Molecular Microbiology and Biotechnology Group

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Development of a Biorefinery Technology that Contributes to Carbon Neutrality

1. Introduction

In October 2020, the Japanese government declared "2050 carbon neutral." Therefore, the government will reduce greenhouse gas emissions by 46% by 2030 and virtually zero greenhouse gas emissions by 2050. The aim was to attain a carbon-free society in 2050.

As a policy to realize this declaration, the "Green Growth Strategy Through Achieving Carbon Neutrality in 2050" was developed in December 2020 and revised in June 2021. Fourteen fields have also been set to achieve this goal. These fields are expected to grow as an industry and are considered indispensable for reducing greenhouse gas emissions.

Among these, ⑪ "The Carbon Recycling Industry" can be mentioned as a field in which biotechnology can influence. Specifically, it is the production field of carbon-recycled fuels, such as alcohol-to-jet (ATJ) and bio-alternative aircraft fuels using microalgae and carbon-recycled chemicals. Biomass resources, CO_2, waste plastics, and waste rubber are used as raw materials.

Biotechnological fields, such as synthetic biology and genome editing technology, have rapidly and recently advanced. In addition, the technological innovation of "bio-digital technology," a fusion of biotechnology and information technology (digital), such as IoT and AI, is rapidly developing. Therefore, "bio-manufacturing," using this bio-digital technology and bio-resources, is expected to significantly contribute to carbon neutrality and carbon negative.

Our group has been developing biorefinery technologies, which are technologies to produce biofuels and green chemicals using renewable resources (biomass) as raw materials using microorganisms, with the aim of balancing the global environment and the economy.

These are technologies that can contribute to ⑪ "The Carbon Recycling Industry" above. In this development, we have observed that although coryneform bacteria (typical industrial microorganisms) suppress their growth under reducing conditions, they maintain metabolic functions. They can metabolize saccharides to produce organic acids and related compounds efficiently. Therefore, we developed a growth-independent bioprocess "RITE Bioprocess®" and established "the complete and simultaneous use of mixed sugars derived from non-food biomass" and "high resistance to fermentation inhibitors" that are essential and elemental technologies for industrialization (see Chapter 2).

With these technologies, we are reporting the world's highest level of high-efficiency biofuel products, including ethanol, butanol, green jet fuels, biohydrogens, and green chemicals, including lactic acid, succinic acid, alanine, valine, shikimic acid, protocatechuic acid, 4-aminobenzoic acid, and 4-hydroxybenzoic acid. Furthermore, we are currently focusing on developing production technologies for aromatic compounds, raw materials for higher value-added fragrances, cosmetics, pharmaceuticals, and so on (see Chapter 3).

Alternatively, we participated in the New Energy and Industrial Technology Development Organization (NEDO) "Smart Cell" project, SIP Strategic Innovation Creation Program, and NEDO "Bio-Manufacturing" project to develop the latest technology, integrating biotechnology and digital technology. Through these national projects, we are progressing with R&D to improve the efficiency of biosynthesizing high-performance chemicals, which were difficult to produce through conventional synthetic methods. Likewise, since 2020, we have participated in the NEDO "Moonshot" project and are working on researching and developing multi-lock type biopolymers that can be decomposed into the ocean using non-food biomass as a raw material (see Chapter 4).

This overview explains our core technology, the "RITE Bioprocess®," and its features. Next, we will introduce these developments, highlighting their target uses. Then, as crucial technological development, national projects based on the "bio-digital technology" that has made remarkable progress will be described. Finally, we want to commercialize our efforts.

2. The core technology of our group "RITE Bioprocess®"

Our group has established the innovative bioprocess "RITE Bioprocess®" based on a new concept, "core technology of RITE." This concept has achieved outstanding results in developing technologies for the highly efficient production of biofuels and green chemicals, such as amino acids and aromatic compounds. It has also been significantly evaluated in Japan and overseas (Fig. 1).

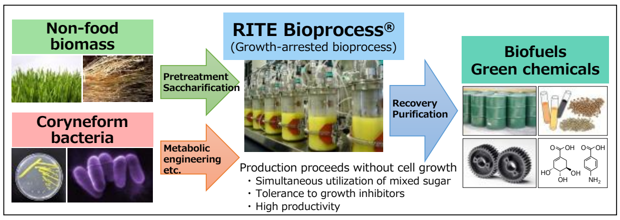

Fig. 1 Biorefinery concept using the "RITE Bioprocess®"

2.1. Features of the "RITE Bioprocess®"

2.1.1. Feature 1: Growth-independent bioprocess

In the "RITE Bioprocess®," first, many coryneform bacteria that are highly and metabolically designed to help efficiently produce the target substance are cultivated. Then, after densely filling the reaction vessel with cells, the reaction is conducted in a state where cell division is stopped by removing anaerobic conditions and factors essential for proliferation (Fig. 2).

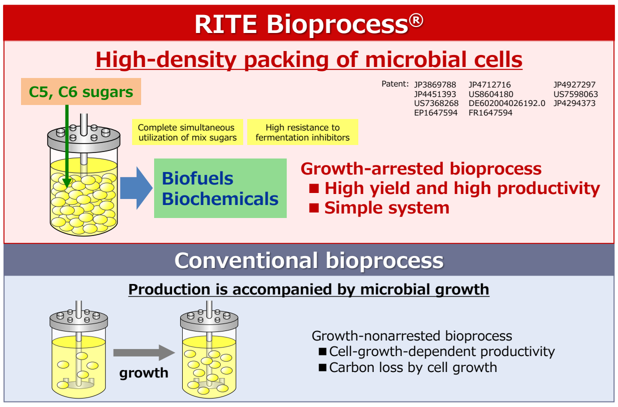

Fig. 2 Feature 1 of the “RITE Bioprocess®”

(Growth-independent bioprocess)

The key to its high efficiency lies in the growth-independent bioprocess of producing compounds while suppressing the growth of microorganisms, which eliminates the need for nutrients and energy required for the development. Specifically, although raw sugar materials are not used for growth, they produce target substances, making it possible to use microbial cells remarkably and efficiently like a chemical catalyst. This method also achieved a bioprocess with high productivity equal to or higher than that of a typical chemical process.

2.1.2. Feature 2: Complete simultaneous use of C5 and C6 mixed sugars

Cellulose-based biomass comprises C5 sugars, such as xylose and arabinose, and C6 sugars, such as glucose. Therefore, simultaneously using C5 and C6 sugars is indispensable for efficient production processes using cellulosic biomass.

Coryneform bacteria into which a C5 glucose metabolism gene has been introduced have slower usage rates with xylose (C5 sugar) and arabinose (C5 sugar) than glucose (C6 sugar) (see the graph on the left in Fig. 3). Therefore, when the raw materials are continuously added, the C5 sugar accumulates, eventually decreasing the production efficiency of the compound.

Alternatively, by further introducing a C5 sugar transporter gene, we succeeded in increasing the usage rate of the C5 sugar to the same level as the C6 sugar (see the graph on the right in Fig. 3). Therefore, C5 and C6 saccharides can be used simultaneously, and cellulosic raw materials can be used efficiently.

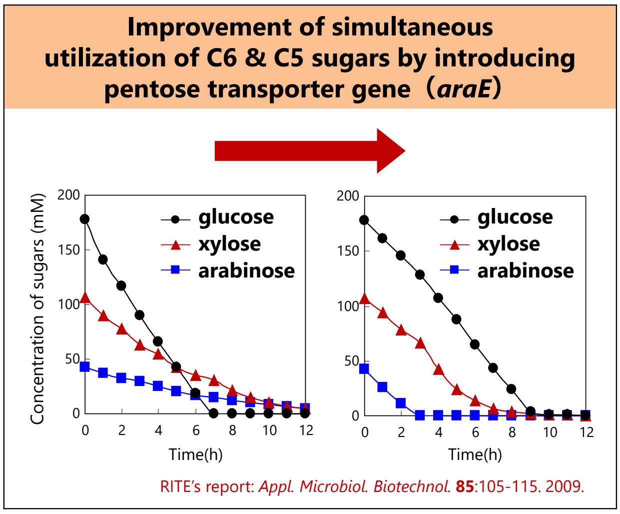

Fig. 3 Feature 2 of the “RITE Bioprocess®”

(Simultaneous usage of mixed sugars)

2.1.3. Feature 3: High tolerance to fermentation inhibitors

Fermentation-inhibiting substances, such as phenols and furans produced during the pretreatment of lignocellulosic biomass, sturdily inhibit creating the target substance. Therefore, increasing the microorganisms (bacteria) resistance to the fermentation inhibitor is indispensable to produce the target substance efficiently. The “RITE Bioprocess®” demonstrates that it is highly resistant to fermentation inhibitors because it does not grow as described above (Fig. 4).

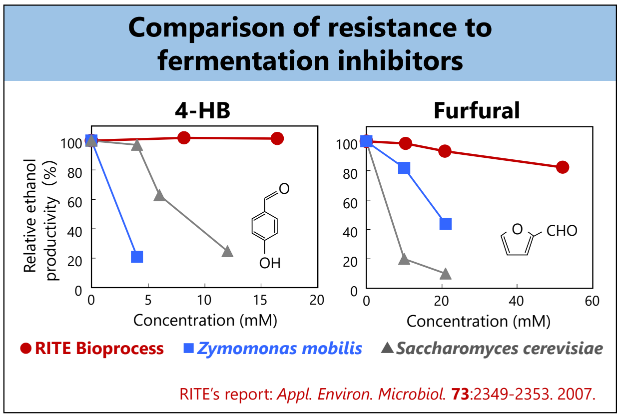

Fig. 4 Feature 3 of the "RITE Bioprocess®" (High tolerance to fermentation inhibitors)

2.2. Main substances produced by the "RITE Bioprocess®"

Figure 5 shows some substances currently achieving high production by the group. As mentioned above, many materials have reached the world's highest level of productivity. Nevertheless, we are expanding from ethanol and biohydrogen to butanol and high-performance bio-jet fuel materials in biofuels. We also expanded from L-lactic acid, D-lactic acid, and amino acids to high-performance chemicals, such as aromatic compounds in green chemicals.

Biofuels	Green chemicals
■ Gasoline additives ・Ethanol* ■ Bio-jet fuels ・Isobutanol* ・n-butanol* ・C9~C15 Saturated 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) ■ Alcohols ・Isopropanol (Propylene raw materials) ・Xylitol (Sweetener) *: Polymer raw materials Red character: World's highest productivity achieved

Fig. 5 Examples of substances produced by the "RITE Bioprocess®"

3. Development of target products

3.1. Biofuel

3.1.1. 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 a base material for producing bio-jet fuels using conventional chemical reactions. In turn, the bio-jet fuel synthesized from biobutanol can be used in airplanes. Airlines and aircraft manufacturers have paid considerable attention to the importance of bio-jet fuel as 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 called "alcohol-to-jet" (ATJ) fuel. In 2016, it cleared the standards of the America Society for Testing and Materials (ASTM) and became available for commercial flights.

Prior to these movements, we have been developing a highly efficient biobutanol production process using "RITE Bioprocess®". As part of this development, we conducted the "International Joint Research and Development Project for Innovative Energy Technology" by the Ministry of Economy, Trade, and Industry. In this project, through joint research with the US National Renewable Energy Laboratory (NREL), we developed biobutanol production technology using mixed sugar derived from non-food biomass as a raw material. In addition, through joint research with the US Pacific Northwest National Laboratories (PNNL), we proceeded with the development of technology to convert butanol into drop-in fuel such as jet fuel. Furthermore, our group improved the butanol resistance of the production strains, optimized the metabolic pathways of the production strains, and developed energy-saving butanol recovery technology. As a result, we have achieved the world's highest level of high productivity in the bioproduction of butanol (Fig. 6).

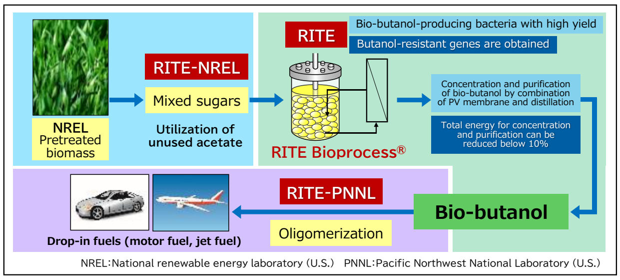

Fig. 6 Production of biobutanol and bio-jet fuel using the "RITE Bioprocess®"

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 manufactured bio-jet fuels from used clothes collected in cooperation with JAL and JEPLAN, Inc., and our technology was selected for it.

The Green Earth Institute Co., Ltd. (GEI), a venture company originating from RITE, participated in this project together with RITE. Consequently, isobutanol was produced by the "RITE Bioprocess®" using coryneform bacteria developed by RITE. Hence, 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. The first flight equipped with domestic bio-jet fuel was realized on February 4, 2021 on JAL's Haneda-Fukuoka route.

Therefore, in the future, by combining these elemental technologies and various procedural knowledge, we propose to produce jet fuels from biobutanol for use and commercialization.

3.1.2. Green jet fuel

There is an urgent need to reduce the aviation industry's environmental load. As part of the reduction measures, electric or hydrogen aircraft are being developed, but considerable technical issues have been observed. Thus, disseminating drop-in biofuels derived from renewable feedstocks is unavoidable.

Petroleum-based jet fuels are mixtures of hydrocarbons comprising *n*-paraffins, isoparaffins, cycloparaffins, and aromatic compounds with 9–15 carbon atoms. So far, ASTM International has approved seven production pathways for bio-jet fuels, such as producing HEFA fuel by hydroprocessing fatty-acid esters and ATJ fuel by oligomerizing ethanol or isobutanol. In 2020, ATJ fuel coupled with our biobutanol production technology was also approved. However, these certified bio-jet fuels primarily consist of isoparaffins and lack other essential components: cycloparaffins and aromatics. Therefore, they do not meet ASTM standards on their own and should be blended with petroleum-based jet fuel to 50% or less when used.

To overcome this blending ratio limitation of certified bio-jet fuels, we are developing a high-performance green jet fuel containing cycloparaffins, aromatics, and isoparaffins. The novel jet fuel is expected to be used alone without being blended with petroleum-based jet fuel. In the R&D of high-performance bio-jet fuel, we achieved promising results, such as a novel biocatalyst that enabled cross-coupling reactions between C2 and C8 compounds to synthesize C9–C15 branched and cyclic compounds, which can then be chemically converted to jet fuel components (Fig. 7).

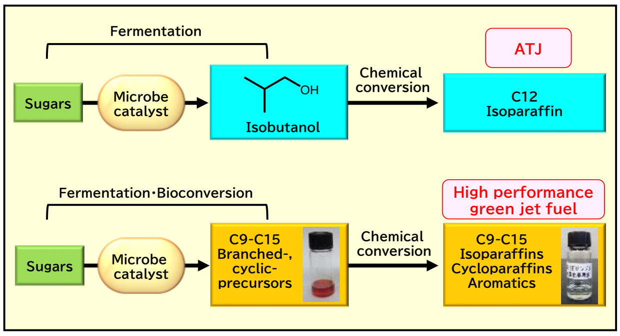

Fig. 7 R&D of a novel high-performance bio-jet fuel

3.1.3. Biohydrogen

Hydrogen is an energy carrier key to realizing carbon neutrality because (i) its combustion generates only water; (ii) it can be produced from diverse energy sources, including renewable ones; (iii) it can be stored in large quantities for long periods; and (iv) it can be distributed and used for power generation, transportation, and various industrial processes. However, CO_2 emissions during hydrogen production processes currently used are a problematic issue because fossil resources are used as feedstock. Therefore, a Basic Hydrogen Strategy was drawn up at the Ministerial Council meeting on Renewable Energy, Hydrogen, and Related Issues in 2017. The conference stated the importance of developing innovative technologies for hydrogen production, storage, and distribution to realize a hydrogen society over the medium to long term up to 2050. Therefore, hydrogen strategies around the world have moved. As noted, the US Department of Energy launched the "Energy Earthshots Initiative," in which the first "Hydrogen Shot" was set as a challenging goal, i.e., reducing the cost of CO_2-free hydrogen by 80% in one decade.

Furthermore, although bioprocesses have significant potential for CO_2-free hydrogen production, innovative improvements in technology should establish a cost-effective process for producing biohydrogen. Therefore, along with Sharp Corporation, our group has developed a biohydrogen production process with an overwhelming production rate (max 300 L H_2/h/L) using the hydrogen production pathway via formate during dark fermentation. On the basis of this achievement, our group is now working on improving hydrogen yield from biomass by integration with photofermentation.

The formate-dependent hydrogen production pathway uses only a part of the reducing power generated by degrading sugars, such as glucose, derived from biomass. Subsequently, a high-yield hydrogen production pathway using both NADH and ferredoxin (Fd) as the reducing power was engineered and demonstrated to work. Our group also elucidated a unique regulatory mechanism of acetate metabolism in photosynthetic bacteria for use in photofermentation, which has been successfully applied to improve the acetate-hydrogen conversion efficiency (Fig. 8).

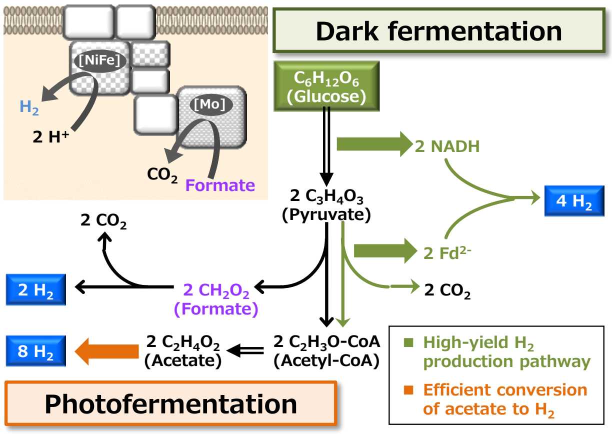

Fig. 8 Metabolic engineering of dark fermentative and photofermentative hydrogen-producing microorganisms

3.2. Amino acids (alanine and valine)

Usually, amino acid fermentation is conducted under aerobic conditions, where high productivity requires the aeration and agitation of the system to be adequately controlled. However, this process is difficult to achieve in large-scale fermenters because their internal oxygen concentration is not homogeneous. To overcome this problem, we have developed a new and genetically modified *Corynebacterium* strain using the "RITE Bioprocess®" to produce amino acids under anaerobic conditions. Furthermore, under anaerobic conditions, the technological hurdle for amino acid production 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 microbial cells, 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 industrializing 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. Subsequently, 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. We aimed to produce this amino acid from renewable resources to reduce the life cycle carbon footprint.

In 2016, we succeeded in demonstrating the feasibility of L-alanine's production technique using 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. After an evaluation by the Food Safety Committee in August 2017, the safety of the L-alanine produced by our strain 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 to produce other amino acids.

3.3. Green-aromatic compounds

Aromatic compounds are essential industrial chemicals used for synthesizing polymers and various value-added chemicals for use in 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 to create 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. 9). Therefore, by employing the metabolically engineered *C. glutamicum*, we successfully established a highly efficient bioprocess for producing the following aromatic compounds from non-food feedstocks: shikimate, an essential building block of the anti-influenza drug, Tamiflu; 4-aminobenzoate, the building block of a potentially useful functional polymer; and aromatic hydroxy acids, having potential applications in polymer, pharmaceutical, cosmetic, adhesive material, and flavor (vanillin) industries. Currently, we are seeking to develop new strains to produce useful aromatic compounds that the wild-type *C. glutamicum* is unable to produce. These strains 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 also help to accelerate the development of strains and improve their productivity.

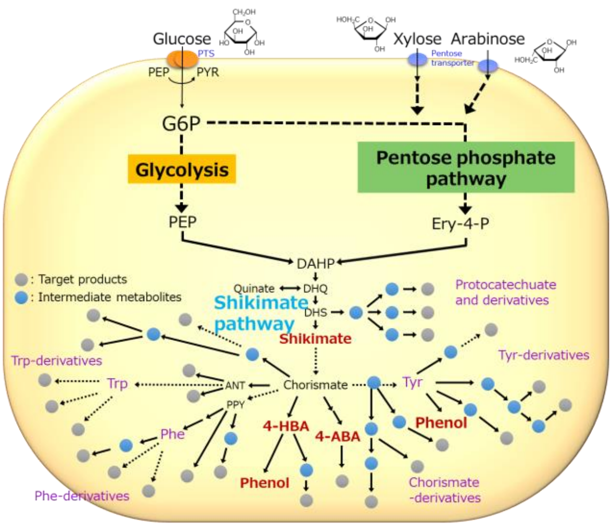

Fig. 9 The biosynthetic pathway for various aromatic compounds

4. Core technologies

4.1. The NEDO Carbon Recycle Project

On a national strategy, realizing the world's most advanced bioeconomy society in 2030, the development of material production technologies as the basis of the bio-manufacturing industry is an urgent issue. By utilizing biological functions, it is possible to produce materials from biomass without depending on fossil resources as raw materials. These technologies will contribute to the realization of a carbon recycling society.

In response to this social situation, NEDO launched a new project in 2020, "Development of Bio-based Production Technology to Accelerate Carbon Recycling." Participating institutions will develop and validate next-generation production technologies based on the fermentation technologies cultivated in Japan so far or that did not involve conventional methods.

Our Group participated in the predecessor of this project, "Development of Production Techniques for Highly Functional Biomaterials Using Plants and Other Organisms" (NEDO Smart Cell Project). Along with project participants (universities, research institutes, and companies), we developed technologies to design a Smart Cell (defined as a finely designed and expression-controlled cell). We also validated the technologies by breeding production strains in a short period of time.

In the Carbon Recycling Project, in addition to further improving the technologies developed during the Smart Cell Project, production process technologies, including scale-up and refining, will be developed. Furthermore, advanced bioproduction system platforms and peripheral technologies will be developed by creating information analysis technologies that can control production processes. These results will accelerate the social implementation of bio-based materials.

Our group participated in this project from the first year. Our target is catechol, a highly toxic aromatic compound. The aim is to develop "Industrial Smart Cell Creation Technology" to solve problems associated with the practical application of fermentative production technologies using production strains developed in the Smart Cell Project and accumulated omics data (metabolome, transcriptome, proteome). We will also validate the technologies (Fig. 10).

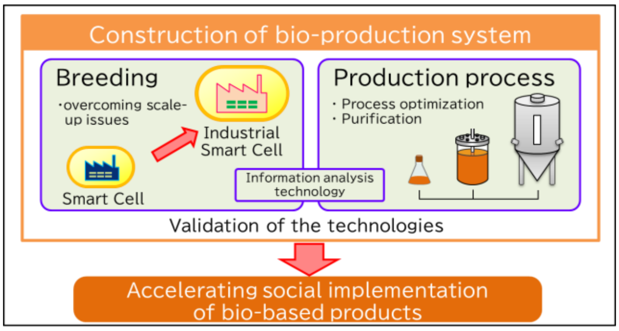

Fig. 10 Construction of an advanced bioproduction system platforms

4.2. The Cross-ministerial strategic innovation promotion program (SIP) (Development of biomonomers for high-performance plastics)

RITE is participating in the theme "Technologies for Smart Bio-industry and Agriculture" in the SIP, which 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 aims to realize a sustainable growing society that uses manufacturing technologies developed through biotechnology and digital resources.

In the program, RITE participates in the consortium "Development of Technologies for Functional Design and Production of Innovative Biomaterials," which aims for synthesizing polymers with new functions desired by markets using monomers biosynthesized from cheap raw materials, such as biomass. Thus far, polymers with ultra-high heat resistance and those for battery materials have been developed (Fig. 11). To design the biosynthetic route for monomers comprising these polymers, the group led by RITE has been developing and validating technologies for functional modification of enzymes using enzymes involved in monomer precursor biosynthesis as targets. The technology enables the efficient extraction of combined functional mutations by providing a machine learning algorithm with data of many mutant enzymes to train it. The technology for seeking novel enzymes with desired functions using multiple amino acid sequence data from enzyme databases is also being developed. These technologies allowed us to modify the substrate specificity of target enzymes and improve their activities. In the fiscal year of 2021, through collaboration between the consortia, RITE has been working on the task to improve the productivity of the target monomer biosynthesized by a bacterial strain by optimizing its culture conditions. Thus far, the productivity of the monomer has successfully been improved twofold by seeking optimal culture conditions. Subsequently, RITE will evaluate the monomer's productivity using biomass-derived raw materials provided by a collaborative consortium.

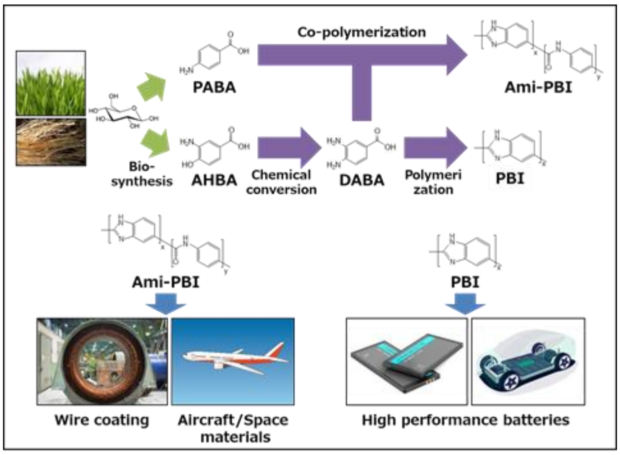

Fig. 11 Synthesis of developing monomers and polymers and polymer applications

4.3. The NEDO moonshot-type R&D project

Plastics are lightweight, inexpensive, and durable polymer materials indispensable for daily life. However, because they are chemically stable, they are not easily decomposed in the natural environment.

It has been reported that there is a typical trade-off between biodegradability and durability/toughness of polymers, such as plastics. Hence, biodegradable polymers are not durable because they degrade quickly in the natural environment and their mechanical properties are insufficient. Thus, only limited applications are possible. Although conventional plastics have excellent durability and toughness, their biodegradability is poor.

Suppose that it is possible to create new plastics with durability and toughness with biodegradability, these plastics can be used in several applications as environmentally friendly plastic. They can also be recycled. Notably, plastic litters scattered in the marine environment are challenging to recover and have a negative effect on the ecosystem.

Therefore, developing plastics that can control the timing and speed of biodegradation (multi-lock biopolymers) is critical for resource recycling.

In the NEDO moonshot-type R&D project "Development of Multi-Lock Biopolymers Degradable in Ocean from Non-Food Biomasses", we will introduce a "multi-lock mechanism" for plastic degradation to break the trade-off relationship between plastics. Hence, through multiple and simultaneous stimuli, such as light, heat, oxygen, water, enzymes, microorganisms, and catalysts during decomposition, the multi-lock mechanism will suppress the decomposition to maintain durability and toughness and prevent degradation. Products to be commercialized in this project are plastics, tires, textiles, fishing nets, and fishing gears made from non-food biomass. Material design guidelines for multi-lock biodegradable plastics will also be established through collaboration between industry, academia, and the government. In this context, our group is promoting R&D on the bioproduction of various monomers that can be used as raw materials for these products from non-food biomass materials. This project includes the functionalization of plastic-degrading enzymes that can be used in the multi-lock mechanism (Fig. 12)

(Here is the HP of the project: http://www.moonshot.k.u-tokyo.ac.jp/en/index.html).

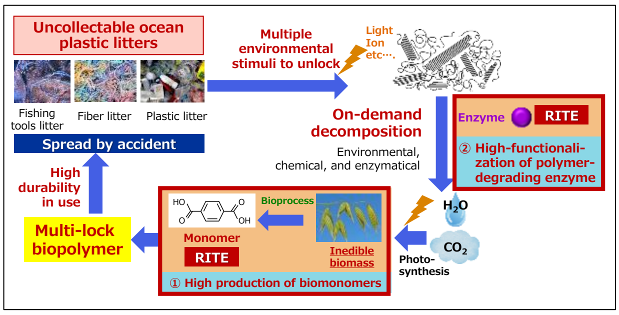

Fig. 12 R&D of marine-degradable multi-lock biopolymers made from non-food biomass for resource recycling

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.)

(Click here for GCC)

Currently, commercial phenol can only be derived from petroleum. We have taken on the challenge of developing the world's first bio-manufacturing process for biomass-derived phenol. The aim is to aid global environmental conservation and greenhouse gas reduction.

In February 2010, Sumitomo Bakelite Co., Ltd. and RITE established Green Phenol/High Performance Phenolic Resin Manufacturing Technology Research Association (GP Union).

In May 2014, GP Union was reorganized into Green Phenol Development Co., Ltd. (GPD), to accelerate the industrialization of our biomass-derived phenol-producing technology, named the "Two-Stage Bioprocess." This was the first example in demutualization of a technology research association. Then, in April 2018, GPD changed its name to Green Chemicals Co., Ltd. (GCC).

Since GCC's phenol-producing technology and knowledge apply to the production of various other aromatic compounds, establishing a bioprocess for each higher value-added chemical and commercializing products that meet customer needs are in progress (see Section 3.3).

The present three significant GCC products are shown in Fig. 13. In 2021, several companies started quality evaluation of 4-hydroxybenzoic acid (4-HBA).

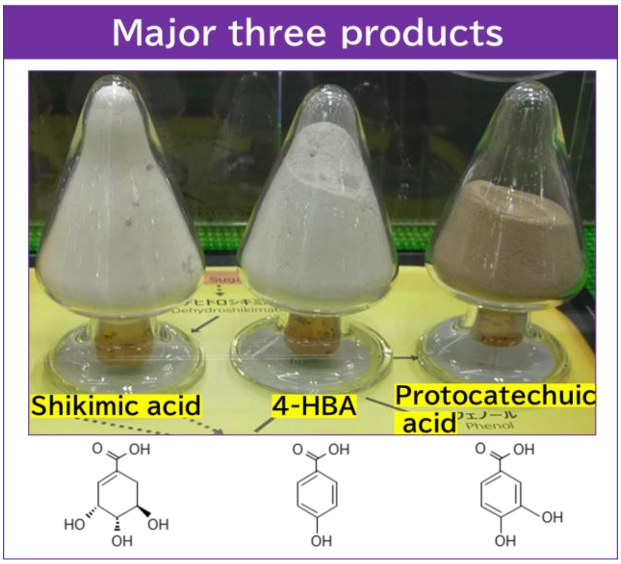

Fig. 13 Three major products of Green Chemicals Co., Ltd.

5.2. Green Earth Institute Co., Ltd.

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

(Click here for GEI homepage)

GEI is a RITE-launched venture company established on September 1, 2011, to facilitate the quick commercialization of research results based on those above-mentioned innovative "RITE Bioprocess®." GEI is currently conducting joint research and activities aimed at commercialization with RITE to realize the practical uses of green chemicals and biofuel production technologies manufactured using microorganisms. The company was listed on TSE Mothers in December 2021.

With 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 (see Section 3.2). In addition, the Ministry of Health, Labor and Welfare confirmed the safety of L-alanine as a food additive, paving the way for its use in the food industry.

However, based on 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. Due to this initiative, we succeeded in producing "Japan's first purely domestic bio-jet fuel flight" on February 4, 2021, for the JL319 flight (from Haneda Airport to Fukuoka Airport) (see Chapter 3.1).

Furthermore, GEI is developing green chemicals in cooperation with RITE for marketing, commercialization, and scaling up the mass production.

GEI will continue to influence developing biorefinery businesses toward realizing a society that does not rely on fossil resources.

5.3. Joint research with companies

In response to requests from companies, we are conducting joint research on the production of many substances other than the biofuels and green chemicals introduced in this overview. Although these contents cannot be presented here, they include the following:

A) At an early stage, we will take measures to change the substance, the company's main product, from fossil resource-derived to bio-derived, toward "2050 carbon neutral." Here, it is being implemented as a medium- to long-term R&D project.

B) At an early stage, we will take measures to change the major raw materials purchased from other companies from fossil resource-derived materials to bio-derived materials toward "2050 carbon neutral." This project is also being implemented as a medium- to long-term R&D goal.

C) Changing the substance, a product of the company, or the substance and raw material from fossil resources to bio-derived ones is another goal. With this project, substances that can be commercialized quickly at high unit prices are being implemented as short-term R&D goals.

6. Closing remarks

Due to the abovementioned technological innovation of "bio-digital technology," the understanding of life phenomena by the "data-driven" approach of discovering the law from huge life information has recently progressed. Under such a background, research on the biology of synthesis that accumulates data and understands biological functions by repeating the Design-Build-Test-Learn (DBTL) cycle is also rapidly developing.

All the national projects introduced in Chapter 4 also use these technological innovations, new knowledge, and methods to achieve dramatic improvements in development efficiency. In these projects, the biorefinery technology using the abovementioned smart cell also plays a significant role as a core technology and is expected to have a tremendous ripple effect in the industrial (manufacturing) and energy fields (Fig. 14).

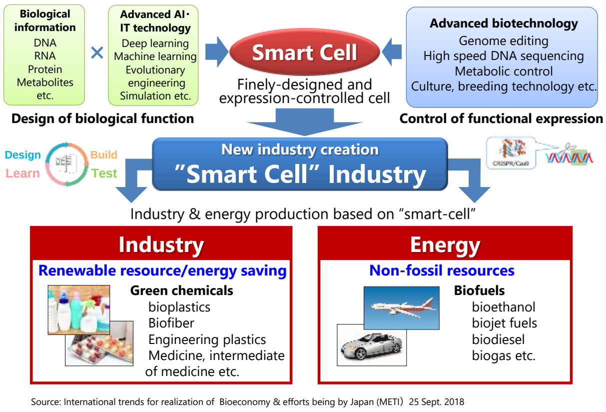

Fig. 14 Fusion of industrial/energy fields changed by bio and digital processes

In 2022, the group will continue to research and develop green chemicals, such as aromatic compounds, and biofuel production using the "RITE Bioprocess®" and "industrial smart cell design system." In addition, we also want to focus on developing practical production technologies and contribute to the "realization of carbon neutral by green bioprocesses."

Since the declaration of the "2050 carbon neutral" in October 2020, inquiries from companies have increased, and the number of joint research projects with companies has also increased. However, the group is looking for research partner companies, including cases other than those introduced in Chapter 5, Section 3. There is also a possibility that compounds that are difficult to produce microorganisms can be highly manufactured using the latest elemental technologies' developmental results, such as the abovementioned aromatic compounds. Therefore, if there is a compound that you want to make bio-derived, please contact us.

※ "RITE Bioprocess®" is a registered trademark of RITE.