A microbial saccharification method for efficient production of high-concentration sugar solutions from rice straw

Rice straw is generated in large quantities across Asia, and open-field burning remains a common disposal method that contributes to air pollution, public health concerns, and transboundary haze problems. Converting rice straw into fermentable sugars provides an environmentally sound alternative and enables the production of biofuels and bio-based chemicals. However, practical application requires high-solids saccharification to obtain concentrated sugar solutions that improve downstream fermentation and purification efficiency. Conventional saccharification methods rely heavily on externally supplied enzymes, creating cost and supply constraints. This study aimed to develop an enzyme-independent microbial saccharification process capable of stably producing high-concentration sugar solutions under high-solids conditions by optimizing culture operation and incorporating surfactant-based stabilization.

To address instability issues associated with high-solids saccharification, such as poor mixing, non-productive enzyme adsorption, and enzyme inactivation, we examined the effect of adding Tween 20 (a non-ionic surfactant) to a co-culture system comprising *Clostridium thermocellum* and the β-glucosidase-producing strain *Thermobrachium celere* A9. Tween 20 improved the retention of cellulase and β-glucosidase activities, resulting in enhanced and more sustained saccharification performance. In parallel, a semi-continuous operation strategy was developed in which pretreated rice straw was added stepwise during cultivation. Combining Tween 20 with this feeding strategy effectively prevented saccharification stagnation, even at high total solids. Under optimized conditions, a total substrate loading of 250 g L^{-1} yielded 140 g L^{-1} of glucose, corresponding to 70% of the theoretical yield, without the addition of external enzymes. These results demonstrate that both biochemical stabilization and operational design are essential for maintaining saccharification activity at high solids.

This microbial saccharification approach provides a cost-effective and enzyme-independent method for producing concentrated sugar solutions from underutilized biomass such as rice straw. It supports the development of sustainable biomass-to-chemicals pathways in Asia, where large volumes of agricultural residues are generated and environmentally sound disposal options are urgently needed. The semi-continuous feeding concept offers practical insights for scaling up high-solids saccharification, including considerations for substrate handling, mixing, and process control. Further evaluation of alternative low-cost non-ionic surfactants and their impacts on downstream processes will aid in refining industrial applicability. Overall, the system provides a promising foundation for expanding biomass utilization while reducing environmental burdens associated with current disposal practices.

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Table 1. Effect of Tween 20 (surfactant) addition on microbial saccharification of rice straw

Tween 20 (% v/v)	Accumulated glucose in the medium (gL^{-1})	Glucose yield from the cellulose of rice straw[*1] (%)	Cellulase activity (U/mL)	β-Glucosidase activity (U/mL)
None	78.8 ± 0.2	65.7 ± 0.2	2.5 ± 0.3	3.0 ± 0.2
0.1	82.5 ± 0.3	68.8 ± 0.3	3.3 ± 0.2	4.5± 0.1
0.5	**90.8 ± 0.2**	**75.7 ± 0.2**	**5.2 ± 0.1**	**5.3 ± 0.2**
1.0	80.8 ± 0.5	67.4 ± 0.5	2.8 ± 0.3	4.5 ± 0.3
1.5	54.8 ± 0.5	45.3 ± 0.5	1.3 ± 0.3	1.1 ± 0.1

[*1]Alkali-pretreated rice straw was used (see Nhim et al. (2025) for the pretreatment conditions). Microbial saccharification was carried out using a co-culture of *Clostridium thermocellum* and *Thermobrachium celere* A9, with a rice straw loading of 150 g L^{-1}. Tween 20 was added at the indicated concentrations on a volume/volume basis (% v/v). Values represent the mean ± standard deviation of three independent experiments.

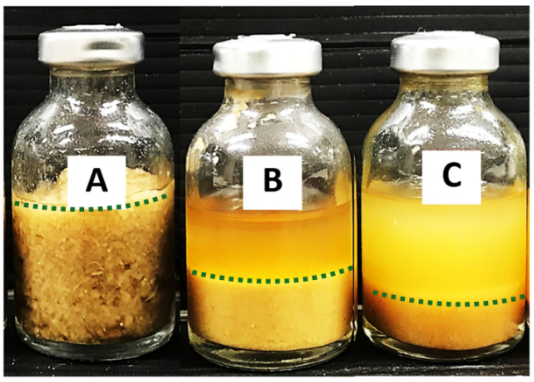

Fig. 1. The effect of Tween 20 (a surfactant) addition on the saccharification of rice straw at a solid loading of 150 g L^{-1} using microbial saccharification

(A) No saccharolytic microorganisms inoculated; (B) Microbial saccharification without Tween 20 addition; (C) Microbial saccharification with 0.5% Tween 20 addition. Lines indicate the remaining amount of substrate during microbial saccharification.

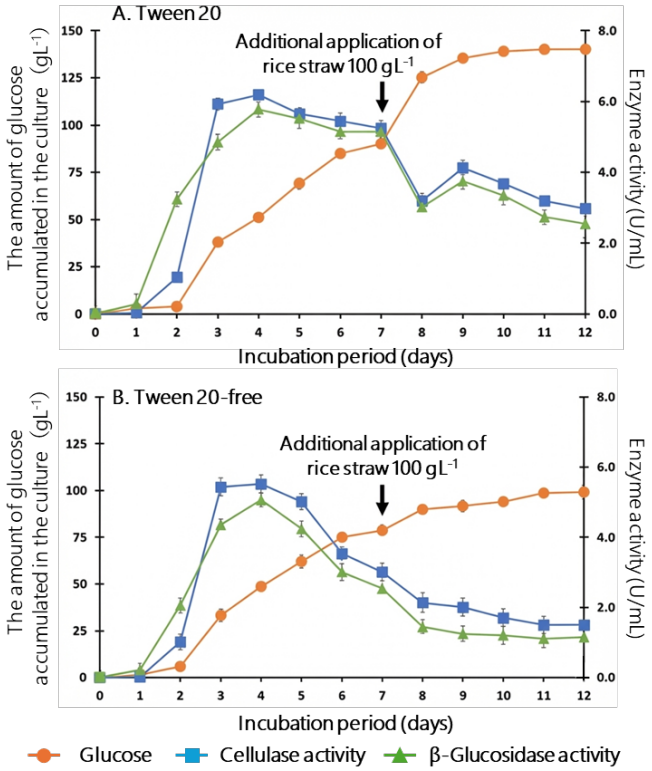

Fig. 2. Semi-continuous microbial saccharification with rice straw

Microbial saccharification was initiated with an initial solid loading of 150 g L^{-1}, followed by the addition of pretreated rice straw at a concentration of 100 g L^{-1}. (A) With Tween 20; (B) Without Tween 20. Arrows indicate the time points at which the rice straw was added. Data are means ± SD (n = 3).

Reference: Nhim et al. (2025) *Frontiers in Microbiology* 15, 1519060.