TOKYO METROPOLITAN UNIVERSITY
首都大学東京

Friday May 31st – June 1st, 2019
Symposium in Honor of P. Leslie Dutton
The Structure and Function
of Natural Proteins and Maquettes
Univ. of Pennsylvania, Philadelphia

Electron transfer through hydrogen, sulfur, and nitrogen in thermophilic photosynthetic and chemosynthetic microbial communities

Arisa Nishihara, Shigeru Kawai, Hiroyo Otaki, Katsumi Matsuura

Tokyo Metropolitan University

Katsumi Matsuura 1980-1982 at Leslie Dutton's Lab.

The recognition and redox properties of a component, possibly a quinone, which determines electron transfer rate in ubiquinone-cytochrome c oxidoreductase of mitochondria

K Matsuura, NK Packham, P Mueller, PL Dutton

A reevaluation of the events leading to the electrogenic reaction and proton translocation in the ubiquinol-cytochrome c oxidoreductase of *Rhodopseudomonas sphaeroides*

K Matsuura, DP O'Keefe, PL Dutton

Reduction of cytochromes b_6 and f in isolated plastoquinol-plastocyanin oxidoreductase driven by photochemical reaction centers from *Rhodopseudomonas sphaeroides*

RC Prince, K Matsuura, E Hurt, G Hauska, PL Dutton

Inhibition of electron transfer by 3-alkyl-2-hydroxy-1, 4-naphthoquinones in the ubiquinol-cytochrome c oxidoreductases of *Rhodopseudomonas sphaeroides* and mammalian mitochondria. Interaction with a ubiquinone-binding site and the Rieske iron-sulfur cluster

K Matsuura, JR Bowyer, T Ohnishi, PL Dutton

Impressive and useful words from Les to me.
(I remember the meaning but not the exact words.)

Good experiments are important, but good thinking is more important.

The conclusion and the significance, not the details, are important for a wide audience.

Don’t hurry.
Be patient.
Don’t worry.
Take it easy.
(when Les was leaving to home at in front of the dark room.)

Katsumi Matsuura 1985-2019 at Tokyo Metropolitan University

Comparison of the binding sites for high-potential iron−sulfur protein and cytochrome c on the tetraheme cytochrome subunit bound to the bacterial photosynthetic reaction center
A Osyczka, KVP Nagashima, S Sogabe, K Miki, K Shimada, K Matsuura

Quinones in chlorosomes of green sulfur bacteria and their role in the redox-dependent fluorescence studied in chlorosome-like bacteriochlorophyll c aggregates
NU Frigaard, S Takaichi, M Hirota, K Shimada, K Matsuura

Roseiflexus castenholzii gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes.
S Hanada, S Takaichi, K Matsuura, K Nakamura

Horizontal transfer of the photosynthesis gene cluster and operon rearrangement in purple bacteria
N Igarashi, J Harada, S Nagashima, K Matsuura, K Shimada, K Nagashima

Nitrogen fixation in thermophilic chemosynthetic microbial communities depending on hydrogen, sulfate, and carbon dioxide
A Nishihara, S Haruta, SE McGlynn, V Thiel, K Matsuura

Symbiotic growth of a thermophilic sulfide-oxidizing anoxygenic photosynthetic bacterium and an elemental-sulfur disproportionating bacterium and cooperative dissimilatory oxidation of sulfide to sulfate
S Kawai, N Kamiya, K Matsuura, S Haruta

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Tokyo Metropolitan University

Recent research interests

1. Early evolution of chemosynthesis to photosynthesis
2. Early evolution of electron transfer through hydrogen, sulfur, and nitrogen

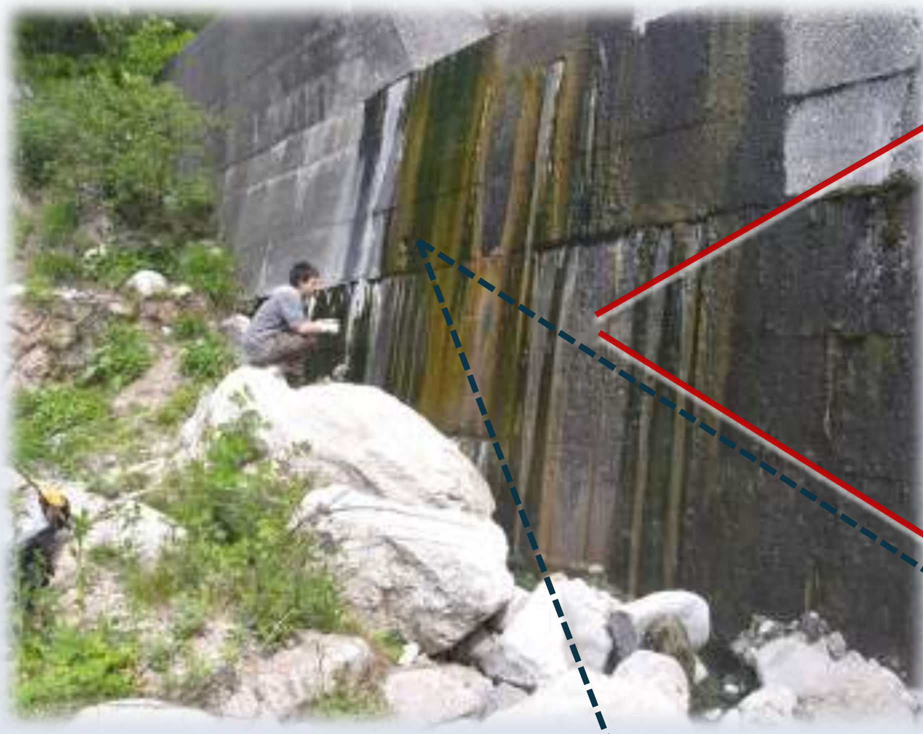
Outline of the talk

1. Hydrogen is produced in thermophilic photosynthetic microbial communities. (Otaki)
2. Hydrogen is a good electron donor for the photoautotrophic and chemoautotrophic growth of *Chloroflexus*. (Kawai)
3. Sulfide is also a good electron donor for *Chloroflexus* only when produced elemental sulfur is consumed by a disproportionating bacterium. (Kawai)
4. Nitrogen fixation was found in thermophilic chemosynthetic communities depending on hydrogen and sulfate. (Nishihara)
5. Nitrogen fixation in isolated bacteria at the highest temperature, so far, were observed, with hydrogen as the electron donor and oxygen and possibly elemental sulfur as the acceptors. (Nishihara)

Study area: Nakabusa Hot Springs, Japan

pH 8.5, 0.3 mM sulfide

Flow of hot spring water



75 ℃

- 75 ℃
- Pale-tan mats
- Chemosynthetic (*Aquificae*)

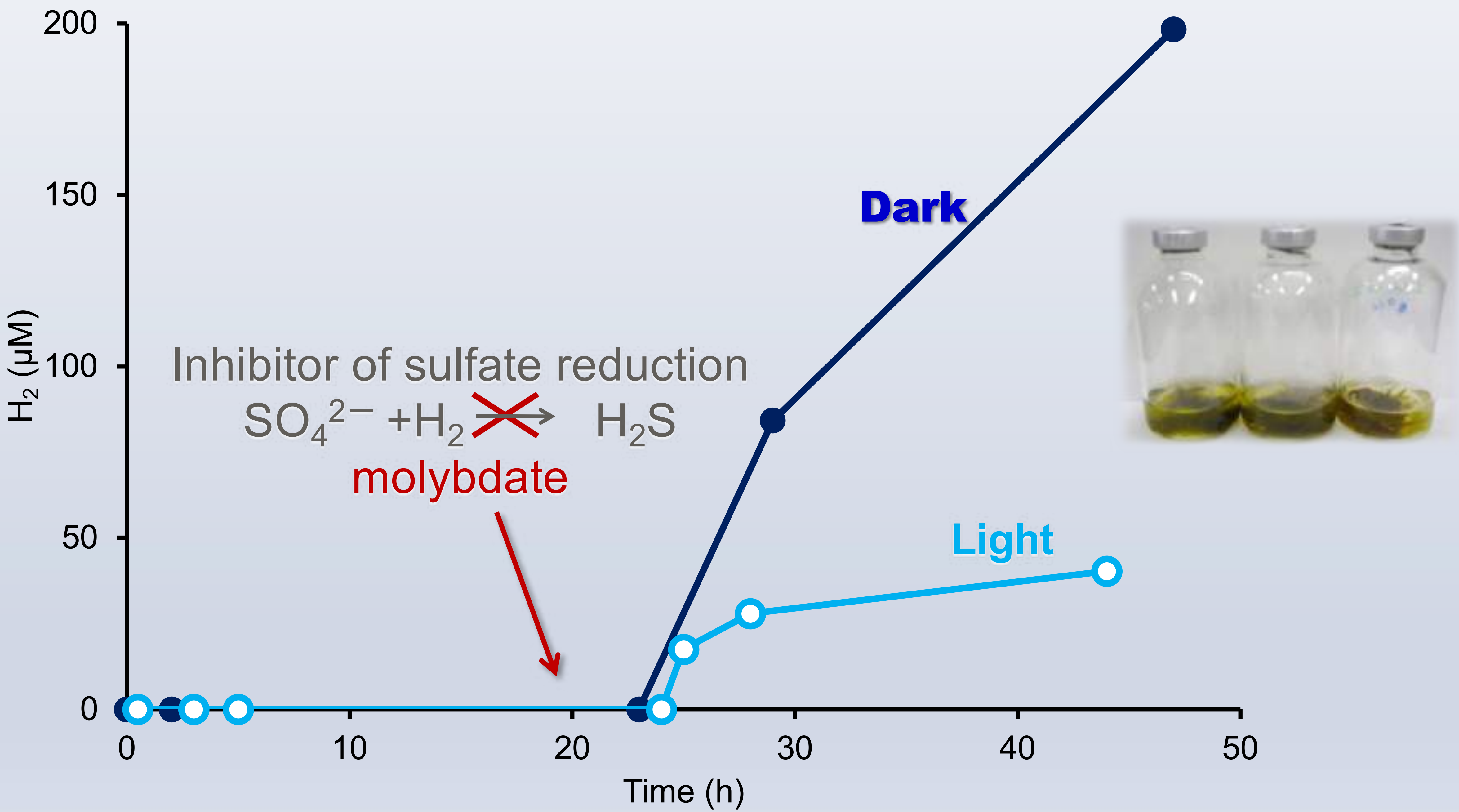
60℃ 65 ℃

- 60 ℃
- Dark-green mats
- Cyanobacteria (*Synechococcus* spp.)

- 65 ℃
- Olive-green mats
- Filamentous annoxygenic phototroph (*Chloroflexus aggregans*)

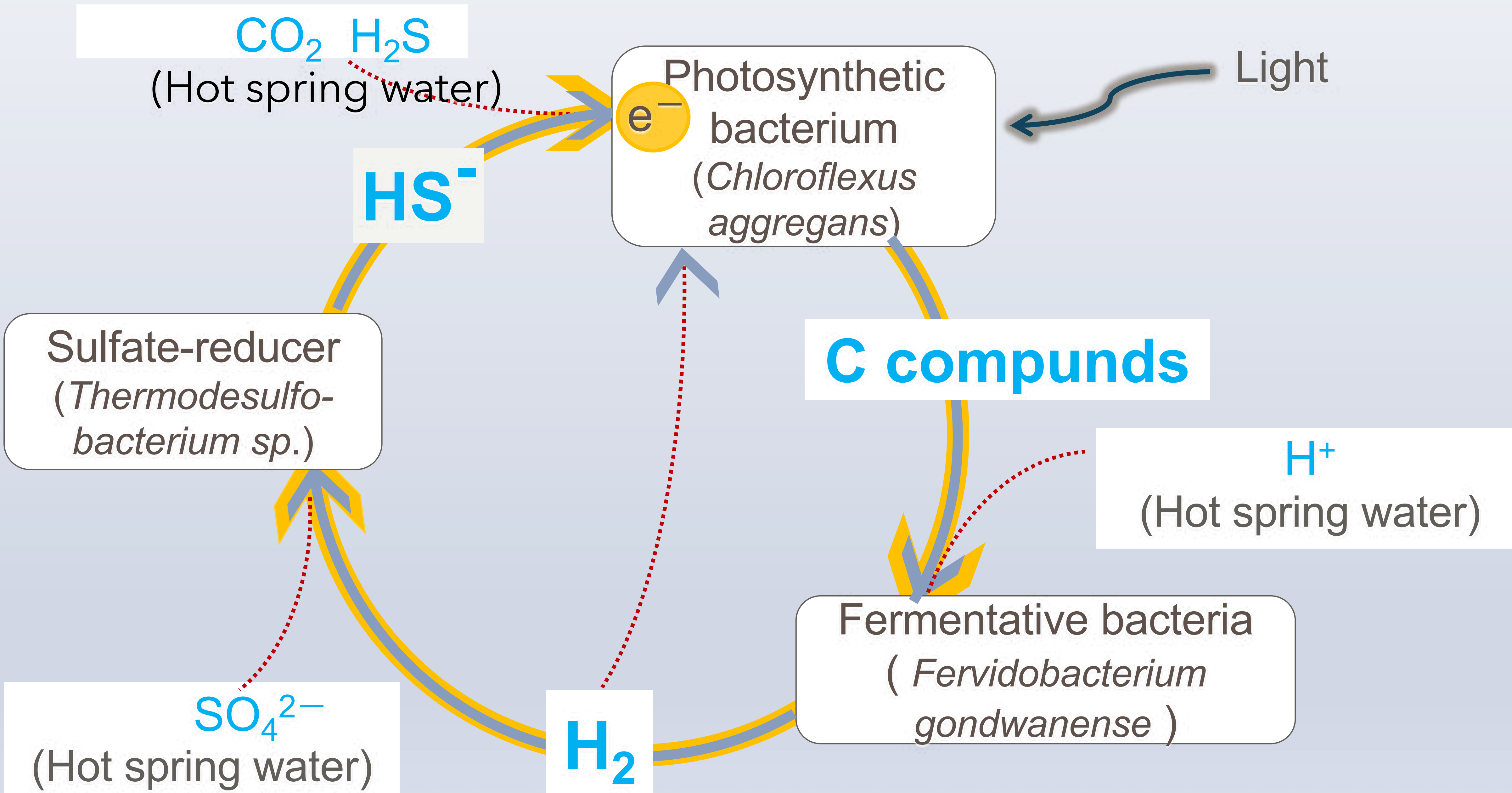
1. Hydrogen is produced in thermophilic photosynthetic microbial communities. (1/2)

Hydrogen production in *Chloroflexus* dominated mats at 65 °C



1. Hydrogen is produced in thermophilic photosynthetic microbial communities. (2/2)

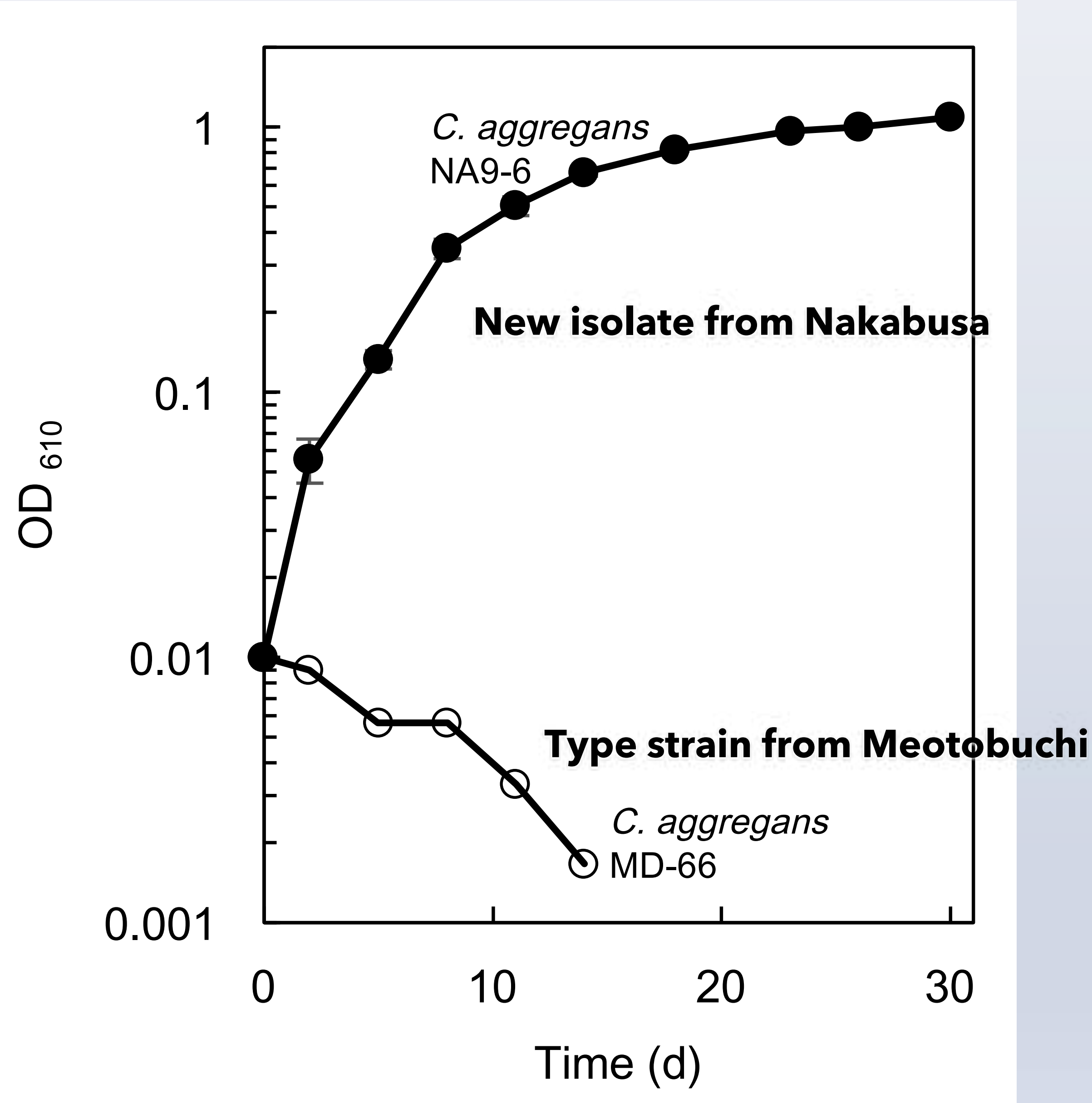
Electron cycling in thermophilic photosynthetic communities with changing chemicals



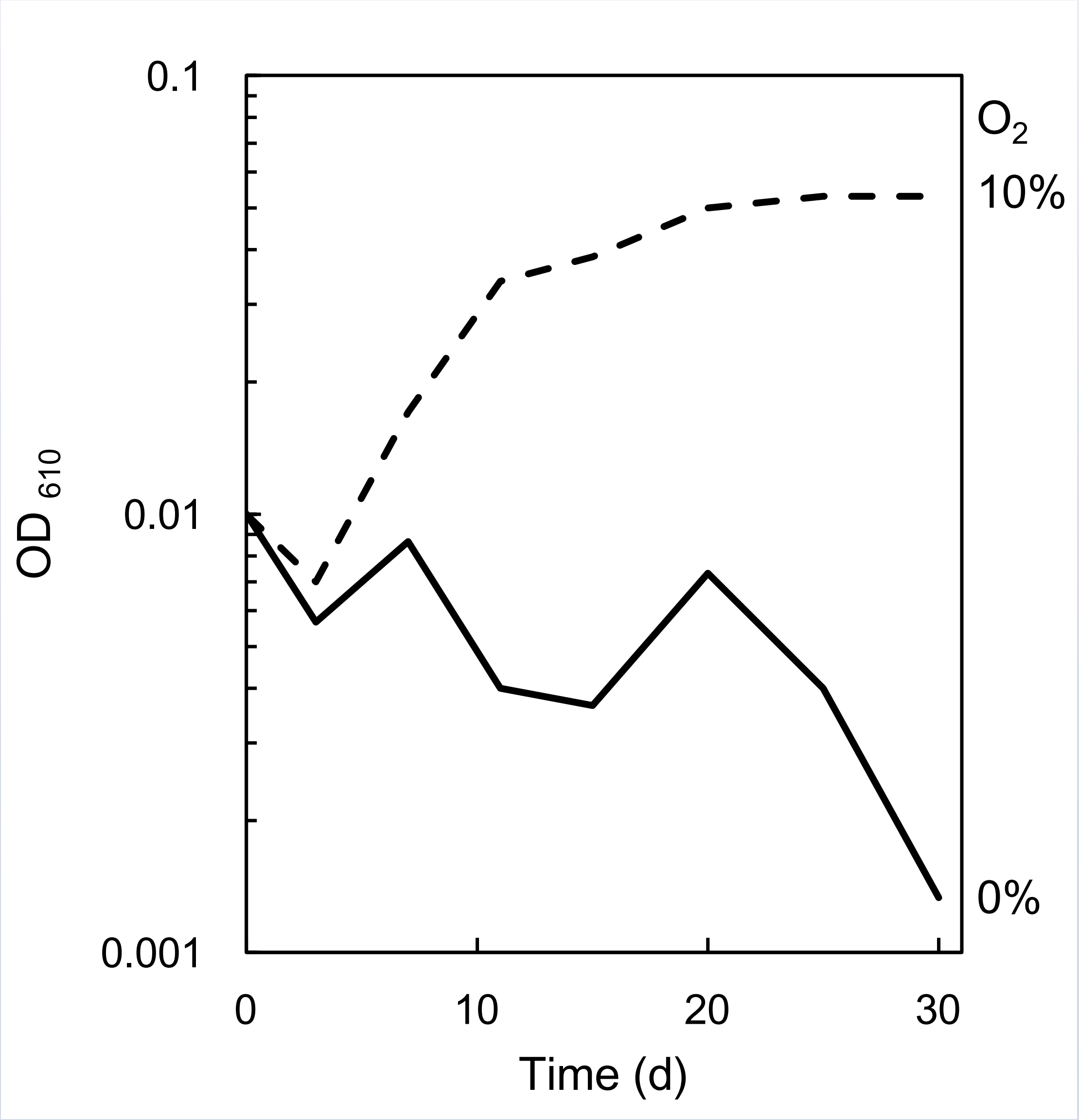
2. Hydrogen is a good electron donor for the photoautotrophic and chemoautotrophic growth of *Chloroflexus*. (1/1)

A new isolate of *C. aggregans* from Nakabusa

Hydrogen dependent photo-autotorophic growth in the light



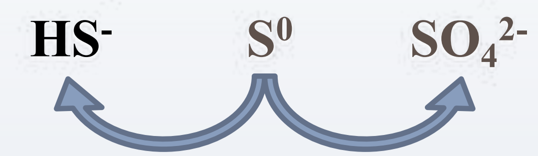
Hydrogen dependent chemo-autotorophic growth in the dark



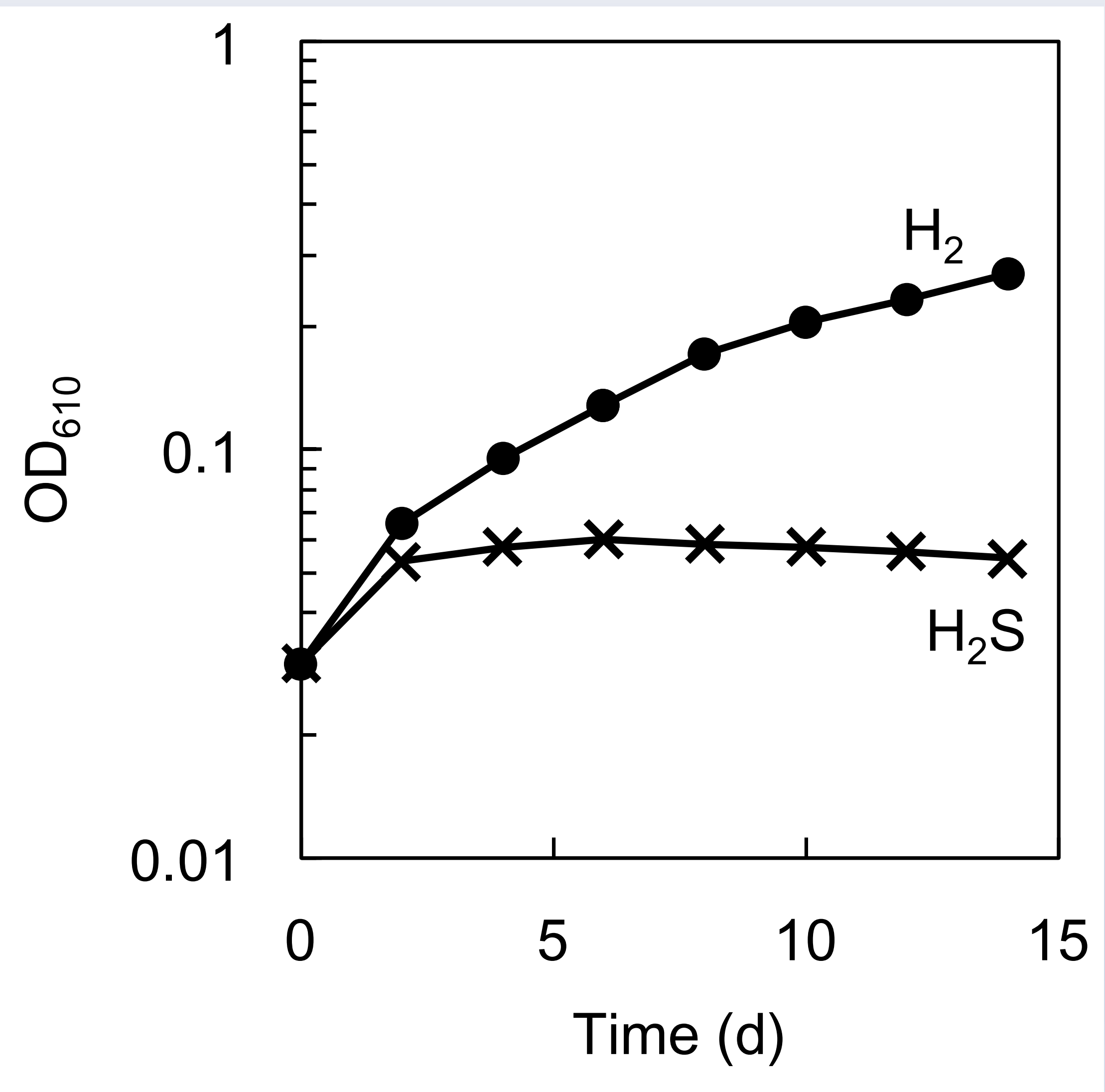
3. Sulfide is also a good electron donor for *Chloroflexus* only when produced elemental sulfur is consumed by a disproportionating bacterium. (1/2)

Caldimicrobium thiodismutans

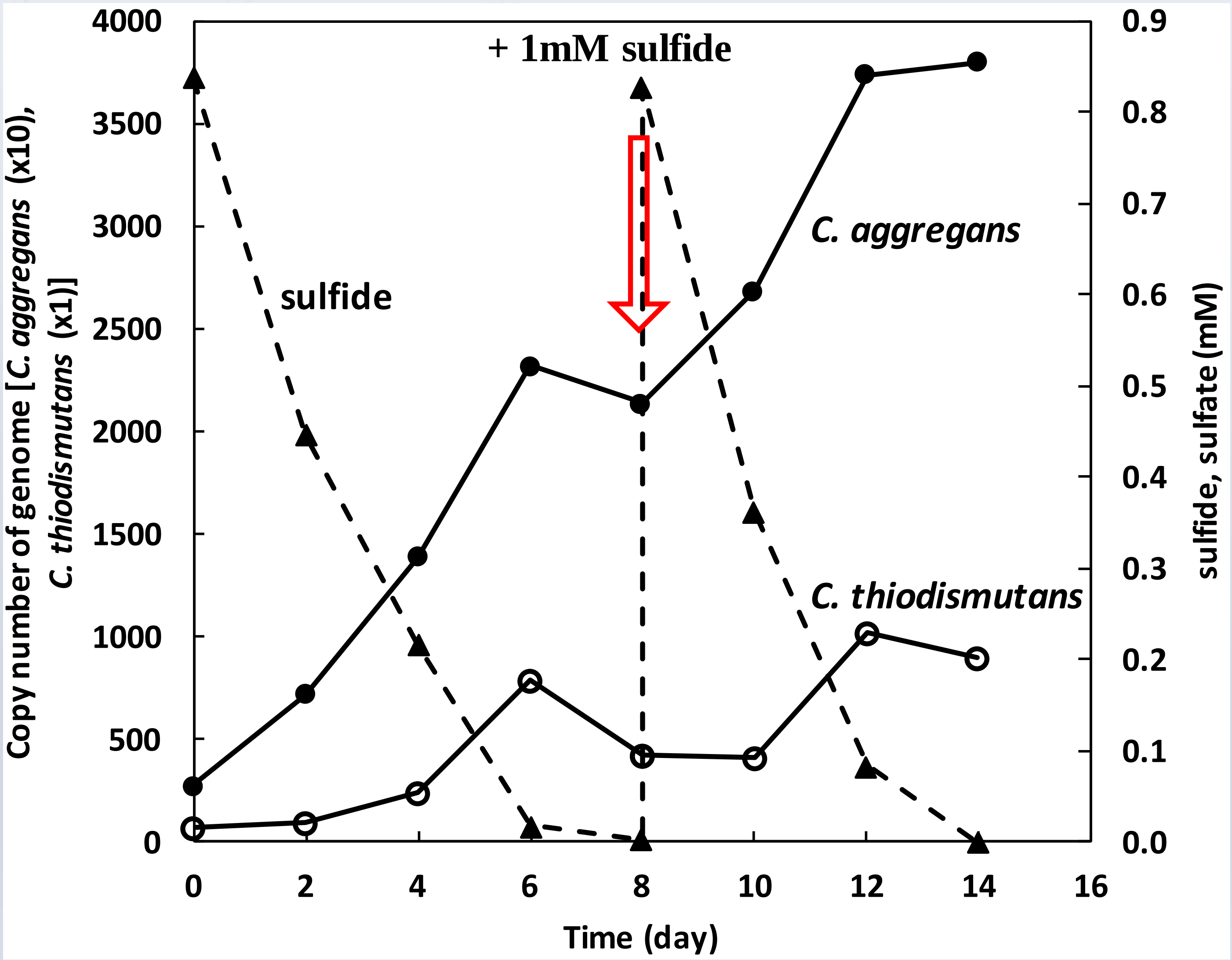
$$4S^0 + H_2O \rightarrow 3HS^- + SO_4^{2-} + 5H^+$$



Sulfide did not support photosynthetic growth in pure culture

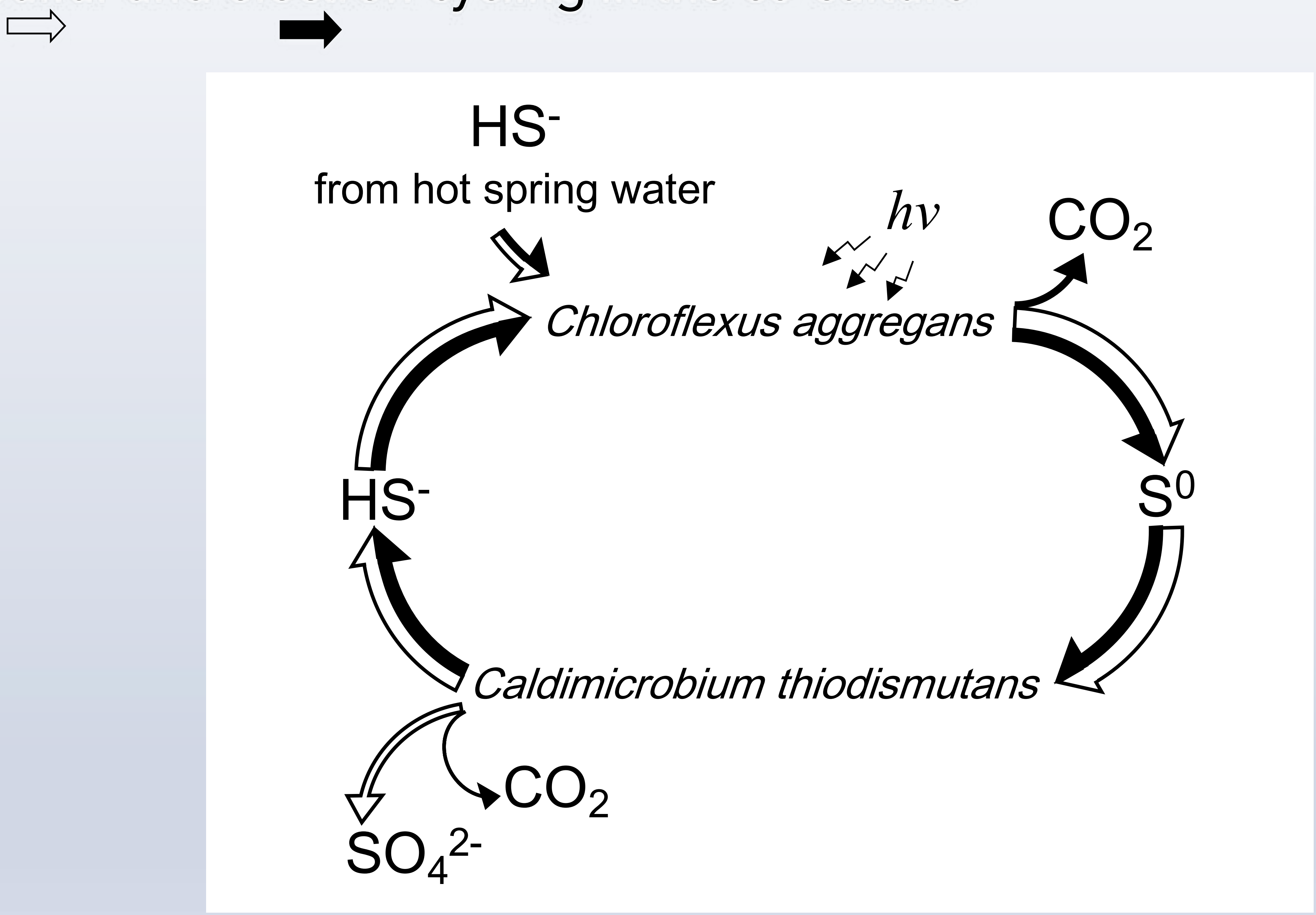


With elemental-sulfur disproportionating bacteria, sulfide supported photosynthetic growth



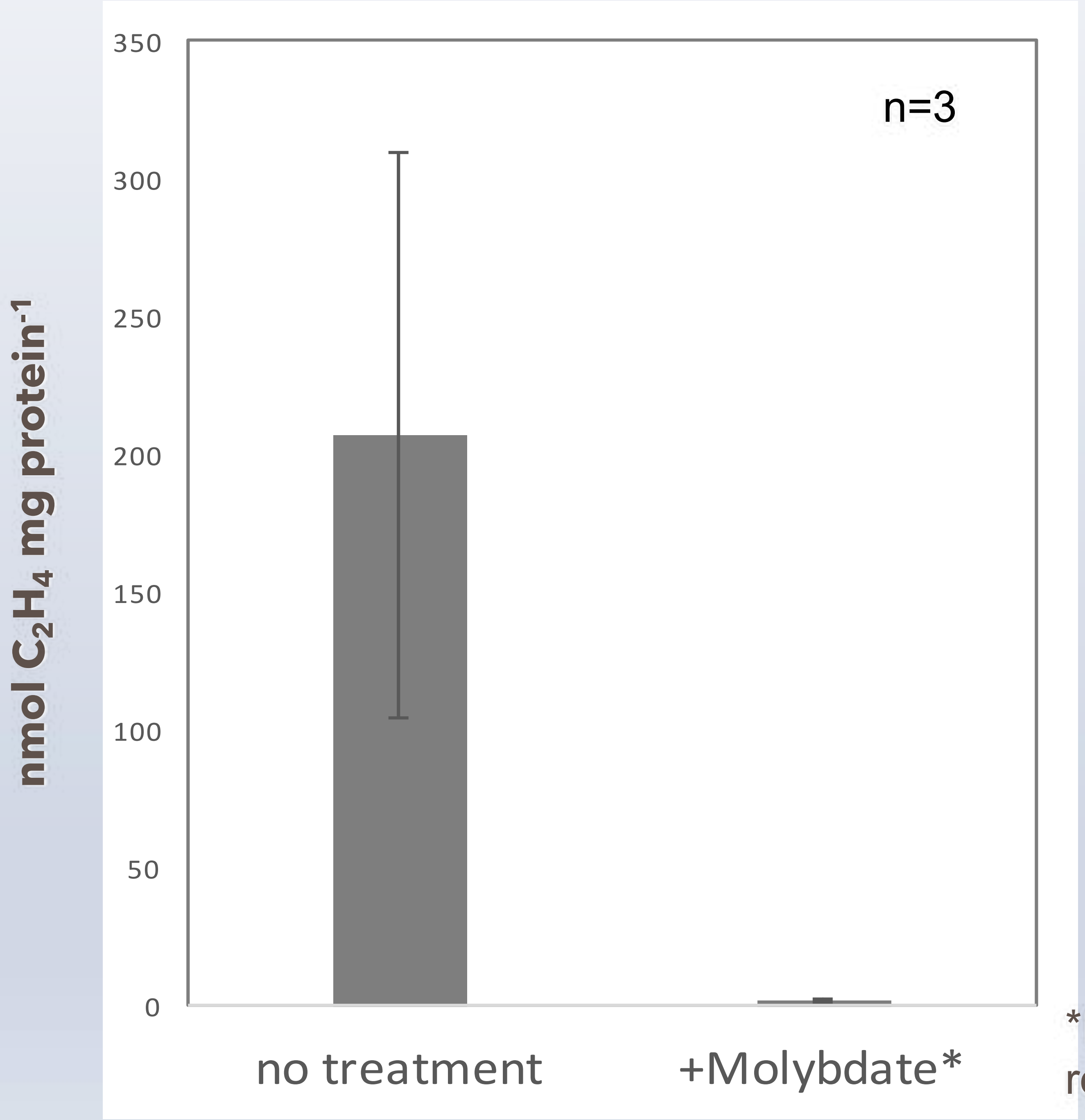
3. Sulfide is also a good electron donor for *Chloroflexus* only when produced elemental sulfur is consumed by a disproportionating bacterium. (2/2)

Sulfur and electron cycling in the co-culture



4. Nitrogen fixation was found in thermophilic chemosynthetic communities depending on hydrogen and sulfate. (1/2)

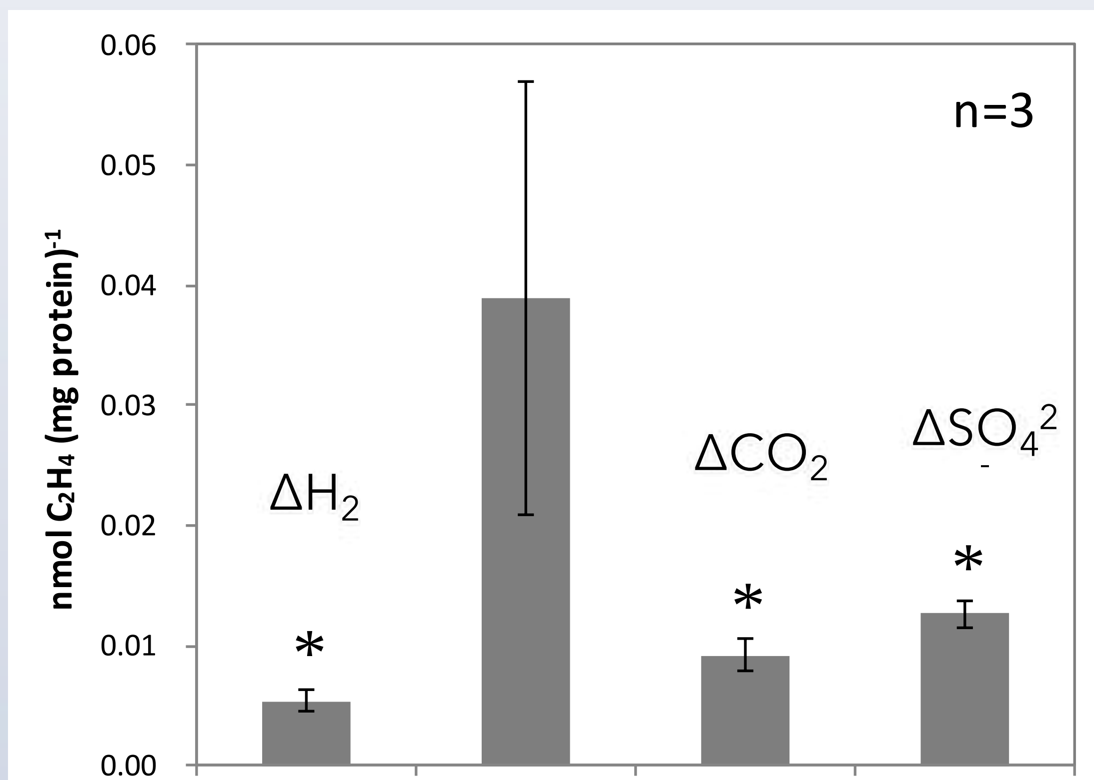
Nitrogenase activity was detected and decreased with the inhibitor of anaerobic sulfur metabolism



*Molybdate, an inhibitor for sulfate reduction and sulfur disproportionation

4. Nitrogen fixation was found in thermophilic chemosynthetic communities depending on hydrogen and sulfate. (2/2)

Effects of H_2, CO_2 and sulfate on nitrogen fixation in homogenized mats



	1	2	3	4
H_2	-	+	+	+
CO_2	+	+	-	+
SO_4^{2-}	+	+	+	-

Artificial hot spring water without sulfide (pH8.5):
1mM NaCl
1mM $NaH_2PO_4 \cdot 2H_2O$
0.5mM Na_2SO_4
1.0mM $NaHCO_3$

5. Nitrogen fixation in isolated bacteria at the highest temperature, so far, were observed, with hydrogen as the electron donor and oxygen and possibly elemental sulfur as the acceptors. (1/3)

Thermophilic chemolithoautotrophic bacteria in *Aquificae* were isolated under semiaerobic and nitrogen-fixing conditions

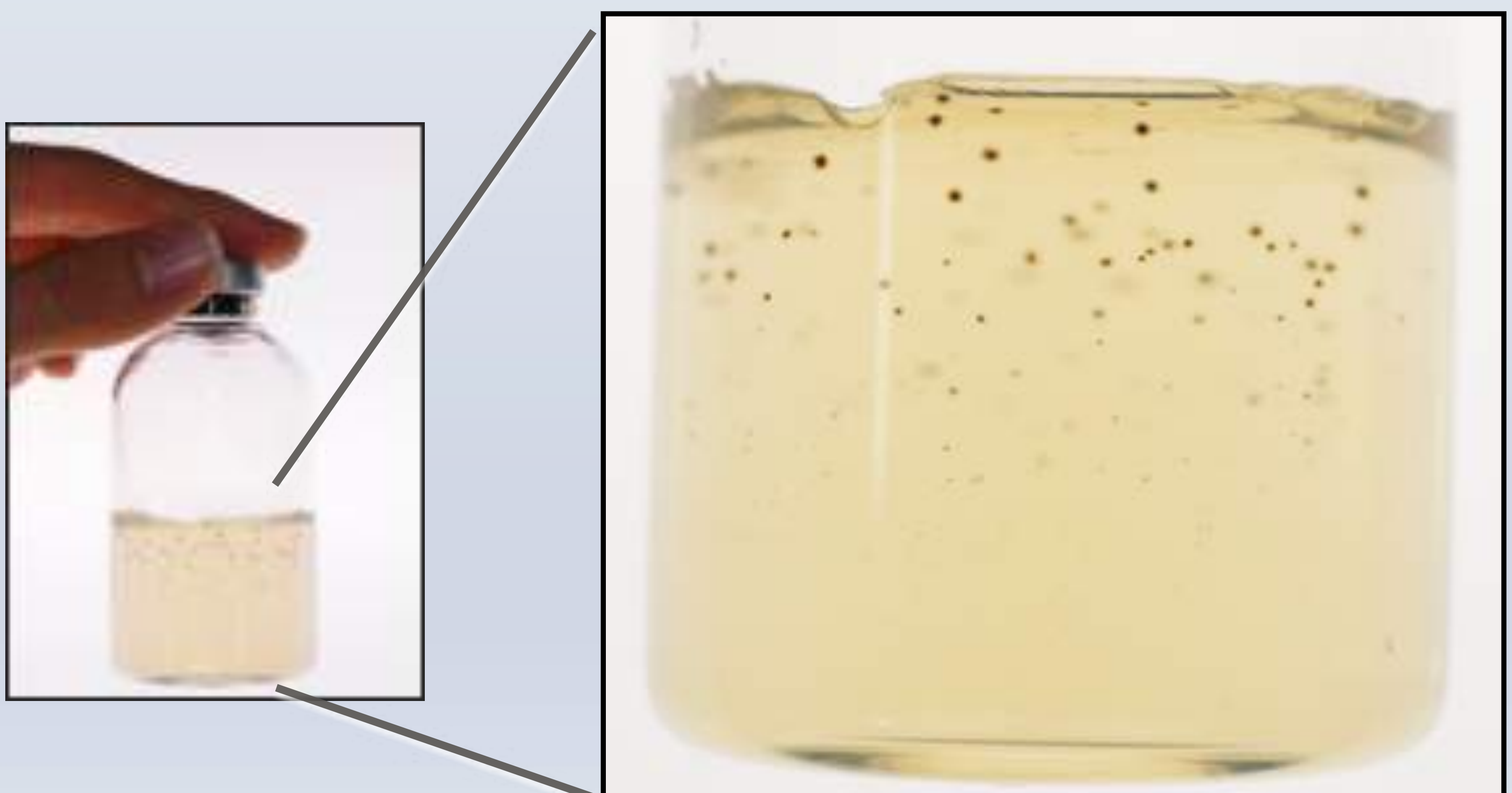
Cultivation conditions:

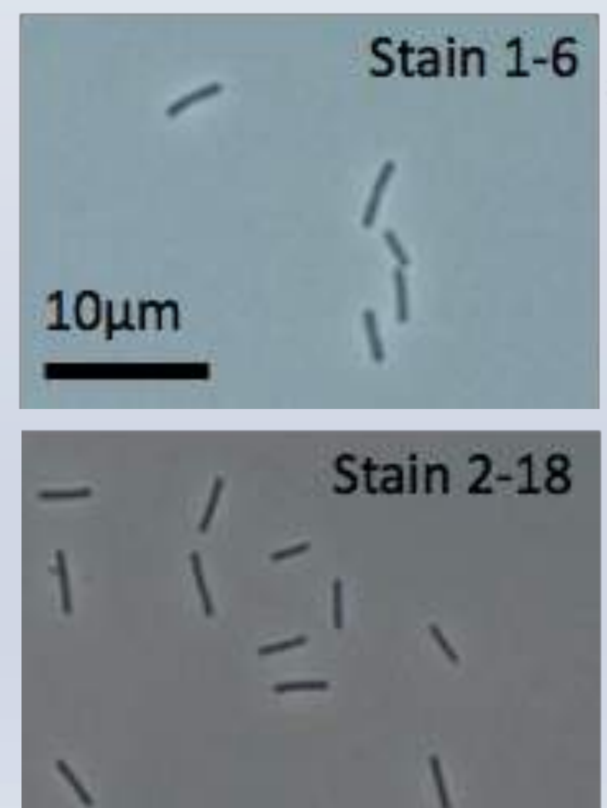
TK-6 modified medium (pH7.0) + Thiosulfate (0.1%)

Headspace: $N_2/CO_2/H_2$ (4/1/2, v/v/v) + O_2 (1-10%)

Incubation at 70°C

Electron donor: H_2 & Thiosulfate
Electron acceptor: O_2
Carbon sources: CO_2
Nitrogen sources: N_2



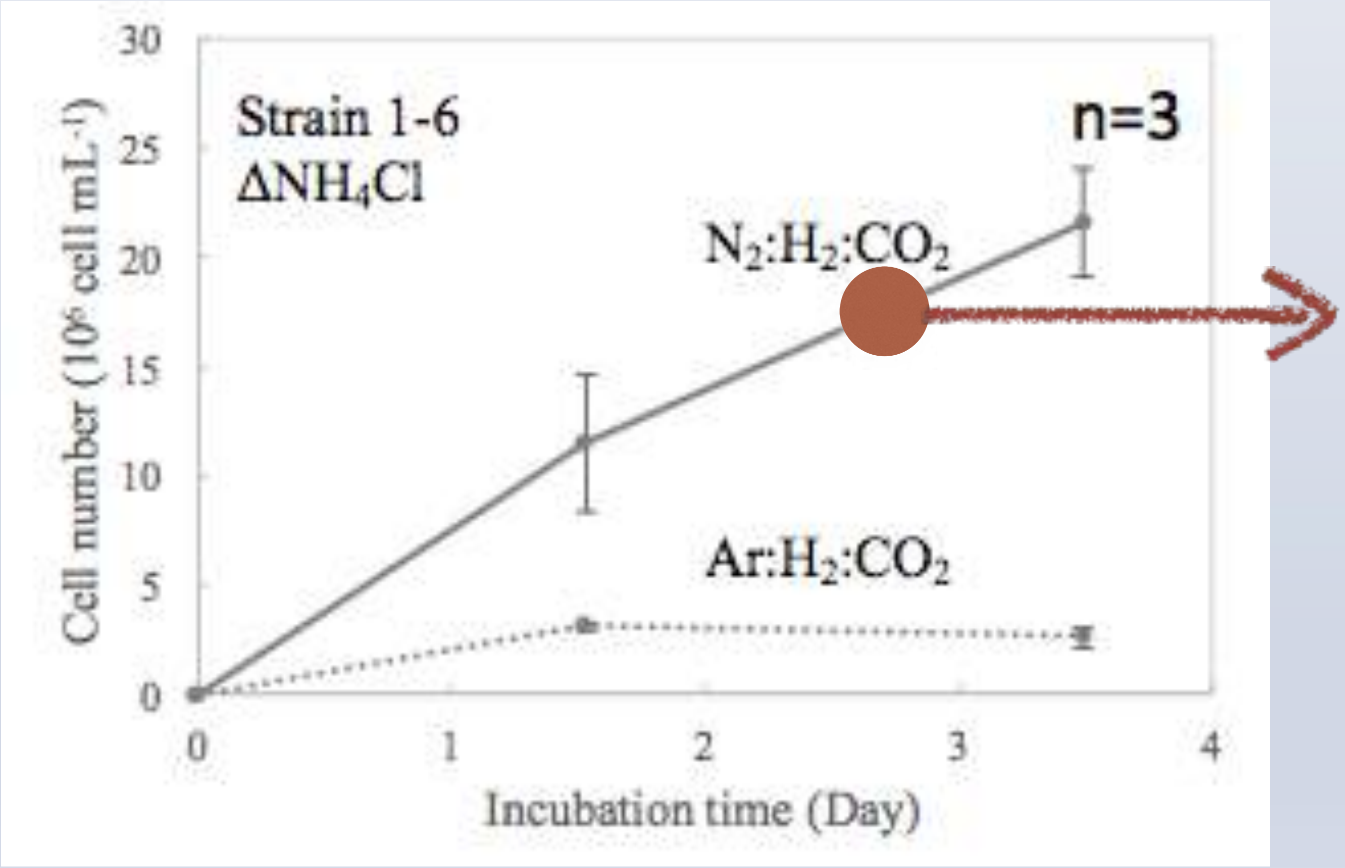


strains

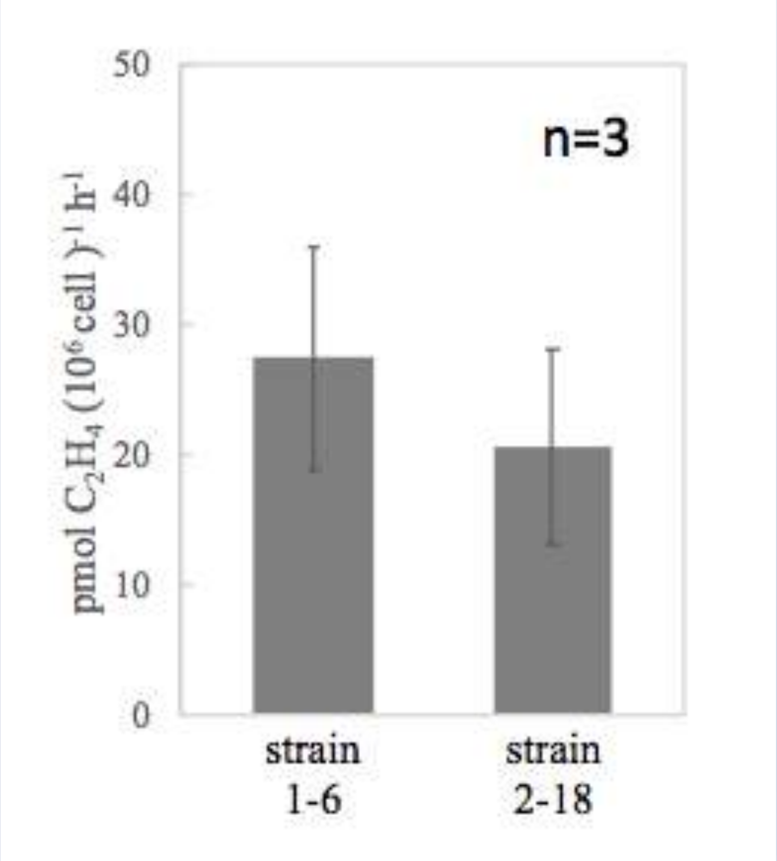
5. Nitrogen fixation in isolated bacteria at the highest temperature, so far, were observed, with hydrogen as the electron donor and oxygen and possibly elemental sulfur as the acceptors. (2/3)

Nitrogenase activities were detected in new *Aquificae* isolates at 70°C, highest teperature in bacteria, so far.

Culture under nitrogen-fixing conditions



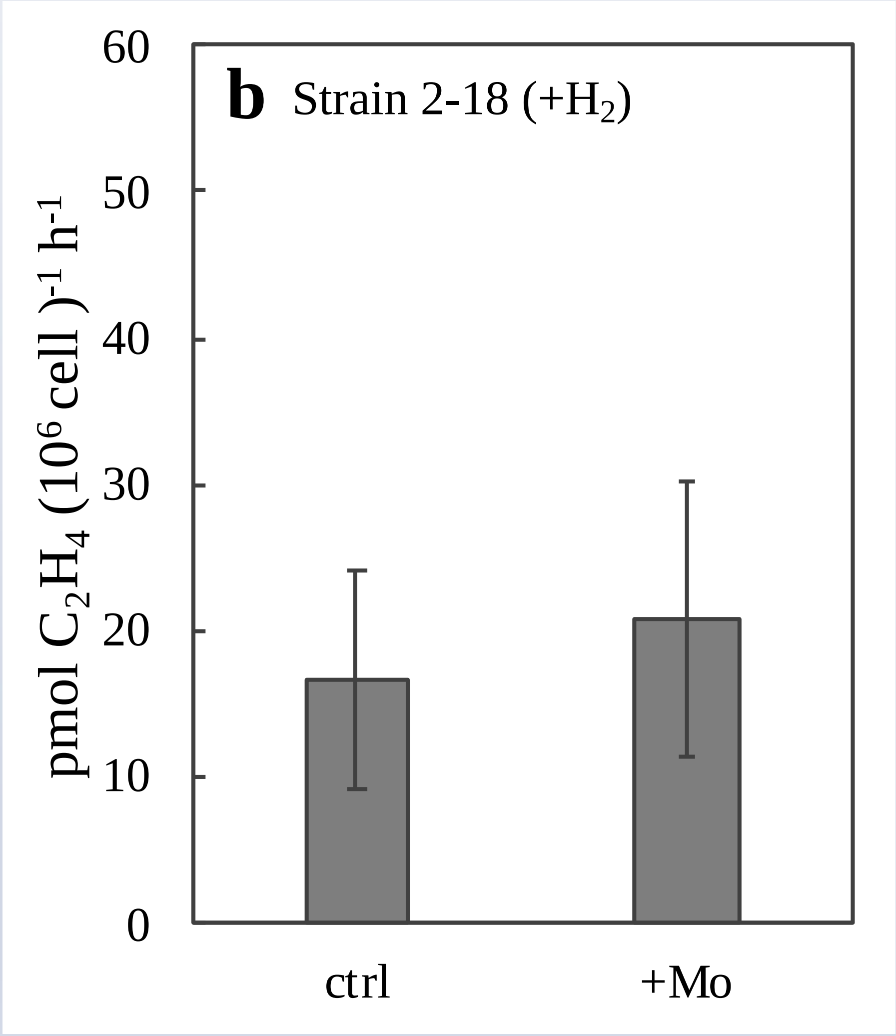
Nitrogenase activities



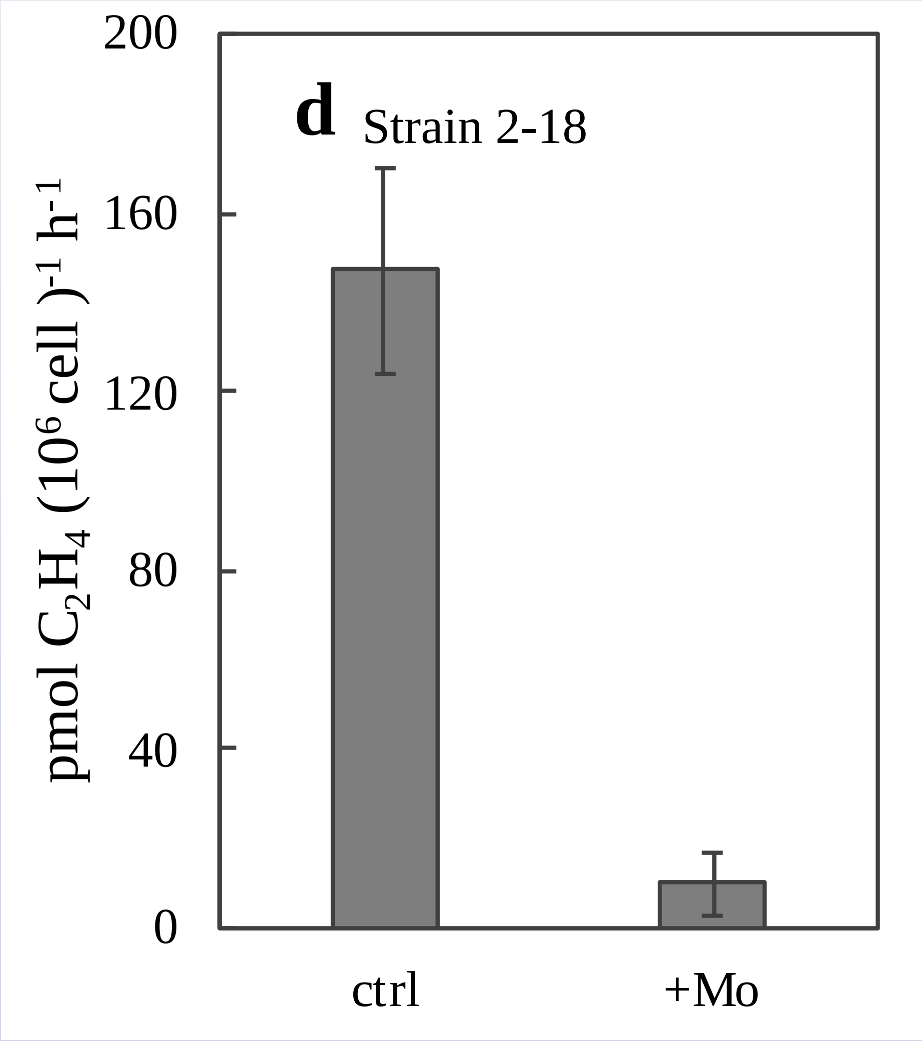
5. Nitrogen fixation in isolated bacteria at the highest temperature, so far, were observed, with hydrogen as the electron donor and oxygen and possibly elemental sulfur as the acceptors. (3/3)

Nitrogen fixation in isolated Aquificae was increased under anaerobic conditions, and the activity became molybdate sensitive

Aerobic conditions



Anaerobic conditions



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Summary

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Katsumi Matsuura retired from Tokyo Metropolitan University In March 2018 after 33 years. Followings are titles from his retiring symposium by former students and young collaborators.

Fukui, M.: Microbial sulfur cycle in aquatic environments
McGlynn, S.: APS reductase: Constraints from enzyme kinetics on the long term evolution of the biogeochemical sulfur cycle
Frigaard, N. U.: The amazing little photothiotroph: *Chlorobaculum tepidum*.
Shimizu, T.: Persulfide-responsive transcriptional repressor SqrR regulates sulfide-dependent photosynthesis.
Nishihara, A.: Nitrogen fixation and hydrogen/sulfur metabolism in hyperthermophilic chemosynthetic microbial communities.
Takabe, Y.: How are aerobic anoxygenic phototrophic bacteria living in the ocean?
Hirose, S.: Diversity of aerobic anoxygenic photosynthetic bacteria in a river
Harada, J.: Chlorosomal pigment biosynthesis of brown-colored green sulfur bacteria
Masuda, S. The blue-light photoreceptor BLUF controls various light-dependent physiology in photosynthetic bacteria and cyanobacteria
Nagashima, K. V. P.: Study on photosynthetic apparatuses of purple bacteria through use of heterogeneous expression system
Nagashima, S.: Sharing of electron donors to utilize the reducing power of photosynthesis for nitrite respiration
Kawai, S.: Photo- and chemolitho-autotrophic growth and hydrogen/sulfur metabolism in anoxygenic photosynthetic bacteria in the genus *Chloroflexus*
Fukushima, S.: Direction of gliding movement driven by individual cell movements in a multicellular filamentous bacterium *Chloroflexus aggregans*
Tank, M.: Hot springs are hotspots on the hunt for novel and unusual chlorophototrophic bacteria
Thiel, V.: Diel meta-omics and microsensor analyses of cyanobacterial hot spring mats in Nakabusa, Japan
Kanno, N.: The survivability of purple non-sulfur bacteria under non-growing conditions
Kubo, K.: A sidelight shining on microbial hydrocarbon degradation under anoxic conditions
Everroad, R. C.: Evolutionary innovation and ecological interactions in microbial systems
Osyczka, A.: Photosynthetic bacteria linking Tokyo, Philadelphia and Kraków
Sakuragi, Y.: Tracking biological carbon fixation and carbohydrate biosynthesis

He is currently a school principal of a Japanese language school for Asian students, as well as in charge of national biology curriculum standard of high schools in Japan.