

Supplementary for: Developing soluble inducer for robust production of cellulases by *Trichoderma reesei* through submerged fermentation

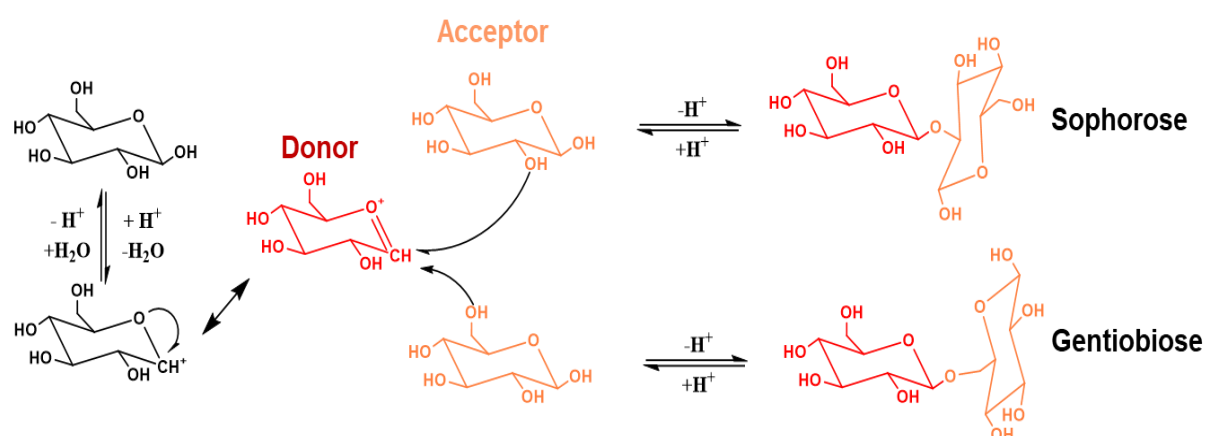


Fig. S1 Mechanism underlying reversible glycosylation to form β -1,2 and β -1,6 glycosidic bonds catalyzed by acid.

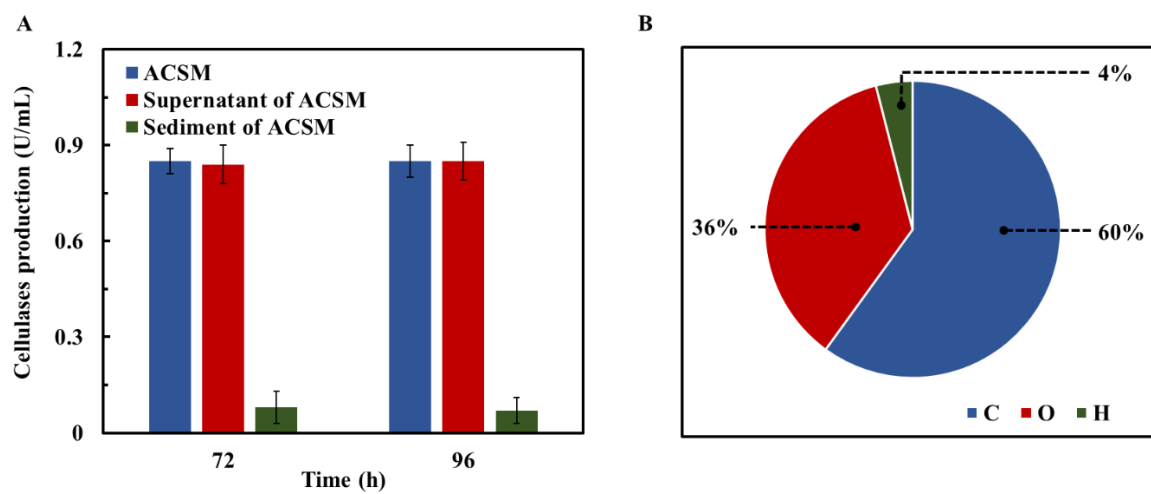
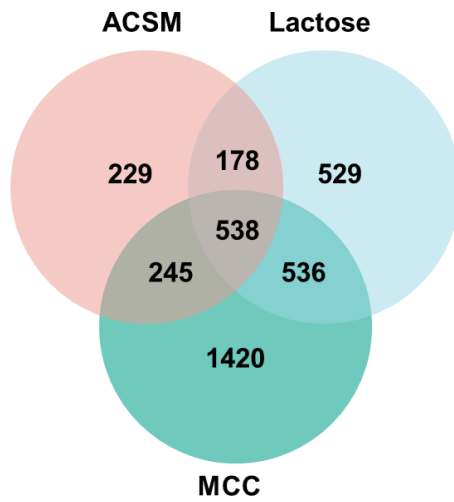


Fig. S2 Cellulases activity in ACSM, supernatant and sediment (A), and elemental analysis for the solid sediment of ACSM (B).

A



B

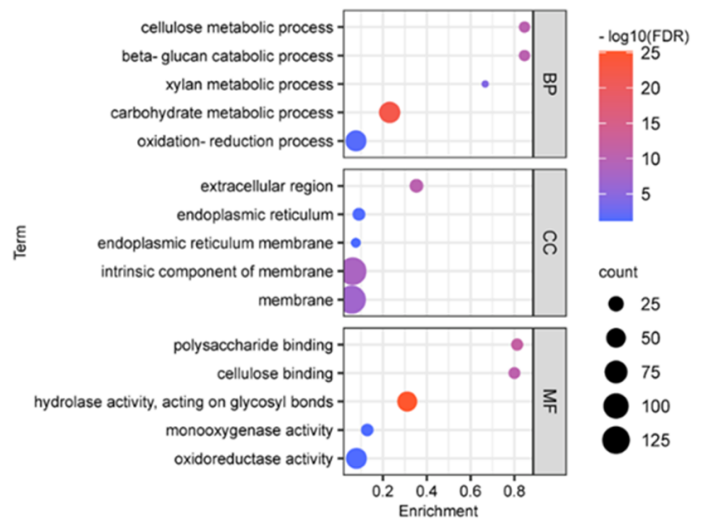


Fig. S3 The number of differentially expressed genes (DEGs) of samples using ACSM, lactose, and microcrystalline cellulose (MCC) as the inducer by referring to the sample using glucose (A), and the main classification of these DEGs were represented by the bubble plot through Gene Ontology (GO) enrichment analysis (B).

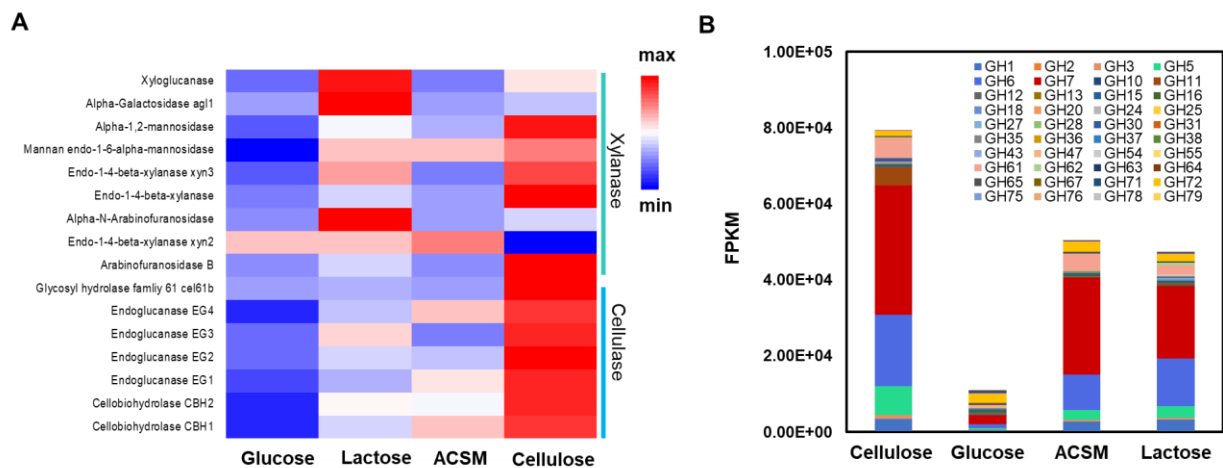


Fig. S4 Expression profiles of genes encoding carbohydrate-active enzymes (CAZymes). A: Expression of genes encoding cellulases and xylanases in *T. reesei* RUT-C30 cultivated by different carbon sources; B: Overview of genes expression in each glycolic hydrolase (GH) family.

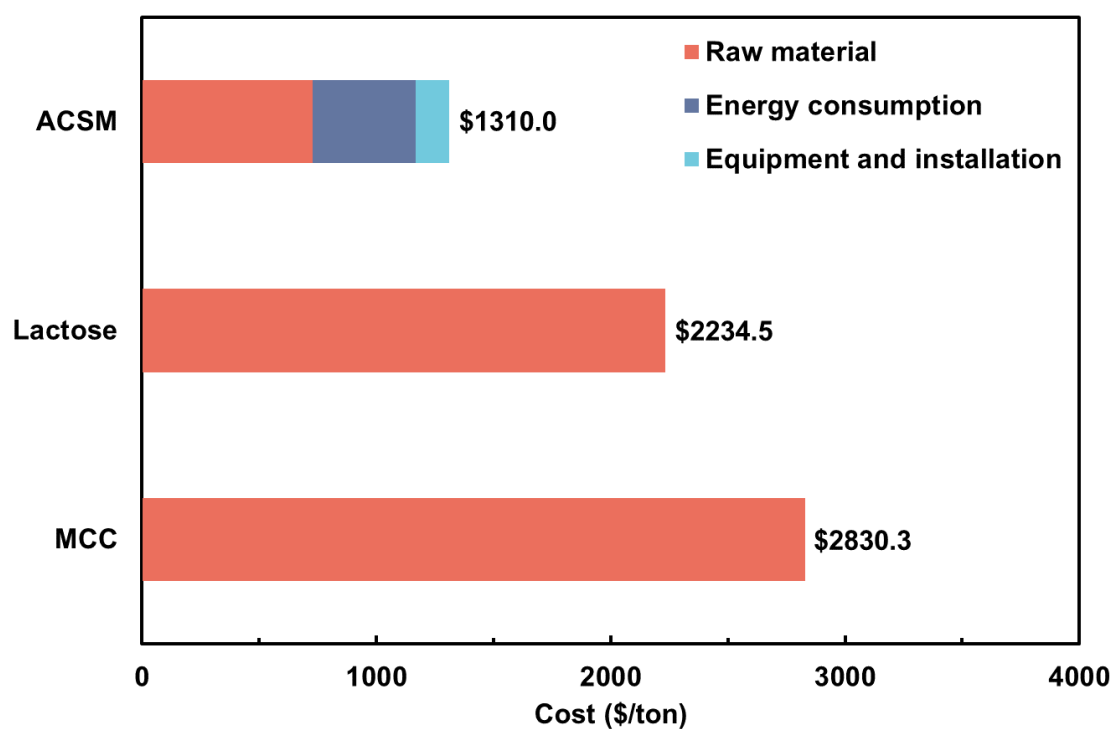


Fig. S5 Comparison between the cost of different inducers. The price of lactose and microcrystalline cellulose (MCC) was referred to the bulk supplier and the production cost of ACSM was simulated by Aspen plus.

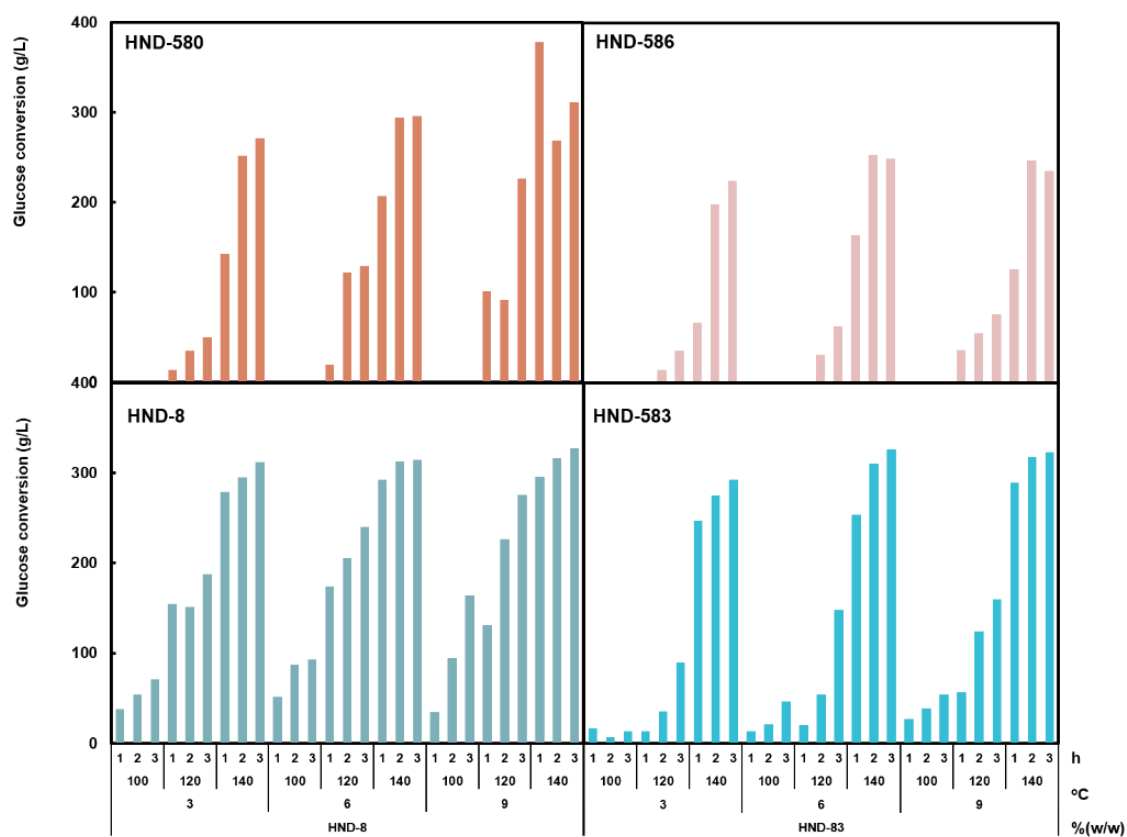


Fig. S6 Content of glucose conversion under different conditions using different solid acids, temperatures, and reaction times.

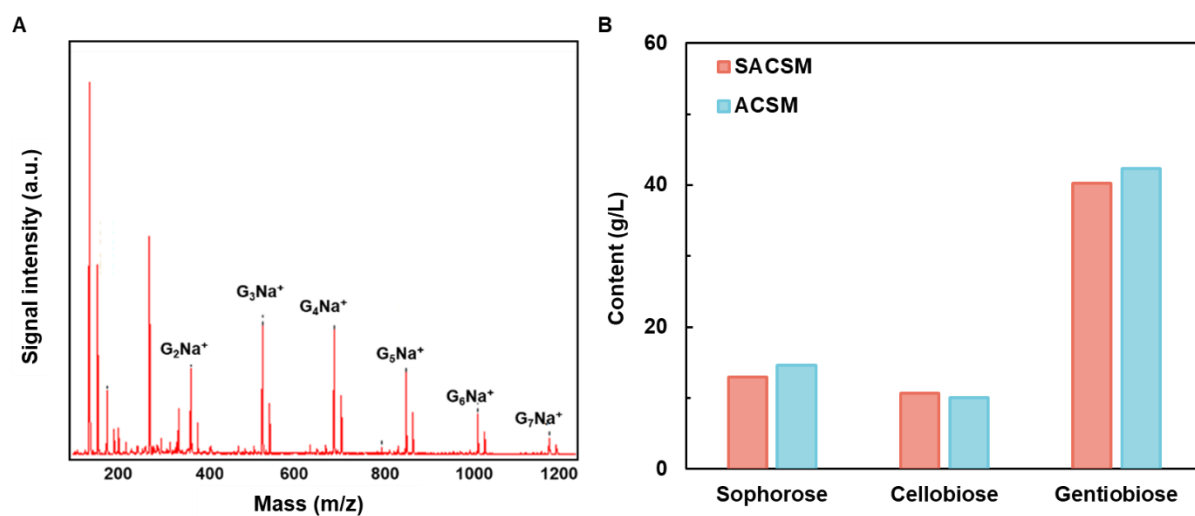


Fig. S7 Characterization of SACS in identification of polymerization degree (A) and quantification of sophorose, cellobiose, and gentiobiose (B).

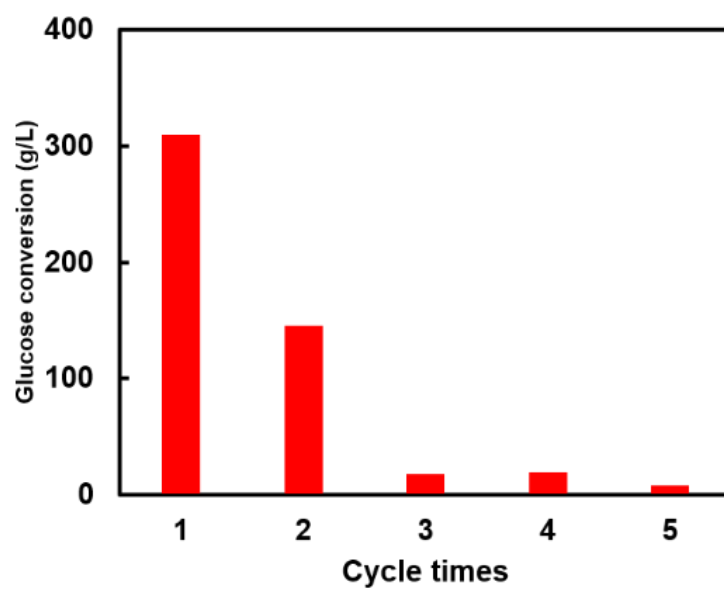


Fig. S8 The recyclability of the solid acid (HND-83) evaluated by glucose conversion.

Table S1 Primer sequence of cellulases encoding genes for RT-PCR.

Genes		Primers
<i>sar1</i>	F	CGTCTTGTCGTCTTTGGGTCT
	R	CGTCTTGTCGTCTTTGGGTCT
<i>xyl1</i>	F	CCATCAACCTTCTAGACGAC
	R	AACCCTGCAGGAGATAGAC
<i>cbh1</i>	F	ACGAGTTCTCTTTCGATGTTGATG
	R	CGGTGTTGGTGGGATACTTG
<i>cbh2</i>	F	CTCACAAACGCAAACCCATC
	R	GCGACAGACAACCAAAGTAGACA
<i>eg1</i>	F	CTGGTGGCTAGTGTTGAGGG
	R	CCGAGTGATCTGTTCCAGAATGT
<i>eg2</i>	F	AACAAGTCCGTGGCTCCATT
	R	TCCGCTCCAACCAATACCTC
<i>ace3</i>	F	TCCAGCTCTACAGGCCCTC
	R	GCTGCAGCTACAGAGCCC

Table S2 Comparison of fold change between RT-PCR and RNA-seq results on major cellulases encoding genes over *sar1* as the reference.

Gene	qRT-PCR	RNA-seq
<i>sar1</i>	-	-
<i>xyl1</i>	6.19	4.02
<i>cbh1</i>	10.5	10.2
<i>cbh2</i>	2.28	10.05
<i>egl</i>	2.67	8.02
<i>eg2</i>	4.84	7.12
<i>ace3</i>	1.67	3.13

Table S3 Fold change of transcriptional factors explored in common DEGs*

Protein ID**	ACSM/Glucose	MCC/Glucose	Lacost/Glucose
98788 (XYR1)	4.02	4.51	3.59
98455 (ACE3)	3.13	5.66	3.62
76250 (CLR2)	7.98	8.26	8.84
78051	0.43	0.14	0.31
105117	0.23	0.17	0.23
73370	0.21	0.24	0.21
7117	0.42	0.34	0.44
9219	0.38	0.38	0.22
141745	0.47	0.43	0.44
78902	5.74	7.77	2.91
97398	2.86	8.43	4.30
97256	3.34	9.97	3.68
135338	2.43	3.53	2.10

*Common DEGs, Common differentially expressed genes from transcriptome results among ACSM, microcrystalline cellulose (MCC) and lactose groups referring to glucose group.

**Gene ID was assigned based on the *T. reesei* RUT-C30 genome database.

Table S4 Fold change of genes encoding transporters explored in common DEGs*

Protein ID**	ACSM/Glucose	ACSM/MCC	ACSM/Lactose
7623	8.89	52.52	6.43
136272	2.36	6.51	3.68
134530	2.26	3.78	6.11
24454	0.17	18.08	2.96
91130	4.40	3.40	15.10
25063	23.62	2.65	32.33
79544	0.09	3.19	0.26
126296	4.66	7.73	7.14
84142	13.55	4.68	3.77
7422	2.51	2.24	2.79
94554	2.83	0.05	0.05
90646	0.45	0.08	0.37
26904	0.12	0.13	0.05
102402	0.34	0.34	0.19
90069	4.44	0.04	0.20
132413	5.49	0.22	0.32

*Common DEGs, Common differentially expressed genes from transcriptome results between ACSM samples to Glucose, MCC, and Lactose, respectively.

**Gene ID was assigned based on the *T. reesei* RUT-C30 genome database.