Carbohydrate degradation mechanisms and compounds from pretreated biomass

The formation of inhibitors during pretreatment of lignocellulosic feedstocks is a persistent problem, and notably the compounds that retard enzymatic cellulose conversion represent an obstacle for achieving optimal enzymatic productivity and high glucose yields. Compounds with many chemical functionalities are formed during biomass pretreatment, which gives possibilities for various chemical reactions to take place and hence formation of many new potential inhibitor compounds. This somehow overlooked contemplation formed the basis for the main hypothesis investigated in this work:

Hypothesis 1) Liquors from biomass pretreatment contain an array of hitherto unidentified cellulase* inhibitors that are believed to be reaction products from carbohydrate degradation.

(*cellulases include endo-cellulases, cellobiohydrolases, LPMO, and beta-glucosidase enzyme activities)

Furthermore the two following two hypotheses were tested.

Hypothesis 2). Formation of these inhibitor compounds can be prevented by protection of reactive chemical functionalities as revealed from their mechanisms for formation.

Hypothesis 3) Process parameters influence the amount and type of reaction products (from hypothesis 1) that are formed and in turn change inhibition.

In order to point out potent cellulase inhibitors, a solvent extraction based fractionation method was developed to separate compounds in liquid from pilot plant hydrothermal pretreatment of wheat straw. Via 2-butanone extraction a group of potent cellulase inhibitors were identified with LC-MS/MS to be oligophenolic compounds. 26 of the compounds were new and by considering the reaction mechanisms and synthesis routes for their formation it was revealed that xylose was heavily involved in their formation. The new oligophenolic cellulase inhibitors were suggested to be formed during hydrothermal pretreatment by xylose self-condensation reactions involving aldol condensations, 1,4 additions to α,β unsaturated carbonyl compounds, 3-keto acid decarboxylations and oxidations. In addition xylose reactions with phenolic lignin components were suggested.

The identification of the central role of xylose in the reaction routes for oligophenolic inhibitor formation led to the solution to protect the reactive anomeric center in xylose. Protection of the anomeric center in in situ generated xylose with ethylene glycol monobutyl ether, during pretreatment of wheat straw, reduced the level of oligophenolic compounds with 73 % compared to the original pretreatment and 41 % compared to the control.

When pretreatment severity was increased the amount of xylooligosaccharides decreased whereas the amount of oligophenolic compounds increased. No new degradation compounds were formed although the profile of the oligophenolic inhibitors changed. New dipentoses with hydroxylated oxane bicyclic moieties and feruloylated tripentoses are suggested also to play a role in inhibition, because LC-MS/MS analysis revealed the presence of these components in the liquid from hydrothermal pretreated wheat straw after enzymatic treatment.

It was found that formation of the oligophenolic degradation compounds were common across biomass sources as sugar cane bagasse and oil palm empty fruit bunches. These findings were in line with that the oligophenolic compounds arise from reactions involving xylose from hemicellulose in the biomass. Even though oligophenolic degradation compounds were common across biomasses, variations were found in biomass structural elements that were released during pretreatment. Pentoseoligosaccharides from sugar cane bagasse had a more acetylated substitution pattern than wheat straw, and in oil palm empty fruit bunches 4-hydroxybenzoic acid was identified to be a variation from a lignin structural elements released during pretreatment.

In conclusion it was found that the reactions taking place during pretreatment of biomass are complex and involve both degradation compounds and biomass structural elements. The present work has shed some light over the reactions and from this new insight a new type of pretreatment with anomeric protection was proposed and tested. The results open up for implementation of new types of pretreatments that hinder monosaccharide degradation to inhibitor compounds in lignocellulosic biomass processing.

General information
Publication status: Published
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering
Contributors: Rasmussen, H.
Number of pages: 152
Publication date: 2016

Publication information
Publisher: Technical University of Denmark, Department of Chemical and Biochemical Engineering
Original language: English
Electronic versions:
Carbohydrate_degradation_mechanisms.pdf
Source: PublicationPreSubmission
Source ID: 127442012