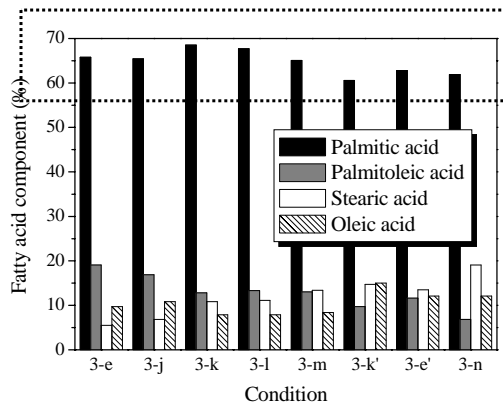
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Cellular components production



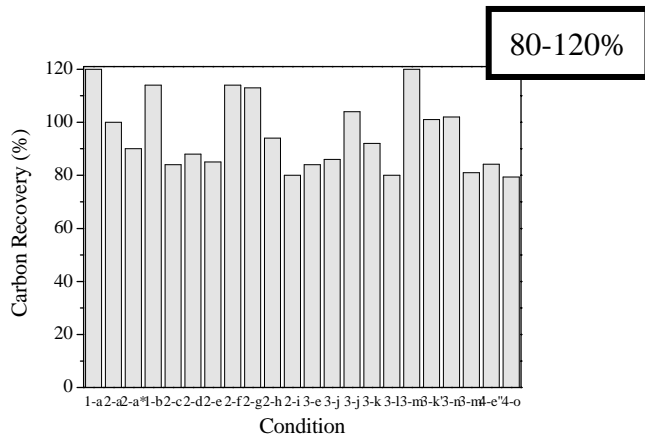
Condition	DIN (mM)
3-e	5.8
3-j	4.4
3-k	2.9
3-l	1.45
3-m	0.725
3-k'	2.9
3-e'	5.8
3-n	11.6
3-m'	0.725

Fatty acids





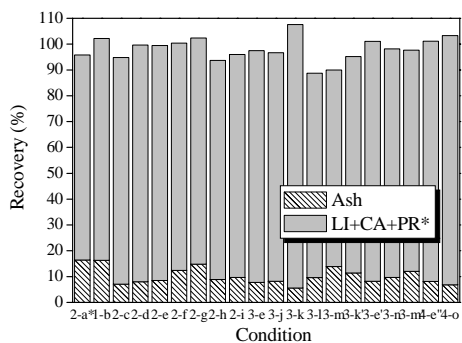
Carbon recovery



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Cellular component recovery (2/2)

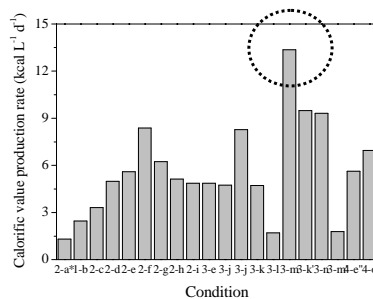
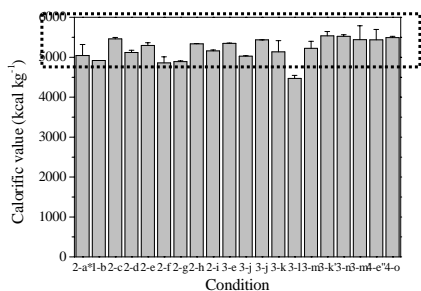


LI: lipid; CA: carbohydrate; PR: protein

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Calorific value & Calorific value production rate



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Conclusions (1/3)

- We have developed a system integrated alkaline absorption with microbial photosynthesis to remove CO₂ from flue gases.
- A hot spring cyanobacterium has a high affinity for DIC under alkaline conditions has been isolated. This strain is named as *Thermosynechococcus sp.* TCL-1.
- Regarding the performance of this system, CO₂ removal efficiencies, 13%, can be obtained under the condition that TCL-1 can grow well. This efficiency is about 5-fold of the case without adding any sodium hydroxide.

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Conclusions (2/3)

- Carbohydrate content can be increased under enough DIC or poor DIN concentrations in a batch cultivation.
- In a continuous culture, the optimal DIC uptake rate is between 2,000-2,750 mg L⁻¹ d⁻¹.
- In a continuous culture, the highest lipid content of TCL-1 is about 30% (lipid production rate is 513 mg L⁻¹ d⁻¹) and its accumulated palmitic acid (60% of fatty acid) has potential for biodiesel production.



Conclusions (3/3)

- The highest carbohydrate content of TCL-1 is over 50% and carbohydrate production rate is 660 mg L⁻¹ d⁻¹.
- The calorific value of TLC-1 is high enough, hence can be taken as fuels for combustion directly.



Thank you for your attention!

