Development of a Bioprocess That Uses Electrical Energy to Fix Atmospheric CO_2

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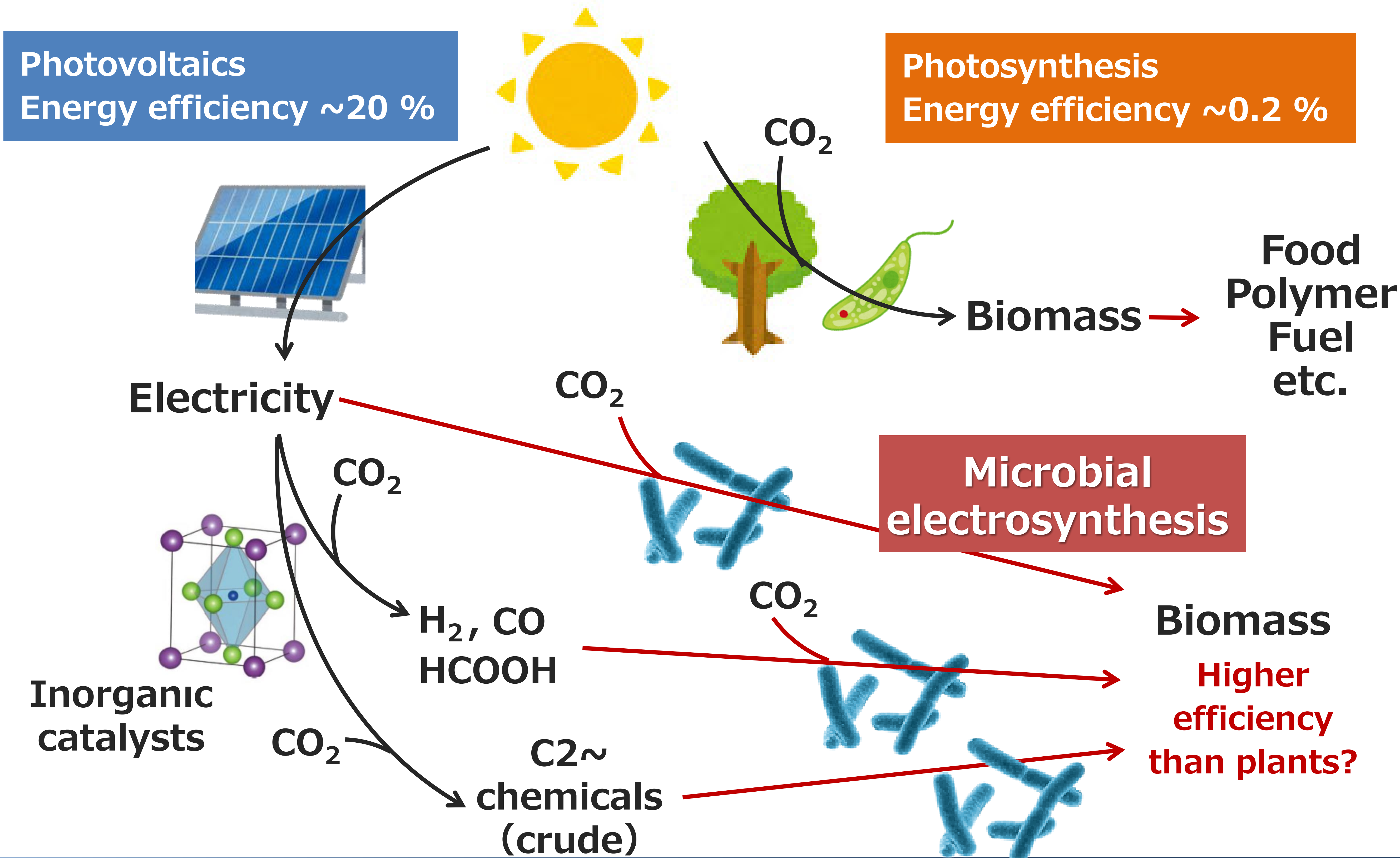
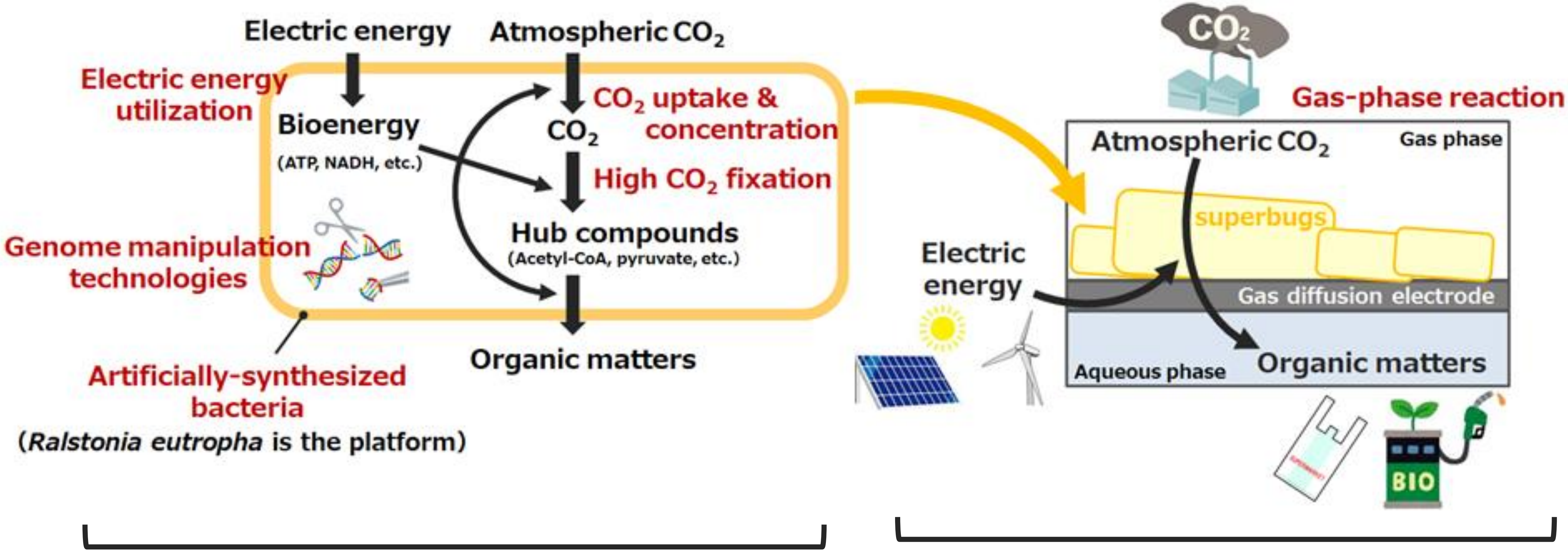
Implementing organizations :
National Institute of Advanced Industrial Science and Technology (AIST)
Tokyo Institute of Technology
Nagoya University
Osaka University
Kobe University

Concept | Material science × Biotechnology



Summary of our project

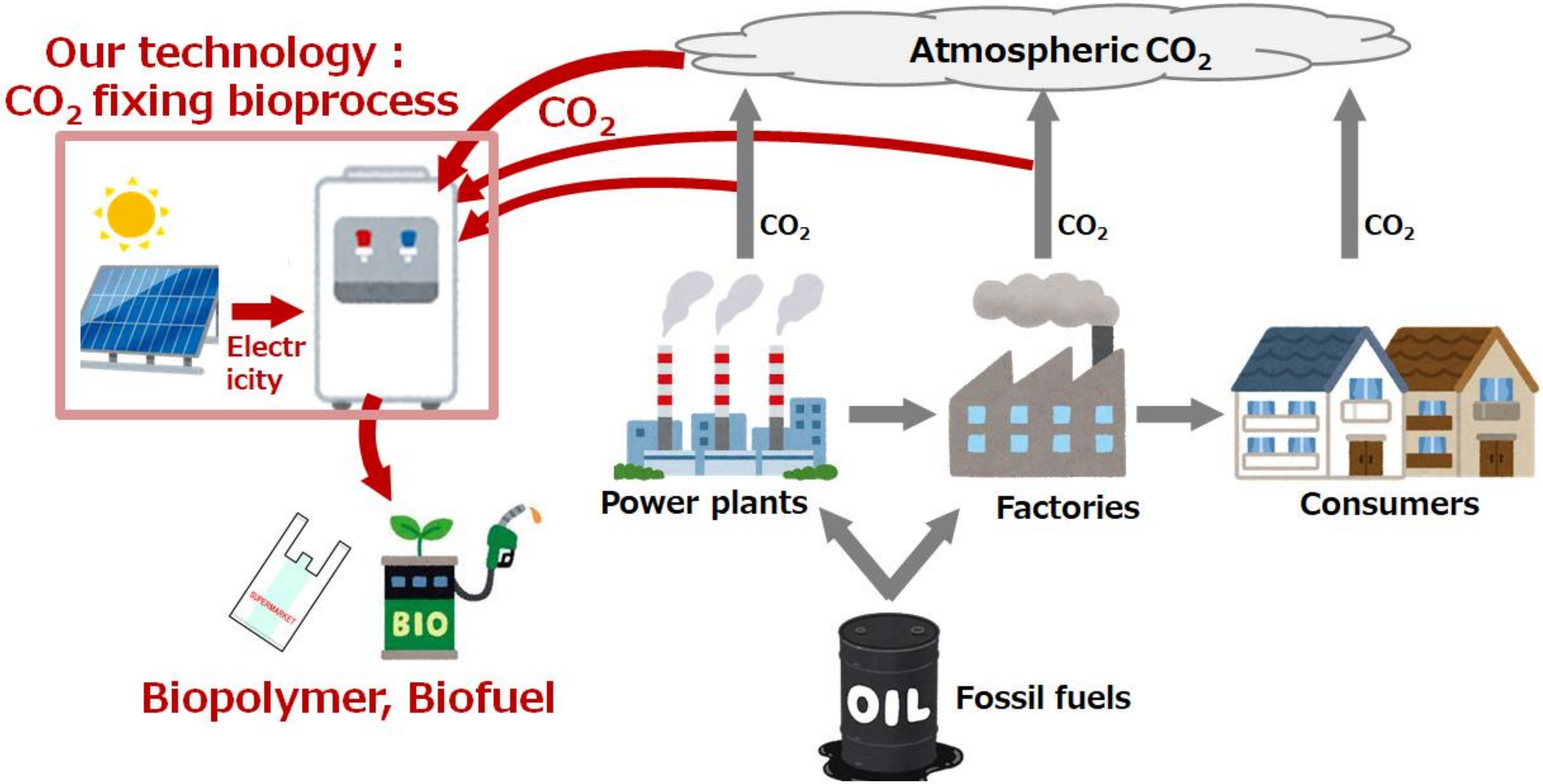
- Development of an innovative biotechnology for negative emission
- Utilizing electric energy to convert atmospheric CO_2 into organic matters
- More than 50 times more efficiently than plants (>50 kg-CO_2/m^2/year)



"superbugs"
that use electric energy, uptake & concentrate atmospheric CO_2, and fix CO_2 with high efficiency.

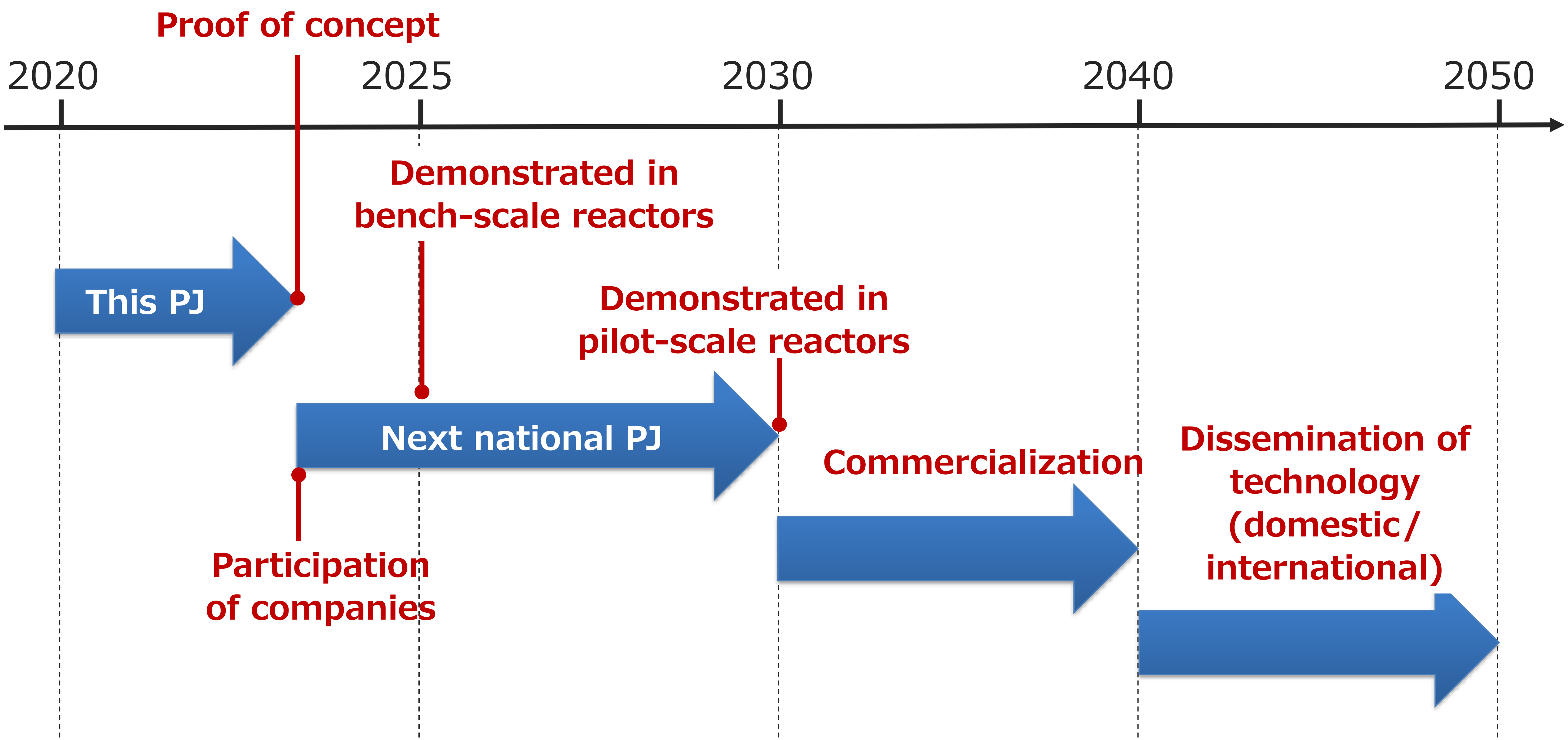
"gas-phase reaction process"
that can effectively supply electricity, nutrients and CO_2 to superbugs.

Social implementation



∗Reduction of atmospheric CO_2 (applicable to high conc. CO_2)
∗Use electricity from renewable energy sources (Light, etc.)
∗New sources of biopolymers and biofuels

Development Schedules



- 500,000 tons of CO_2 fixed per year (3% of the negative emission target)
- 210,000 tons of organics production per year
  (10% of domestic bioplastics and fuels production)

R&D Items & Cooperation

■ Achievement goal (2022FY) : Demonstrate the feasibility of microbial CO_2 fixation by electricity using a gas-phase reactor

Project management, Synthetic microbiology (AIST)

①Genome manipulation technology

・Long DNA transfer technology

・Promoter library

②-4. Synthetic microbiology

・Create bacteria that can use electricity to efficiently produce organics

Genome manipulation technology

Gene info.

Provide bacteria

Provide reactor technology

Development of each property

②-1. Electricity utilization (Tokyo Tec. Inst.)

②-2. CO_2 uptake/concentration (Kobe Univ.)

②-3. CO_2 fixation (Tokyo Tec. Inst.)

Development of a gas-phase reactor

③-1. Reactor engineering (Nagoya Univ.)

③-2. Gas diffusion electrodes (Osaka Univ.)

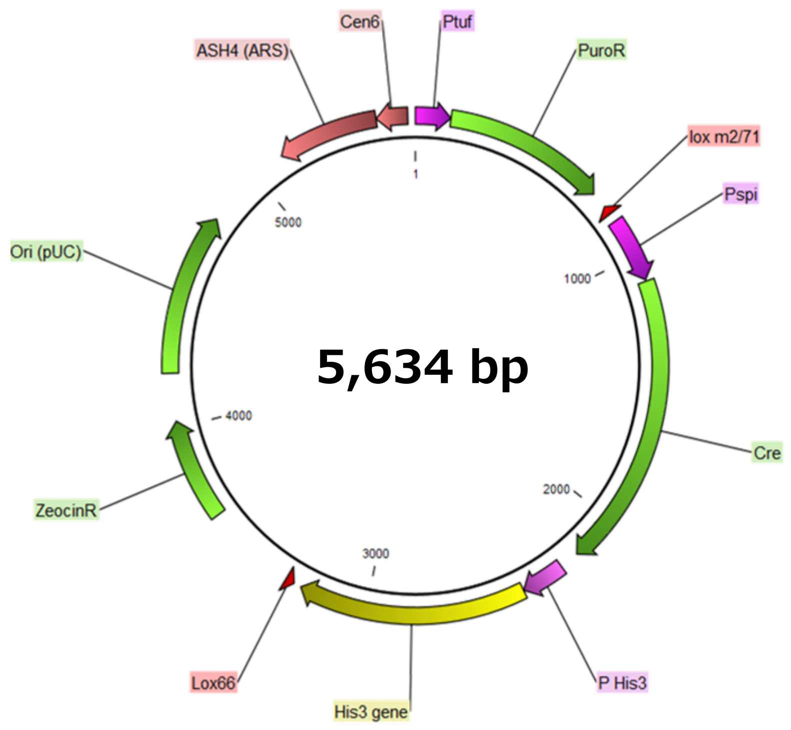
1. Genome manipulation method

■ **Target in this PJ : Development of genome manipulation method for *Ralstonia***

＊Genome manipulation method

Objective: Develop a method that can introduce long DNA into the genome.

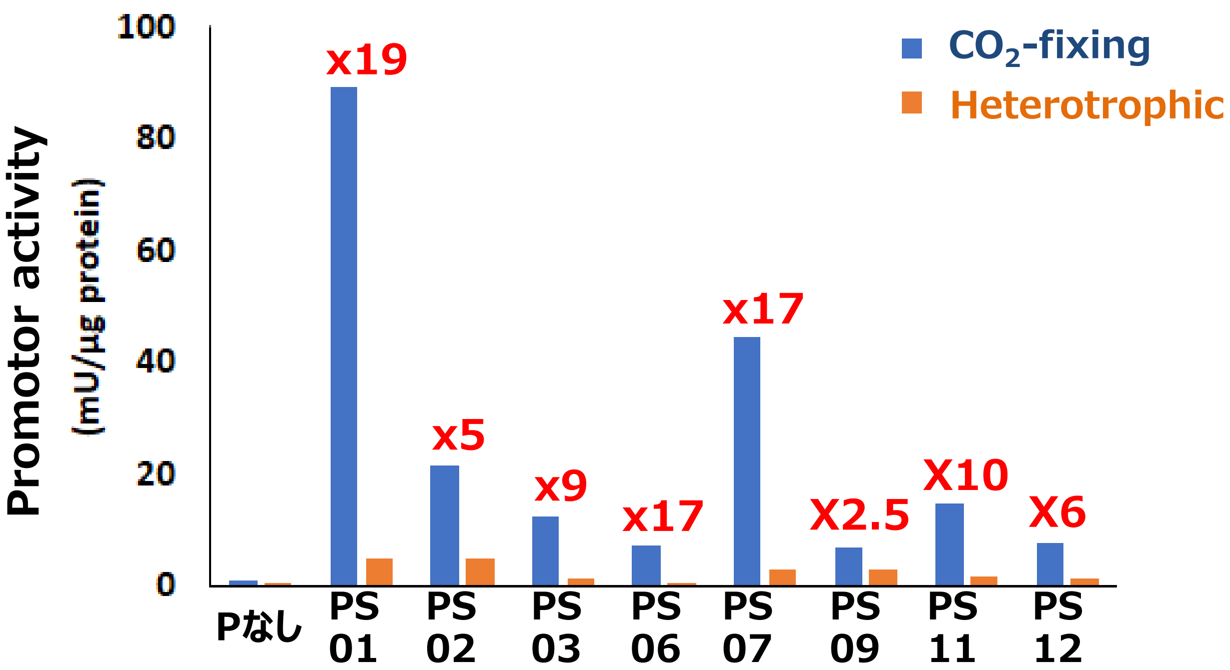
Achievements:
- ・Design a vector based on yeast artificial chromosome
- ・Gene introduction into the genome was achieved by CreLoxP method



＊Promotor library

Objective: Obtain promoters necessary to appropriately express the transgenes

Achievements:
- ・A simple promoter activity evaluation system was developed
- ・Identify 8 promoters that function specifically under CO_2-fixing conditions



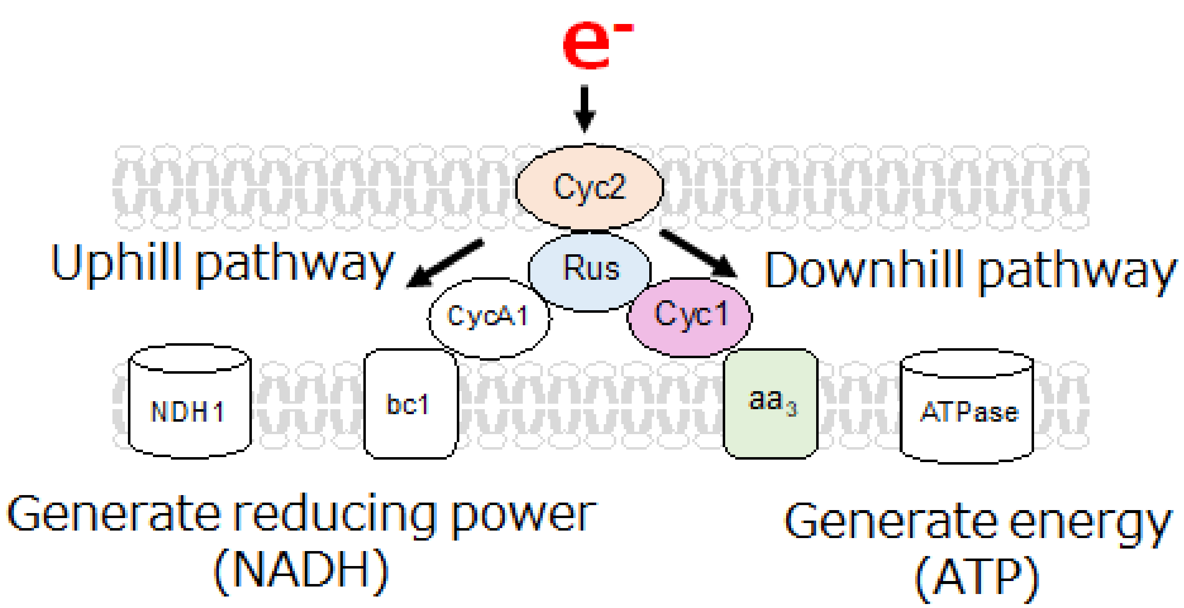
2-1. Electricity-utilizing activity

■ **Target in this PJ: Introducing a heterogeneous microbial electron transfer path in *Ralstonia* to confer electricity-utilizing activity**

＊Introduction of electron transfer path

Objective: Introduce electron transfer path genes of *Acidithiobacillus*

Achievements:
- **Uphill path & Up/Downhill paths were introduced into *Ralstonia***
- **The expression was confirmed at RNA and protein level**

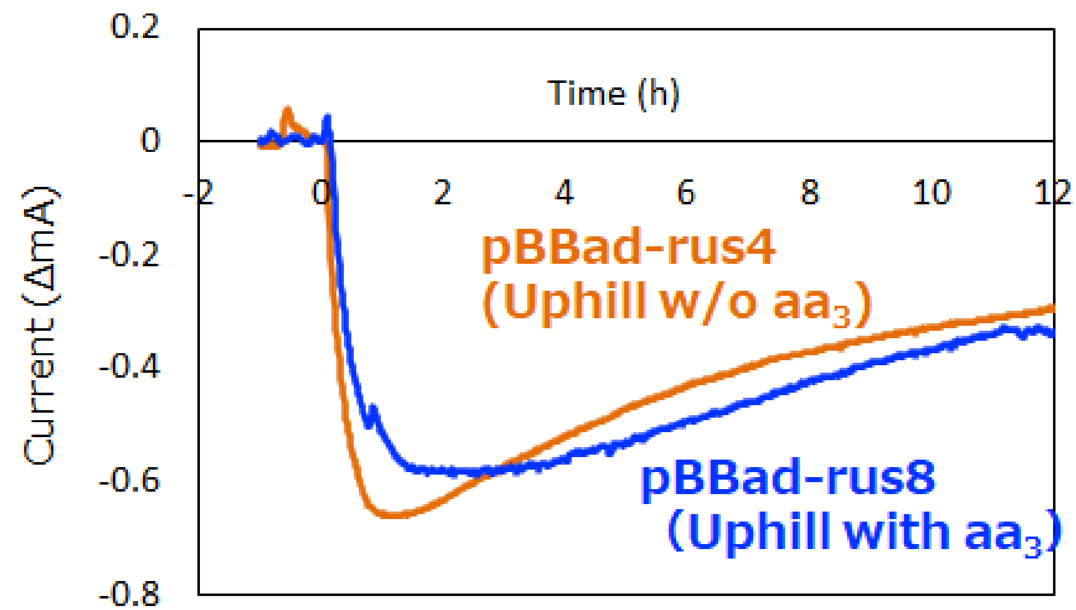


Electron transfer path of *Acidithiobacillus*

＊Electrochemical measurement

Objective: Demonstrate the electricity-utilizing activity of the *Ralstonia* strains

Achievements:
- **Current consumption was observed in Uphill pass-introduced *Ralstonia* strains**
- **On-going for Up/Downhill paths mutants**



50 mM futarate buffer (pH4.5)
-0.1 V vs. Ag/AgCl

2-2. CO_2 uptake/concentration

■ **Target in this PJ: Introducing CO_2-fixing enzyme/-enrichment systems into *Ralstonia* to henhance their activities**

＊Introduction of CO_2 enrichment system

Objective: Introduce CO_2 enrichment systems of cyanobacteria into *Ralstonia*

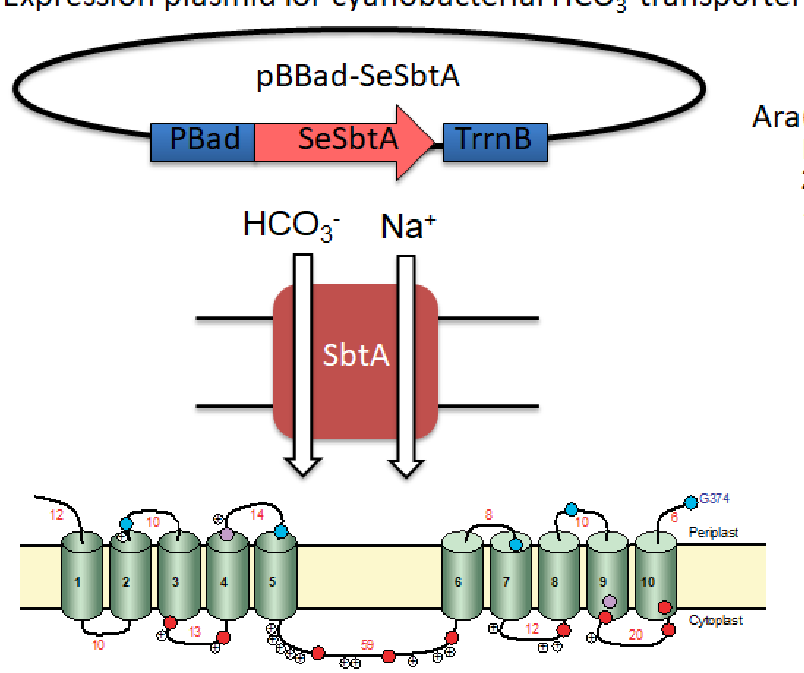
Achievements:
- **A bicarbonate transport protein was adequately expressed in *Ralstonia***
- **On-going for its activity measurements**

＊High expression of CO_2-fixing enzyme

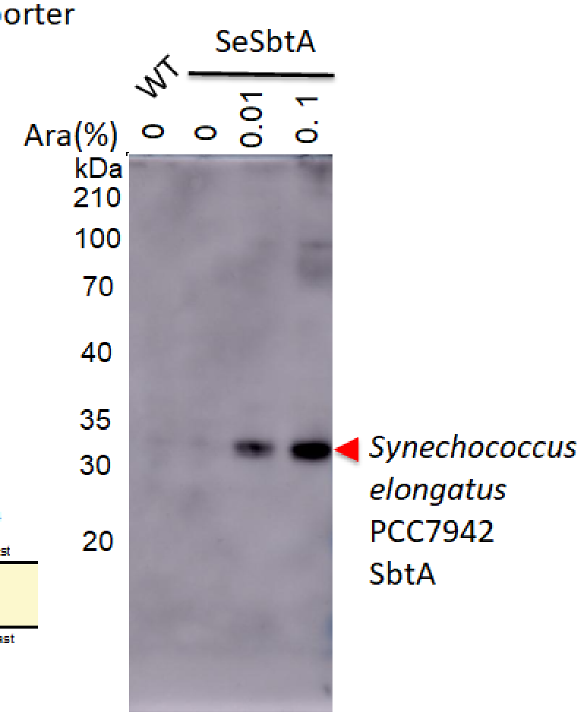
Objective: High expression of endogenous and exogenous CO_2-fixing enzyme (RuBisCO)

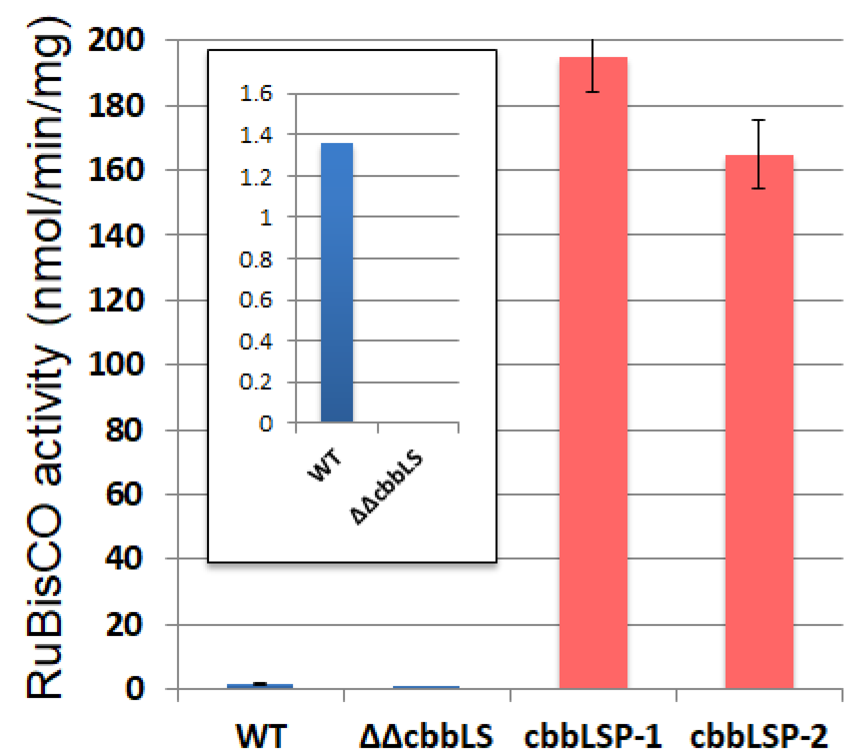
Achievements:
- **High expression of endogenous RuBisCO resulted in higher activity/growth**
- **On-going for exogenous ones**

Expression plasmid for cyanobacterial HCO_3^- transporter



Price et. al., 2011





RuBisCO-introduced strains

2-3. Enhancement of CO_2 fixation

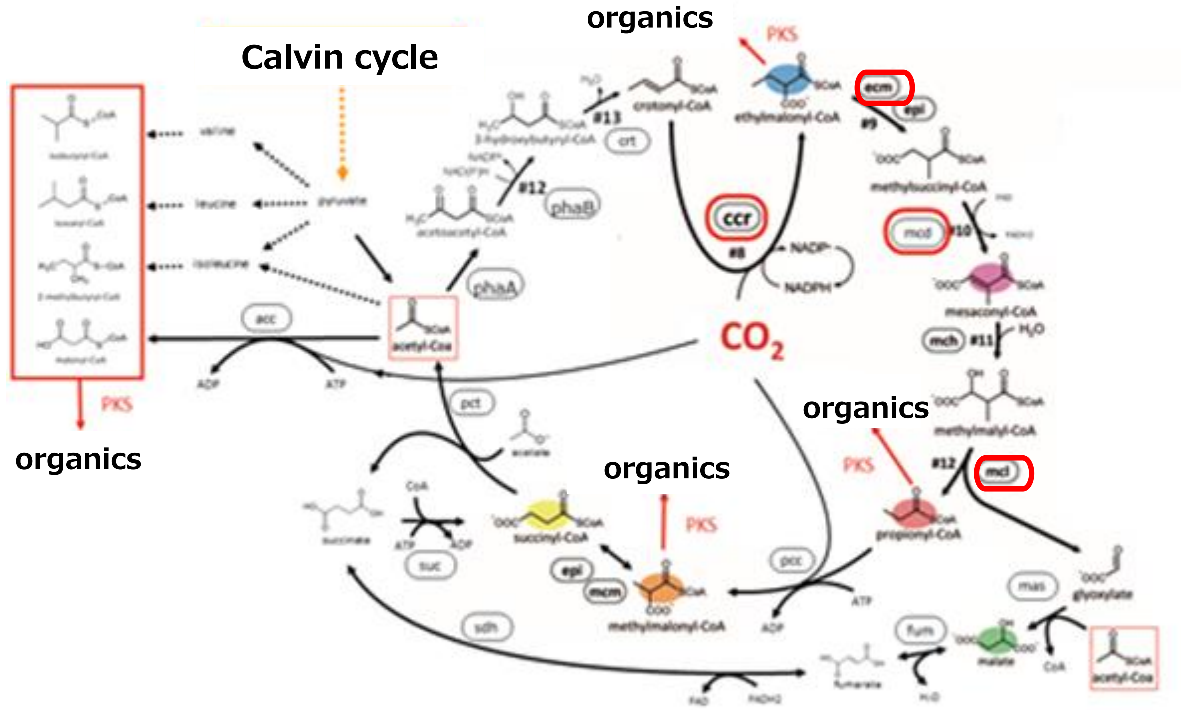
■ Target in this PJ: Enhancing CO_2 fixation by introducing a semi-artificial pathway

＊Construction of semi-artificial pathway

Objective: Introduce exogenous enzymes to make a semi-artificial CO_2 fixation pathway

Achievements:
・The Em-rTCA pathway that functions by introducing 4 enzymes
・CO_2 fixation by the Em-rTCA pathway was confirmed by isotope experiments.

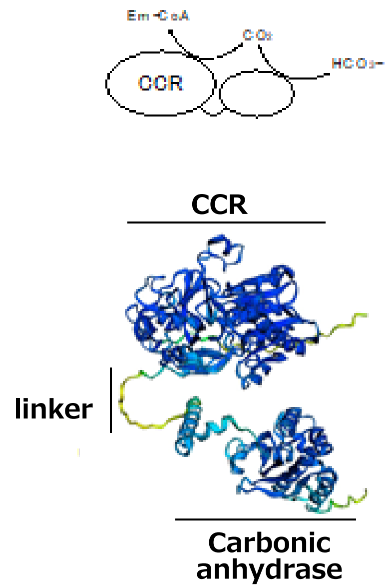
Em-rTCA pathway



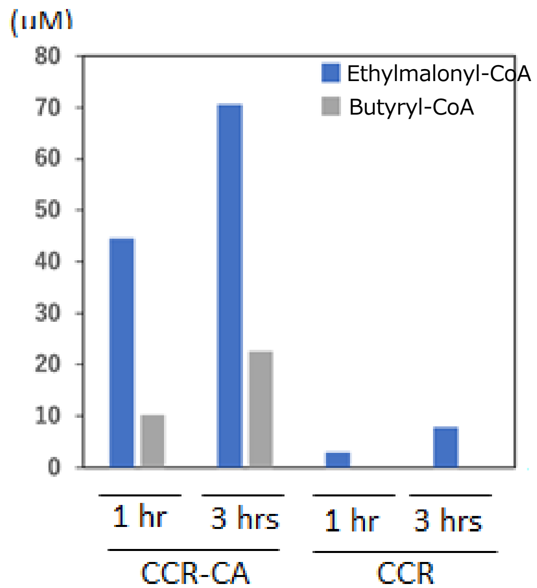
＊Improvement of CO_2-fixing enzymes

Oblective: Modify two CO_2-fixing enzymes to enjance Em-rTCA pathway

Achievements:
・Activity of CCR was enhanced by fusion with carbonic anhydrase
・Activity of PCC was enhanced by fusion with other bacterium’s domain



CCR-CA fusion protein



CCR-CA has higher CO_2-fixing activity than original enzyme

3. Gas-phase reactor (1)

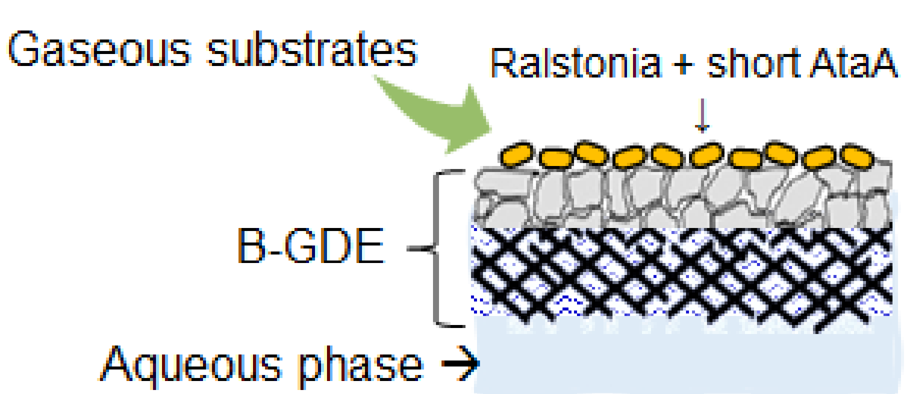
■ **Target of this PJ : Establishing a gas-phase reactor to enhance CO_2 fixation**

＊Gas diffusion electrode

Objective: Develop electrodes capable of supplying electricity, gas (CO_2), and liquid (nutrients) to *Ralstonia*

Achievements:

- **Gas diffusion electrodes used in fuel cells were modified for bio-reactions**
- **Adjustment of resin/carbon mixing ratio in microporous layer, etc. enabled appropriate gas and liquid diffusivity**



[A]: Applied microporous layer (MPL) (Ketjen Black (KB) + PVDF)
[B]: Backing paper (Toray carbon paper + 5% PTFE)

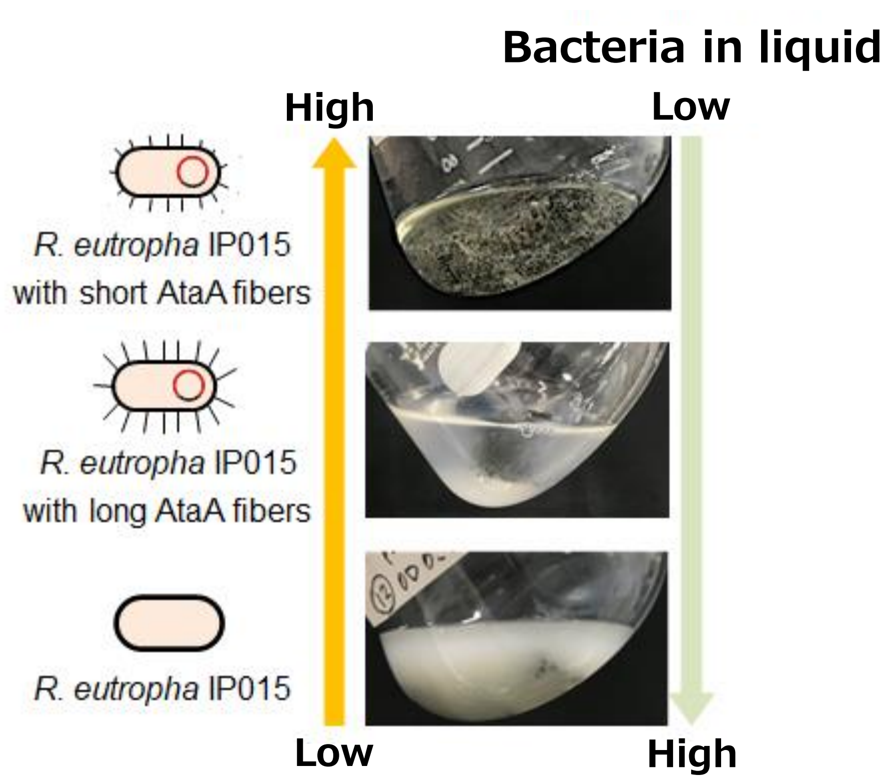
Gas diffusion bio-electrode (B-GDE)

＊Adhering ability of *Ralstonia*

Objective: Improve electrode adhesion in *Ralstonia* by introducing adhesive fibers

Achievements:

- **Adhesiveness of *Ralstonia* was improved by introduction of *Acinetobacter*-derived adhesive fiber protein (Ata)**



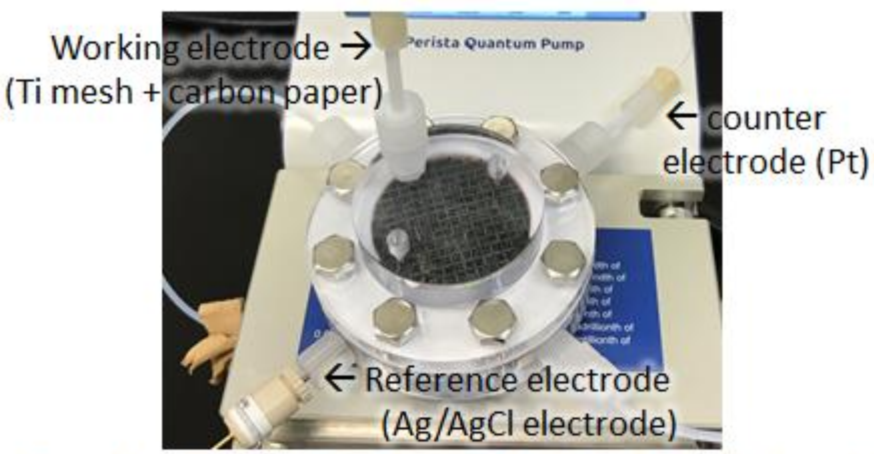
3. Gas-phase reactor (2)

■ **Target of this PJ : Establishing a gas-phase reactor to enhance CO_2 fixation**

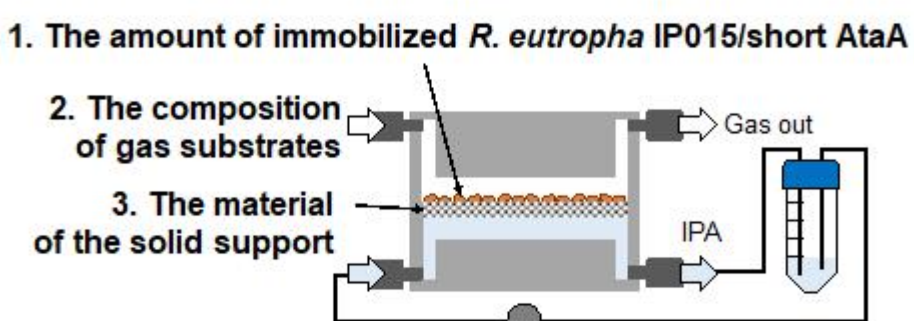
＊Development of a gas-phase reactor

Objective: Develop electrodes capable of supplying electricity, gas, and liquid

Achievements:
・A lab-scale gas-phase reactor was developed to meet requirements



Gas-phase bioreactor integrated with a 3-electrode system

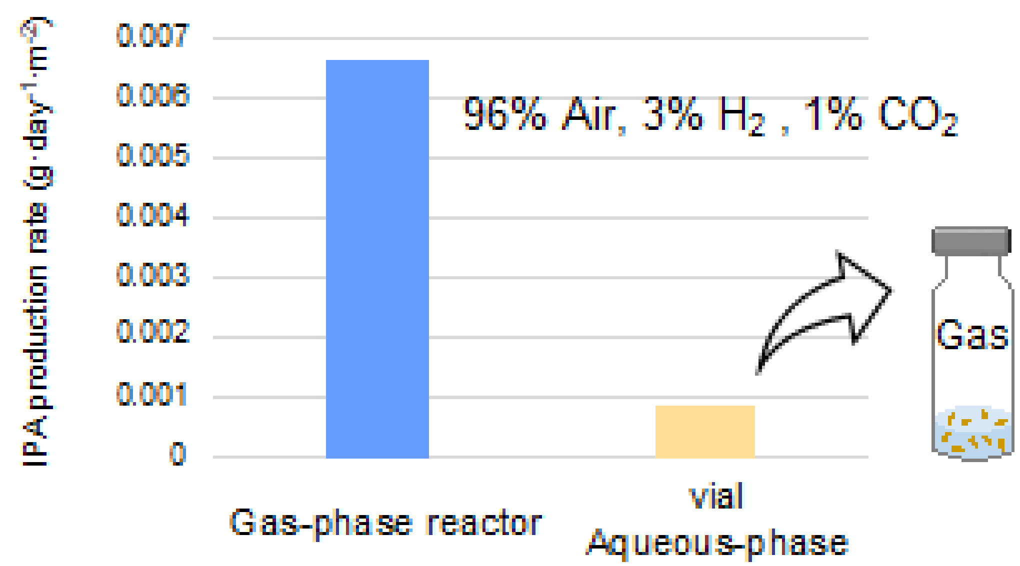


Optimizing parameters in the gas-phase bioreactor

＊Superiority of gas-phase reactors

Objective: Demonstrate a gas-phase reactor can enhance *Ralstonia* CO_2 fixation

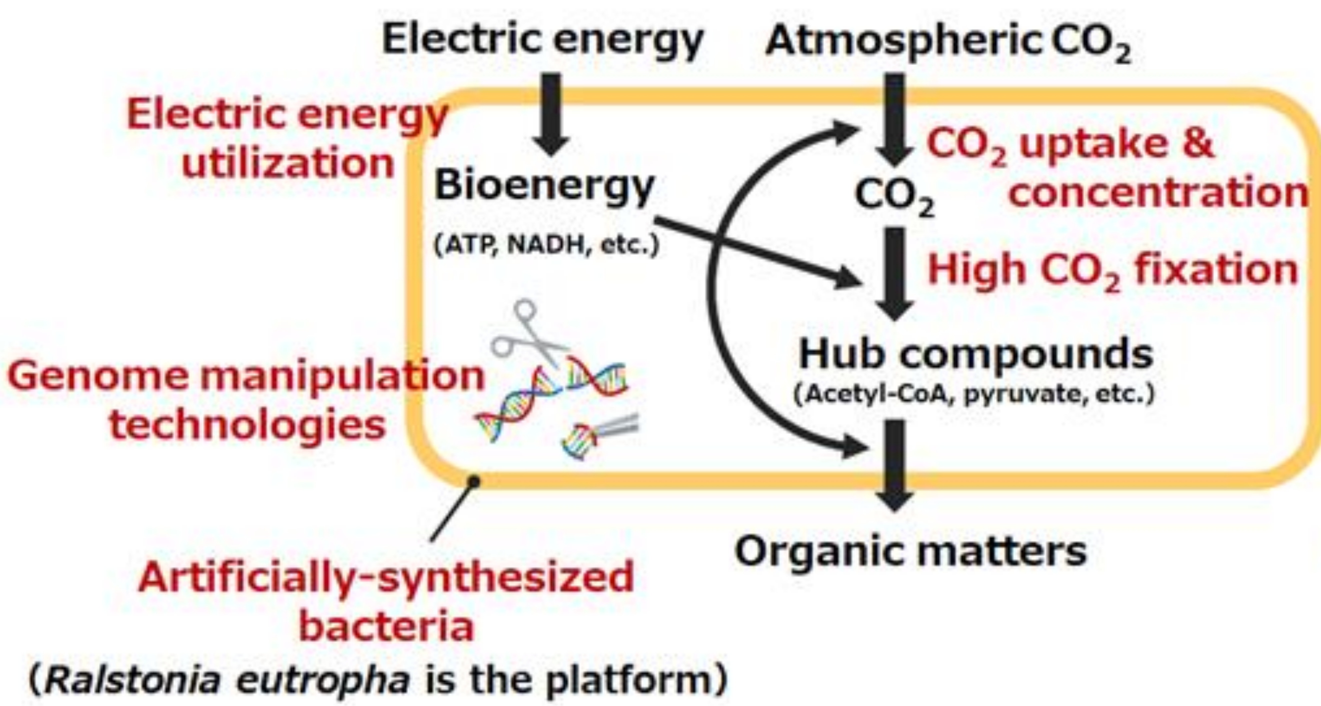
Achievements:
・The production of isopropanol (IPA) from H_2/CO_2 was significantly increased by the gas-phase reaction

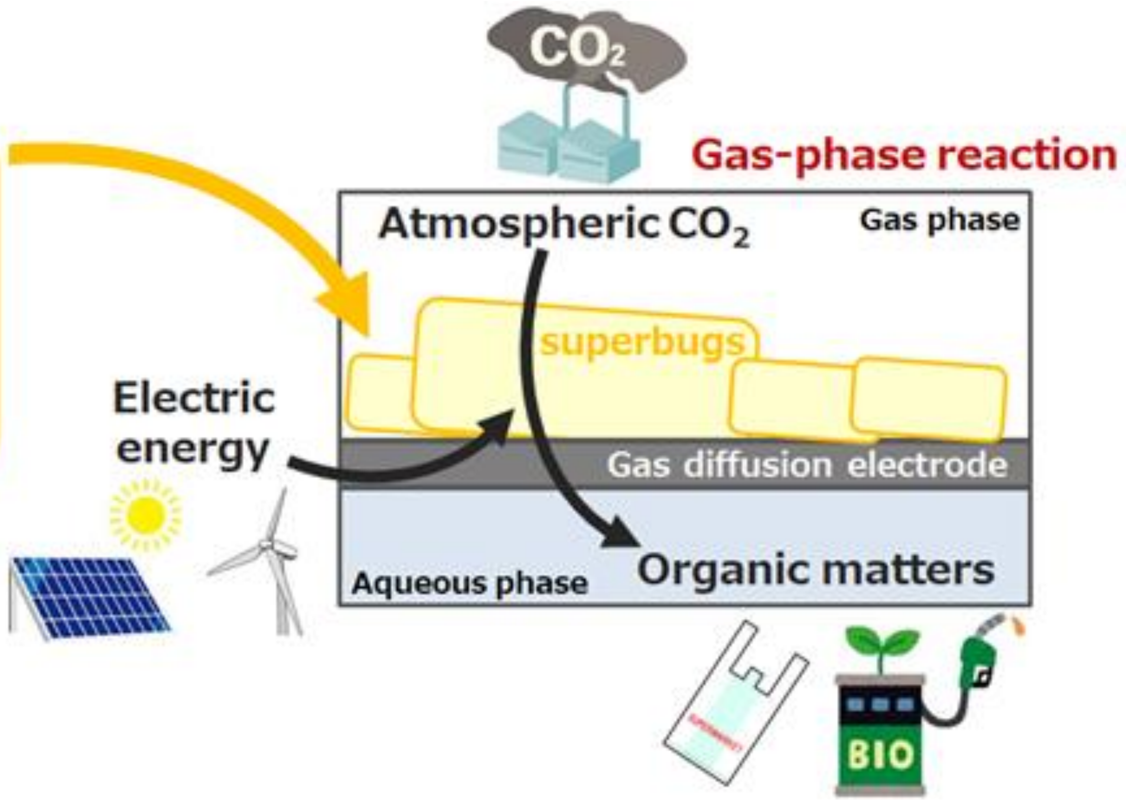


Comparison of IPA production in the gas-phase reaction and in the aqueous phase reaction

For achieving project goals

PJ goals (FY2022):
To achieve "artificial synthesis of electricity-utilizing CO_2-fixing microorganisms" and "construction of a gas-phase reactor", and clearly demonstrate the feasibility of this technology.





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"gas-phase reaction process" that can effectively supply electricity, nutrients and CO_2 to superbugs.