

# Molecular Microbiology and Biotechnology Group

## Global Biorefinery Trends and Research Overview

### 1. Introduction

The biorefinery is a relatively recent concept proposed by, among others, the U.S. Department of Energy (DOE) to encompass technologies and industries that enable production of chemicals and liquid fuels from biomass instead of from fossil feedstocks (Figure 1). The biomass is of plant origin, enabling a virtuous carbon-neutral cycle of plant growing, processing, harvesting, and burning, meaning that its use does not contribute to net changes in the level of atmospheric CO<sub>2</sub>. Since the 1990s, the transition from oil petrorefinery to biorefinery has been one of the key strategic scientific missions of the U.S. Consequently, the advancement of technologies relevant to the implementation of the biorefinery vision is a priority U.S. policy to achieve a sustainable society less dependent on fossil resources in the 21st century. The EU, like the U.S., has recognized the importance of biorefinery, and supported the introduction of biofuels and development of biorefinery using tax incentives, etc. According to a new biofuels roadmap report published last year by the International Energy Agency (IEA), biofuels can provide up to 27% of world transportation fuel by 2050. Biofuel production in 2050 will be 10 times the present production but require only three times the existing area of cultivated land for biofuel plants because future technological improvements are expected to enhance the overall productivity.

Recent trends where competition between biofuel feedstocks and food supply posed a serious downside of

the present biorefinery have elevated the shift to non-food-based biomass resources to the status of the most important subject of the biorefinery sector. Agricultural Outlook 2011 report jointly by OECD and FAO (Food and Agriculture Organization of the United Nations), says that higher prices of agriculture commodities such as crops will persist in the 2011-2020 period due to the expanding consumption by an increasing population and economic growth of emerging and developing countries (Figure 2).

### 2. Biofuels

The 2011 world ethanol production was estimated to be 23.4 billion gallons. This represents more than 20% growth since 2009 and was essentially achieved by converting corn starch or sugarcane-derived sucrose. The production in the U.S. accounts for ca. 60% of the global production and its demand will continue to grow due to the approval of E15 (ethanol-gasoline blends containing up to 15 percent ethanol by volume) in 2010. As much as 40% of the U.S. corn crop is going into ethanol production, therefore material conversion of biofuels into cellulosic biomass from grain is expected without loss of time. The U.S. government has strongly backed biofuel projects which can use agricultural residues such as corn stover or so-called energy grasses such as switchgrass. Cellulosic biofuels are able to eliminate competition with foods, and effectively reduce CO<sub>2</sub> emissions as demonstrated by Life Cycle Assessment (LCA) analyses. At present, the con-

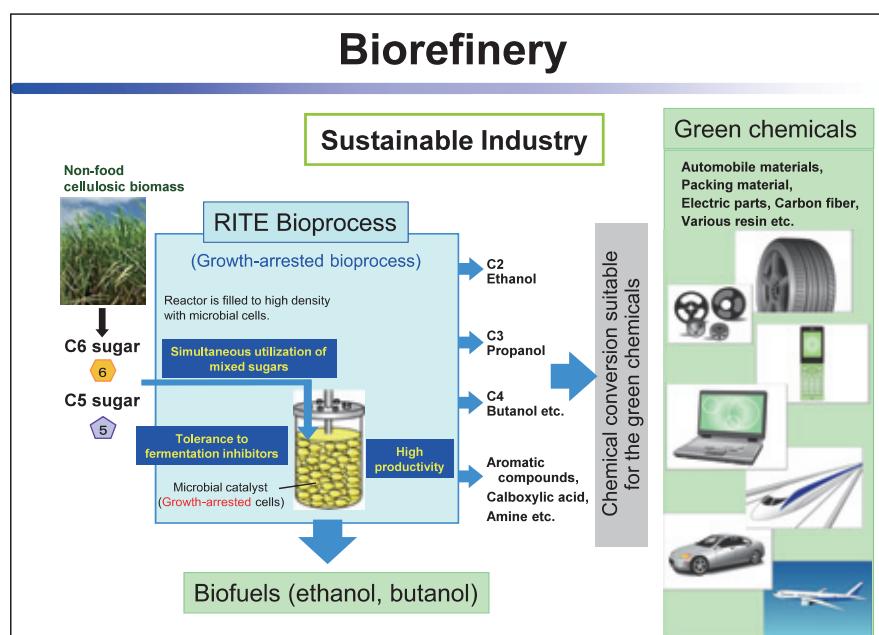


Figure 1 Productions of biofuels and green chemicals from non-food biomass

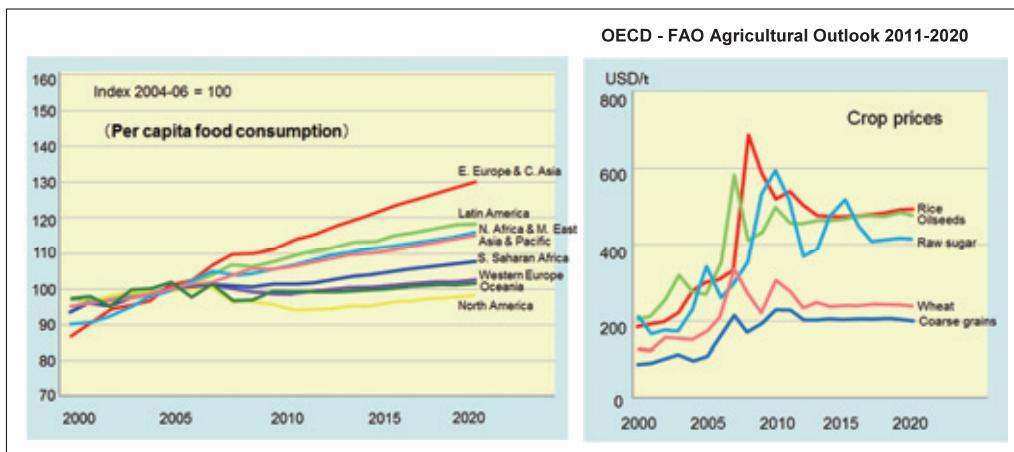


Figure 2 Increase of global food consumption (left) and rising crop prices (right)

struction and operation of large scale cellulosic ethanol plants scheduled earlier in the U.S. seems to be behind schedule due to technological constraints associated with fermentation inhibitors derived from the pre-treatment of lignocellulosic biomass.

The 2011 global production capacity of biofuels, estimated at 44.6 billion gallons, will expand up to 20% by 2015 (Lux Research). In Europe, biofuel consumption in forms such as biodiesel has slowed down due to tax increases on biofuels resulting from concerns of environmental destruction in biofuel production processes etc., or the economic recession of recent years.

However, energy consumption and investment in biofuels sector have continued to expand in emerging countries such as China and India as well as East Asian and South American countries. In Japan, the Basic Energy Plan revised by the Ministry of Economy, Trade and Industry established the promotion of biofuels for transportation in 2010, aiming at a biofuels use of around 3% of the gasoline consumption by 2020.

### 3. Green chemicals

The growth of green (renewable) chemical production via bioprocessing is expected lead to a sustainable industry. Although more advanced technologies are necessary for the production relative to those for biofuels, a variety of product groups and integrated market size are predicted in the biorefinery sector. The use of non-food feedstocks like corn stover or switchgrass is also an essential requirement. As a target of green products, a new trend puts emphasis on producing commodity chemicals rather than fine chemicals such as 1,3-propanediol, which have been the early focus of biochemical engineering. The green production of acrylic acid and isoprene, products which comprise large industrial markets, as well as carboxylic acids, amines and aromatic compounds have begun to grow. Various joint ventures between companies have been established in these green chemical businesses. The future market size of green chemical is

estimated to have the potential to generate \$70B at 2015 or \$100B at 2020.

### 4. Technology development: the RITE Bioprocess (Growth-Arrested Bioprocess)

Our group has developed an efficient biomass utilization technology based on intrinsic characteristics of coryneform bacteria. The process was named "RITE Bioprocess" (a growth-arrested bioprocess), and it has so far enabled elevated productivities of organic acids and biofuels. This pioneering technology enables the simultaneous utilization of mixed sugars from cellulosic biomass in biorefinery settings. In collaboration with a private company, we applied it in a cellulosic ethanol production system, earning the Grand Prize at the 18th Nikkei Global Environment Award (see RITE Today 2009). What is more, our process has evoked the interest of international academia and their researchers. Our group leader was awarded the 2011 fellowship award from The Society for Industrial Microbiology (SIM), the first Japanese scientist to receive the award (see Topics). Moreover a German group has been following our footsteps and carried out additional research using coryneform bacteria; they independently confirmed the capabilities and attributes of our innovative bioprocess, which is characterized particularly by a clear separation between product production and the growth phase of the bacteria catalysts. The main technological features of the RITE Bioprocess are given in the following paragraphs.

#### 4-1. Technological attributes of the RITE Bioprocess

In the RITE Bioprocess, coryneform bacteria are engineered to have an optimum metabolic pathway for a particular target chemical. The cells are grown at a large scale and packed to very high densities in a reactor in order to maximize the catalyst/volume ratio at the production stage. Sugars are subsequently added to initiate bioconversion as a substrate under oxygen deprivation; this has the effect to cease the growth of these bacteria while

keeping them metabolically active (Figure 3). As a result, the target chemical is produced by growth-arrested cells, with a larger share of the substrate being converted into useful products without any additional natural rich medium or external energy. The key to achieving high efficiency and high productivity is the effective separation of the microbial growth phase from the production phase of the target compound.

This manner of using bacterial cells as if they were simple chemical catalysts enables one to produce large amounts of chemicals in short periods of time, and unlike conventional bioprocesses, the productivities reached, expressed as space-time-yield (STY), are comparable to those of chemical processes.

#### 4-2. Simultaneous utilization of C6 and C5 sugars

Lignocellulosic biomass hydrolysates constitute complex mixtures of different sugars. They compose of pentoses (C5 sugars such as xylose and arabinose) derived from hemicelluloses, as well as hexoses (C6 sugars such as glucose and fructose). By comparison, starch from food grains such as corn, wheat etc. and sugar from sug-

arcane contain only hexoses. Therefore, for achieving a high yield per substrate, it is essential for microorganisms used in biofuel processes to exhibit the ability to simultaneously utilize both pentoses and hexoses. We introduced several genes involved in the catabolism of C5 sugars into coryneform bacteria, and applied the resultant recombinant bacteria to our bioprocess. These modifications allowed for efficient utilization of cellulosic materials, and faster conversions thus became possible since we could achieve, without any lag phase, the simultaneous utilization of all the sugars present in the reaction medium.

#### 4-3. Tolerance against fermentation inhibitors

Fermentation inhibitors include phenols, furans and organic acids such as acetic acid. These compounds are by-products formed during the pre-treatment of lignocellulosic biomass. As exemplified by hydrothermal treatment, such treatments are typically very harsh but are necessary to break the recalcitrant biomass fiber and thereby facilitate enzymatic hydrolysis. Their strong inhibition has been known for many years to be a cause of

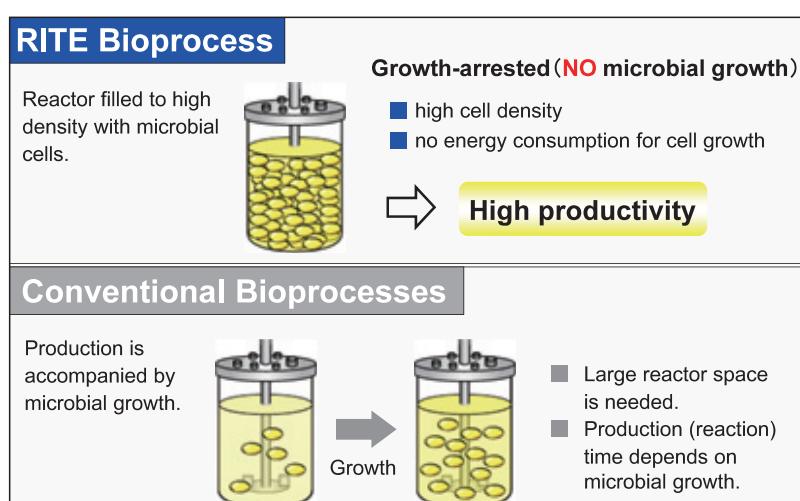


Figure 3 Comparison of RITE Bioprocess with conventional bioprocess

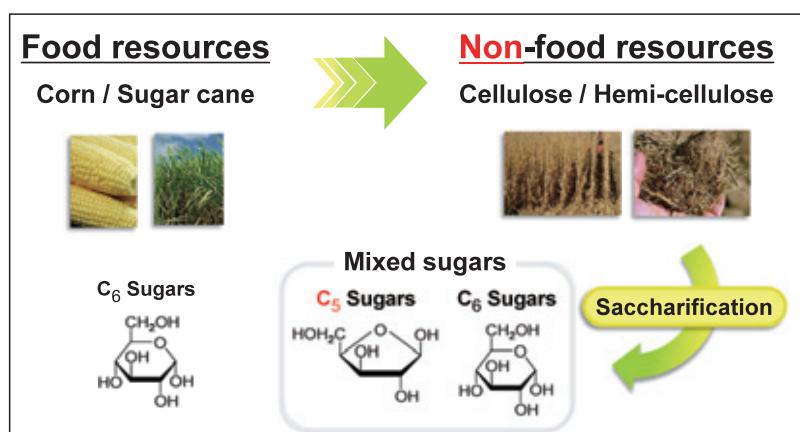


Figure 4 Expanding usage of mixed sugars from non-food biomass

concern to the biofuel manufacturer and they represent one of the biggest problems associated with conventional bioprocesses. However, we demonstrated that these fermentation inhibitors essentially do not affect the RITE Bioprocess, since their action is to inhibit cell growth while our process separates the cell growth phase from the product production phase. Furthermore, we extensively demonstrated that the main metabolic pathways necessary to produce compounds of interest on the one hand remain active under the conditions of the growth arrested RITE Bioprocess and on the other hand are virtually not affected by the presence of fermentation inhibitors in quantities that would hinder conventional processes.

#### 4-4. Future technology development

We are constantly expanding the range of product options that the RITE Bioprocess can support. To this end, we implement global analysis tools including system biology based on metabolome analysis, metabolic pathway design, and genome engineering based on the genome database of coryneform bacteria. In addition to the successful production of ethanol, or L- and D-lactic acids and succinic acid, we are developing a whole range of new targets addressing large market needs or high value added compounds comprising butanol, aromatic compounds, and aminoacids.

Although the economical production of aromatic compounds has been a challenge when using conventional fermentation technologies, their production by industrial biotechnology is still an important aim since these materials, once made from sustainable raw materials such as biomass, are expected by leading Japanese companies to become building blocks for advanced products such as electric devices, hardware, and automobiles. In addition, the RITE Bioprocess shows higher cost performance than conventional fermentation processes such

as amino acids manufactured by aerobic fermentation. Because aerobic processes require air compressors and agitation motors to ventilate and mix liquids, respectively, aerobic processes involve additional equipment and expensive operational cost. We have already begun to develop production processes for several amino acids by using the RITE Bioprocess.

#### 4-5. Development for industrialization

In 2009, we established two technology research associations with private companies to accelerate our research and development, in addition to collaborative work for a cellulosic ethanol production system (see RITE Today 2008~2010). The association has corporate status and joint research with private companies and public R&D agencies is now possible. Successful collaborative work is going on under the auspices of "Green Phenol Technology Research Association" and "Bio-Butanol Technology Research Association". For further acceleration towards industrialization, we established "Green Earth Institute Co., Ltd." last year to provide biofuels and green chemicals by using our RITE Bioprocess (see Topics). The concept for its establishment is the industrialization of our RITE-Bioprocess to contribute to the preservation of global environment by employing efforts against global warming and the realization of a post-fossil resources sustainable society.

#### 5. Ending remark

Intense competition in the development of technology which contributes towards global warming prevention and environmental protection at a global scale will persist into the future. To achieve early establishment of commercial-scale biorefinery, we hope to continue our collaborative research development with domestic as well as overseas companies to further expand the RITE Bioprocess platform technology.

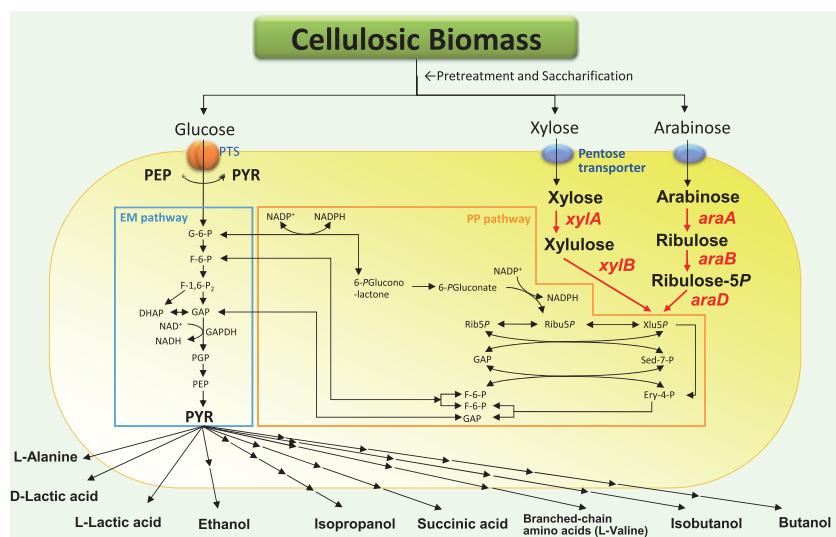


Figure 5 Production pathways of coryneform bacteria designed for acyclic chemicals and biofuels