Molecular Microbiology and Biotechnology Group

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Development of Bio-manufacturing Technologies that Contribute to Carbon Neutrality

1. Introduction

Since bioprocesses can be manufactured under ambient temperature and pressure, they are expected to reduce CO_2 emissions, unlike chemical processes, which are manufactured under high temperature and pressure. This also has an advantage over chemical processes in producing complex compounds with high carbon counts because they are generally synthesized by multistep reactions in the cell. Thus, biomanufacturing is attracting attention as an innovation that enables both economic growth and solutions to global-scale social issues such as resource autonomy and a breakaway from dependence on fossil resources. With the development of "bio x digital technology" through the fusion of biotechnology (e.g. synthetic biology and genome editing technology) with digital technology (e.g., the internet of things artificial intelligence) and the growing awareness of global environmental issues, such as the need to disengage from fossil resources for resource autonomy, the practical application of this technology is expected to accelerate and the market size to expand rapidly in the future. Major investments are being made in this field in the U.S. and China, and international competition is intensifying. In Japan, the Green Innovation (GI) Fund Project and the Biomanufacturing Revolution Promotion Project were launched this fiscal year as large-scale projects to develop and socially implement technologies for the design and development of new microorganisms and the advancement of manufacturing processes using microorganisms. These projects aim to be carbon neutral and carbon negative by switching from petroleum as a raw material to atmospheric CO_2 and unused resources.

Against this background, the Research Institute of Innovative Technology for the Earth (RITE) has been developing biorefinery technology to produce biofuels and green chemicals from non-food biomass at high efficiency through microbial bioprocesses. RITE discovered that coryneform bacteria, a typical industrial microorganism, maintain metabolic functions under reducing conditions despite growth inhibition and that they efficiently metabolize sugars and produce organic acids. Consequently, RITE developed the growth-independent bioprocess "RITE Bioprocess"[*1]. In addition, we established elemental technologies essential for industrialization, such as "complete simultaneous use of mixed sugars derived from non-food biomass" and "high tolerance to fermentation inhibitors" (see Chapter 2, Section 1). Using these technologies, we reported the world's highest efficiency production of ethanol, butanol, green jet fuel, and biohydrogen as biofuels, and lactic acid, succinic acid, alanine, valine, tryptophan, shikimic acid, protocatechuic acid, 4-aminobenzoic acid, and 4-hydroxybenzoic acid as green chemicals. Currently, RITE is focusing on developing production technologies for aromatic compounds that can be used as raw materials for high-value–added fragrances, cosmetics, pharmaceuticals, fibers, and polymers, as well as bio-manufacturing technologies that directly use CO_2 as a raw material.

So far, we have participated in the New Energy and Industrial Technology Development Organization (NEDO) "Smartcell" project and the "Data-driven Integrated Bioproduction Management System" project and have been developing "Smart Cell Creation Technology," a bio x digital technology (see Chapter 2, Section 2). We are also participating in the NEDO "Bio-Manufacturing Demonstration" project as a joint development with private companies using the same technology and are conducting research and development to commercialize bioproduction of carotenoids and flavors (see Chapter 3, Section 4 and Chapter 3, Section 5). In this fiscal year, the company began participating in the GI Fund Project and the Bio-manufacturing Revolution Promotion Project to develop bioproduction technology for high-performance adhesive raw materials from CO_2 and bio-upcycling technology to produce useful chemicals from unused resources (see Chapter 3, Section 1 and Chapter 3, Section 2). In addition, RITE is participating in the NEDO "Moonshot" project to research and develop ocean degradable multilock biopolymers made from non-food biomass (see Chapter 3, Section 6).

In this overview, we first explain our core technologies such as "RITE Bioprocess"[*1] and "Smart Cell Creation Technology." Next, we discuss a national project based on bio x digital technology innovation, which has been making remarkable progress in recent years as a basic technology development, and finally, we introduce our efforts for commercialization.

2. The Core Technologies of RITE

2.1. "RITE Bioprocess"[*1]

"RITE Bioprocess"[*1], developed by RITE, is a proprietary technology that enables highly efficient production of biofuels and green chemicals, such as amino acids and aromatic compounds (Fig. 1). The three features of "RITE Bioprocess"[*1] are described below (for details, see RITE Today 2022).

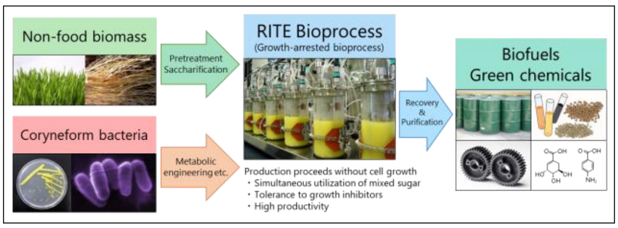

Fig. 1 Biorefinery concept using the "RITE Bioprocess"[*1]

Feature 1: Growth-arrested bioprocess

Anaerobic conditions and removing factors essential for proliferation allow the desired series of reactions to occur while cell division is arrested (Fig. 2). In other words, nutrients and energy previously used for multiplication are now used to produce the target substance. This has enabled microbial cells to be used extremely efficiently similar to a chemical catalyst, realizing a bioprocess with high productivity equal to or greater than that of ordinary chemical processes.

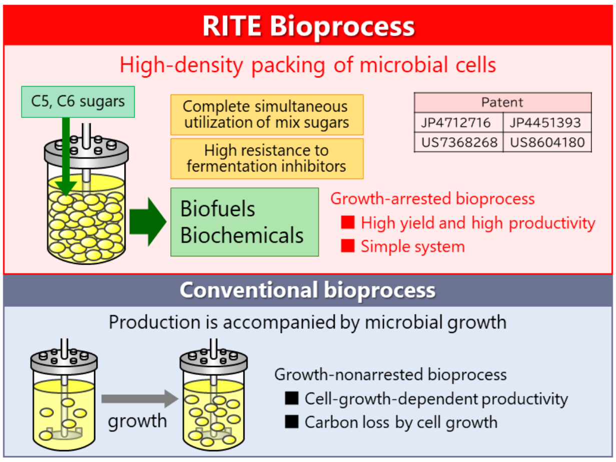

Fig. 2 Feature 1 of the "RITE Bioprocess"[*1]

(Growth-arrested bioprocess)

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

Most inedible biomass (cellulosic biomass) comprise a mixture of C5 sugars such as xylose and arabinose and C6 sugars such as glucose.

RITE has succeeded in increasing the utilization rate of C5 sugars to that of C6 sugars by introducing a C5 sugar transporter gene in addition to the C5 sugar metabolism gene (Fig. 3). This enables the full simultaneous use of C5 and C6 sugars and efficient use of cellulosic (non-food biomass) feedstock.

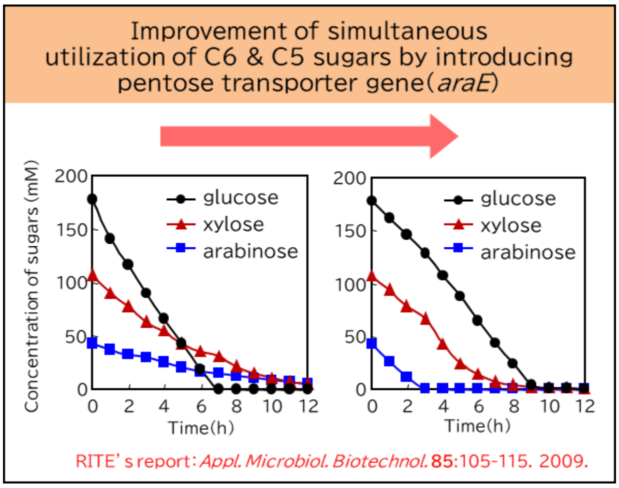

Fig. 3 Feature 2 of the "RITE Bioprocess"[*1]

(Simultaneous usage of mixed sugars)

Feature 3: High tolerance to fermentation inhibitors

"RITE Bioprocess"[*1] has demonstrated high resistance to fermentation inhibitors because the microorganisms do not grow as described above (Fig. 4). Therefore, this can be applied to using saccharification liquids containing various fermentation inhibitors and even to producing fermentation inhibitors.

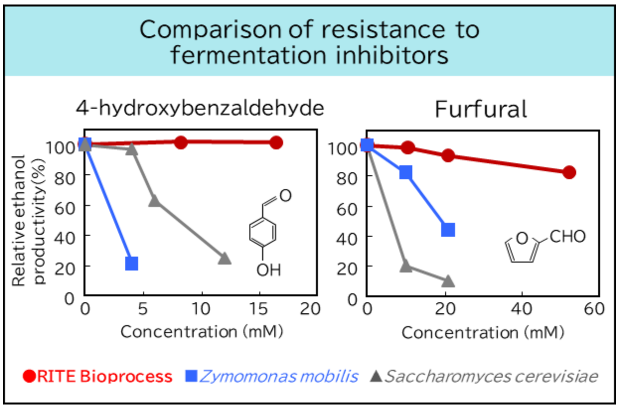

Fig. 4 Feature 3 of the "RITE Bioprocess"[*1]

(High tolerance to fermentation inhibitors)

2.2. Smart Cell Creation Technologies

With the fusion of cutting-edge biotechnology and digital technology, the potential of biological cells can be maximized to create "smart cells" that are optimized for biomanufacturing. This type of technology is called

Smart Cell Creation Technology and has made the development of smart cells dramatically more efficient. RITE is involved in developing these technologies by participating in the NEDO Smart Cell Project (2016–2020). During the project, we demonstrated the effectiveness of the Smart Cell Creation Technology by efficiently creating high-producing strains for the target chemical. By incorporating these technologies, RITE has succeeded in upgrading the production strain–breeding technology and fermentation-production technology (Fig. 5). Additionally, the Smart Cell Creation Technologies have been overtaken by the NEDO Bio-manufacturing project, and improvements are being made for the practical application of microbial production.

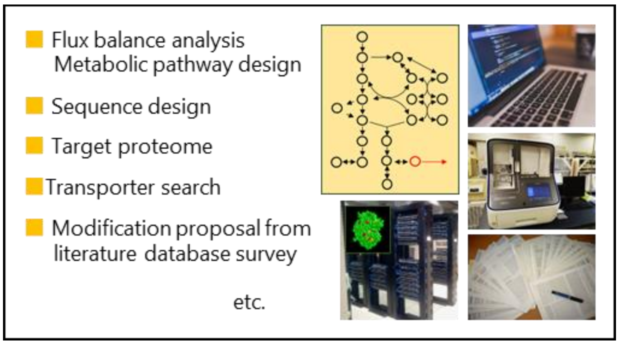

Fig. 5 Smart Cell Creation Technologies

2.3. Continuous Reaction System

RITE has developed biotechnological processes for producing various compounds. In this development activity, we have faced the problem that several target compounds have extremely strong cytotoxic effects, and production is halted because of the toxicity of the compounds that have accumulated during production. For example, catechol production, which is being developed in the NEDO Smart Cell Project and the NEDO Bio-manufacturing Project, plateaued after reaching a specific concentration in the conventional batch method. To avoid cytotoxicity and achieve high production, we constructed a continuous reaction system that selectively removes and recovers the target compound from the reaction system (e.g., by constructing a continuous reaction system that combines resin adsorption and membrane separation, Fig. 6). By applying this system to catechol production, a dramatically high production of catechol was achieved.

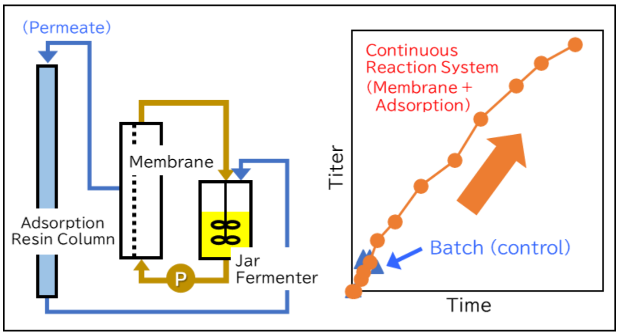

Fig. 6 A continuous reaction system using membrane separation and resin adsorption

2.4. Substances Produced by the "RITE Bioprocess"*1

Several materials currently in high production by RITE are shown in Figure 7. As mentioned above, the company has achieved world-class productivity in many substances. In biofuels, the company is expanding into production of butanol and high-performance biojet fuel materials in addition to that of ethanol and biohydrogen. Furthermore, in green chemicals, RITE is developing a wide range of high-performance chemicals such as aromatic compounds in addition to L-lactic acid, D-lactic acid, and amino acids.

Biofuels	Green chemicals
■ Gasoline additives ・Ethanol *	■ 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)
■ Bio-jet fuels ・Isobutanol * ・n-butanol * ・C9-C15 Saturated hydrocarbon + Aromatics	■ Organic acids ・D-lactate *, L-lactate * (Stereo-complex PLA) ・Succinate *
■ Biohydrogen	■ Amino acids ・Alanine (Chelators) ・Valine (Next-generation feed-use amino acids) ・Tryptophan (Next-generation feed-use amino acids)
* : Polymer raw materials Red character : World's highest productivity achieved	■ Alcohols ・Isopropanol (Propylene raw materials) ・Xylitol (Sweetener)

Fig. 7 Substances produced using the "RITE Bioprocess"*1

3. Fundamental Technology Development (National Projects)

3.1. NEDO GI Fund Project[*2]

The GI fund project entitled “Promotion of carbon recycling using CO_2 as a direct raw material through biomanufacturing technology” aims to contribute to realizing carbon neutrality by developing new biomanufacturing products using CO_2 as a raw material and implementing them in society to transform the industrial structure by using CO_2 as a resource and to achieve “carbon neutrality by 2050.”

In this context, RITE, in collaboration with Sekisui Chemical Co., Ltd, has started a project entitled “Commercialization of high-value–added chemicals using CO_2 as raw material through bioproduction technology” from FY2023 and is currently ongoing (project period: eight years from FY2023 to FY2030). (For details, see Special Feature)

3.2. Research and Development of Technologies to Promote Biomanufacturing[*2]

This project aims to solve both social issues such as environmental problems and economic growth by using unused resources such as discarded domestic biomass as raw materials and converting them into useful substances using biotechnology. (For details, see Special Feature).

3.3. NEDO Bio-manufacturing Project[*2]

The NEDO project “Development of bio-based product production technology to accelerate the realization of carbon recycling,” also known as the Bio-manufacturing project, conducts research and development that combines biotechnology and digital technology to produce materials from biomass that does not depend on fossil resources (from 2020). The Smartcell Project is the predecessor of this project. This project aims to expand new bioresources, develop bioproduction processes including separation, purification, and recovery, and accelerate the creation of bioderived products by demonstrating industrial production systems. Specifically, teams in this project are conducting research and development in three areas: “Development of basic technology to promote the utilization of bioresources,” “Development of basic technology for biofoundry production processes,” and “Demonstration of industrial material production systems.”

RITE has been involved in this project since the first year and is currently developing a new group of technologies (Industrial Smart Cell Creation Technology) to solve the problems associated with the practical application of biomanufacturing technology (Fig. 8). In FY2023, RITE mainly worked with partner research institutions to develop technology that eliminates productivity declines caused by uneven culture environments in large fermenters. RITE contributed to constructing and validating a fermentation-production simulation model by acquiring and providing detailed gene expression data and metabolite data in an environment that reproduces culture stress. By creating technologies to solve problems that may occur during large-scale production, we aim to eliminate rework in developing production strains and accelerate the social implementation of bio-based products.

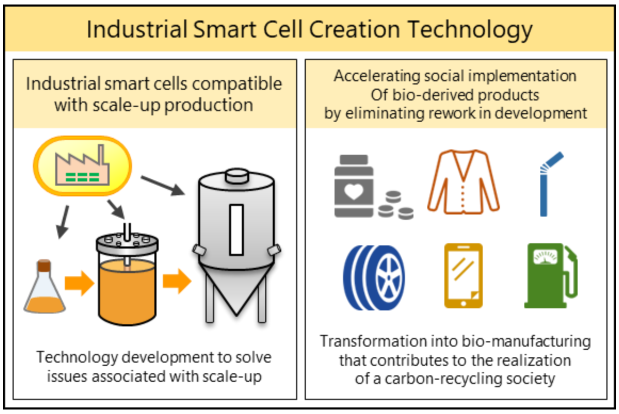

Fig. 8 Bio-manufacturing project: Development of Industrial Smart Cell Creation Technology

3.4. NEDO Bio-manufacturing Demonstration Project (Carotenoid)[*2]

Carotenoids are natural pigments produced by plants and some bacteria. They are popular as functional ingredients for lifestyle disease prevention and antiaging because of their high antioxidant activity, and their market size is growing. However, the carotenoid content in natural sources is low, and most of marketed carotenoids are produced by chemical synthesis from petroleum. Moreover, most carotenoids are characterized by low-absorption because of their chemical structure, and their bioavailability is also low. Several chemical processes that converting carotenoid structures from low- to high-adsorption types have been devised, but their conversion rates are unsatisfactory. Recently, as new sources of carotenoids, smart cells producing high amounts of carotenoids have been reported, but their carotenoids are also low-absorption types.

Since 2022, RITE has participated in the NEDO “Bio-manufacturing” project with Harima Chemicals, Inc. for social implementation of a bio-based mass production system for highly bioavailable carotenoids (Fig. 9). We have developed a smart cell that specifically produces high-adsorption types of carotenoids. We are currently improving the smart cell and developing other processes such as fermentation, carotenoid-extraction, and purification.

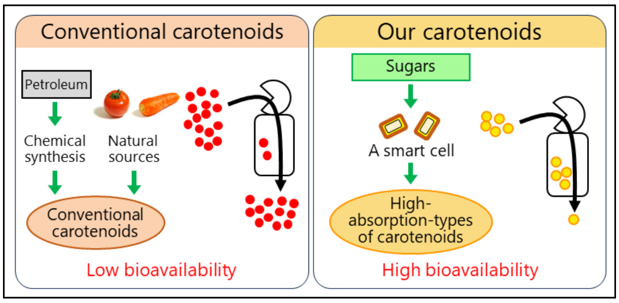

Fig. 9 Outline of our project for bio-based production of highly bioavailable carotenoids

3.5. NEDO Bio-manufacturing Demonstration Project (Rose Aroma Ingredient)[*2]

Since 2022, RITE has participated in the NEDO “Bio-manufacturing” project with Takasago International Corporation. We are driving the development of an "industrial smart cell" that can produce a rose aroma ingredient and a bioproduction system that can avoid microbial “product inhibition” derived from the fragrance materials (Fig. 10).

In FY2023, RITE succeeded in increasing the productivity by metabolic engineering and optimizing production conditions, by using the production system described above. We will continue with the improvement of the smart cells, testing of scale-up, and modification of the equipment. We aim to achieve Japan’s first social implementation of production of a fragrance ingredient by precision fermentation.

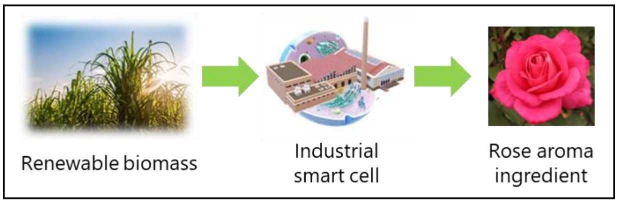

Fig. 10 Production of a rose aroma ingredient

3.6. NEDO Moonshot-type Research and Development Project[*2]

The project entitled “Development of Multilock Biopolymers Degradable in Ocean from Nonfood Biomasses” is performing research and development to introduce a "multilock mechanism" for plastic degradation (Fig. 11). Multiple stimuli such as light, heat, oxygen, water, enzymes, microorganisms, and catalysts can be used as triggers to start degradation of plastics, but avoid their degradation and maintain their durability and toughness while the plastics are in use. However, when accidentally dispersed into the marine environment, the multilock mechanism is unlocked to enable fast on-demand degradation.

Products targeted for practical application in this project include tires and textiles, which generate secondary fine debris when used, as well as plastic bottles, fishing nets and fishing tackle that contribute to ghost fishing, all of which negatively impact the environment due to runoff into the ocean.

In FY2023, we focused on developing a technology that enables the artificial control of the initiation timing of the degradation of multilocked plastics (Developments of new technologies using degradative enzymes). First, the thermostability of the enzyme was dramatically improved by electrostatic binding of the thermostable-plastic–degrading enzyme to a biodegradable carrier.

Next, we produced a film where the enzyme was mixed into plastic by thermal melting, exposed this to seawater, and succeeded in proving that rapid enzymatic degradation at the laboratory level (on-demand degradation) occurred.

We will aim to achieve faster on-demand degradation by improving the functionality of the plastic–degrading enzyme and optimizing the mixing conditions with the plastic. In addition to degradation tests in marine fields (e.g. Ainan Town, Ehime Prefecture), an international joint research with the U.S. Department of Energy's ARPA-E will be launched.
(The HP of the project can be found at:
http://www.moonshot.k.u-tokyo.ac.jp/en/index.html).

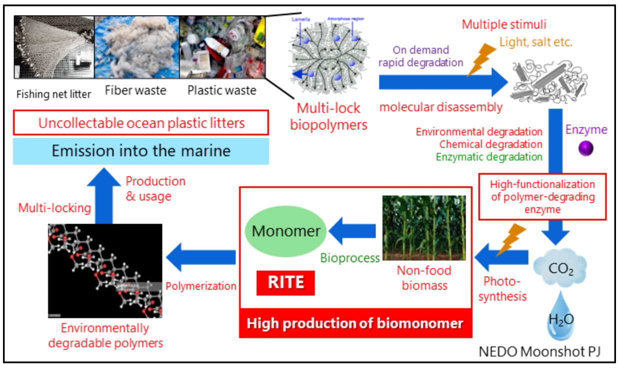

Fig. 11 Marine-degradable multilock biopolymers from nonfood biomass and their circulation

3.7. Japan Science and Technology Agency COI-NEXT

RITE has participated in the program on open innovation platform (COI-NEXT) commissioned by the Japan Science and Technology Agency. In the platform "Carbon Cultivation Hub Challenging the Limits of Carbon Negativity" started in 2023, we are working on developing biohydrogen production and liquid biofuel production technologies for establishing carbon-cultivation–based fuel-production technology. In this project, we will develop biological conversion technologies to efficiently produce fuels (hydrogen/ liquid fuel) from various biomass feedstocks in collaboration with the participant organizations working on developing biomass cultivation technologies, enabling an increase in CO_2 fixation by photosynthesis (Fig. 12). Hydrogen is expected to be the ultimate clean energy and is key in realizing carbon neutrality/negativity. In this context, a medium- to long-term theme is to develop CO_2-free hydrogen production processes. A short- to medium-term theme is to develop liquid fuel-production processes that use the same fundamental technologies as the other long-term theme. One of the key challenges for the social implementation of biomass fuel-production technology is reducing production costs. In addition, the components of biomass feedstock are diverse, and their composition considerably varies depending on the type of feedstock, making it difficult to meet a wide range of demands with uniform technology. To solve these issues, this project will promote the development of technologies in different fields, including various thermochemical and biological conversion technologies in an integrated manner for enabling the construction and expansion of a flexible biomass fuel supply system tailored to regional and feedstock needs.

RITE has developed a biohydrogen production process with high production rate. Building on this achievement, we are developing a microbial catalyst for improving the hydrogen yield from biomass-derived sugars to a large extent. We constructed a genetically engineered microorganism with a novel hydrogen production pathway enhanced by metabolic engineering. In addition, RITE has established a bioprocess that efficiently converts mixed C6 and C5 sugars to ethanol. Using this technology, we will develop an alcohol to jet process to produce a sustainable aviation fuel using various biomass feedstocks, such as energy crops, rice with high CO_2 fixation capability, and microalgae with high carbohydrate productivity.

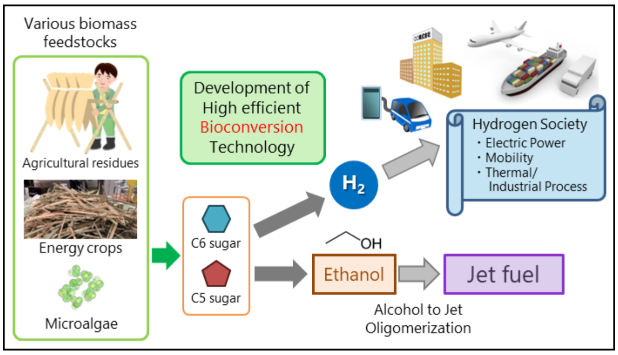

Fig. 12 Development of biohydrogen/bioethanol production technologies

4. Future Industrialization of Our Technologies

4.1. Green Chemicals Co., Ltd. (GCC)

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

In February 2010, RITE established the "Green Phenol and High-Performance Phenolic Resin Production Technology Research Association" (GP Association) with and Sumitomo Bakelite Co., Ltd. to develop fundamental technologies related to phenol production and phenolic resin production through the application of bioprocesses that use cellulosic raw materials (non-food biomass).

In May 2014, the GP Association was reorganized as "Green Phenol Development Co., Ltd." (GPD). This was the first example of demutualization of a technology research association.

In April 2018, given that GPD technology can produce useful compounds in parallel with phenol production, the trade name of Green Phenol Development Corporation was changed to Green Chemicals Co., Ltd., (GCC).

Since the phenol-producing technology and knowledge of Green Chemicals Co., Ltd can be applied to producing other aromatic compounds (Fig. 13), we are developing a bioprocess for other high-value–added chemicals and commercializing products that meet customer needs.

In FY2023, approval for industrial use of the two production strains of GCC’s products (4-HBA, PCA) was obtained by the Ministry of Economy, Trade and Industry.

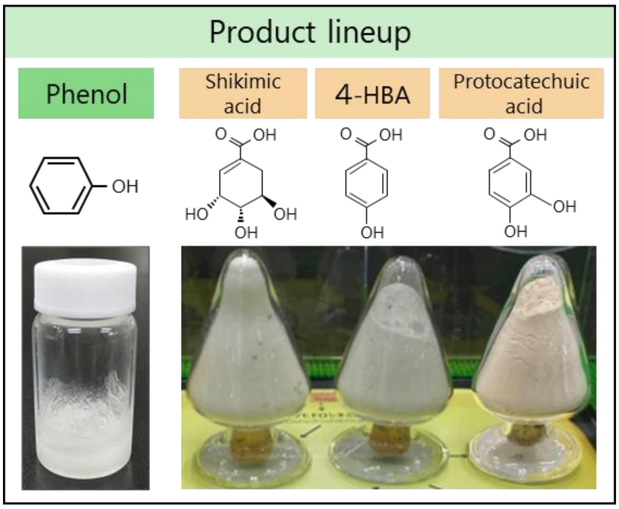

Fig. 13 Major product lineup of Green Chemicals Co., Ltd.

4.2. Green Earth Institute Co., Ltd.

(Head office: 6F Q Plaza Shinjuku 3-chome, 3-5-6 Shinjuku, Shinjuku-ku, Tokyo, Laboratory: 2-5-9 Kazusa-kamatari, Kisarazu-shi Chiba)

(Click here for the Green Earth Institute Inc. web site)

In September 2011, RITE established the Green Earth Institute Inc. to commercialize "RITE Bioprocess"[*1]. In December 2021, due to successful business results, the company was listed on the Tokyo Stock Exchange (Mothers), and in April 2022, moved to the Tokyo Growth Market due to market reorganization.

Currently, the company is promoting research and development with domestic and overseas partner companies, including "Biofoundry Base" (based on the government's "Biostrategy 2020), NEDO's "Green Innovation Fund Project," and NEDO's "Bio-manufacturing Revolution Promotion Project."

4.3. Joint Research with Companies

In addition to the main substances produced (see Chapter 2, Section 4) introduced in this overview, we are conducting joint research on many other substances in response to requests from companies.

In addition to Harima Chemicals, Inc. and Takasago International Corporation, in the NEDO Bio-manufacturing Demonstration Project, we are also conducting joint research with many other companies. These include research and development to convert a company's product (fossil resource-derived material) to bioderived materials at an early stage, as well as research and development to convert company's main products or main raw materials (fossil resource-derived materials) to bioderived materials in the medium to long term.

5. Closing remarks

In recent years, technological innovations and new knowledge and methods in the aforementioned "bio x digital technology" have dramatically improved the efficiency of smart cell development, especially in the national projects described in Chapter 3. And production demonstration efforts are vigorously underway for social implementation of the smart cells created by these results. These are expected to create a new industry (smart cell industry) and have a significant ripple effect on the industrial sector (manufacturing) in addition to the energy sector (Fig. 14).

RITE will continue to promote the development of bio-manufacturing technologies that contribute to carbon neutrality by utilizing the "Smart Cell Creation Technology" and "RITE Bioprocess"[*1] to focus on research and development using unused resources and CO_2 in the atmosphere as raw materials, as well as the development of practical production technologies for green chemicals.

Since the declaration of "carbon neutrality by 2050" in October 2020, inquiries from companies have been increasing, and the number of joint research projects with companies has also been increasing. RITE is still looking for companies to collaborate with. Compounds that have generally been considered difficult to produce by microorganisms may be produced at high levels by using the latest elemental technology development results. If you have a compound you would like to biologize, we would like to hear from you.

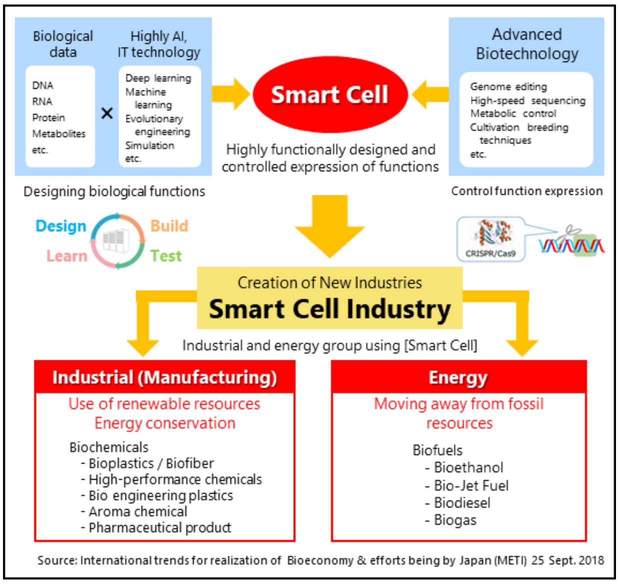

Fig. 14 Fusion of industrial / energy fields impacted by new bio and digital technologies

[*1] "RITE Bioprocess" is a registered trademark of RITE.

[*2] This article is based on results obtained from a project commissioned or subsidized by the New Energy and Industrial Technology Development Organization (NEDO).