New Method for Fast Detection of Improved Degradability in Genetically Modified Plants

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Abstract

Plant genetic engineering is considered a potential approach to reduce costs for biofuel production from lignocellulosic material. The most successful efforts to date have focused on the modification of lignin quantity and/or quality, in an effort to obviate the need for expensive pretreatment processes. Here we report a method for rapid detection of improved degradability in genetically modified plants that vary in lignin content and/or composition. For this purpose, only 50 mg of ground material is needed for liquid hot water pretreatment, and the method allows the pretreatment of at least 500 samples per day. Enzyme hydrolysis in the presence of commercial cellulases and β-glucosidase is performed in a final volume of 1 mL for 30 min, at 50 °C, and pH 4.8. The amount of glucose liberated is analyzed via a microplate assay. Using this approach, we have been able to rapidly and reproducibly identify genetically modified plants with improved biodegradability.

Introduction

The ability to control cell-wall composition without compromising plant performance is a challenge and key objective of bioenergy crop improvement. Identification of successful plant modifications entails metabolic profiling methods applied to greenhouse- and field-grown wild-type and transgenic plants. While profiling gives insights on effects of the up- or down-regulation of the main enzymes known to impact lignin synthesis, comparison of changes in genetics to changes in bioprocessing properties that enhance the production of ethanol is needed.

Methods

Composition of poplar samples was analyzed by using NREL standard analysis procedures. Monomeric sugars were analyzed by HPLC after acid hydrolysis, and then used to calculate polysaccharide composition.

Pretreatment was carried out by pressure cooking 50 mg cellulosic material in water at 200 °C for 10 min (Kim et al., 2009). The samples are then cooled, commercial cellulase at 50 FPU/g glucan (Spezyme CP, Genencor, Palo Alto, CA) and β-glucosidase at 200 CBU/g glucan (Novo Nordisk, Denmark) are added, and hydrolysis carried out for 30 min at 50 °C and pH 4.8. The liquid is assayed for the amount of glucose formed using a commercial kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland).

All the experiments were done in triplicate and error bars represent 95% CI.

Project Objectives

1. Develop a method for rapid detection of glucose in plant extracts.

2. Identify lignin-modified transgenic plants with improved hydrolysis.

Method for fast detection of hydrolysis in plants with genetically modified lignin

Sample preparation

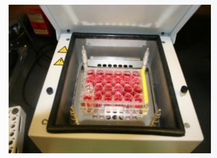
50 mg of ground plant material is transferred to small tube reactors (length of 2.8 inches, outside diameter of 0.3 inch, and wall thickness of 0.035 inch), and 1.5 mL water is added

Pretreatment

10 min and 30 sec

Enzyme Hydrolysis

30 min



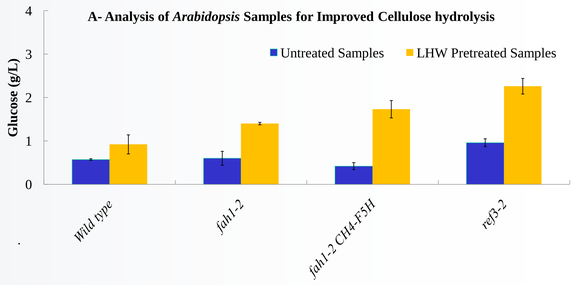
Glucose detection

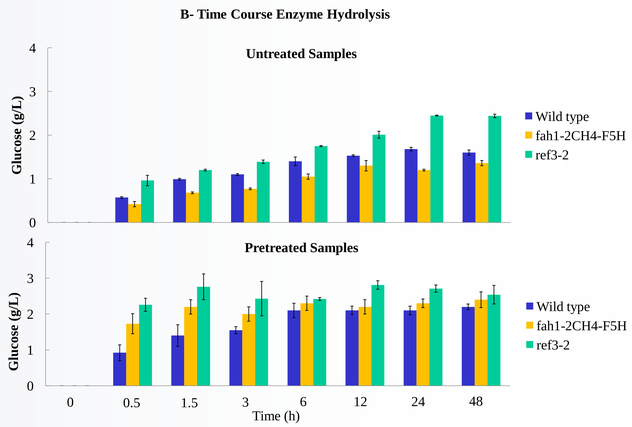
20 min

Elapsed time (min) 0 30 60 90

Results

A) *Arabidopsis*

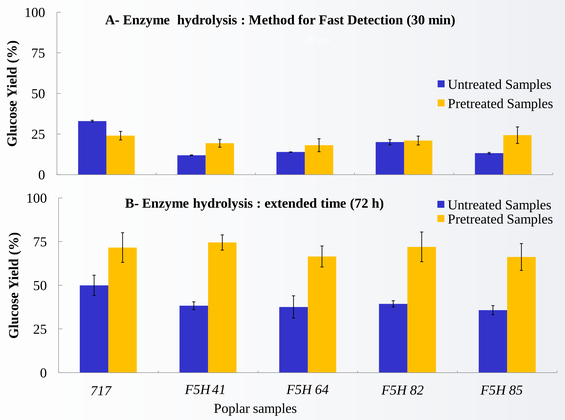




B) *Populus*

Table 1. Composition of poplar (wild type and genetically Modified) by cellulosic biomass compositional analysis.

Poplar Samples	Glucan (%)	Xylan (%)	Arabinan (%)	Acetyl (%)	Total Lignin (%)
Wild type 717 1B4	41.4	16.2	2.7	6.4	22.1
F5H 41	40.6	18.5	3.0	5.6	24.7
F5H 64	41.3	19.3	3.0	6.0	26.0
F5H 82	41.4	17.7	1.75	5.5	24.3
F5H 85	42.3	19.6	3.1	5.9	24.0



Conclusions

We have:

1) developed a method for fast detection of improved hydrolysis in lignin-modified transgenic plants, which rapidly and reproducibly identify genetically modified plants that have improved biodegradability; and

2) shown that extended hydrolysis times (48 to 72 h) have resulted in conversions approaching 80% for liquid hot water pretreated poplar *Populus*.

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Acknowledgements

This work is supported by DOE BES Project 0012846: Manipulation of Lignin Biosynthesis to Maximize Ethanol Production from *Populus* Feedstocks, DOE grant # DE-FG36-04G01417, #GO12O26-174, a grant from the Energy Center, Discovery Park, Purdue University, and USDA IFAFS contract # 00-52104-9663.