

Advanced Energy Research Section

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1. Summary

During the 2025 academic year, the author continued his research as a Visiting Associate Professor at the Institute of Advanced Energy (IAE), Kyoto University, in collaboration with Professor Masato Katahira's group. Building upon previous work on integrated biorefineries, this year's research focused on the development of next-generation engineered baker yeast "*Saccharomyces cerevisiae*" strains capable of multi-carbon co-fermentation and enhanced stress tolerance.

2. Introduction

The transition toward a sustainable bioeconomy requires efficient utilization of diverse and low-cost carbon sources derived from lignocellulosic biomass and industrial byproducts. While conventional *S. cerevisiae* efficiently ferments glucose, its inability to utilize substrates such as glycerol, xylose, and acetic acid limits its applicability in next-generation biorefineries.¹

To overcome these limitations, this study expanded previous engineering strategies²⁻⁴ by integrating multi-substrate fermentation capability with redox balancing and stress tolerance mechanisms. In addition, transcriptomic and ultrastructural analyses were employed to understand the global cellular adaptations associated with enhanced fermentation performance under industrially relevant conditions, including hypoxia and acetic acid stress.

3. Results and Achievements

(1) Engineering baker yeast for multi-carbon co-fermentation

A major outcome of this study is the successful construction of a metabolically engineered *S. cerevisiae* strain capable of co-utilizing glycerol, xylose, acetic acid, and glucose within a single fermentation system (Fig. 1). This was achieved through CRISPR-based genome editing combined with heterologous pathway introduction and redox pathway optimization.

Specifically, the introduction of a xylose assimilation module, together with the replacement of native redox-associated pathways and enhancement of acetate uptake mechanisms, enabled efficient flux redistribution toward ethanol production. The resulting strain exhibited stable fermentation performance under microaerobic conditions and achieved a theoretical ethanol yield exceeding 95%, representing, to the best of our knowledge, the first

demonstration of simultaneous co-fermentation of these four substrates in a single *S. cerevisiae* strain.⁵

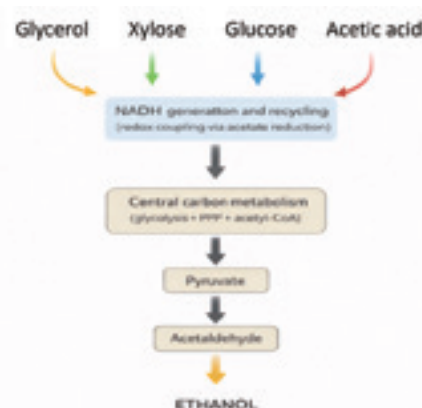


Fig. 1. Conceptual framework of multi-carbon co-fermentation and redox-balanced ethanol production in engineered *Saccharomyces cerevisiae*. Glycerol, xylose, glucose, and acetic acid are simultaneously assimilated through engineered metabolic pathways. Redox balancing via NADH generation and recycling, coupled with acetate assimilation, enables efficient carbon flux through central carbon metabolism toward ethanol production.

(2) Transcriptomic analysis of engineered strains under glucose–acetic acid co-fermentation

To investigate the molecular basis of improved fermentation performance, comparative transcriptomic analysis was conducted between engineered strains and the parental control. The results revealed extensive transcriptional remodeling, particularly in the advanced engineered strain, where approximately one-third of the genome exhibited differential expression.

Genes associated with glycolysis, alcohol metabolism, membrane transport, and cell wall organization were upregulated, while genes related to mitochondrial respiration and amino acid biosynthesis were generally downregulated. Functional enrichment analysis further indicated significant involvement of biological processes related to transmembrane transport, metabolic adaptation, and redox activity (Fig. 2), which suggesting a coordinated reprogramming of cellular metabolism in response to the engineered pathways and environmental conditions.⁶

(3) Ultrastructural characterization and flocculation behavior

In addition to transcriptomic changes, significant alterations in cellular ultrastructure were observed using transmission electron microscopy and fluorescence imaging. Engineered strains displayed enlarged cells, expanded vacuoles, and the accumulation of intracellular lipid droplets. Moreover, a distinct restructuring of the cell envelope was observed, characterized by a porous and flocculent outer layer.

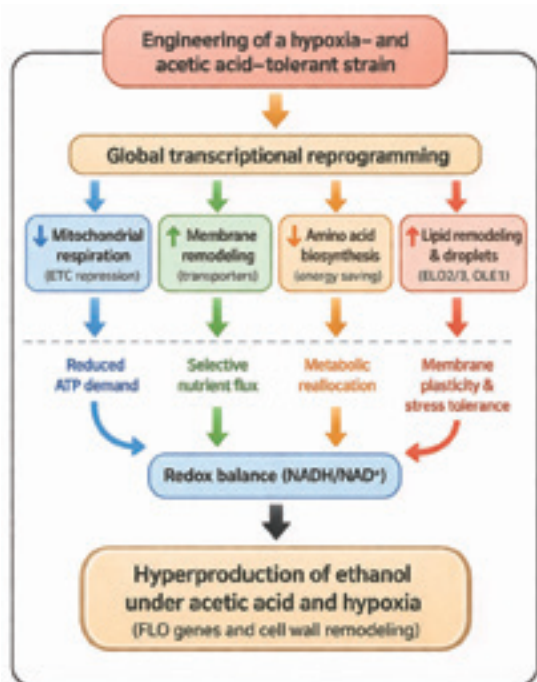


Fig. 2. Mechanistic model of transcriptional and physiological adaptation in engineered *S. cerevisiae* under acetic acid and hypoxic conditions. Global transcriptional reprogramming is characterized by repression of mitochondrial respiration and amino acid biosynthesis, together with activation of membrane remodeling and lipid metabolism. These coordinated changes promote reduced ATP demand, selective nutrient flux, metabolic reallocation, and enhanced membrane plasticity, collectively supporting redox balance (NADH/NAD⁺) and driving hyperproduction of ethanol under acetic acid and hypoxia, accompanied by flocculation-associated cell wall remodeling.

These structural features were particularly pronounced in the advanced engineered strain and were consistent with the observed flocculation phenotype under ethanol-producing conditions. The enhanced flocculation is likely associated with increased expression of subtelomeric gene families involved in cell adhesion and stress response. These findings indicate that metabolic engineering not only affects intracellular pathways but also induces substantial modifications in cell morphology and surface properties, which may contribute to improved fermentation performance and downstream processing.⁶

(4) Concluding Remarks

The present study demonstrates the successful integration of metabolic engineering, systems-level analysis, and structural characterization to develop a robust yeast platform for sustainable bioethanol production. The ability to co-utilize multiple carbon sources, including inhibitory compounds such as acetic acid, represents a significant advancement toward the realization of efficient and flexible biorefinery systems.

Future work will focus on scaling up the developed strains, optimizing fermentation conditions under industrially relevant settings, and further refining metabolic pathways to enhance productivity and robustness. These efforts are expected to contribute to the development of economically viable and environmentally sustainable aviation fuel production technologies.

References

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Advanced Energy Research Section

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1. Summary

The author spent three months (October 1, 2025 – December 31, 2025) as a guest associate professor at the Uji Campus of Kyoto University, hosted by Prof. Kazunobu Nagasaki of the Heliotron J research group. During this period, the author conducted collaborative research, and this report summarizes the progress and outcomes of the studies related to Heliotron J.

2. Introduction

Magnetic confinement devices such as stellarators and heliotrons require effective control of particle and heat transport in order to achieve improved plasma confinement. In the medium-sized heliotron device Heliotron J, several studies have focused on plasma performance improvement through optimized fueling schemes. Previous experiments have shown that efficient core fueling techniques, such as conventional gas puffing (GP) and high-intensity gas puffing (HIGP), can significantly increase the electron density and produce highly peaked core density profiles, which enhance stored energy in the plasma [1]. In addition, experiments using electron cyclotron heating (ECH) have demonstrated the formation of an electron internal transport barrier (e-ITB), characterized by a steep electron temperature gradient in the plasma core. Under such conditions, a hollow density profile is often observed, reflecting the modified particle and energy transport associated with improved confinement [2]. It can be seen in the results in Ref. [2], the time evolution of plasma parameters in Heliotron J comparing HIGP and GP discharges during ECH startup and neutral beam injection heating (NBI). While the density profiles remain similar for both cases, the HIGP discharge produces a higher and more peaked electron temperature in the core, indicating improved electron heat confinement, whereas the ion temperature changes only slightly. These results highlight the importance of understanding transport barriers and fueling methods for optimizing plasma confinement in Heliotron J.

3. AXUV Signals under Different Magnetic Field Strengths

In this work, the temporal evolution and spatial distribution of plasma radiation during the formation of the e-ITB in HIGP discharges are examined using

an AXUV spectrometer. The analysis of these signals provides valuable information for understanding plasma transport processes and the confinement improvement associated with the e-ITB regime. Figure 1 shows the AXUV spectrometer installed on Heliotron J. The system consists of 16 lines-of-sight that cover the plasma region from the core to the edge.

The AXUV signals were analyzed under different magnetic field strengths, B_T , ranging from 0.63 T to 1.41 T to investigate their influence on plasma radiation and confinement properties. Variations in the magnetic field configuration can significantly affect plasma stability, transport processes, and the formation of the e-ITB. By comparing the AXUV signals obtained at different B_T , the characteristics of plasma radiation and the associated e-ITB behavior in HIGP discharges can be evaluated.

4. Line-Integrated Signal Profile

Figure 2 shows the temporal evolution of the discharge parameters and the line-integrated AXUV signals for channels #1–#16 during HIGP discharges at different B_T . The upper panels present the operational conditions, including ECH power, NBI power, gas puff with the HIGP at $t = 230$ – 240 ms, and H_a emission, while the lower panels illustrate the spatiotemporal evolution of the AXUV radiation signals. Around $t = 245$ – 250 ms, a noticeable change

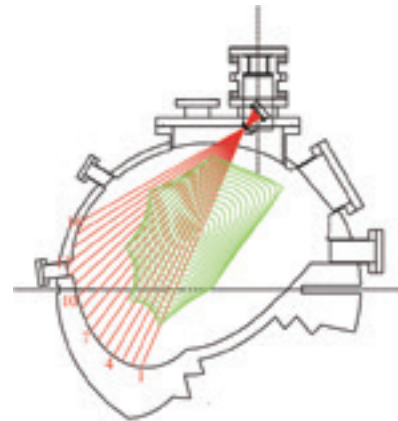


Figure 1: AXUV spectrometer system installed on Heliotron J, consisting of 16 lines-of-sight covering the plasma region from the core to the edge.

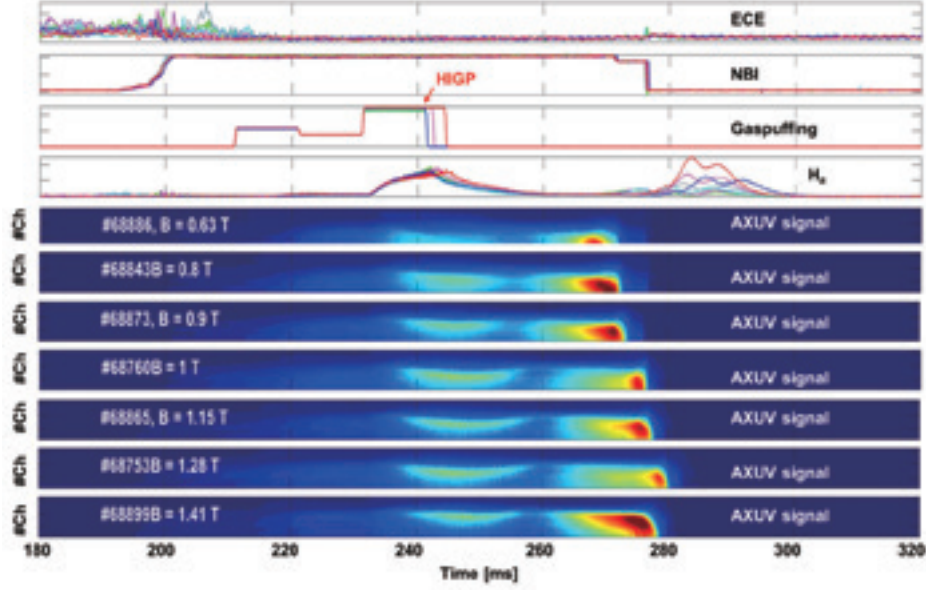


Figure 2: Time evolution of the discharge parameters and the line-integrated AXUV signals for channels #1–#16 during HIGP discharges with different toroidal magnetic field strengths.

in the AXUV signal distribution is observed, indicating the formation of the e-ITB at around $t = 255$ ms. During the e-ITB formation, the radiation intensity becomes more localized in specific channels, suggesting a modification of the radial radiation profile associated with improved confinement. Furthermore, the AXUV intensity generally increases with increasing B_T , indicating enhanced plasma radiation and improved confinement characteristics under stronger magnetic field conditions. These results suggest that the magnetic field strength plays an important role in the formation and evolution of the e-ITB in HIGP discharges.

Figure 3 shows the channel distribution of the line-integrated AXUV signals (#1–#16) at $t = 255$ ms for HIGP discharges with different B_T . The AXUV intensity gradually increases from the outer channels toward the central channels and reaches a maximum

around channels #7–#10, indicating that the dominant plasma radiation originates from the core region. After the peak region, the signal rapidly decreases toward the outer channels (#12–#16), corresponding to the plasma edge where the radiation level is lower. In addition, the peak intensity and profile shape vary with B_T , suggesting that the magnetic field strength influences the radiation distribution and confinement characteristics of the plasma. The pronounced peak observed near the central channels at $t = 255$ ms is consistent with the formation of the e-ITB, which leads to improved confinement and enhanced core radiation.

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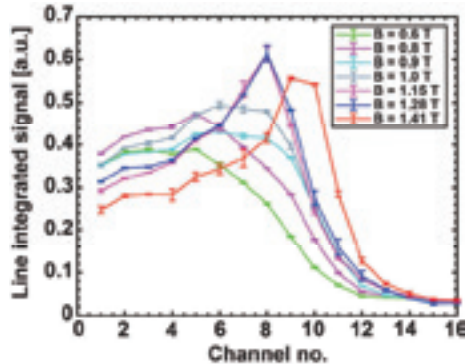


Figure 3: Channel distribution of the line-integrated AXUV signals (#1–#16) measured by the AXUV spectrometer at $t = 255$ ms during HIGP discharges for different toroidal magnetic field strengths B_T , representing the spatial distribution of plasma radiation at the time of e-ITB formation.