Molecular Microbiology and Biotechnology Group

Members (As of Dec. 2020)

Masayuki Inui, Group Leader, Chief Researcher
Haruhiko Teramoto, Associate Chief Researcher
Kazumi Hiraga, Associate Chief Researcher
Hajime Terasaki, Associate Chief Researcher
Masato Miyamoto, Associate Chief Researcher
Yuya Tanaka, Senior Researcher
Masako Suda, Senior Researcher
Yukihiro Kitade, Senior Researcher
Koichi Toyoda, Senior Researcher
Naoto Kato, Senior Researcher
Satoshi Hasegawa, Senior Researcher
Akira Watanabe, Senior Researcher
Takahisa Kogure, Senior Researcher
Takeshi Kubota, Senior Researcher
Toshihiro Oshima, Senior Researcher
Shinichi Oide, Senior Researcher
Tetsu Shimizu, Researcher
Tomoaki Hara, Researcher
Akiyoshi Higo, Researcher
Natalia Maria Theresia, Researcher
Norimasa Kashiwagi, Researcher
Jyumpei Kobayashi, Researcher
Ryoma Hashimoto, Researcher
Naoki Saruya, Researcher
Yuki Nozaki, Researcher
Junko Watanabe, Assistant Researcher
Yuko Ikenaga, Assistant Researcher
Shoko Minakuchi, Assistant Researcher
Miyuki Nagamori, Assistant Researcher
Kae Naito, Assistant Researcher
Noriko Ikeda, Assistant Researcher
Kazuyo Yoneda, Assistant Researcher
Mayu Koizumi, Assistant Researcher
Junko Nishi, Assistant Researcher
Kayoko Mori, Assistant Researcher
Motomi Iwashima, Assistant Researcher
Kayo Yoshida, Assistant Researcher
Aya Okada, Assistant Researcher
Kimiyo Kishi, Assistant Researcher

Development of Biorefinery Technology to Realize a Sustainable Society

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has had a great impact on economic and social activities worldwide. Meanwhile, environmental problems such as global warming and marine plastic pollution remain are becoming more serious. Under these circumstances, the promotion of the bioeconomy is becoming increasingly important for economic recovery after the convergence of infectious diseases and for solving environmental problems.

The concept of "bio-economy," which aims to expand a sustainable, renewable and recycling-oriented economic society while solving global issues by utilizing biotechnology and renewable biological resources (Fig.1).

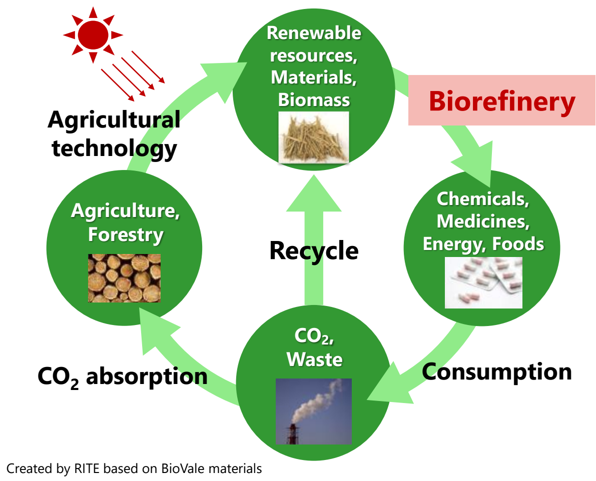
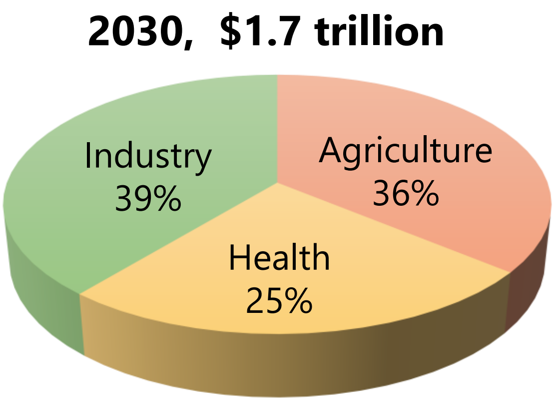


Fig. 1 Economic contribution of biotechnology

According to the Organisation for Economic Co-operation and Development (OECD), "the biotechnology market in 2030 will expand to about 190 trillion yen in all member countries and the manufacturing sector will reach about 40%" (Fig. 2). It is said that all of these industries are bio-based.



Source：The Bioeconomy to 2030. OECD(2009), NEDO

Fig. 2 Economic contribution of biotechnology

Under such circumstances, the government formulated “Bio-Strategy 2019” in June 2019, with the overall goal of “achieving the world's most advanced bioeconomy society in 2030.” This was to be promoted while being updated every year, and in June 2020, "Bio-Strategy 2020 (Basic Measures)" was announced, followed by "Bio-Strategy 2020 (Final version of Market Area Measures)" in January 2021.

Our group is promoting the development of biorefinery technology, the core of bioeconomy, by using microorganisms. Biorefinery technology is used for producing biofuels and green chemicals, using renewable resources (biomass) as raw materials.

This first section provides an overview of the current status of biofuel and bioplastic (a substitute for general-purpose plastics) research and development (R&D).

1.1. Biofuels

Bioethanol, a major biofuel, is produced from raw materials such as corn (in the U.S.) and sugarcane (in Brazil). It is mixed with 10%–25% gasoline for use in automobile engines. The highest production and consumption of bioethanol occurs in the United States (U.S.) where corn is a major crop. According to estimates provided by the U.S. Energy Information Administration, 15.8 billion gallons (59.81 million kL) of bioethanol was produced in the U.S. in 2019. Similarly the Agricultural Outlook 2020–2029 report, published jointly by the OECD and the Food and Agriculture Organization, states that 129 million kL of bioethanol was produced worldwide in 2019, with the U.S. accounting for approximately half of this production.

Cellulose ethanol, a second-generation biofuel, is produced from raw materials that do not compete with food resources, such as the agricultural waste corn stover. On the basis of Renewable Fuel Standard (RFS) rules, the Environmental Protection Agency's final volume for the production of cellulosic biofuel in 2020 was 590 million gallons (2.23 million kL), which was slightly more than 5.6% of the RFS target set in 2007. The acceleration of biofuel commercialization is required in the future. The Research Institute of Innovative Technology for the Earth (RITE) is developing a bioprocess that can efficiently utilize cellulosic biomass (see Chapter 2).

With regard to aviation fuel, the International Civil Aviation Organization decided at their 2010 General Assembly not to increase total greenhouse gas emissions after 2020. At the 2016 General Assembly, a decision was made to introduce a greenhouse gas reduction system using market mechanisms (GMBM: global market-based measures) after 2020. On the basis of this decision, the International Air Transport Association has formulated a concrete action plan, including the uses of bio-jet fuel and emissions trading (Fig. 3). The GMBM are expected to be launched in 2021. Along with these, bio-jet fuel has been widely spread mainly in Europe and the U.S. every year, and commercial flight has been continued as a sustainable aviation fuel using cooking waste oil and the like.

Our group is also conducting R&D on biofuels. In a project sponsored by Japan Airlines Co., Ltd. (JAL), we have succeeded in producing domestic bio-jet fuel for the first time in Japan, and this bio-jet fuel was refueled on a regular domestic flight (see Section 4.1).

We are also conducting R&D on a green jet fuel that can be used as is, that is, without limitation of mixing ratio to petroleum fuels.

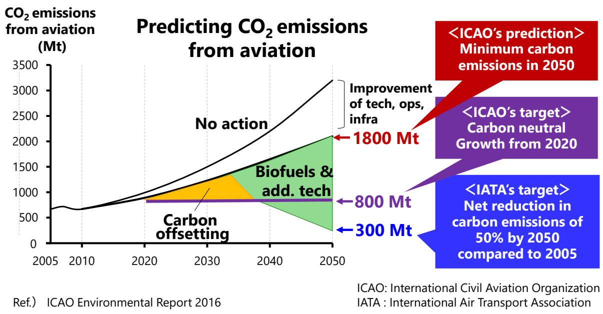


Fig. 3 Emissions reduction roadmap

With regard to marine fuels, Japan's International Shipping Greenhouse Gas Zero Emission Project, established in collaboration with the Japan Ship Technology Research Association, aims for a zero emissions target by 2050 through the promotion of technological innovations, such as alternative low-carbon fuels of the next generation like biohydrogen. The Project has developed a roadmap toward accelerating efforts to achieving zero emissions.

1.2. Bioplastic

Efforts to reduce greenhouse gases by spreading and generalizing the use of plastics that do not depend on fossil resources have stagnated, and the environmental pollution caused by marine plastic wastes has become a serious global issue. Against this background, there are great expectations from bioplastics and biodegradable plastics made from biomass, which is a renewable resource.

“Bio-Strategy 2020” places great importance on the immediate and continuous promotion of bioplastics (a general-purpose plastic alternative), which is one of the nine market areas shown in “Bio-Strategy 2019.”

Our group is involved in the Moonshot R&D project (Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses) of the New Energy and Industrial Technology Development Organization (NEDO). Participation in the project “Production of Bio-monomers from Non-food Biomass and Development of Polymer-degrading Enzymes” will be undertaken.

2. Core technology of our group

Our group has established an innovative bioprocess on the basis of a new technological concept. This trademark, registered RITE Bioprocess®, has achieved great results in the development of technologies for producing green chemicals (e.g., biofuels, amino acids, and aromatic compounds), exhibiting high efficiency. Furthermore, it has received high praise both in Japan and abroad (Fig. 4).

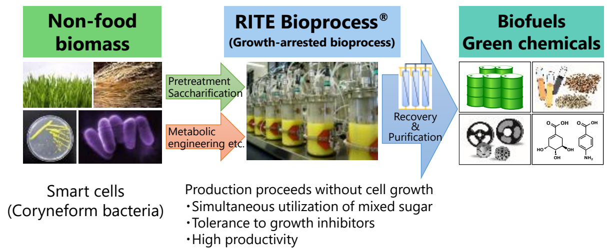


Fig. 4 Biorefinery concept using the RITE Bioprocess®

2.1. Features of the RITE Bioprocess®

2.1.1. Feature ①: Growth-independent bioprocess

The RITE Bioprocess® involves cultivating a large amount of coryneform bacteria (smart cells) that are metabolically designed to produce the target substance efficiently; filling a reaction tank with a high density of these cells; and finally, creating anaerobic conditions. Alternatively, the reaction is performed in a state where cell division is stopped by removing the factors essential for cell proliferation (Fig. 5).

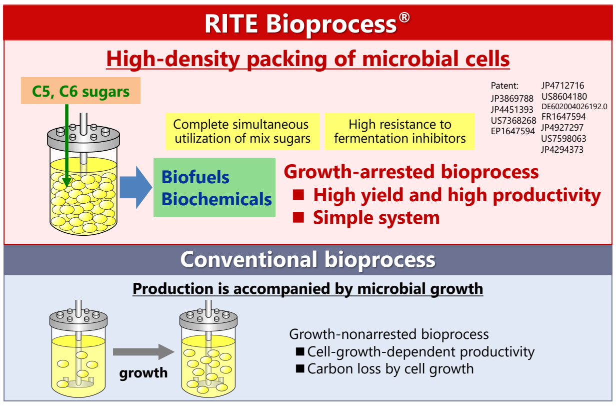


Fig. 5 Features of the RITE Bioprocess® ①
(Growth-independent bioprocess)

The key to high efficiency is to produce compounds in a state in which the growth of the microorganisms is suppressed (i.e., a growth-independent bioprocess) thereby eliminating the need for nutrients or energy to achieve microbial growth. As a result, it is possible to use microbial cells extremely efficiently. The RITE Bioprocess® has succeeded in realizing a bioprocess with high productivity equal to or higher than that of a normal chemical process.

2.1.2. Feature ②: Simultaneous utilization of mixed sugars

A cellulosic biomass consists of mixtures of hemicellulose-derived pentoses (C5) and hexoses (C6). The simultaneous utilization of both pentoses and hexoses is essential for microbial biofuel production.

However, wild coryneform bacteria use xylose (C5 sugar) and arabinose (C5 sugar) at a slower rate than they do glucose (C6 sugar) (see the graph on the left side of Fig. 6). When raw materials are continuously added, the C5 sugars accumulate, and their production efficiency eventually decreases.

Our group has succeeded in improving the metabolic system of coryneform bacteria by introducing into them several genes involved in the utilization of C5 sugar thereby increasing its utilization rate to the same level as that of C6 sugar (see the graph on the right side of Fig. 6).

As a result, C5 and C6 saccharides can be used at the same time (simultaneous utilization of mixed sugars), and cellulosic raw materials can be used efficiently.

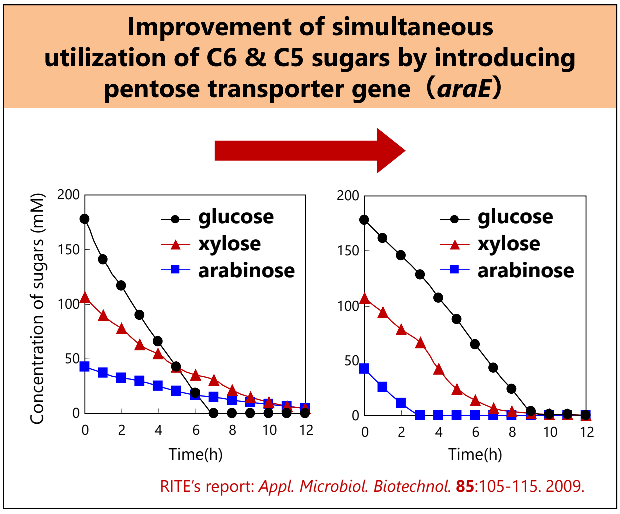


Fig. 6 Features of the RITE Bioprocess® ②
(Simultaneous utilization of mixed sugars)

2.1.3. Feature ③: High tolerance to fermentation inhibitors

Fermentation inhibitors (phenols, furans, etc.) that are formed during the pretreatment of lignocellulosic biomass are known to exhibit strong inhibitory activity in the bioethanol production process. Therefore, in order to be able to produce the target substance efficiently, an increase in the tolerance of microorganisms (bacteria) to the fermentation inhibitors is indispensable.

The coryneform bacterium developed by our group has been demonstrated to have high tolerance to fermentation inhibitors rendered through the introduction of several genes (Fig. 7).

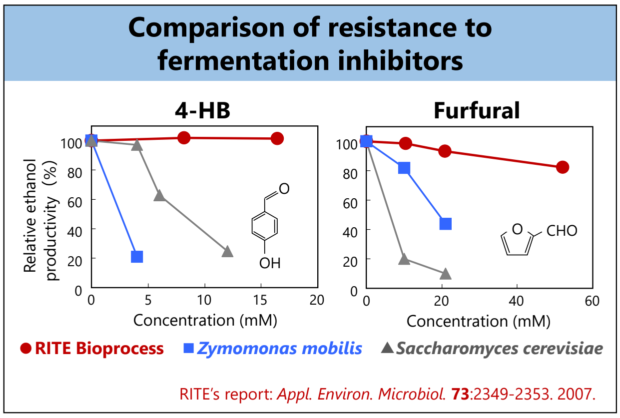


Fig. 7 Features of the RITE Bioprocess® ③

(High tolerance to fermentation inhibitors)

2.2. Examples of substances produced by the RITE Bioprocess®

Currently, in addition to the highly efficient production of various substances, such as ethanol, L-lactic acid, D-lactic acid, and amino acids, we are expanding our business to the production of high-performance chemicals, such as butanol, jet fuel materials, and aromatic compounds (Fig. 8).

Biofuels	Green chemicals
■ Gasoline additives ★ Ethanol * ■ Bio-jet fuels ★ Isobutanol * ☆ n-butanol * ★ C9~C15Saturated hydrocarbon + Aromatics ■ ★ Biohydrogen	■ Aromatics ★ Shikimic acid (Anti-influenza drug; Tamiflu raw materials) ★ Phenol * (Phenolic resins, Polycarbonates) ★ 4-hydroxybenzoic acid * (Polymer raw materials) ★ Aniline * (Natural resource tire (Age resistor)) ★ 4-aminobenzoic acid * (Pharmaceutical raw materials) ★ Protocatechuic acid * (Cosmetic raw materials) ■ Organic acids ★ D-lactate *, L-lactate * (Stereo-complex PLA) ☆ Succinate * ■ Amino acids ★ Alanine (Chelators) ★ Valine (Next-generation feed-use amino acids) ☆ Tryptophan (Next-generation feed-use amino acids) ☆ Methionine (Feed-use amino acids, Seasoning) ■ Alcohols ☆ Isopropanol (Propylene raw materials) ★ Xylitol (Sweetener) * : Polymer raw materials ★ Red character : World's highest productivity achieved

Fig. 8 Examples of substances produced by the RITE Bioprocess®

In the following sections, we describe the efforts in the national projects in which our group is participating, the development of production technology for green chemicals containing biofuels and aromatic compounds, which are the main targets, and introduce the efforts for their practical application.

3. Next core technology

3.1. NEDO Smart Cell Project

NEDO launched the project "Development of Production Techniques for Highly Functional Biomaterials Using Smart Cells of Plants and Other Organisms" (Smart Cell Project) in 2016. Our group has participated in this project since its inception. Members of the project develop technologies for designing the Smart Cell (defined as a finely designed and expression-controlled cell) and validate these Smart Cell design systems.

We have selected catechol as our target compound in the project. Catechol has not been produced in high concentration by fermentation until now, even though it is in high demand in various industrial fields. It is presumed that the reasons are its high toxicity to microorganisms and the high complexity of the metabolic pathways from glucose to catechol. Furthermore, wild-type *Corynebacterium glutamicum* does not have its own genes for catechol production. To solve these problems, we applied the Smart Cell design systems to create a *C. glutamicum* catechol overproducer in a short period of time.

In collaboration with universities, research institutes, and one company, which all have their own original Smart Cell design systems, several genetic modification points were proposed for improving catechol productivity. The proposals were integrated into a *C. glutamicum* strain to realize its high catechol-producing potential. As a result, the concentrations of catechol were gradually increased in stages, and the final strain achieved a very high production concentration, far exceeding the highest reported worldwide (Fig. 9).

This fiscal year (FY 2020) is the final year of the project, and we have already achieved productivity that exceeds its target value of the project. Continued efforts towards development for practical use is being undertaken, which will contribute to the realization of a new bio-based industry, the Smart Cell industry.

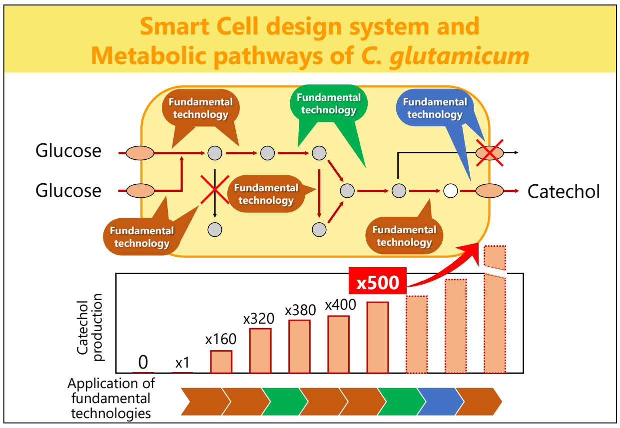


Fig. 9 Development of catechol production by applying Smart Cell design systems

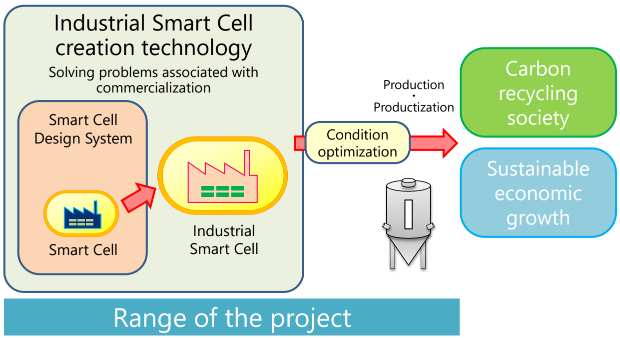


Fig. 10 Development of bio-derived material production technologies

3.2. NEDO Carbon Recycle Project

NEDO has launched a new project, "Development of Bio-derived Material Production Technologies that Accelerate the Realization of Carbon Recycling." In the ongoing Smart Cell Project (from 2016 to 2020), Smart Cell design systems have been developed to create smart cells. On the basis of this achievement, the development of industrial material production technology using biological functions is being carried out in the new project. The aim is to accelerate the social implementation of bio-derived products by developing technologies related to production processes for industrialization, including bioreactor scale-up and refining processes (Fig. 10).

Our group participated in this project with the goals of identifying problems associated with the practical application of microbial fermentation and contributing to the development of "Industrial Smart Cell creation technology" to solve the problems. Furthermore, we selected terpenoids as our target chemical compounds and started developing industrially applicable overproducers.

4. Development of target products

4.1. Biofuel production research and development

4.1.1 Biobutanol

Butanol is more suitable as a gasoline additive than ethanol owing to its better physicochemical properties, including higher energy content, lower vapor pressure, and lower water solubility. It can also be used as the base material for the production of bio-jet fuel using conventional chemical reactions. The bio-jet fuel synthesized from biobutanol can be used in airplanes. Airlines and aircraft manufacturers have paid great attention to the importance of bio-jet fuel, which has been recognized as being critical for reducing CO_2 emissions because it uses plant-based materials as feedstock instead of petroleum. The bio-jet fuel synthesized from butanol is often referred to as "alcohol-to-jet" (ATJ) fuel and has been approved by the American Society for Testing and Materials (ASTM) and is ready for use in commercial aircraft.

We have developed a genetically engineered *C. glutamicum* strain that is highly efficient in producing biobutanol. Furthermore, we had conducted a research project from 2015 to 2019 to investigate cellulosic butanol production (see Topics in RITE Today, 2016). The project was funded by the Ministry of Economy, Trade and Industry. The advantages of our production process are as follows: (i) cellulosic biomass-derived mixed sugars can be used as feedstock, and (ii) production is fast and generates a high product yield (Fig. 11).

Because butanol is highly toxic, we have developed a genetically engineered *Corynebacterium* strain that has high tolerance to its toxicity. The strain has further enhanced the high productivity of the RITE Bioprocess®. Through collaboration with the U.S. National Renewable Energy Laboratory, we have accelerated the R&D of biobutanol production from non-food biomass.

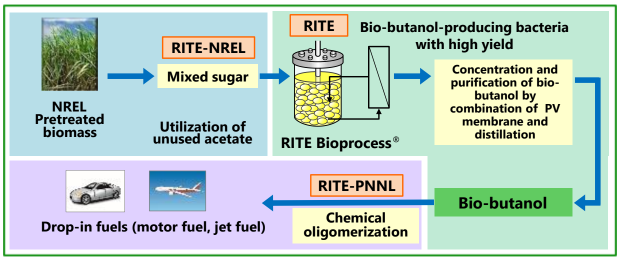


Fig. 11 Production of biobutanol and bio-jet fuel using the RITE Bioprocess®

Since 2017, we have also collaborated with the U.S. Pacific Northwest National Laboratories in researching and developing the production of jet fuels by the chemical oligomerization of biobutanol. This was based on a new idea that if acetic acid is included in mixed sugars from a pretreated biomass, it can be converted to ethanol by an engineered *C. glutamicum* strain into which new genes have been introduced. Therefore, the mixtures are utilized for the bioproduction of butanol and ethanol, which can then be subjected to chemical oligomerization for use as jet fuels.

The distillation of a biobutanol solution requires a large amount of energy. To reduce the amount of energy needed, we developed an energy-saving biobutanol recovery process using a combination of distillation and pervaporation. This resulted in energy savings of up to 90% and the achievement of the highest biobutanol productivity in the world. The project successfully achieved the original goals of improving the butanol tolerance and optimizing the metabolic pathway of the producing bacterial strain, and developing an energy-saving butanol recovery technology.

Meanwhile, as an initiative for commercialization, RITE provided technical cooperation in the "Let's Fly by Recycling 100,000 Clothes!" project (2018–2020) sponsored by JAL. This project aimed to manufacture bio-jet fuel from used clothes collected in cooperation with JAL and JEPLAN, Inc.

Green Earth Institute Co., Ltd. (GEI), a venture company originating from RITE, also participated in this project, and isobutanol was produced by the RITE Bioprocess® using coryneform bacteria developed by RITE. In 2020, the bio-jet fuel produced from this isobutanol passed the international standard ASTM D7566 Annex5 Neat for the first time as a purely domestic bio-jet fuel. On February 4, 2021, it was used as the first domestically produced bio-jet fuel on a JAL commercial flight, JL319, from Tokyo Haneda to Fukuoka Airport.

In the future, by combining these elemental technologies and a variety of procedural knowledge, we aim to produce jet fuel from biobutanol and put it into application and commercialization.

4.1.2. Green jet fuel

Petroleum-based jet fuels are mixtures of hydrocarbons that consist of *n*-paraffins, isoparaffins, cycloparaffins, and aromatic compounds with 9–15 carbon atoms. Any jet fuel must meet strict standards with regard to its physical properties, such as a specified freezing point and density.

So far, ASTM International has approved six production pathways for bio-jet fuels, such as the production of HEFA fuel by the hydroprocessing of fatty acid esters, FT-SPK fuel by the Fischer–Tropsch synthesis of hydrocarbons from syngas, and ATJ fuel by the oligomerization of alcohol. In 2020, ATJ fuel coupled with our biobutanol production technology was approved. However, these certified bio-jet fuels consist mostly of isoparaffins and are lacking in other essential components: cycloparaffins and aromatics. They do not meet ASTM standards on their own and are required to be blended with petroleum-based jet fuel so as to be 50% or less in total when used. Therefore, even when the production capacity of bio-jet fuels catches up with demands, more than 50% of fuel demands will still be occupied by petroleum-based jet fuel.

To overcome this blending ratio limitation of the certified bio-jet fuels, we are additionally developing a high-performance green jet fuel that contains cycloparaffins and aromatics in addition to isoparaffins. The novel jet fuel meets ASTM standards and is expected to be used alone. In the R&D of the high-performance bio-jet fuel, we have achieved some promising results, such as a novel biocatalyst that enables the cross-coupling reactions between C2 and C8 compounds for the synthesis of C9–C15 branched and cyclic compounds, which can then be chemically converted to jet fuel components.

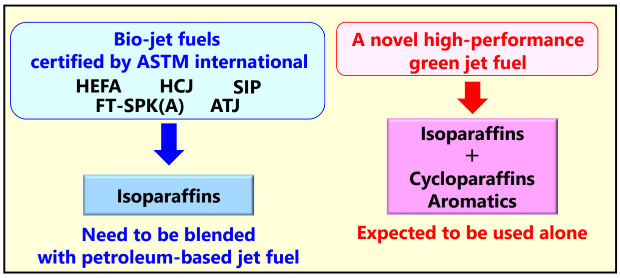


Fig. 12 Research and development of a novel high-performance bio-jet fuel

4.1.3. Biohydrogen

Hydrogen, the combustion of which generates only water, is considered the ultimate clean energy source. However, CO_2 emissions during the hydrogen production processes currently in use are a problematic issue, because fossil resources are used as the feedstock. The Basic Hydrogen Strategy drawn up at a meeting of the Ministerial Council on Renewable Energy, Hydrogen and Related Issues in 2017 states the importance of the development of innovative CO_2-free hydrogen production technologies to realize a hydrogen society over the medium to long term. The aim is to achieve this by 2050, based on the goals set to develop commercial-scale hydrogen supply chains by circa 2030.

Although bioprocesses have significant potential for CO_2-free hydrogen production, innovative improvements in technology are necessary to establish a cost-effective process for producing biohydrogen. In collaboration with the Sharp Corporation, our group has developed a biohydrogen production process. The hydrogen production rate achieved by our process is two orders of magnitude higher than that of conventional fermentation processes. On the basis of this achievement, our group is now working on the metabolic engineering of hydrogen-producing microorganisms to improve hydrogen yields from cellulosic biomass.

Photosynthetic bacteria produce hydrogen gas (H_2) using nitrogenase (a nitrogen-fixing enzyme) and the reducing power generated from the degradation of organic compounds (Fig. 13). Although H_2 is a byproduct of the nitrogenase reaction that generates ammonia (NH_3) from nitrogen gas (N_2), only H_2 is produced by the reduction of H^+ in the absence of N_2. This photofermentative process can produce hydrogen from acetate (a thermodynamically unfavorable reaction) using light energy. Thus, a major improvement in hydrogen yield is expected by integrating this process with the dark fermentative process of hydrogen production, which produces acetate as a byproduct. However, in photosynthetic bacteria, reducing power is consumed by CO_2 fixation and polymer synthesis for carbon storage, thereby limiting its use for hydrogen production. Moreover, hydrogen is reused by uptake hydrogenase for providing cells with reducing power. On the basis of these findings, the metabolic engineering of H/C/N metabolic pathways has resulted in a marked increase in the hydrogen yield from acetate.

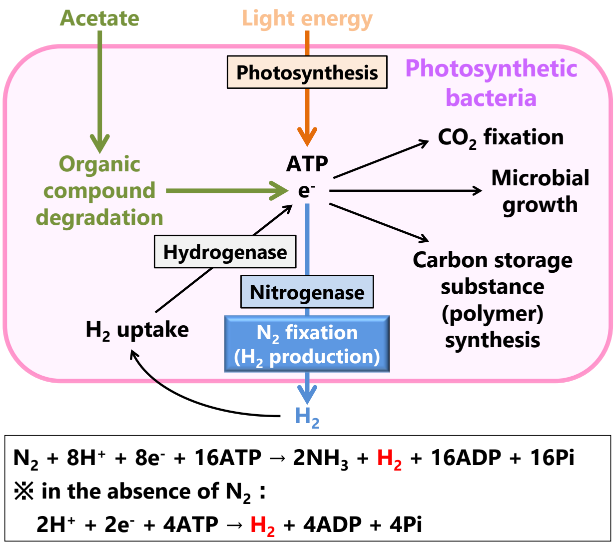


Fig. 13 Competition with H_2 production (N_2 fixation) for reducing power (e^-) in photosynthetic bacteria

4.2. Amino acids (alanine and valine)

Normally, amino acid fermentation is carried out under aerobic conditions, where high productivity requires the aeration and agitation of the system to be properly controlled. However, this is often difficult to achieve in large-scale fermenters because their internal oxygen concentration is not homogeneous. To overcome this problem, we have developed a new, genetically modified *Corynebacterium* strain with the RITE Bioprocess® that allows the production of amino acids to be carried out under anaerobic conditions. The technological hurdle for amino acid production under anaerobic conditions is to balance the redox reaction without oxygen as an electron acceptor. To this end, we successfully introduced an artificial pathway for amino acid biosynthesis into the microbial cells thereby solving the technological hurdle. Our group published this accomplishment in an international journal in 2010 (Appl. Microbiol. Biotechnol. 87: 159–165).

GEI was established in 2011 for the industrialization of the RITE Bioprocess®. In 2011, RITE and GEI began collaborative research on amino acid production using the RITE Bioprocess® and developed technologies for scaling up production, growing efficient production strains, and reducing production costs. In 2019, RITE succeeded in producing a strain that yielded the world's highest production concentration of L-valine with the best production efficiency. Furthermore, RITE has completed commercialization projects with GEI and overseas partners to achieve the commercial production of these amino acids. Our goal was to produce this amino acid from renewable resources thereby reducing the life cycle carbon footprint.

In 2016, we succeeded in demonstrating the feasibility of L-alanine production technique by using the commercial-scale facilities of our partner company, which was an important milestone for its industrialization. One of our group members also participated in the first operation and worked with local employees to lead the project to a successful conclusion. As the result of an evaluation by the Food Safety Committee in August 2017, the safety of the L-alanine produced by our strain for use as a food additive was confirmed, allowing it to be made commercially available for this purpose besides its use for industrial applications. We are now working on a joint research project for the production of other amino acids.

4.3. Green-aromatic compounds

Aromatic compounds are important industrial chemicals used for the synthesis of polymers as well as a diverse group of value-added chemicals that are applied in the pharmaceutical, nutraceutical, flavor, cosmetic, and food industries. Although they are currently derived from petroleum or natural plant resources, their environmentally friendly biotechnological production from renewable feedstocks is desirable from the viewpoint of creating a sustainable society that is no longer dependent on petroleum resources and has efficient production processes. Bacterial cells synthesize various aromatic compounds, including amino acids (phenylalanine, tyrosine, and tryptophan), folate (vitamin B9), and coenzyme Q, all of which are derived from the shikimate pathway (Fig. 14). By employing the metabolically engineered *C. glutamicum*, we have successfully established a highly efficient bioprocess for producing the following aromatic compounds from non-food feedstocks: shikimate, a key building block of the anti-influenza drug Tamiflu; 4-aminobenzoate, which is used as a building block of a potentially useful functional polymer; and aromatic hydroxy acids, which have potential applications in the polymer, pharmaceutical, cosmetic, and adhesive material industries. Currently, we are seeking to develop new strains for the production of useful aromatic compounds that the wild-type *C. glutamicum* is unable to produce. This will be achieved by introducing genes derived from versatile biological resources into the bacterium. The techniques developed in the Smart Cell Project, as described earlier, will help to accelerate the development of strains and improve their productivity.

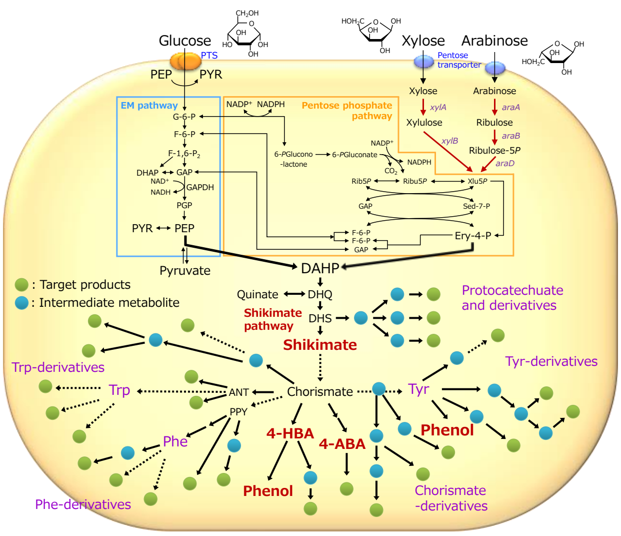


Fig. 14 Biosynthetic pathway for various aromatic compounds

4.4. Strategic Innovation Promotion Program (SIP)

The cross-ministerial Strategic Innovation Promotion Program seeks to promote R&D, from the basic research stage to the final outcome, in a seamless manner by endeavoring to strengthen cooperation among industries, academia, and governments beyond the framework of government ministries and traditional disciplines. The theme "Technologies for Smart Bio-industry and Agriculture", one of the 12 themes in the SIP at Second Phase, aims to realize a sustainable growing society that uses manufacturing technologies developed through the integration of biotechnology and digital resources.

RITE is participating in "Development of Technologies for Functional Design and Production of Innovative Biomaterials (Fig. 15)," a consortium comprising the theme. This consortium consists of two groups: the polymer group for designing polymers with marketable properties and predicting the function of polymers consisting of particular monomers; and the monomer group for selecting biosynthesizable monomers and designing biosynthetic pathways and enzymes for the monomers required for polymer synthesis. As a leader of the monomer group, RITE is evaluating enzyme candidates and enzyme modifications that are predicted to be required for the synthesis of target monomers by bioinformatics teams in the group. The enzymes involved in the synthesis of an aromatic diol and a precursor of an aromatic diamine are current targets to be modified. Thus far, the predicted modification of amino acid sequences has successfully improved the activity of the enzymes and altered their substrate specificity. We are improving the accuracy of the technologies by evaluating more enzymes.

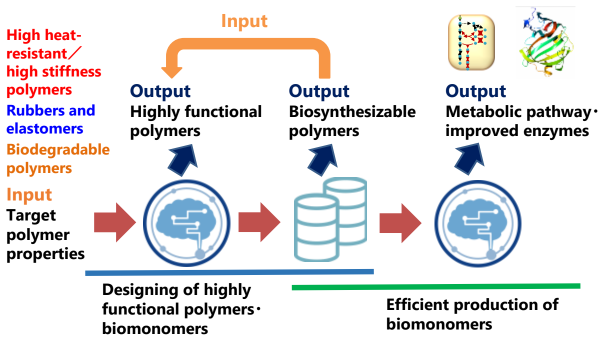


Fig. 15 Integrated design and production technology system for biomaterials

4.5. NEDO Moonshot-type R&D Project: Development of Multi-lock Biopolymers Degradable in Ocean from Non-food Biomasses

This project aims to develop a "multi-lock-type biopolymer" that is as tough as conventional petroleum-derived polymers when in use but can be rapidly degraded by external stimuli in the marine environment after its use (Fig. 16). We aim to simultaneously solve the problems of global warming and environmental pollution in the field of polymers. Generally, there is a trade-off between plastic toughness and marine degradability (biodegradability), but this project aims to achieve both.

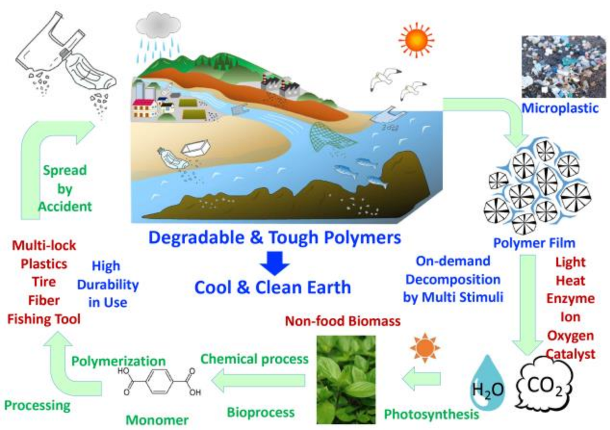


Fig. 16 Research and development of marine-degradable multi-lock biopolymers made from non-food biomass

https://www.nedo.go.jp/english/news/ZZCA_100007.html

In this project, RITE will promote the production of biomonomers and the development of polymer-degrading enzymes from non-food biomasses. Specifically, (1) in order to establish a bioprocess that enables the high production of biomonomers, which are the raw materials for multi-lock biopolymers, RITE will promote the construction of a high biomonomer-producing strain and the development of scale-up production technology. Additionally, (2) for the practical use of multi-lock biopolymers, we will promote the development of highly functionalized polymer-degrading enzymes for efficient enzymatic degradation in the multi-lock mechanism and of high production technologies for these enzymes.

5. Toward the industrialization of our technologies

5.1. Green Chemicals Co., Ltd.

(Head Office・Laboratory: in Kyoto headquarters, RITE; Shizuoka Laboratory: in Shizuoka plant, Sumitomo Bakelite Co., Ltd.)

Currently, commercial phenol can only be derived from petroleum. We have taken on the challenge of developing the world's first biomanufacturing process for biomass-derived phenol, with the ultimate goal being to aid global environmental conservation and greenhouse gas reduction.

In May 2014, Sumitomo Bakelite Co., Ltd., and RITE established Green Phenol Development Co., Ltd. (GPD), to accelerate the industrialization of our biomass-derived phenol-producing technology, named the "Two-Stage Bioprocess." In April 2018, GPD changed its name to Green Chemicals Co., Ltd. (GCC).

Because GCC's phenol-producing technology and knowledge are applicable to the production of various other aromatic compounds, the establishment of a bioprocess for each higher value-added chemical and the commercialization of products that meet customer needs are in progress (see Section 4.3).

The present product lineup of GCC is shown in Fig. 17. In 2020, using the pilot-scale facilities of GCC, we succeeded in demonstrating the high-concentration production of two target compounds, protocatechuic acid and shikimic acid, which was an important milestone for industrialization.

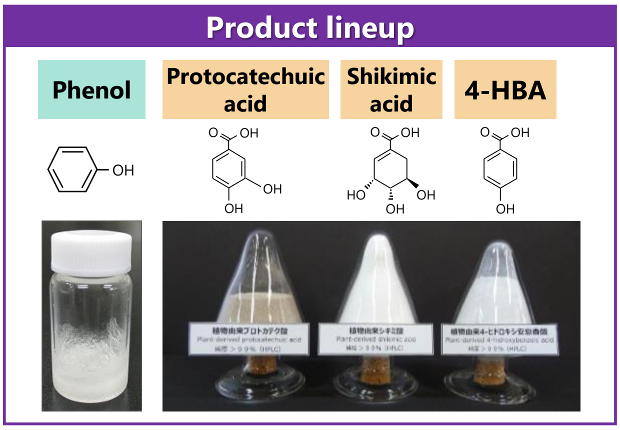


Fig.17 Product lineup of Green Chemicals Co., Ltd.

5.2. Green Earth Institute Co., Ltd.

(Headquarters: Bunkyo-ku, Tokyo, Japan; Research Institute: Kazusa, Kisarazu City, Chiba, Japan)

GEI is a RITE-launched venture company that was established on September 01, 2011, to facilitate the quick commercialization of the research results of the aforementioned innovative RITE Bioprocess®. GEI is conducting both joint research and activities aimed at commercialization with RITE in order to realize the practical uses of the green chemicals and biofuel production technologies produced using the microorganisms (coryneform bacteria) generated in the RITE Bioprocess®.

With regard to amino acids, as mentioned earlier, GEI has succeeded in producing L-alanine and L-valine on a commercial scale using the production strain developed by RITE. Currently, commercial production is being realized through license agreements with domestic and overseas partner companies (see Section 4.1). Additionally, the safety of L-alanine as a food additive has been confirmed by the Ministry of Health, Labour and Welfare, paving the way for its use in the food industry.

With regard to bio-jet fuels made from non-food biomass, which are highly expected to reduce CO_2 emissions from aircraft, GEI is continuously conducting joint research with RITE in this area and is working toward commercialization (see Section 4.1).

Additionally, GEI is developing green chemicals (e.g., cosmetic materials) in cooperation with RITE, and the marketing for commercialization and the scaling up for mass production are under way.

As a venture company that realizes the commercialization of RITE-originated technology and by contributing to the development of the biorefinery industry, GEI will continue toward the realization of a society that does not rely on fossil resources.

6. Closing remarks

As mentioned earlier, in anticipation of a rapid economic recovery after the COVID-19 pandemic has subsided, the promotion of the bioeconomy is expected to become increasingly important in the future.

Under such circumstances, in recent years, the understanding of life phenomena by the "data-driven" approach of discovering the law from a large amount of life information has been progressing.

Against this background, research on the biology of synthesis (fusion of bio and digital processes) that accumulates data and understands biological functions by repeating the cycle of design (Design), build (Build), evaluation (Test), and learning (Learn) (i.e., the DBTL cycle) is developing rapidly. Our group also participates in multiple national projects (see Section 3).

In these projects, the biorefinery technology based on the Smart Cell described earlier is expected to play a major role as a core technology and have a large ripple effect on the industrial (manufacturing) and energy fields (Fig. 18).

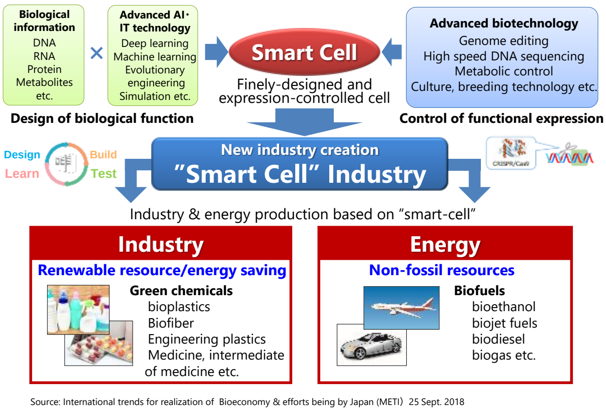


Fig. 18 Fusion of industrial/energy fields changed by bio and digital processes

In 2021, we will continue to develop innovative biorefinery production technologies centered on the cutting-edge biotechnology of the Smart Cell and contribute to the construction of a sustainable low-carbon society.

※ RITE Bioprocess® is a registered trademark of RITE.