Japan's bioeconomy: "smart cells," optimized production processes and a shared infrastructure

Rolf Schmid, Bio4Business, Stuttgart, Germany
www.bio4business.eu

Introduction

Japan has retained a strong manufacturing sector, maintaining a firm place as a production location in a global economy increasingly dominated by the service sector. Among the characteristics of Japanese industry, three are particularly noteworthy:

- the pursuit of perfection, including in production (kaiseki、解析)
- openness to innovation, combined with a willingness to diversify into profitable new markets, and
- a willingness to collaborate with competitors in the development of fundamental technologies.

Starting from a strong classical fermentation industry, grown on the mass production of sake, beer, soy sauce, glutamate, enzymes, and many other bioproducts, new methods of biotechnology have rapidly entered Japanese industry. The Ministry of Industry and Trade, Meti, is promoting this through its project house, Nedo, through public-private partnerships between academia and industry ("Research Associations," 研究組合). Hundreds of companies, startups and universities have joined forces to form such research alliances over the years. This has led to rapid diffusion of new technologies and an excellent network of personal relationships. Table 1 shows a small selection of the topics in the industrial biotechnology sector.

Table 1: Public-private partnerships in the field of biotechnology

Duration	Name of Program
1981 - 1988	Bioreactors
1981 - 1989	Cell cultivation on a large scale
1981 - 1989	Adding value with recombinant DNA
1998 - 2002	Development of genome-based bioinformatics
2002 - 2010	Production of high-value materials through transgenic plants
2005 - 2012	Minimal Genome Factories
2016 - 2021	Smart cells
2020 - 2026	Smart cell industries

Nikkei Biotech, a service of Nikkei Publishing specializing in biotechnology, estimates that the Japanese bioproducts market will be worth about ¥6.654 trillion (€46.4 billion) in 2022.[1] Of that, biopharmaceutical products accounted for nearly 40%, devices and services for 30%, but chemical industry bioproducts such as amino acids and detergent enzymes for only 10%. The Japanese government wants to fundamentally change that with its 2019 strategy to build "the

[1] https://bio.nikkeibp.co.jp/atcl/report/16/082400016/121500265/

world's most advanced bioeconomy" and thus advance the decarbonization of the Japanese economy.[2] There is still a lot of potential for sustainable biotech processes, especially among medium-sized companies.

1. The Smart Cell Industry Project (2020 - 2026).

Japan's 2019 bioeconomy strategy paper includes "bio-digital integration" as a core element: by combining genetic methods with bioinformatics, microorganisms, plants and enzymes are to be developed into high-performance biocatalysts in a short time for an industry that is increasingly shifting its basic material requirements to bio-based feedstocks.

To this end, the project house Nedo has drawn up a project plan for the period 2020 - 2026, for which around JP¥15 billion in mainly government grants is available (around €105 million); this is intended to create bioeconomy products worth around €50 billion, the production of which will save 3.67 million tons of CO_2 annually.[3] The project involves a large number of companies, universities and government research institutes. It is tightly structured in the form of stage-gate processes, which also include life cycle assessments (LCA), and consists of five clusters that are available to companies and their academic partners for project work and training in the spirit of open innovation

Table 1: Five clusters in the project, and one training module

Cluster 1	Kansai BioFoundry[4], a bioinformatics and robotic platform for rapid molecular genetic optimization of microorganisms.	Kobe University
Cluster 2	Development team for transgenic plants for the production of valuable materials	Bioproduction Research Institute, Sapporo
Cluster 3	Establishment of a joint information and management system for bioproduction with 70 partners	Kyoto University
Cluster 4	Pilot plants for optimizing production processes in the greater Osaka area (Kansai)	Osaka Institute of Technology, Kyoto University and Chitose
Cluster 5	Pilot plants for the optimization of production processes up to 3000 L in the Tokyo area (Kanto)	Green Earth Institute and Kyowa Hakko Bio
Cluster 6	Training of bioengineers at Osaka Institute of Technology, Kyoto University and other consortium institutions.	Special budget from Nedo

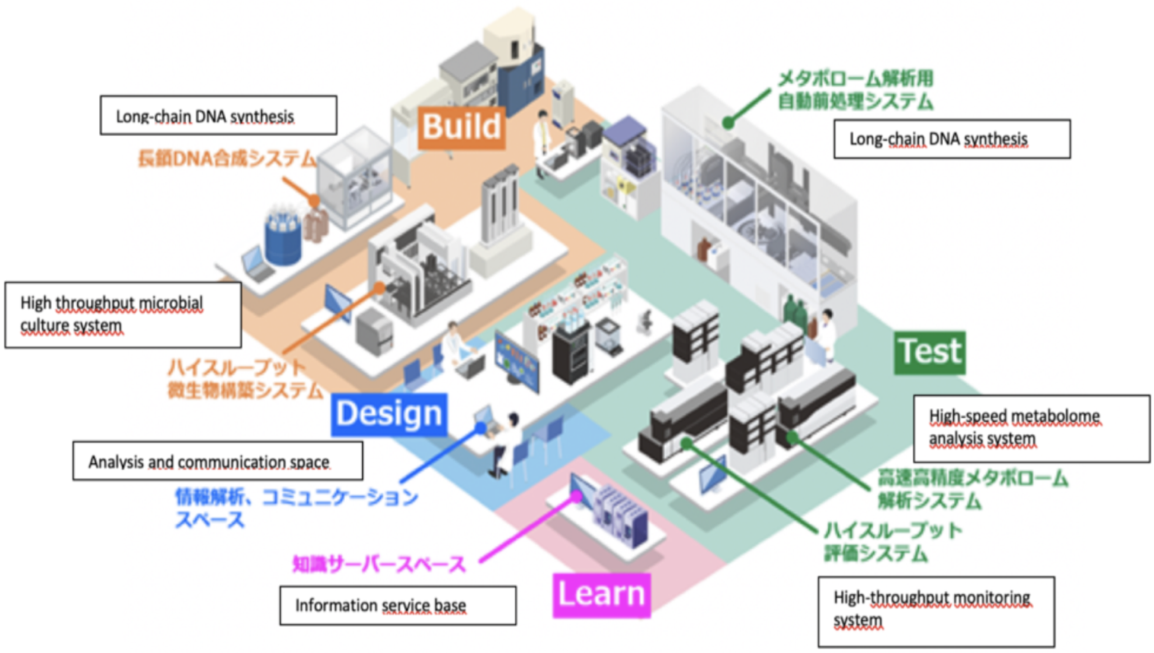
[2] Rolf Schmid, Nachrichten aus der Chemie 67, September 2019
[3] https://www.nedo.go.jp/activities/ZZJP_100170.html
[4] a BioFoundry is a workbench for optimizing microorganisms, plant cells or enzymes that serve as biocatalysts for the production of valuable substances. It integrates the modern methods of molecular genetics and bioinformatics

The Japan Bioindustry Association has published an overview of the project in English.[5] More complete and well-illustrated overviews in Japanese outline its core elements.[6,7]

2. The "Kansai BioFoundry" in Kobe: synthetic biology and genome design

High throughput is used here to develop optimized genomes of microorganisms for the desired implementation, or alternatively a gene coding for an improved enzyme. This involves four steps: a design based on the principles of bioinformatics (Design), the synthesis of the gene sequences designed in this process in high throughput (Build), the success control in biological tests (Test) and the improvement of the genome or the gene in a next optimization cycle (Learn). These workflows are performed at very high throughput, resulting in optimized biocatalysts in a short time.

Fig. 1: The "BioFoundry" at Kobe University[8]



The workbench is located at Kobe University, where a proprietary technology for the synthesis of long-chain DNA is available. It allows high-throughput production of recombinant microorganisms and also measures their metabolic products very quickly. The director, Professor Akihiko Kondo, runs a second research laboratory for cell factories at Japan's major research institution RIKEN in a personal capacity. Companies can use both facilities as pilot laboratories, and Table 3 summarizes which companies are working on this issue at the BioFoundry.

[5] https://www.jba.or.jp/nedo_smartcell/en/proof/
[6] https://www.nedo.go.jp/content/100948488.pdf
[7] https://www.nedo.go.jp/content/100951572.pdf
[8] https://www.kobe-u.ac.jp/research_at_kobe/NEWS/news/2021_02_17_02.html

Table 3: Topics of the Kansai BioFoundry

Topic	Organism	Major partner
Optimization of enzymes for the saccharification of biomass	Trichoderma reesei	Kao Corporation
Catechol and shikimic acid with resting cells	Corynebacterium glutamicum	RITE*
(omega-3) fatty acids from fatty yeasts	Lipomyces starkey	Fuji Oil
Microbial synthesis of the alkaloid reticuline	E. coli	Kobe University
Ergothioneine, an amino acid	E. coli	Nagase
Adipic acid for nylon		Toray
Bell pepper carotenoids ß-cryptoxanthin, lutein from microorganisms	E. coli and S. cerevisiae	Ezaki Glico
Lipase for the enantioselective cleavage of menthol esters and P450 monooxygenase for the production of .omega.3 fatty acids.	E. coli	Amano Enzymes
Cholesterol esterase: 30-fold increase in yield due to secretion of the enzyme	Burkholderia stabilis	Asahi Kasei Pharma

*Research Institute of Innovative Technology for the Earth, Kyoto

3. Data-driven methods for bioproduction

Cell factory development includes protein function prediction, metabolic model improvement, missing kinetic parameter estimation, non-natural biosynthetic pathway design, and metabolic engineering optimization. Two consortia have formed to establish a nationwide knowledge base and management system in this area. The first is headed by Jun Ogawa at Kyoto University and includes 27 universities and 32 companies. The second, under Akihiko Kondo at Kobe University, focuses on bioinformatics with 5 universities and 6 companies, looking for enzyme sequences that do not occur in nature but have the selectivity we are looking for - they can then be synthesized at the chassis of natural enzymes.

4. The Kansai and Kanto Biofoundry Base: "Production optimization through craftsmanship" (ものづくり、monozukuri).

High-performance strains are a necessary but not yet sufficient condition for economic bioproduction: the production process in the bioreactor must also be optimized according to the rules of the craft. In good Japanese tradition, Meti is therefore promoting the "development of production technologies for biobased products to accelerate carbon recycling" both in the Kansai region at the Osaka Institute of Technology https://www.oit.ac.jp/english/index.html and at the Kanto Biofoundry Base. The project leader in the Kanto region is Green Earth Institute, Inc. https://gei.co.jp/en/ , which operates a microbial bioproduction process demonstrator at two sites in Chiba Prefecture, with support from Kyowa Hakko Bio. Processes for media optimization (upstream) and processing (downstream) are to be optimized, as is product formation through "smart cells" in the bioreactor. The aim is to upgrade at least 16 bioprocesses for commercialization by fiscal 2026. Processes currently being worked on are summarized in Table 4.

Table 4: Demonstration processes

Target compound	Industrial partner
Project phase 1, 2021 - 2023	
oxidized glutathione with E. coli	Kaneka
Polyamide raw materials (adipic acid)	Toray
Natural human-type ceramide as anti-aging product from Aspergillus oryzae	Fukuoka Soysauce Brewing Association
hatching accelerator for potato cyst nematode (PCN)	Hokusan, an Agro-Enterprse
Microbial production of glycyrrhetinic acid and analogs	Sumitomo Chemicals
Microbial production of 10-hydroxy-cis-12-octadecenoic acid with lactobacilli to lower postprandial glucose levels	Noster
Methanation of waste gas	Taisei Corporation
Epigenetic optimization of a sugar production system	Aquaplanta, a Manufacturer of Agrochemicals
2nd generation sugar production in a paper mill	Mitsubishi Paper
Hyperstable thermococcus proteases for infection control	Saraya, a Producer of Cosmetics
Pesticide production with filamentous fungi	MMAG, an Agro-Enterprise
Cyclic lipopeptides with Bacillus spec.	Kaneka
Imidazole dipeptides with carnosine and anserine using L-amino acid.alpha.ligase	Tokai Bussan, a Producer of food additives
Isopropanol with corynebacteria	Green Earth Institute
Project phase 2, 2023 - 2025	
Production system for single-cell oil from biomass with fatty yeasts	Idemitsu Kosan
Production system for rose aroma with dormant cells	Takasago International
Production system for flavor synthesis intermediates	Ogawa Flavor and Fragrances
Production system for cannabinoid compounds with actinomycetes	Digzyme
Production system for highly absorbable natural carotenoids	Harima Chemical
Mass production of highly modified proteins using plant cells	Chiyoda Corporation

5. Smart plant cells for production

In a future bioeconomy, genetically modified plants will also be used to produce valuable materials, such as for the synthesis of therapeutic humanized antibodies, human and veterinary vaccines, drug precursors, cosmetics, and food additives. This part of the project is led by Norihiko Fukuzawa at AIST's Bioproduction Institute, a Meti research center in Sapporo, Hokkaido Prefecture. The goal is to build a pilot plant for the production of useful substances that can process up to 100 kg of plant material per day under Cartagena rules****. The "smart cell" used is the tobacco plant Nicotiniana benthamiana, native to Australia, in which formation of the desired product is induced by transient expression of a recombinant CMV vector. To date, two companies and two universities are involved in this subproject.

6. Training of bioengineers

One of the challenges to building a bioeconomy is that smaller companies usually lack the R&D environment and skilled personnel for biomanufacturing. With a special program, Nedo has therefore been supporting human resource development since the end of 2022, with an initial 61 participants. The biofoundries are also expected to take on trainees at a later date.

Outlook

Japan is serious about building a bioeconomy and has established an extensive research network to this end, involving larger and smaller companies, universities and many large-scale research institutions. Data is accessed in a widely accessible network, biocatalysts are produced in one center for microorganisms and one for transgenic plant cells, and bioprocess development is carried out in two facilities in the metropolitan areas of western and eastern Japan.

From 2020 to the end of 2022, 27 Japanese patents, 8 foreign patents, and 10 PCT patents have already been published by the project partners, along with 48 scientific publications and 159 conference proceedings. It is reasonable to assume that these numbers will increase sharply in the coming years - even beyond 2026, because the project is an important building block for the decarbonization of the Japanese economy and help to achieve the goals of the Kyoto Protocol.