

# Molecular Microbiology and Biotechnology Group

## Global Biorefinery Trends and Research Overview

### 1. Introduction

The biorefinery is a relatively recent concept proposed among others by the U.S. Department of Energy (DOE) to encompass technologies or industries that can produce chemicals and liquid fuels from biomass (instead of from fossil feedstock) (Figure 1). Biomass, since it originates from plants and microorganisms, enables a virtuous carbon neutral cycle of harvesting via plant growing, processing, burning, and recycling of CO<sub>2</sub>, thus contributing to no net changes in the level of atmospheric CO<sub>2</sub>. Since the 1990s, the transitioning from oil petrorefineries to biorefineries has been one of the strategic scientific missions of the U.S. The advancement of technologies relevant to the implementation of the biorefinery vision is therefore a key federal policy to achieve a post-fossil resources sustainable society in the 21<sup>st</sup> century. The problem is even more urgent that according to the International Energy Association (IEA), the global energy consumption has continued its expansion in spite of the global economical stagnation of 2008-2009, driven to a large extent by the continued strong and fast increasing energy demand of emerging economies such as China, India, Brazil, and Russia (BRIC countries). As a result, the implementation of cost-effective biorefineries constitutes a pressing development need to enable the production of biofuels and green chemicals from biomass. Notably,

it is important to make use of non-food biomass resources in order to avoid the food-vs.-fuel/chemicals dilemma.

### 2. Biofuels

The 2010 world ethanol production is estimated to be 23 billion gallons. This represents more than 15% growth since 2009 and has essentially been achieved by converting corn starch or sugarcane-derived sucrose. However, important technology developments to enable the use of non-food biomass have significantly been achieved in recent years as a means to decrease environment deterioration risks and risks of competition with the global food supply (Figure 2). For example, agricultural residues such as corn stover or so-called energy grasses such as switchgrass, are now expected to represent useful options to produce clean fuels. Notably, their uses can eliminate the competition with foods and effectively reduce CO<sub>2</sub> emissions as demonstrated by Life Cycle Assessment (LCA) analyses. The U.S. DOE has been strongly backing biorefinery projects using non-food biomass. As a result, several demonstration scale cellulosic ethanol plants will be open after 2011 in the U.S. Notably, to promote the market penetration of biofuels, the U.S. government has recently released new regulations, Renewable Fuel Standard 2 (RFS2), and increased to 36 billion gallons the vol-

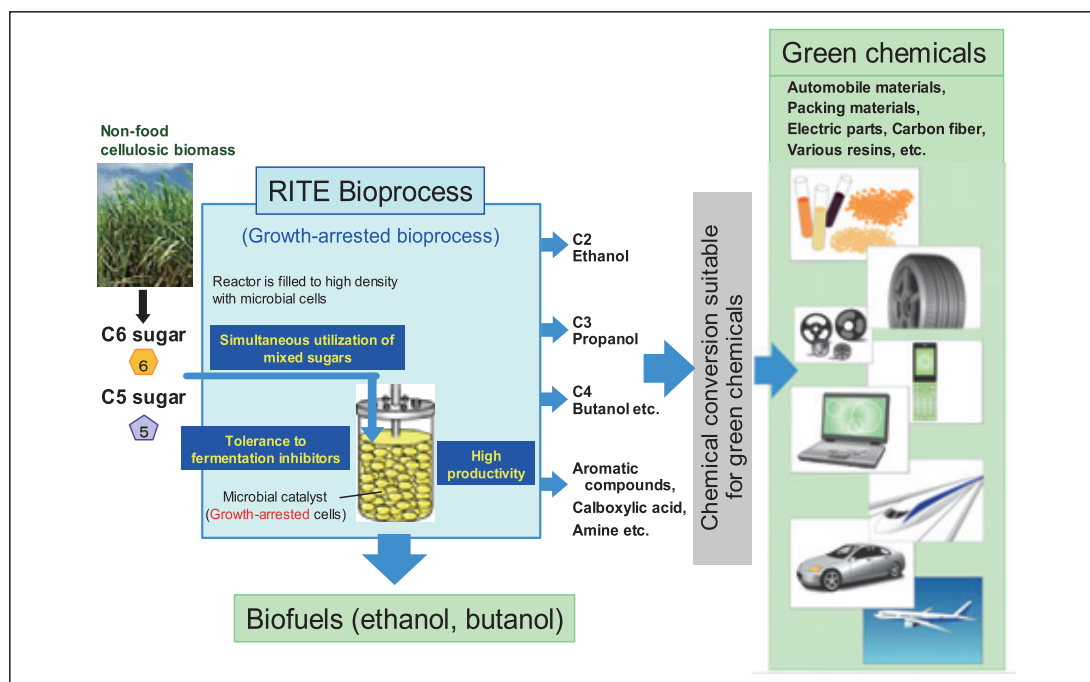


Figure 1 Biorefinery: production of biofuels and green chemicals from non-food biomass

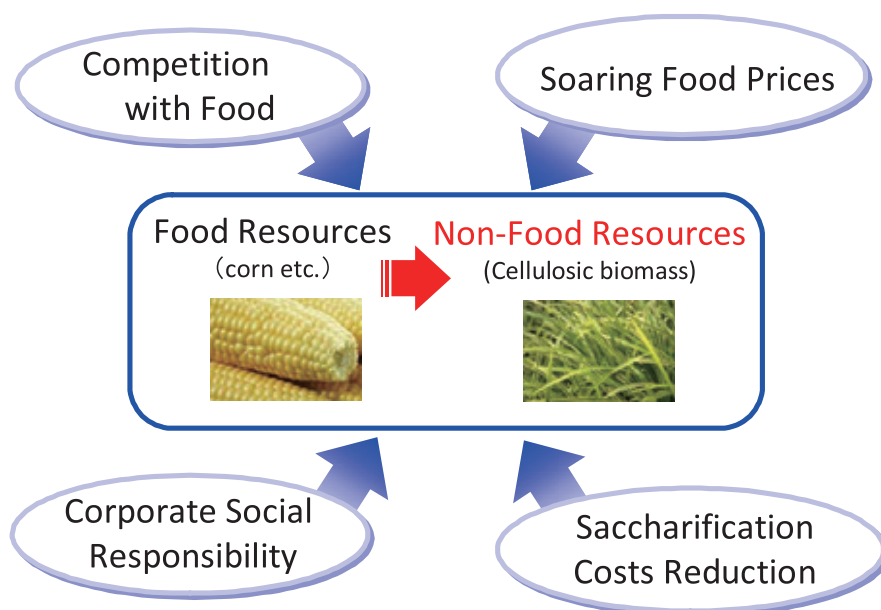


Figure 2 Shift to non-food resources

ume of renewable fuel that is required to be blended into transportation fuels by 2022. Moreover, the use of ethanol-gasoline blends containing up to 15 percent ethanol by volume (E15) was also decided in October 2010 for all vehicles built in 2007 and newer. On the other hand, in Europe, the slow economy and low profitability of 2010 have decreased the growth of biodiesel consumption, which accounts for approximately 80% of the European biofuels consumption. Nonetheless, it is also worth noting that the European Union has set the target to reach 10% biofuels in its transportation energy mix by 2020. In Japan, the Basic Energy Plan revised by the Ministry of Economy, Trade and Industry has established the promotion of renewable energies such as biofuels for transportation, and biofuel targets are included in the key targets for 2030.

### 3. Green chemicals

In addition to biofuels, strong growth is expected in the field of green (renewable) chemicals produced from biomass. The global market of green chemicals has been growing at around 30%, and will continue to expand to more than \$10 billion in sales after 2015. The use of non-food feedstock like corn stover or switchgrass is also an essential requirement for manufacturers (Figure 2). Since saccharification costs have been greatly decreasing with the decrease of cellulose hydrolyzing enzyme (cellulase) costs through technology improvements achieved by enzyme manufacturers, the use of cellulosic biomass hydrolysates that contain mixed sugars has become a promising possibility. Presumably, this prospect is one of the

reasons accounting for the fast market growth that is observed today. Green chemicals have sprung an industry-transforming technology trend of substituting sustainable chemicals for the most common oil-derived platform chemicals. Remarkably, the new trend places the emphasis on producing commodity chemicals rather than fine chemicals, which have been the early focus of biochemical engineering. As a result, several manufacturing platforms can be envisaged, such as a C-2 platform from bioethanol, a C-3 platform from biopropanol, or a C-4 platform from biobutanol. In the future, more complex structures comprising for example carboxylic acids, amines, and aromatic compounds should also be amenable to similar platform chemical production processes.

### 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” (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 18<sup>th</sup> Nikkei Global Environment Award (see RITE Today 2009). What is more, our process has evoked the interest of international public and private researchers. In particular, a German

group has been following our footsteps and carried out additional researches 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

The RITE Bioprocess addresses fundamental problems of conventional bioprocesses. In the first step that occurs in the laboratory, coryneform bacteria are designed and engineered to have an optimum metabolic pathway for a particular target chemical. At the production plant stage, cells are grown at a large scale and packed to very high densities in a reactor in order to maximize the catalyst/volume ratio. The substrate is subsequently added to initiate bioconversion under oxygen deprivation; this has the effect to cease the growth of these bacteria while keeping them metabolically active. As a result, the target chemical is produced by growth-arrested cells, with a larger share of the substrate being converted into useful products. 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 that of chemical processes.

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

Lignocellulosic biomass hydrolysates constitute complex mixtures of different sugars. They comprise pentoses (C5 sugars such as xylose and arabinose) derived from hemicelluloses, as well as hexoses (C6 sugars such as glucose and fructose); whereas corn starch and sugarcane-derived sucrose hydrolysates 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 relative to the catabolism of C5 sugars into coryneform bacteria, and applied these recombinant bacteria to our bioprocess. These modifications made the utilization of cellulosic materials efficient 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 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. Figure 3 shows the synthetic pathways from pyruvate of a variety of compounds using cellulosic biomass hydrolysates as raw materials. 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, comprising butanol, aromatic compounds, and amino acids. 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 Japanese leading companies to become basic materials for advanced products, such as electric device, hardware, and automobiles. In addition, we revisit with the RITE Bioprocess the production of compounds conventionally manufactured by aerobic fermentation such as amino acids. There is an important cost component that can be exploited here: the production of amino acids by conventional fermentation processes requires air compressors and agitation motors for carrying out forced ventilation and mixing liquids, respectively. These equipments and their operations result in significant capital and variable expenses. We have already begun to develop anaerobic production processes for several amino acids by using the RITE Bioprocess. We will announce these in the future.

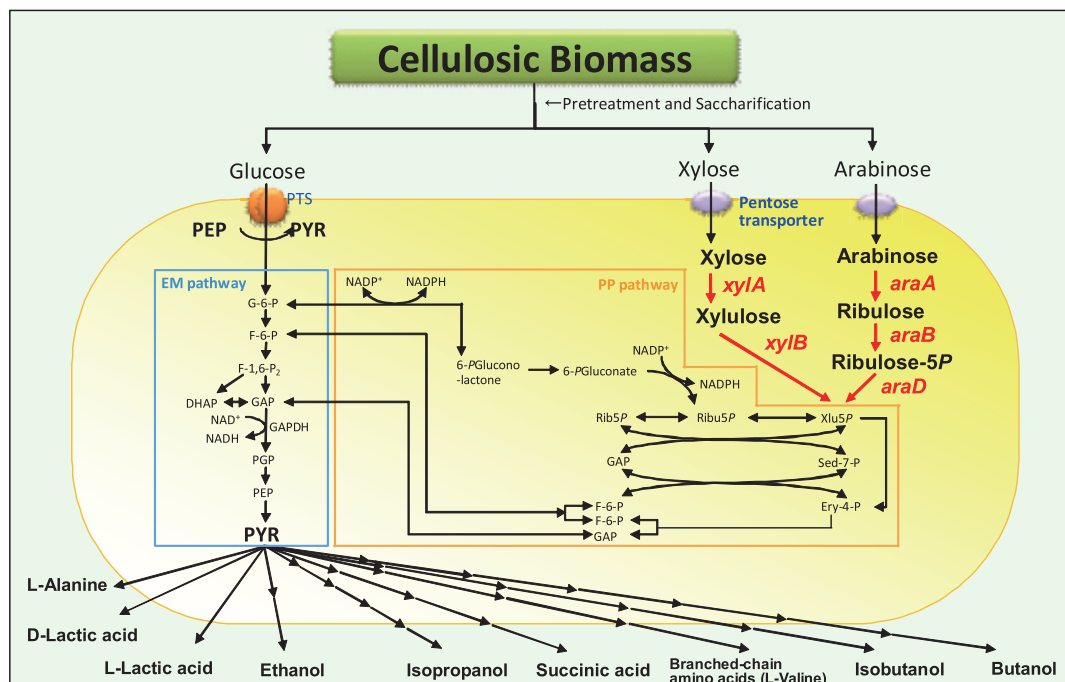


Figure 3 Production pathways of coryneform bacteria engineered for acyclic chemicals and biofuels biosynthesis

#### 4-5. Development for industrialization

Last year, we have established several partnerships with private companies to accelerate our research and development, in addition to the collaborative work for the cellulosic ethanol production system described elsewhere (see RITE Today 2008~2010). A recent legal change in Japan makes that no more than 2 unrelated legal entities are necessary to establish a venture having corporate status, with the important specification that joint research is now also possible between companies and public R&D agencies. The ability to pursue such partnerships will help us develop and evolve cooperative entities to quickly commercialize our developments. In addition to the associations established last year, "Green Phenol Technology Research Association" and "Bio-Butanol Technology Research Association", new associations with other goals are in preparation.

#### 5. Ending remark

Last November, the World Meteorological Organization announced that, globally, the average atmospheric CO<sub>2</sub> concentration reached a record level in 2009. Although the reasons why the levels of warming gases have steadily increased in the atmosphere over the past few years are still being debated, the competition for technology development in the field of biorefinery (one of the conceptually very effective means against global warming) is intensifying worldwide. As reported in the preceding paragraphs, in the field of biofuels, demonstration-scale cellulosic ethanol plants have been and are being constructed, and their productions are scheduled

to go on line in the U.S. after 2011. Green chemicals such as aromatic compounds are expected to become essential materials; a strong know-how in this arena will undoubtedly help boost the competitiveness of Japanese leading industries. To contribute in achieving early the establishment of a competitive commercial-scale biorefinery, we hope to continue to aggressively expand our base of collaborative researches with industry to further the chemical scope and industrial deployment of the RITE Bioprocess platform technology.