Enzymatic generation of short chain cello-oligosaccharides from Miscanthus using different pretreatments

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Supplementary information

Table S1: DNA sequences for the enzymes used in this study, codon optimised for P. pastoris. Uppercase lettering represents coding sequence.

Gene name	Sequence (5'-3')
TfCel9a	GAACCAGCTTTCAATTACGCTGAGGCCTTGCAAAAGTCCATGTTCTTCTACGAGGCTCAGAGATCC GGTAAGTTGCCAGAGAACAACAGAGTCTCTTGGAGAGGTGACTCTGGTTTGAACGATGGTGCTGA TGTTGGTTTGGACTTGACTGGTGGTTGGTACGATGCTGGTGATCACGTTAAGTTCGGTTTCCCAAT GGCTTTCACTGCTACTATGTTGGCTTGGGGTGCTATTGAATCTCCAGAGGGTTACATTAGATCCGG TCAGATGCCTTACCTGAAGGACAACTTGAGATGGGTCAACGACTACTTCATCAAGGCTCACCCATC TCCAAACGTCTTGTACGTTCAAGTTGGTGATGGTGACGCTGATCATAAGTGGTGGGGTCCAGCTG AAGTTATGCCAATGGAAAGACCATCCTTCAAGGTCGATCCATCTTGTCCAGGTTCTGACGTTGCTG CTGAAACTGCTGCTGCTATGGCTGCTTCTTCTATCGTTTTCGCTGATGACGATCCAGCTTACGCTGC TACTTTGGTTCAACACGCTAAGCAGTTGTACACTTTCGCCGACACTTACAGAGGTGTTTACTCCGAT TGTGTTCCAGCTGGTGCTTTCTACAACTCTTGGTCTGGTTACCAGGACGAATTGGTTTGGGGAGCT TACTGGTTGTACAAGGCTACTGGTGACGACTCCTACTTGGCTAAGGCTGAATACGAGTACGACTTC TTGTCCACTGAGCAGCAGACTGACTTGAGATCCTACAGATGGACTATTGCCTGGGACGACAAGTC CTACGGTACTTACGTTTTGCTGGCCAAAGAGACTGGTAAGCAAAAGTACATCGACGACGCCAACA GATGGTTGGACTACTGGACTGTTGGTGTCAACGGTCAGAGAGTTCCATACTCTCCAGGTGGTATG GCTGTTTTGGATACCTGGGGTGCTCTTAGATACGCTGCCAACACTGCTTTCGTTGCTTTGGTTTACG CCAAGGTTATCGACGACCCAGTCAGAAAGCAAAGATACCACGACTTCGCCGTCAGACAGATCAAC TACGCTTTGGGTGATAACCCAAGAAACTCCTCCTACGTTGTCGGTTTCGGTAACAACCCACCAAGA AACCCACATCACAGAACTGCTCATGGTTCTTGGACTGACTCTATTGCTTCTCCAGCTGAGAACAGAC ACGTTCTGTACGGTGCATTGGTTGGTGGTCCTGGTTCTCCAAATGATGCTTACACTGACGACAGAC AGGACTACGTTGCTAACGAAGTTGCTACTGACTACAACGCTGGTTTCTCTTCCGCTTTGGCTATGTT GGTTGAAGAGTACGGTGGTACTCCATTGGCTGATTTCCCACCAACTGAAGAACCAGACGGTCCAG AGATTTTCGTTGAGGCCCAGATTAACACTCCAGGTACTACTTTCACTGAGATCAAGGCCATGATCA GAAACCAGTCTGGTTGGCCTGCTAGAATGTTGGACAAGGGTACTTTCAGATACTGGTTCACCTTGG ACGAAGGTGTTGACCCAGCTGATATTACTGTTTCCTCCGCCTACAATCAGTGTGCTACTCCTGAAG ATGTTCACCACGTTTCCGGTGACTTGTACTACGTTGAGATCGACTGTACCGGTGAGAAGATTTTCC CAGGTGGTCAATCTGAGCACAGAAGAGAGGTTCAGTTCAGAATTGCTGGTGGTCCAGGATGGGA TCCATCTAACGATTGGTCCTTCCAAGGTATCGGTAACGAATTGGCTCCAGCTCCTTACATCGTGTTG TACGATGACGGTGTTCCAGTTTGGGGTACTGCACCTGAAGAGGGTGAAGAACCTGGTGGTGGTG AAGGTCCAGGTGGCGGTGAGGAACCAGGTGAGGATGTTACTCCACCATCTGCTCCAGGATCTCCA GCAGTTAGAGATGTTACTTCTACCTCCGCTGTTTTGACTTGGTCTGCTTCCTCTGATACTGGTGGAT CTGGTGTTGCTGGTTACGACGTTTTTTTGAGAGCCGGTACTGGTCAAGAGCAGAAGGTTGGTTCTA CTACCAGAACCTCCTTCACCTTGACTGGATTGGAACCAGACACTACTTACATTGCTGCCGTTGTTGC TAGAGACAACGCCGGTAACGTTTCCCAAAGATCTACCGTTTCCTTCACTACCTTGGCTGAAAACGG TGGTGGACCAGATGCTTCTTGTACTGTTGGTTACTCTACCAACGACTGGGACTCCGGTTTTACTGCT TCCATCAGAATCACCTACCACGGTACTGCTCCATTGTCCTCTTGGGAGTTGTCTTTCACTTTCCCTGC TGGTCAACAGGTTACTCATGGTTGGAACGCTACTTGGAGACAAGACGGTGCTGCTGTTACTGCTAC TCCAATGTCTTGGAACTCTTCCTTGGCTCCTGGTGCTACTGTTGAGGTTGGTTTTAACGGTTCTTGG TCCGGTTCTAACACTCCTCCAACTGACTTCACTTTGAACGGTGAGCCATGTGCTTTGGCTTAA
CcCel9m	GCTGGTACTCACGATTACTCTACTGCTTTGAAGGACTCCATCATTTTCTTCGACGCCAACAAGTGTG GTCCACAAGCTGGTGAAAACAACGTTTTCGATTGGAGAGGTGCTTGTCACACTACTGACGGTTCTG ATGTTGGTGTTGACTTGACTGGTGGTTACCACGATGCTGGTGATCACGTTAAGTTCGGTTTGCCAC AAGGTTACTCCGCTGCTATTCTTGGTTGGTCCCTGTACGAGTTCAAAGAGTCTTTTGACGCTACCGG TAACACCACCAAGATGTTGCAGCAGCTGAAGTACTTTACCGACTACTTCCTGAAGTCTCACCCAAA CTCCACCACCTTCTACTACCAAGTTGGTGAAGGTAACGCTGACCATACTTACTGGGGTGCTCCAGA AGAACAGACTGGTCAAAGACCATCTCTGTACAAGGCTGACCCTTCTTCTCCTGCTTCCGATATTTTG TCTGAGACTTCCGCTGCCTTGACCTTGATGTACTTGAACTACAAGAACATCGACTCCGCCTACGCTA CCAAGTGTTTGAACGCTGCTAAAGAACTGTACGCCATGGGTAAAGCCAATCAAGGTGTTGGTAAC GGTCAGTCCTTCTACCAAGCTACTTCTTTCGGTGATGACTTGGCTTGGGCTGCTACTTGGTTGTACA CTGCTACCAACGACTCCACTTACATCACTGACGCTGAGCAGTTCATCACCTTGGGTAACACTATGAA CGAGAACAAGATGCAGGACAAGTGGACCATGTGTTGGGACGATATGTACGTTCCAGCCGCTTTGA

	GATTGGCTCAGATTACTGGTAAGCAGATCTACAAGGACGCCATCGAGATCAACTTCAACTACTGGA AAACCCAGGTCACCACTACTCCAGGTGGTTTGAAGTGGTTGTCTAACTGGGGTGTTTTGAGATACG CTGCTGCTGAGTCCATGGTCATGTTGGTTTACTGCAAGCAAAACCCAGACCAGTCCTTGTTGGACT TGGCTAAGAAGCAGGTCGACTACATCTTGGGTGATAACCCAGCTAACATGTCCTACATCATCGGTT ACGGTTCCAACTGGTGTATTCACCCACATCACAGAGCTGCTAACGGTTACACTTACGCTAACGGTG ATAACGCCAAGCCAGCTAAGCACTTGTTGACAGGTGCTTTGGTTGGTGGTCCAGACCAAAACGAT AAGTTCTTGGATGACGCCAACCAGTACCAGTACACTGAAGTTGCTTTGGACTACAACGCTGGTTTG GTCGGTGTTTTGGCTGGTGCTATTAAGTTCTTCGGTGGCACCATCGTTAACCCACCAGTTAAGAAG GGTGACCTGAACAACGACACTTTCATCGACGCTATTGACTTGGCCCTGTGCAAGAACTACATCCTG ACTCAGAACGGTAACATCGACAAGAACAACGCCGATATGAACGGTGATGGTTCCATTGATGCTAT CGACTTCTCACTGCTGAAGAAGGCCATCCTGGGTTAA
CaCel	TTGACTTCTGGTTCCGGTGTTACTACTAGATACTGGGACTGTTGTAAGCCTTCCTGTTCTTGGGGTG GTAAGGCTTCTGTTACCAAGCCAGTTAGAACCTGTAAGGCCAACGGTAACACTACCATCGACTCCA ACACTCAGTCTGGTTGTAACGGTGGTTCTTCCTACGTCTGTAACGACCAACAACCATTCACTCAGG GTAACGTCGGTTACGGTTTTGCTGCTGCTTCTATTTCTGGTCAGCCAGAGTCTCAGACTTGTTGTGC TTGTTACGAGATGACCTTCACCAACACTGCTATCTCCGGTCAGAAGATGATCGTTCAGGTCACTAA CACTGGTTCCGACTTGAACGGTAACCACTTCGACTTGATGATTCCAGGTGGTGGTGTCGGTATCTT CAACGGTTGTCAATCTCAATGGGGTGCTCCATCTAACGGTTGGGGTCAAAGATACGGTGGTATTTC TTCCCAGTCCGAATGCAACCAGTTGCCAACTTCTTTGAGAGCCGGTTGTAATTGGAGATTCGGTTG GTTCAAGAACGCTGACAACCCATCCATGAAGTTCACCCAGGTTAGATGCCCAACCATCTTGACTCA AAAGTCCCAGTGTGTTAGAACCCCAGGTCCATAA
CtCel9r	CCAGTTTTTGCTGCTGACTACAATTACGGTGAGGCCTTGCAAAAGGCCATCATGTTCTACGAGTTC CAGATGTCCGGTAAGCTGCCAGACAACATCAGAAACAATTGGAGAGGTGACTCCTGCCTTGGTGA TGGTTCAGATGTTGGTTTGGACTTGACTGGTGGTTGGTTCGATGCTGGTGATCACGTCAAGTTCAA CTTGCCAATGGCTTACACTGCTACCATGTTGGCTTGGGCTGTTTACGAGTACAAGGATGCCTTGCA GAAGTCCGGTCAATTGGGTTACTTGATGGACCAGATTAAGTGGGCCTCCGACTACTTCATTAGATG TCACCCAGAGAAGTACGTCTACTACTACCAGGTTGGTAACGGTGACATGGATCACAGATGGTGGG TTCCAGCTGAGTGTATTGATGTTCAAGCTCCAAGACCATCCTACAAGGTGGACTTGTCTAACCCAG GTTCCACTGTTACTGCTGGTACTGCTGCTGCTTTGGCTGCTACAGCTTTGGTTTTCAAGGGTACTGA TCCAGCTTACGCCGCCTTGTGTATTAGACATGCTGAAGAGTTGTTCGACTTCGCCGAGACTACTAT GTCTGACAAGGGATACACAGCCGCCTTGAACTTCTACACTTCTCACTCTGGATGGTACGACGAATT GTCTTGGGCTGGTGCTTGGATCTACTTGGCTGATGGTGACGAAACCTACTTGGAGAAGGCCGAGA AGTATGTTGACAAGTGGCCAATTGAGTCCCAGACCACTTACATTGCTTACTCTTGGGGTCACTGTT GGGACGATGTTCATTACGGTGCAGCTTTGTTGTTGGCCAAGATCACCAACAAGTCCCTGTACAAAG AGGCCATCGAAAGACACTTGGACTACTGGACTGTTGGTTTCAACGGTCAGAGAGTCAGATACACC CCAAAAGGTTTGGCTCACTTGACTGACTGGGGTGTTTTGAGACATGCTACTACCACTGCTTTCTTG GCCTGTGTTTACTCTGACTGGTCTGAGTGTCCAAGAGAGAAGGCCAACATCTACATTGACTTCGCT AAGAAGCAGGCCGACTACGCTTTGGGTTCTTCTGGTAGATCTTACGTCGTTGGTTTCGGTGTTAAC CCACCACAACATCCACATCACAGAACTGCTCATTCCTCTTGGTGTGACTCTCAGAAGGTTCCAGAAT ACCACAGACACGTCTTGTACGGTGCTTTGGTTGGTGGTCCAGATGCTTCTGATGCTTACGTTGACG ACATCGGTAACTACGTCACTAACGAGGTTGCCTGTGATTACAACGCTGGTTTCGTCGGTTTGCTGG CTAAGATGTACGAAAAGTACGGTGGTAACCCCATTCCAAACTTCATGGCCATCGAGGAAAAGACC AACGAAGAGATCTACGTTGAGGCTACTGCCAACTCCAACAACGGTGTTGAGCTTAAGACCTACCTG TACAACAAGTCTGGTTGGCCAGCTAGAGTTTGCGACAAGTTGTCCTTCAGATACTTCATGGACCTG ACCGAGTACGTTTCCGCTGGTTACAACCCAAACGACATCACCGTTTCCATCATCTACTCTGCTGCTC CAACTGCTAAGATCTCCAAGCCAATTCTGTACGACGCCTCCAAGAACATCTACTACTGTGAGATTG ACCTGTCCGGCACCAAGATTTTCCCAGGTTCTAACTCCGACCACCAGAAAGAGACTCAGTTCAGAA TTCAACCTCCAGCTGGTGCTCCATGGGATAACACTAACGACTTCTCTTACCAGGGTATCAAGAAGA ACGGCGAGGTCGTCAAAGAGATGCCAGTTTACGAAGATGGTGTCCTGATTTTCGGTGTCGAGCCA AATGGTACTGGTCCTGCTACTCCTACTCCAAAGCCATCTGTTAACCCTTCACCTTCACCAACTCCTAC CTCCGACATCCTGTACGGTGATATTAACCTGGACGGTAAGATCAACTCCTCCGACGTTACTCTGTTG AAGAGGTACATCGTGAAGTCCATCGACGTTTTCCCAACTGCTGACCCAGAGAGATCTTTGATTGCC TCTGACGTTAATGGTGACGGTAGAGTGAACTCCACCGACTACTCTTACTTGAAGAGATACGTCCTG AAGATCATCCCAACTATCCCAGGTAACTCCTAA
TrCel45a	TACAAGGCTACTACTACCAGGTACTACGATGGTCAAGAGGGTGCTTGTGGTTGTGGTTCTTCTTCT GGTGCTTTCCCATGGCAGCTTGGTATTGGTAACGGTGTTTACACTGCTGCTGGTTCCCAAGCTTTGT

	TCGATACTGCTGGTGCTTCTTGGTGTGGTGCTGGTTGTGGAAAGTGTTACCAGTTGACTTCCACTG GTCAAGCCCCATGTTCCTCTTGTGGTACTGGTGGTGCTGCTGGACAATCCATTATCGTTATGGTCAC CAACTTGTGCCCAAACAACGGTAACGCTCAATGGTGTCCAGTTGTCGGTGGTACTAATCAGTACGG TTACTCCTACCACTTCGACATCATGGCTCAGAACGAGATCTTCGGTGACAACGTCGTTGTTGACTTC GAGCCAATTGCTTGTCCAGGTCAAGCTGCTTCTGATTGGGGTACTTGTTTGTGTGTTGGTCAGCAA GAGACTGACCCAACTCCAGTTTTGGGTAACGACACTGGTTCTACTCCACCAGGATCTAGTCCTCCA GCTACTTCTTCTTCACCACCTTCCGGTGGTGGTCAACAAACCTTGTATGGTCAATGTGGTGGTGCA GGTTGGACTGGTCCAACTACTTGTCAAGCTCCAGGTACTTGCAAGGTCCAAAACCAGTGGTACTCC CAGTGTTTGCCATAA
TrCel61a (including signal sequence)	ttaacgacaacttgagaagatcaaaaaacaactaattattcgaaacgATGATCCAGAAGCTGTCCAACTTGTTGGT TACTGCTTTGGCTGTTGCCACCGGTGTTGTTGGTCATGGTCACATTAACGACATCGTCATCAACGGT GTTTGGTATCAGGCTTACGACCCAACTACTTTCCCATACGAATCTAACCCACCAATCGTTGTTGGTT GGACTGCTGCTGATTTGGACAACGGTTTCGTTTCCCCAGACGCTTACCAGAATCCAGACATCATCT GTCACAAGAACGCTACCAACGCTAAGGGTCACGCTTCTGTTAAGGCTGGTGACACTATTTTGTTCC AGTGGGTTCCAGTTCCATGGCCACATCCAGGTCCTATCGTTGATTACTTGGCCAACTGTAACGGTG ACTGTGAGACTGTTGACAAGACCACCTTGGAGTTCTTCAAGATCGACGGTGTTGGTTTGTTGTCTG GTGGTGATCCAGGTACTTGGGCTTCCGACGTTTTGATCTCCAACAACAACACTTGGGTCGTCAAGA TCCCAGACAACTTGGCTCCAGGTAACTACGTTTTGAGACACGAGATTATCGCCTTGCACTCTGCTG GTCAAGCTAACGGTGCTCAAAACTACCCACAGTGCTTCAACATTGCTGTTTCTGGTTCCGGTTCCTT GCAACCATCTGGTGTTTTGGGTACTGACTTGTACCACGCTACTGACCCAGGTGTTCTGATCAACATC TACACTTCCCCACTGAACTACATCATCCCAGGTCCAACTGTTGTTTCCGGTTTGCCAACTTCTGTTGC TCAAGGTTCTTCTGCTGCTACTGCAACTGCTTCTGCTACTGTTCCAGGTGGTGGTTCTGGTCCAACT TCCAGAACTACTACTACCGCTAGAACTACTCAGGCTTCTTCTAGACCATCTTCCACTCCTCCAGCTAC TACTTCTGCTCCAGCTGGTGGTCCTACTCAAACCTTGTATGGTCAATGTGGTGGATCCGGTTACTCC GGTCCAACTAGATGTGCTCCACCAGCTACATGTTCCACTTTGAACCCTTACTACGCCCAGTGCTTGA ACTAAggccgccagctttctagaacaaaaactcatctcagaagaggatctga
OsCbh(-105)	ATGATCCAGAAGCTGTCCAACTTGTTGGTTACTGCTTTGGCTGTTGCCACCGGTGTTGTTGGTCATG GTCACATTAACGACATCGTCATCAACGGTGTTTGGTATCAGGCTTACGACCCAACTACTTTCCCATA CGAATCTAACCCACCAATCGTTGTTGGTTGGACTGCTGCTGATTTGGACAACGGTTTCGTTTCCCCA GACGCTTACCAGAATCCAGACATCATCTGTCACAAGAACGCTACCAACGCTAAGGGTCACGCTTCT GTTAAGGCTGGTGACACTATTTTGTTCCAGTGGGTTCCAGTTCCATGGCCACATCCAGGTCCTATC GTTGATTACTTGGCCAACTGTAACGGTGACTGTGAGACTGTTGACAAGACCACCTTGGAGTTCTTC AAGATCGACGGTGTTGGTTTGTTGTCTGGTGGTGATCCAGGTACTTGGGCTTCCGACGTTTTGATC TCCAACAACAACACTTGGGTCGTCAAGATCCCAGACAACTTGGCTCCAGGTAACTACGTTTTGAGA CACGAGATTATCGCCTTGCACTCTGCTGGTCAAGCTAACGGTGCTCAAAACTACCCACAGTGCTTC AACATTGCTGTTTCTGGTTCCGGTTCCTTGCAACCATCTGGTGTTTTGGGTACTGACTTGTACCACG CTACTGACCCAGGTGTTCTGATCAACATCTACACTTCCCCACTGAACTACATCATCCCAGGTCCAAC TGTTGTTTCCGGTTTGCCAACTTCTGTTGCTCAAGGTTCTTCTGCTGCTACTGCAACTGCTTCTGCTA CTGTTCCAGGTGGTGGTTCTGGTCCAACTTCCAGAACTACTACTACCGCTAGAACTACTCAGGCTTC TTCTAGACCATCTTCCACTCCTCCAGCTACTACTTCTGCTCCAGCTGGTGGTCCTACTCAAACCTTGT ATGGTCAATGTGGTGGATCCGGTTACTCCGGTCCAACTAGATGTGCTCCACCAGCTACATGTTCCA CTTTGAACCCTTACTACGCCCAGTGCTTGAACTAA

Table S2: PCR primers for each enzyme/plasmid used in this study, presented with the annealing temperature for the sections of primers annealing to their original DNA templates. Uppercase lettering represents annealing regions of primers, lowercase lettering represents 5' overhang regions for Gibson assembly.

Gene/ construct		Sequence (5'-3')	Annealing temperature/ °C
pPICZα	Forward	GTTTGTAGCCTTAGACATGACTG	59.8
	Reverse	AGCTTCAGCCTCTCTTTTCT	59.5

TrCel45a	Forward	agaagaaggggtatctctcgagaaaagagaggctgaagctTACAAGGCTACTACTACCAGGTACTAC	59.4
	Reverse	caacttgaactgaggaacagtcatgtctaaggctacaaacttaTGGCAAACACTGGGAG	61.6
CaCel	Forward	agaagaaggggtatctctcgagaaaagagaggctgaagctTTGACTTCTGGTTCCGGT	61.3
	Reverse	caacttgaactgaggaacagtcatgtctaaggctacaaacttaTGGACCTGGGGTTCTAA	61.2
CcCel9m	Forward	agaagaaggggtatctctcgagaaaagagaggctgaagctGCTGGTACTCACGATTACTCTACTG	62.3
	Reverse	caacttgaactgaggaacagtcatgtctaaggctacaaacttaACCCAGGATGGCCTT	62.3
CtCel9r	Forward	agaagaaggggtatctctcgagaaaagagaggctgaagctCCAGTTTTTGCTGCTGACTA	61.1
	Reverse	caacttgaactgaggaacagtcatgtctaaggctacaaacttaGGAGTTACCTGGGATAGTTG	60.4
TfCel9a	Forward	agaagaaggggtatctctcgagaaaagagaggctgaagctGAACCAGCTTTCAATTACGC	61.7
	Reverse	caacttgaactgaggaacagtcatgtctaaggctacaaacttaAGCCAAAGCACATGG	60.4
TrCel61a	Forward	TTAACGACAACTTGAGAAGATCA	60.8
	Reverse	TCAGATCCTCTTCTGAGATGAG	60.7
OsCbh(-105)	Forward	agaagaaggggtatctctcgagaaaagagaggctgaagctACTTCCGCTAGAACTACTAC	60.0
	Reverse	caacttgaactgaggaacagtcatgtctaaggctacaaacttaGAATGGTGGGTTAGC	58.0

Table S3: Biomass composition and yield as well as component recovery and removal of untreated and pretreated Miscanthus.

Conditions	Glucan (w/w %)	Xylan (w/w %)	Lignin (w/w %)	Others (w/w %)	Biomass/pulp yield (w/w %)	Glucan recovered in biomass/pulp (%)	Xylan removal (%)	Lignin removal (%)
Untreated	36.4 ± 0.4	19.5 ± 0.2	22.1 ± 0.5	22.0 ± 0.2	100	N.a.	N.a.	N.a.
[H_3PO_4] [20 min]	34.9 ± 0.3	5.3 ± 0.0	20.9 ± 0.4	27.4 ± 0.1	88.5 ± 2.1	95.9 ± 0.9	76.0 ± 0.1	16.4 ± 1.6
[H_3PO_4] [45 min]	34.9 ± 0.1	1.9 ± 0.1	21.9 ± 1.2	21.5 ± 1.0	80.1 ± 0.5	95.9 ± 0.4	92.2 ± 0.3	20.8 ± 4.3
[SE] + [H_3PO_4] [20 min]	39.3 ± 0.5	2.5 ± 0.1	10.4 ± 1.8	39.4 ± 1.2	91.7 ± 0.2	97.4 ± 1.3	65.8 ± 1.1	64.1 ± 6.3
[SE] + [H_3PO_4] [45 min]	38.1 ± 1.5	1.5 ± 0.0	8.9 ± 2.1	37.6 ± 0.8	86.3 ± 1.0	94.3 ± 3.6	81.3 ± 0.4	71.1 ± 6.7
[C_2mim][OAc]	31.4 ± 0.2	2.3 ± 0.2	5.8 ± 0.4	7.0 ± 0.0	49.9 ± 1.1	86.2 ± 0.7	88.0 ± 0.8	73.7 ± 1.9

Lignin is total lignin (acid insoluble and acid soluble lignin). Others can include water and ethanol extractives, ash and other solids.
Biomass yield (%) = milligram of DM residual solids recovered after pretreatment/100 mg DM untreated biomass.
Glucan recovery in biomass/pulp (%) = (Glucan content in pretreated solids/Total glucan in initial biomass) x 100.
Component removal (%) = 100 - component recovery (%) in pretreated solids.
Component recovery (%) = (Component content in pretreated solids x biomass recovered)/Total component in initial biomass.
± indicates Standard error; N.a, not applicable.

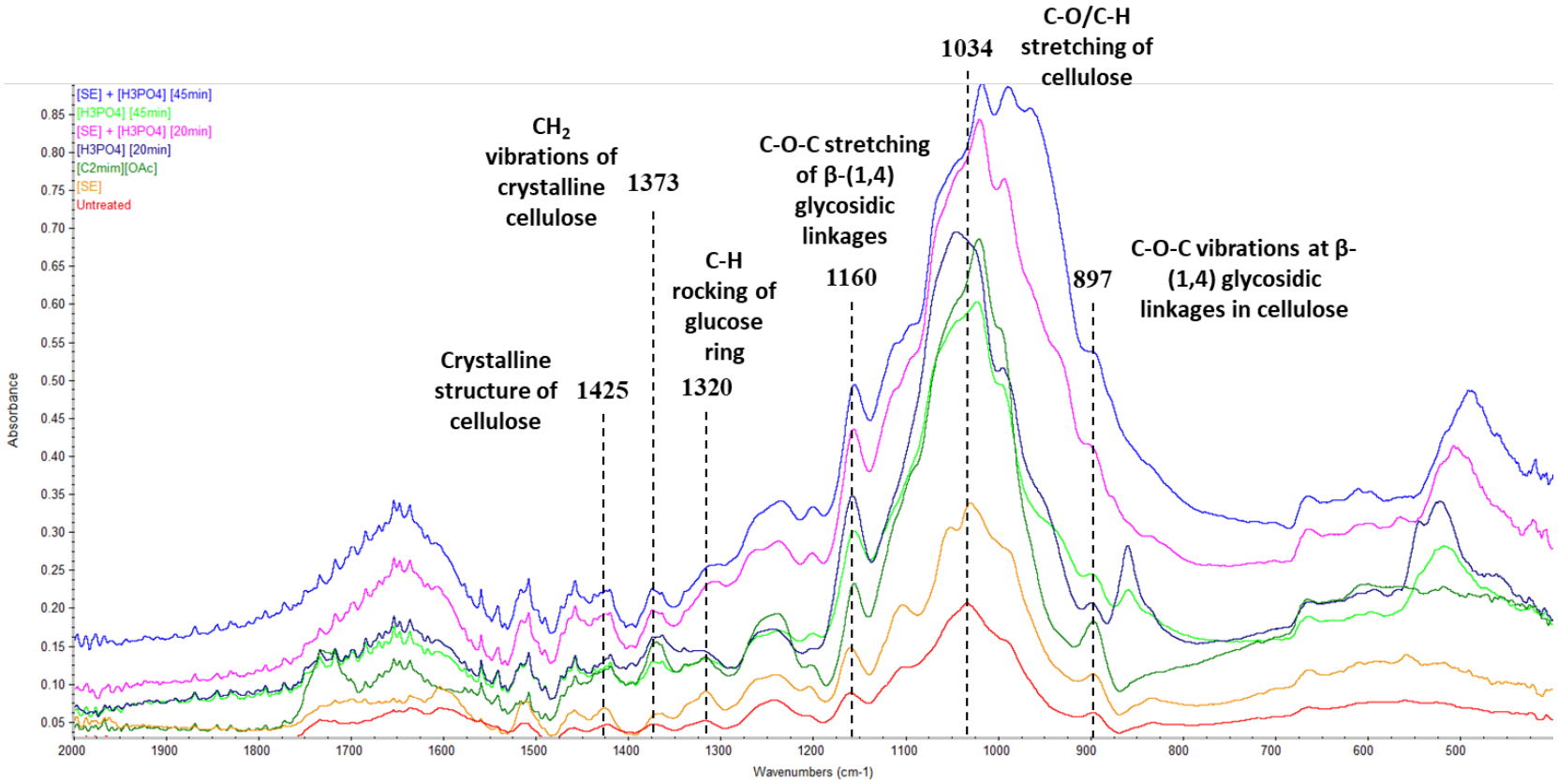

Fig. S1: FTIR analysis of native (untreated) and pretreated Miscanthus samples used in this study, highlighting features associated with the crystallinity of cellulose.

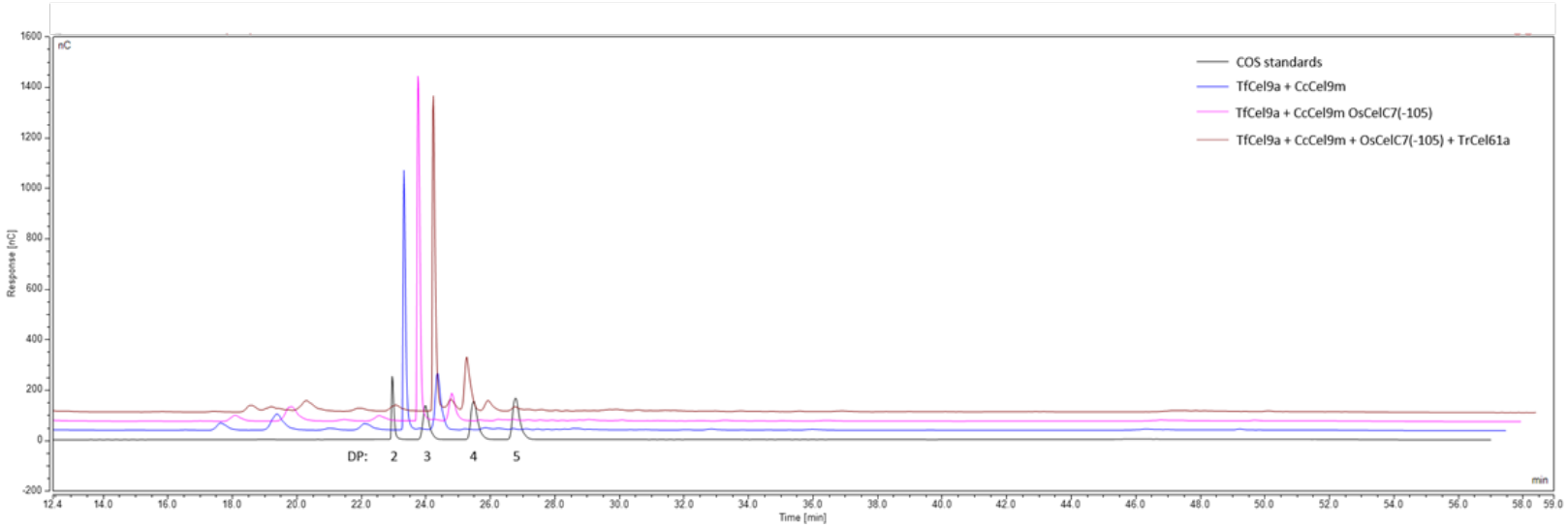

Fig. S2: HPAEC chromatogram of COS formation from Miscanthus Mx2779 following a 45 minute H_3PO_4 swelling pre-treatment step for enzyme combinations of TfCel9a (150 U/g solids), CcCel9m (150 U/g solids), OsCelC7(-105) (150 U/g solids) and TrCel61a (5 mg/g solids, 10 mM ascorbic acid). Positions of DP:2-5 COS are labelled.