

The 4<sup>th</sup>

Bioenergy & Biotechnology

# Symposium



November 25<sup>th</sup> 2010

Bioenergy Research Center, Chonnam National University

## Organized and Sponsored by

- Bioenergy Research Center, CNU
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- 2<sup>nd</sup> Stage of Brain Korea 21
- Biogreen 21

09:30 - 09:50	<b>Registration</b>
09:50 - 10:00	<b>Welcome Address</b>
10:00 - 10:30	<b>Biorefinery for Biofuels and Biomaterials in China</b> Run -Cang Sun (Beijing Forestry University)
10:30 - 11:00	<b>Evaluation of Crambe as a New Source for Biofuel and Feed Stock Production</b> Bui Minh Tri (Nong Lam University)
11:00 - 11:20	<b>Coffee Break</b>
11:20 - 11:50	<b>Major Bottlenecks to Economical and Efficient Bioethanol Production</b> Hyeun-jong Bae (Chonnam National University)
11:50 - 13:30	<b>Lunch</b>
13:30 - 14:00	<b>Fractional Isolation, Structural Characterization, and Chemical Modification</b> Feng Xu (Beijing Forestry University)
14:00 -14:30	<b>Monitoring Lignin-hydrolysate Removal through Bioluminescence</b> Robert J. Mitchell (Ulsan National Institute of Sci. & Tech.)
14:30-14:50	<b>Coffee Break</b>
14:50-15:20	<b>High-speed Atomic Force Microscopy Visualizes Processive Movement of Cellulases on Crystalline Cellulose</b> Kiyohiko Igarashi (The University Of Tokyo)
15:20-15:50	<b>Proteins and Protein Engineering in Bioproduction</b> Darshan H. Patel (Baytech Korea Inc, ENB Group)
15:50	<b>Closing Remark</b>
17:00	<b>Banquet</b>

## **Biorefinery for Biofuels and Biomaterials in China**

**Run-Cang Sun**

Institute of Biomass Chemistry and Technology  
Beijing Forestry University, China

In this review, the biorefinery of lignocellulose for bioenergy such as bioethanol, biodiesel and biogas fuel, biochemicals like lactic acid, succinic acid, xylitol, 1,3-propanediol, malic acid, levulinic acid, glycerol, furfural, 5-hydroxymethylfurfural, acrylic acid, bio-ethylene and acrylamide, and biomaterials and/or bioplastics, for examples PLA from lactic acid, PTT from 1,3-PDO, PBS from succinic acid, and long chain dicarboxylic acid in china today will be reported in details. In particular, the new technologies for producing bioethanol from lignocellulose materials such as wood and cereal straws will be discussed.

# Biorefinery in China

Run-Cang Sun

Institute of Biomass Chemistry and Technology,  
Beijing Forestry University, Beijing, China

and

State Key Laboratory of Pulp and Paper Engineering  
South China University of Technology, Guangzhou, China

## Outline

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1. Background
2. Bioenergy
3. Biobased Chemicals
4. Biomaterials

## **Evaluation of crambe as a new source for biofuel and feed stock production**

**Bui M. Tri**

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Crambe (*Crambe abyssinica*) was introduced into Vietnam recently as a candidate crop for biodiesel production. Belong to Brassicaceae with life cycle only 3 months and fatty acid constitutions is favorable for industrial use, crambe was expected to be a highly potential candidate for technical oils and biodiesel production. However, performance and adaptability of this new plant in Vietnam ecological condition remained unknown. Our research aimed to evaluate growth, development, oil yield and quality of crambe in the circumstance of using it as a source for biodiesel production. Our research indicated that crambe was only productive in areas where temperature is not higher than 25 degree Celsius. In a suitable ecological condition crambe seed yield can be about 2.1 tones per hectare. Optimal plant density for growing crambe in Vietnam is 256 plants/m<sup>2</sup>. Oil content in the seeds ranged from 30-35%. Fatty acid components in the seeds consisted of erucic acid (56.1 - 59 %), oleic acid (15 – 16.6 %), linoleic acid (9.5 – 10.7 %), arachidic acid (6 – 8.2 %), behenic acid (1,9 – 2,3) and palmitic acid (1.86 – 2.15 %). Procedures for oil extraction from seeds and the conversion of oil to biodiesel were optimized and no major obstacle were found even at lab or pilot scale. Based on the obtained results, it indicated that crambe plants grew fairly well but yield and oil content were still low as compared with results recorded in some reports from other countries. New cultivars testing still needs in the coming time in order to assess potential of growing crambe in Vietnam.

# EVALUATION OF NEW CANDIDATE PLANTS & BIOMASS RESOURCES FOR BIOFUEL PRODUCTION

Tri Minh BUI (PhD)  
Department of Plant Biotechnology (Chair)  
Nong lam University (Hochiminh City-VIETNAM)  
CNU, Nov. 25th 2010

Biofuel production consumption and production in Vietnam

## **Major bottlenecks to economical and efficient bioethanol production**

*Hyeun-Jong Bae<sup>1</sup> · In Seong Choi<sup>2</sup>*

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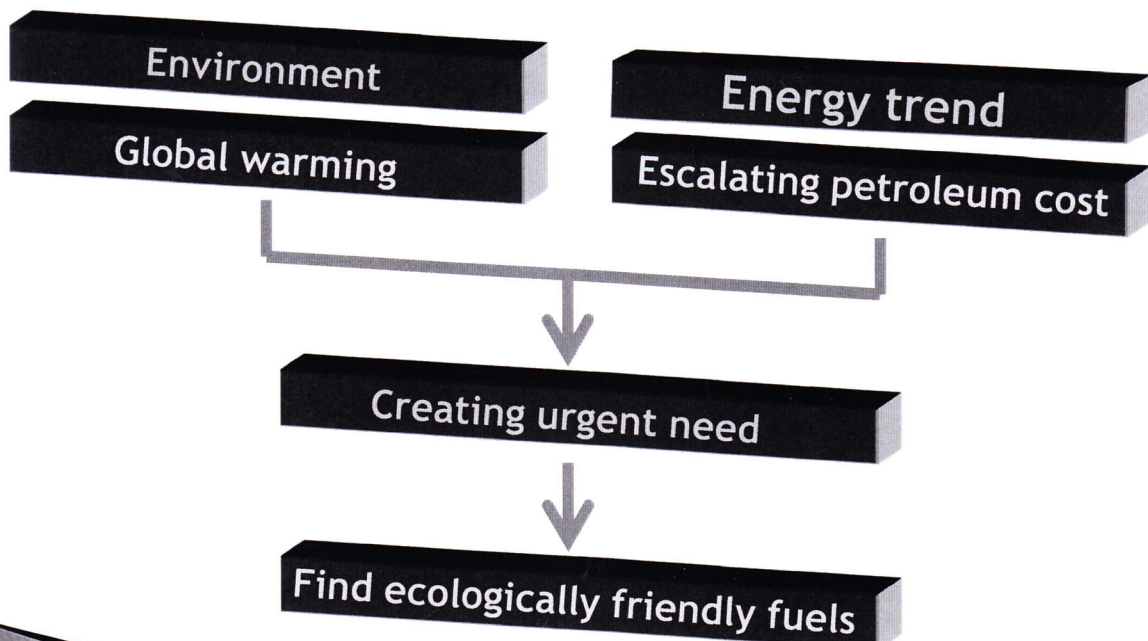
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Issues facing current international bioenergy research and development mainly from concerns over shortage in petroleum supply and global climate change. Today, many countries have tried to develop biofuel because bioenergy is one of the primary strategies to tackle the issue of climate change. However, the successful development of advanced biofuels technologies, using lignocellulosic biomass, has not been satisfactorily resolved. In this seminar, the major bottlenecks in cellulose bioconversion processes will be discussed and reported. Also, different analytical methods have been compared to evaluate the commercial application with the advanced technologies

# Major Bottlenecks to Economical and Efficient Bioethanol Production

Hyeun-Jong Bae

## Why biofuels?





# Fractional Isolation, Structural Characterization, and Chemical Modification of Hemicelluloses from Lignocellulosic Materials for Industrial use

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Hemicelluloses are a group of non-cellulose polysaccharides found in the primary and secondary cell walls of various lignocellulosic materials such as cereal straws and grasses, accounting for about 25-40% of the biomass. They represent an immense renewable resource of biopolymers. They are commonly removed from the straw with dilute alkali and are isolated by alcohol precipitation. Hemicelluloses consist of various different sugar units, arranged in different proportion and with different substituents. The principle sugars are D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent, L-rhamnose, L-fucose, and various *O*-methylated neutral sugars. Hemicelluloses from cereal straws have a backbone of (1→4)-linked β-D-xylopyranosyl units. The chain may be linear, but is often branched and usually has other glycosidically bound sugar units. Some xylan chains have D-glucopyranosyluronic acid units attached, but the most important acidic hemicelluloses are *O*-acetyl-4-*O*-methyl-D-glucuronoxylans and L-arabino (4-*O*-methyl-D-glucurono)xylans. In this review, the fractional isolation, structural characterization, and chemical modification of the hemicelluloses from cereal straws, sugarcane bagasse, shrubs, and grasses will be reported based on the results obtained in our laboratory in recent years. Attention will be also paid to both native and modified hemicellulosic polymers as biomaterials for food and non-food industrial applications.



# Fractional isolation, structural characterization and chemical modification of hemicelluloses from lignocellulosic materials

Feng XU (许凤)

*Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing, China*

## Outline

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- Introduction
- Fractional isolation
- Structural characterization
- Chemical modification
- Acknowledgement



## Monitoring Lignin-Hydrolysate Removal Through Bioluminescence

Robert J. Mitchell

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### Abstract

Lignocellulose offers many benefits in that it is a renewable source of sugar for biofuel production, a non-food feedstock and its use aids in reducing or even reversing the carbon emissions into the atmosphere. However, during its hydrolysis to release the sugars, numerous compounds are formed from the lignin portion, including various phenolic acids and aldehydes. Tests have shown that these compounds can inhibit or kill the microbes involved in the fermentative process when present in even small amounts (0.25 ~ 1 g/L). To remove these compounds, different methods have been devised, including over-liming, which generate a substantial amount of waste that is environmentally unsound.

To address these issues, therefore, our lab is evaluating the use of natural means, *i.e.*, different bacterial isolates, to degrade these compounds and to selectively remove them from the feedstock hydrolysate. Furthermore, by coupling this degradation with the bioluminescent response of a reporter strain, SP4 (*E. coli* BL21, *pinaA::luxCDABE*), which was found to respond to these compounds, we were able to measure both the degradation kinetics and the “apparent” toxicity and concentration through the gene expression levels. The application of both of these novel tools – biodegradation of the phenolics and the bioreporter strain(s) – permit us to both prepare and evaluate lignocellulose samples for downstream fermentations and to prevent potential system failure due to the toxicity of the lignin-based compounds.

# Transcriptomic Responses from E. coli Exposed to Lignin-Hydrolysates Compounds



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# High-speed atomic force microscopy visualizes processive movement of cellulases on crystalline cellulose

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As a result of global warming and the increasing cost of oil and crops, utilization of cellulosic biomass has assumed increasing importance. In nature, filamentous fungi are one of the most potent degraders of cellulose using various cellulose-hydrolyzing extracellular enzymes, generally called cellulases. Although extensive studies have been done, the mechanisms of hydrolysis of celluloses by cellulases are quite ambiguous, because the reaction occurs at a solid/liquid interface, and it is quite difficult to perform the quantitative analysis of cellulases.

Fungal cellobiohydrolases act at liquid-solid interfaces. They have the ability to hydrolyze cellulose chains of a crystalline substrate due to their two-domain structure, i.e., cellulose-binding domain (CBD) and catalytic domain (CD), and unique active site architecture. However, the details of the action of the two domains on crystalline cellulose are still unclear. Here we present real-time observations of *Trichoderma reesei* (*Tr*) cellobiohydrolase I (Cel7A) molecules sliding on crystalline cellulose, obtained with a high-speed atomic force microscope (Fig. 1) [1]. The average velocity of the sliding movement on crystalline cellulose was 3.5 nm/sec, and the catalytic domain without the CBD moved with a velocity similar to that of the intact *Tr*Cel7A enzyme. However, no sliding of a catalytically inactive enzyme (mutant E212Q) or a variant lacking tryptophan at the entrance of the active site tunnel (mutant W40A) could be detected. This indicates that, besides the hydrolysis of glycosidic bonds, the loading of a cellulose chain into the active site tunnel is also essential for the enzyme movement.

## Reference

- [1] K. Igarashi, et. al., *J. Biol. Chem.* **284**: 36186-34190 (2009).

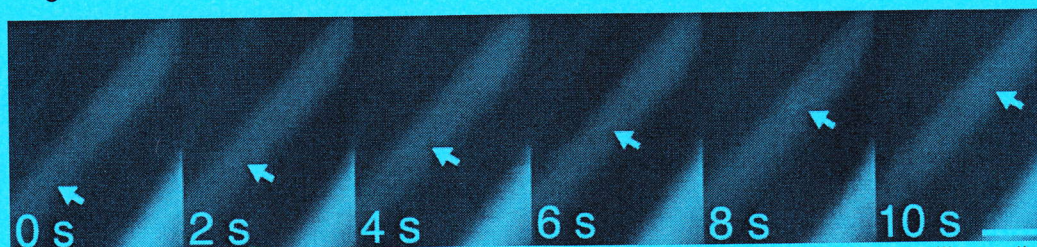


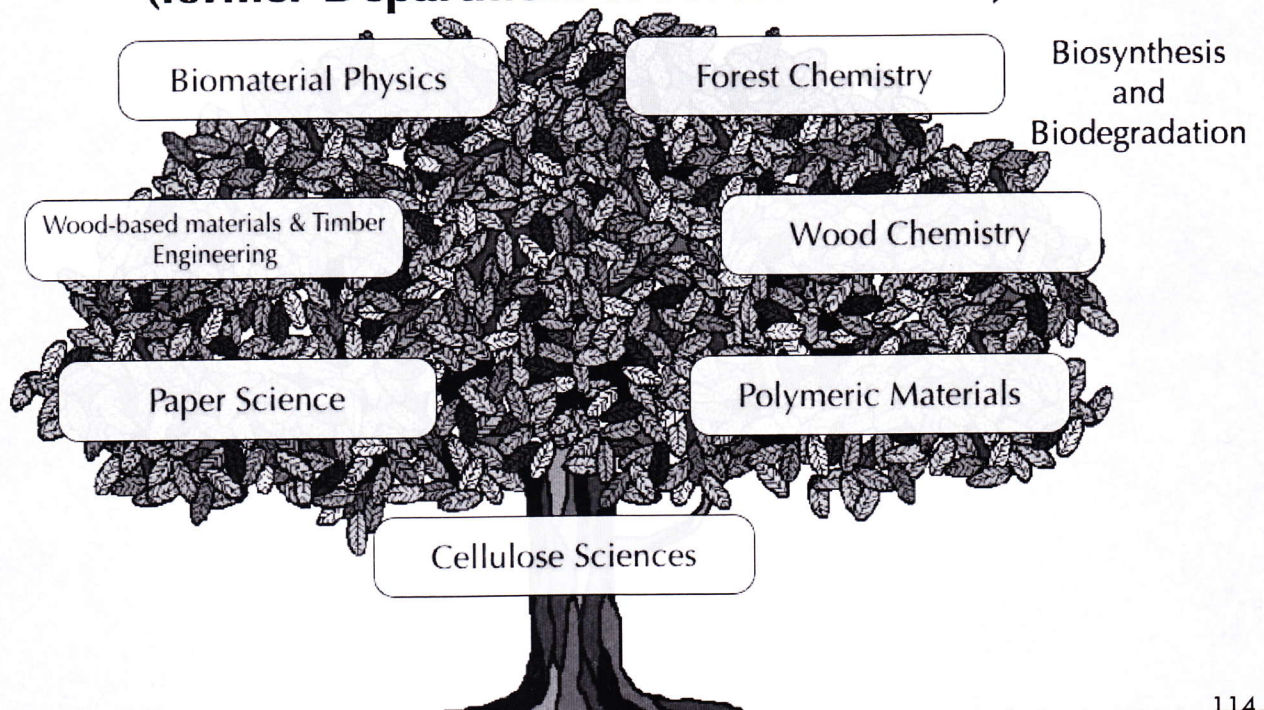
Fig. 1. Time-lapse images of *Tr*Cel7A (white arrow) molecules sliding on a highly crystalline cellulose. Scale bar indicates 50 nm.

# High-speed atomic force microscopy visualizes processive movement of cellulases on crystalline cellulose

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## Department of Biomaterials Sciences (former Department of Forest Products)



## **Protein and protein engineering in bioproduction.**

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Proteins (Enzymes) play a key role in enhancing the industrial process and bioproductions of biochemical. The isomerization of sugars released from biomass not only influence on ethanol yield, can also produce rare and medically important sugar and isomerase makes the process easy and simpler.

Bioconversion of mannose to glucose, enhance the fermentation capacity of ethanol producing organisms and thus will provide the opportunity to utilize mannose rich biomass, especially marine biomass as an alternate to cellulosic biomass. Similarly bioconversion of readily available sugar to rare and medically important sugar will provide the value added products from the sugar released from biomass. Two-step isomerization with the help of isomerases makes process easy for the bioproduction of readily available sugar to rare and highly consumable sugar. Here we have converted mannose to glucose and xylose to lyxose, a starting material for antiviral medicine at nearly 37% production rate with the help of mannose and xylose isomerase.

Since the sugar isomerase has multiple, preferential substrate specificities, protein engineering possibly lead the process in single step rather than two steps isomerization. Xylose isomerase has been studied in details and number of mutations has been created to alter activity. Knowledge based alteration of amino acid in catalytic center of xylose isomerase can function as non preferential sugar isomerase. The presentation will discuss the replacement of a single amino acid in catalytic center of xylose isomerase working on wide range of sugar and produced isomer.

# Proteins and protein engineering in bioproductions

Darshan H Patel  
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