

# Enzyme Wave

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## ODA Urakusai's space "Jo-an".

Jo-an is one of the three teahouses designated Important Cultural Properties of Japan. Built in 1618 by ODA Urakusai, a younger brother of ODA Nobunaga, it was originally constructed on the premises of Kennin-ji Temple in Kyoto. The teahouse was placed at its present location in Uraku-en (Aichi Prefecture) after two relocations. The teahouse interior, arranged in a distinctive style that reflects Urakusai's aesthetic sense, creates a special atmosphere.



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Beta-mannanase is an enzyme widely used in various industries, including food and oil drilling detergents. Amano Enzyme has succeeded in commercializing a beta-mannanase preparation from *Aspergillus niger* that contains very few enzyme side-activities, thereby overcoming the shortcomings of beta-mannanase preparations already in the food market. This report summarizes Amano's new beta-mannanase product and its characteristics.

**Beta-mannanase and its industrial uses**

Beta-mannanase is an endo-type enzyme that hydrolyzes beta-1,4 mannoside bonding in mannan (mannose polymer), which belongs to the group of hemicellulose, a complex polysaccharide, in plants. The cell walls of higher plants are composed of cellulose (approx. 40%), hemicellulose (approx. 30%) and lignin (approx. 30%). Hemicellulose is a collective term for straight or branched polymer chains composed of xylose, galactose, mannose, glucose, arabinose, etc. Beta-mannanase is the enzyme that breaks down galactomannan and glucomannan, whose main chains are of mannose. Well-known sources of beta-mannanase are filamentous fungi such as *Aspergillus niger* and *Trichoderma reesei*, as well as bacteria *Bacilli*.

Beta-mannanase is used in a wide range of industries. One such use in food processing is in the extraction of coffee. When coffee is extracted from roasted coffee beans, problems of low filterability and sediment formation after extraction can occur, due to the insoluble, highly viscous nature of galactomannan. To prevent these problems, beta-mannanase is applied during extraction, to break galactomannan down into low polymers. As another example, since beta-mannanase has some physiological effects, including enhanced bowel movement, it is used to manufacture guar bean-derived galactomannan hydrolysate, which is recognized as FOSHU (food for specified health uses) in Japan. Coffee bean-derived manno-oligosaccharide (mannobiose) is also given the same status. Other reported applications of beta-mannanase include the production of soluble konjac (devil's tongue starch cake) mannan, the decomposition of copra meal (residue after extraction of coconut oil), and the removal of viscous materials from aloe.

Technical applications include pulp bleaching, in which beta-mannanase is used as a type of hemicellulase, and oil-well drilling, during which galactomannan decomposed with beta-mannanase is used as a coagulant. Mannanase is also used in detergents to remove stains. Galactomannan, which is contained in a variety of foodstuffs, cosmetics and personal care products as a viscosity enhancer, is bound strongly with cotton and attracts particles of earth soil, which often stain clothing. Mannanase is therefore contained in detergents to remove such stains.

**Mannanase BGM "Amano" 10**

Mannanase BGM "Amano" 10 has been developed as a beta-mannanase preparation for food use. The new product features much lower enzyme side-activities and higher purity than existing products. As shown in the table below, Amano's product demonstrates less activity in concomitant enzymes (xylanase, beta-mannosidase, alpha-galactosidase and acid protease) than the other two products already in the market.

Table. Comparison of concomitant enzyme activities in mannanase preparations

	Mannanase BGM "Amano"10	Existing product 1	Existing product 2
<Main activity> beta-galactomannanase	11,000	10,920	9,521
<Side-activity> Xylanase	0	9,733	3,654
Alpha-galactosidase	2	191	646
Beta-mannosidase	25	0	108
Acid protease	24	7242	109

**Applications of Mannanase BGM "Amano" 10**

Fig. 1 indicates effects of Mannanase BGM "Amano" 10 on the coffee extraction application: filtration speed increased, while extracted liquid viscosity was lowered. Moreover, sediment formation was restrained during low-temperature storage of extracted liquid. In a taste evaluation, use of the Amano product was found to have improved the coffee taste, with intensified acidity, and a milder taste. This is attributed to a lower content of protease and other enzyme side-activities.

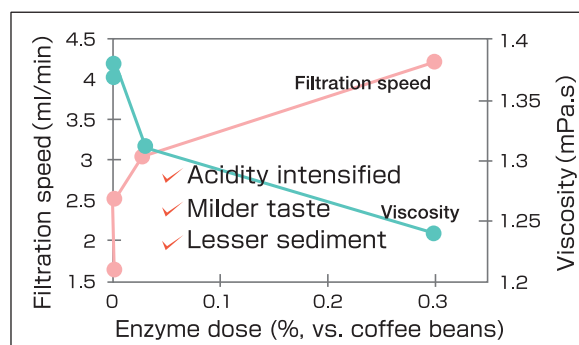


Fig. 1 Application to coffee extraction

Mannanase BGM "Amano" 10 was also tested in the hydrolysis of guar bean-derived galactomannan. The results of analysis of the oligosaccharide generated in galactomannan hydrolysis demonstrated that more low-polymer (hexa- or lower) manno-oligosaccharides were produced with the Amano product than when the other existing products were used. Fig. 2 compares the quantities of disaccharides (mainly mannobiose) produced. Moreover, the amount of monosaccharides produced also decreased with use of Mannanase BGM "Amano" 10. The presence of monosaccharides is not desirable in galactomannan hydrolysates or manno-oligosaccharide products for food use because monosaccharides cause osmotic pressure increase and browning. Use of Mannanase BGM "Amano" 10 reduces monosaccharide formation, presumably because of its lower content of exo-type enzymes releasing monosaccharides, such as alpha-galactosidase and beta-mannosidase. The taste of hydrolysate products obtained using Mannanase BGM "Amano" 10 was also improved.

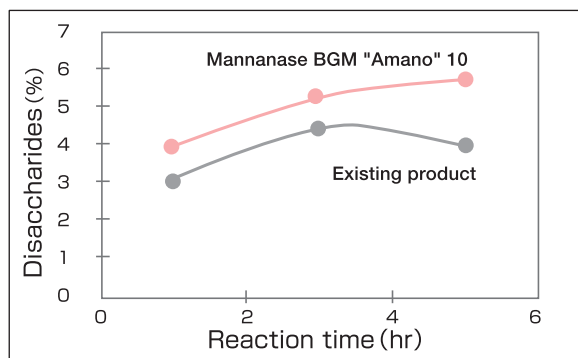


Fig. 2 Disaccharide formation from galactomannan

Mannanase BGM "Amano" 10 is expected to play a significant role in the manufacture of high value-added foods and food ingredients that are advantageously differentiated from other products because of its features, e.g., lower activity of various concomitant enzymes leading to lesser side reactions that can cause off-flavors, compared to conventional enzyme preparations.

The 10th Conference of the Parties (COP10) to the Convention on Biological Diversity (CBD) was held from October 18 through 29, 2010 at the Nagoya Congress Center in Nagoya City. One important theme on the Conference agenda was the issue of access to, and benefit-sharing of, genetic resources (ABS issue). This article explains the ABS issue, the Nagoya Protocol adopted at COP10, and ABS issue-related activities undertaken by the National Institute of Technology and Evaluation (NITE).

### [What is the ABS issue?]

The ABS issue concerns access to genetic resources and sharing of benefits arising from their use. The issue was first recognized in Article 15 of the CBD, "Access to Genetic Resources," which stipulates the following:

- \*States of origin of genetic resources possess sovereign rights to them.
- \*Contracting Parties have the right to institute national legislation concerning use of genetic resources.
- \*Prior informed consent is necessary on accessing and using genetic resources between the countries providing them and those wishing to use them.
- \*Access and use of genetic resources shall be on terms mutually agreed to by the resource providers and users.
- \*Benefits arising from the use of genetic resources shall be shared in a fair and equitable way between providers and users.

This framework has given rise to problems, notably due to dissatisfaction on either side (provider or user). While the provider side insists that benefits arising from the use of genetic resources are not distributed to the providers in a fair and equitable way, the user side maintains that its access to genetic resources that belong to a provider State is not always assured even when attempted access is legitimate. The core of this problem lies in the disparity in the understanding of the two sides as to what constitutes fair and equitable benefit-sharing or legitimate access. At the same time, given the fact that the majority of genetic resource providers are developing countries, whereas genetic resource users are developed countries, the ABS issue is considered a new North-South problem.

Meanwhile, it is essential that we correctly understand the ABS issue. In particular, considering the sovereign rights of respective countries over their genetic resources, transporting genetic resources from another country to Japan without authorization constitutes bio-piracy, which is subject to international sanction.

### [Nagoya Protocol (Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization)]

At the World Summit on Sustainable Development (WSSD) held in Johannesburg in September 2002, it was decided that negotiations would be pursued to establish an International Regime (IR) that would promote and protect fair and equitable sharing of benefits arising from the use of genetic resources. Accordingly, discussions on the IR commenced at the 2nd ABS Working Group (ABS-WG) meeting held in Montreal in December 2003, with no conclusion reached all the way through the 9th ABS-WG meeting in September 2010. The negotiations were carried over to COP10 to be held in Nagoya in October 2010.

At COP10, discussions on the ABS issue commenced on October 18, but no sign of a conclusion was seen even on Day 10. At the Informal Consultative Group (ICG) meeting starting from 7:15 p.m. on October 28, the ABS Co-chairs declared the group's failure to reach a conclusion. At the plenary meeting opened at 7:40 p.m. on the same day, COP10 Chairman Ryu Matsumoto, Minister of the Environment of Japan, requested the ABS Co-Chairs to submit an agreement on ABS by 0:00 that evening or, failing this, a proposal by the Co-Chairs on the following morning. The ABS Co-Chairs failed to comply.

On the morning of October 29, the Chairman's paper on ABS Nagoya Protocol was distributed for debate in the plenary meeting that resumed at 11:10 p.m. Countries that were initially opposed, such as Venezuela and Cuba, were eventually led to state that they did not agree, but would not obstruct adoption of the paper. At 1:29 a.m. on October 30, the legally binding IR on ABS, which ABS-WG had long debated, was unanimously adopted as the "Nagoya Protocol."

### [NITE's microorganism exploratory projects in Asia]

Discussions alone cannot do anything to obtain tangible results with regard to the ABS issue. In 2003, the Department of Biotechnology of the National Institute of Technology and Evaluation (NITE-DOB), to which I belong, commenced a joint project on microorganism exploration with Indonesia. The project continued for six years, until March 2009. During this period, we isolated and selected about 7,000 strains of fungi, yeasts and actinomycetes from soil, fallen leaves and other samples collected in 13 regions in Indonesia and preserved them in Indonesia and at the NITE-DOB. These preserved strains are made available to Japanese companies so that their potential utility can be examined. NITE also commenced similar projects with Myanmar (the project is currently suspended) and Vietnam in 2004, and with Mongolia in 2006. In the projects with Vietnam and Mongolia (which are still in progress), researchers from Japanese companies also participate and collect samples, then isolate microorganisms using their own methods in those provider countries. The potential utility of the isolates are then tested and reviewed in Japan.

For our project with Indonesia, we commenced negotiations in 2000 and on March 20, 2002 signed a Memorandum of Understanding (MOU) about the conservation and sustainable use of microbial resources. We spent another year working out details of the actual project and on April 11, 2003 signed an agreement on the "Joint Research Project on Taxonomic and Ecological Studies of Fungi and Actinomycetes in Indonesia and Japan." I believe that we managed to create a new and functioning scheme through the process of finalizing the MOU and project agreement with Indonesia. It was time-consuming, considering the magnitude of the question involved as to how resource provider and user can build a win-win relationship, overcoming problems relating to genetic resource access and resultant benefit sharing, in compliance with the CBD. Thanks to this experience, we were able to finalize MOU and project agreement in an extremely short period of time for our projects with Vietnam, Myanmar and Mongolia.

### [Epilogue]

The world has shifted from the idea that "biological genetic resources belong to all" to "ownership rests with the country of origin." The Nagoya Protocol adopted at the CBD-COP10 is expected to take effect by the end of next year. Accordingly, the signatory governments are likely to prepare national legislations regarding the provision of their genetic resources to users, along with that regarding utilization of genetic resources obtained from other countries. It is hoped that the national legislative frameworks will be established to further facilitate the use of genetic resources.

Katsuhiko ANDO

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On April 6, 2011, Amano Enzyme will celebrate the 30th anniversary of its operation in the United States. We would like to thank those who have supported us during those 30 years, and helped us expand our business so smoothly.

Milestones in our thirty-year history are as follows

As for future prospects, we have decided to have our own building constructed in 2013 near our present location. In this new building we plan to commence full-scale blending of products for the American market. Having blending facilities in both Japan and the United States will provide us with a timely and stable supply system of greater security. At the same time, AEU will have local manufacturing and quality control divisions, in addition to its already operating sales division, thereby enhancing our responsiveness to diversifying customer needs.

Your continued business with Amano Enzyme, a company committed to continuous improvement in quality service, will be greatly appreciated.



Present AEU Office



A Scene after the Signing Ceremony for the AIEC

- 1981 : Joint venture Amano International Enzyme Corporation (AIEC) established in the State of Virginia
- 1992 : AIEC made wholly owned subsidiary of Amano Enzyme Inc. and renamed Amano Enzyme USA (AEU)
- 1995 : Relocated from Virginia to Lombard City in the suburbs of Chicago, chosen for geographical situation favorable to nationwide business coverage
- 2003 : Relocation to Elgin City (present location) in the suburbs of Chicago to accommodate expansion of R&D capabilities

## Amano News Letter

### ► Enhanced business activities in the Kanto area

In November 2010, our Tokyo Office commenced sales activities to reinforce the Company's sales mechanism in Japan.

Previously, sales activities in Japan were based at the Head Office in Nagoya. The Tokyo Office, now functioning as the second principal base, is expected to realize more rapid and closer responses to customers.

(The Tokyo Office has been relocated from the 6th to the 16th floor of Imperial Hotel Tower.)

### ► Food Ingredients China 2011

We exhibited a booth at Food Ingredients China 2011, held in Shanghai from March 23 to March 25. In particular, we succeeded in arranging a technical seminar with Amano Enzyme China, with whom. We introduced food-technical solutions using Amano's specialty enzymes, such as, protein hydrolysis, starch processing, and baking. We will continue contributing to development and innovation in the Chinese food industry by providing our specialty enzymes and excellent services.



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