SCREEN AND IDENTIFY SUITABLE PLANT FEEDSTOCKS FOR LARGE SCALE PRE-TREATMENTS TO PRODUCE HIGH YIELD SUGAR AND HIGH QUALITY LIGNIN

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EXECUTIVE SUMMARY

Biomass recalcitrance, a collective term describing the resistances of biomass material toward mechanical and/or biochemical deconstructions, is the key barrier hindering the development of an economically viable biomass conversion process. Despite the larger abundance, softwood and its forest residues are still not an economically viable feedstock for biofuel production. The feedstock collection, transportation and processing steps all contribute significantly to the overall cost. One effective means of reducing feedstock cost and subsequent conversion cost is to select biomass with high amount of sugars and low recalcitrance toward deconstruction to release sugar.

Our work carried out in the last two years has clearly demonstrated that there is a significant variation in biomass recalcitrance among different Douglas-fir families. A parameter "recalcitrance factor" is introduced to quantify the level of biomass recalcitrance toward sugar production from different Douglas-fir families. The goal of our research is to develop and implement a selective feedstock breeding methodology to identify and produce "ideal" softwood biomass to maximize sugar yield and reduce conversion (pretreatment and hydrolysis) cost.

INTRODUCTION

Biomass recalcitrance is a major bottleneck in the development of an economically viable process to convert woody biomass into fuels and other valuable chemicals. Selective breeding of trees with low recalcitrance toward biofuel conversion could help significantly reduce the cost of biofuel production, but such efforts would require a greater understanding of the nature of variations in the biomass recalcitrance of softwood species. The complexity of biomass recalcitrance, however, hinders research into determining the viability of breeding programs aimed to improve the recalcitrance of softwoods.

To allow for the screening of potential Douglas-fir feedstock sources, a "multi-level" method was developed to determine biomass recalcitrance at three levels: chemical composition, pretreatment yield, and sugar release from the enzymatic hydrolysis. This method was first tested on a population of 150 trees to measure the variability of parameters and to refine the method. A second population of approximately 300 trees was then tested and analyzed to estimate the genetic parameters associated with biomass recalcitrance and to further understanding of the underlying physical and genetic factors that influence biomass recalcitrance.

TASK 1: ESTABLISH AND TEST A METHOD TO SCREEN BIOMASS RECALCITRANCE OF DOUGLAS-FIR TREES

OBJECTIVE

This task was intended to provide a foundation of methods for future research into the variation and heritability of the biomass recalcitrance of Douglas-fir.

METHODOLOGY

Due to the complexity of the nature of biomass recalcitrance, it is recognized that a single parameter, for example either chemical composition or hydrolysis yield, does not provide a realizable predication of the overall recalcitrance of biomass material. To obtain an aggregated understanding of softwood biomass recalcitrance, a three-step screening procedure was applied based on previous high-throughput analysis procedures (Studer, 2010; Gao, 2013; Ju, 2013). The first step of the process is to determine the chemical composition of biomass material in terms of three components: klason lignin, holocellulose, and extractives. Lignin is considered to be a main component of biomass recalcitrance, hindering both the biomass pretreatment and enzymatic hydrolysis. Holocellulose is determined to represent the total carbohydrate content of the material. In the second step, pretreatment was carried out on each sample, with a solid-liquid separation and washing step. The yield of solid substrates from pretreatment and the holocellulose of this pretreated biomass were measured. In the third step, the pretreated solid substrates were subjected to enzymatic hydrolysis to measure the end-point yield of glucose sugars released into solution. These three steps allow for biomass recalcitrance to be determined considering all three levels of the chemical composition, effect of pretreatment, and the sugar yield of enzymatic hydrolysis.

RESULTS

Considerable variation was found in all traits studied using the method developed. It was also observed that, in general, trees did not follow any major trend between any particular factor and overall degree of recalcitrance (i.e. while a high lignin content is often considered an indicator of high recalcitrance, it is not necessarily the case here), and each tree can demonstrate differing susceptibility to different pretreatment methods. This serves to demonstrate the complexity of the nature of biomass recalcitrance and the advantage of examining multiple factors when screening for and exploring recalcitrance of softwoods.

In order to quantitatively compare the recalcitrance between samples based on the three levels analysis in this study, a “recalcitrance factor” was introduced to measure the overall recalcitrance relevant to biomass subjected to a complete biorefinery process. This factor represents the fraction of raw biomass fed to pretreatment that is ultimately recovered as glucose after the enzymatic hydrolysis process; it does not include carbohydrates solubilized by the pretreatment process. The calculation for this recalcitrance factor can be determined by tracking the total mass fed to a process and the resulting yield. In contrast to the enzymatic hydrolysis yield, which only reports the conversion from the hydrolysis process, the overall recalcitrance factor yield additionally takes into account biomass lost in the liquid fraction of pretreatment and the carbohydrate content of the biomass. This recalcitrance factor is a more practical value for screening biomass recalcitrance of potential feedstocks because it best represents the overall yield of product in a biorefinery. Figure FSD-1.1 shows the recalcitrance factor of the most and least recalcitrant samples using two pretreatment methods (dilute acid and alkaline peroxide).

CONCLUSIONS/DISCUSSION

A three-level (biomass composition, pretreatment yield, hydrolysability) biomass recalcitrance screening method was designed to gather an aggregated understanding of the variation in recalcitrance of different Douglas-fir tree samples. This measurement of recalcitrance allows for the chemical composition and multiple traits influencing recalcitrance to be considered, or the overall recalcitrance to be determined using a combined “recalcitrance factor”. This method was then applied to a test group of 150 Douglas-fir trees to demonstrate the capability for observing variations and potential applicability of the different levels of recalcitrance tested.

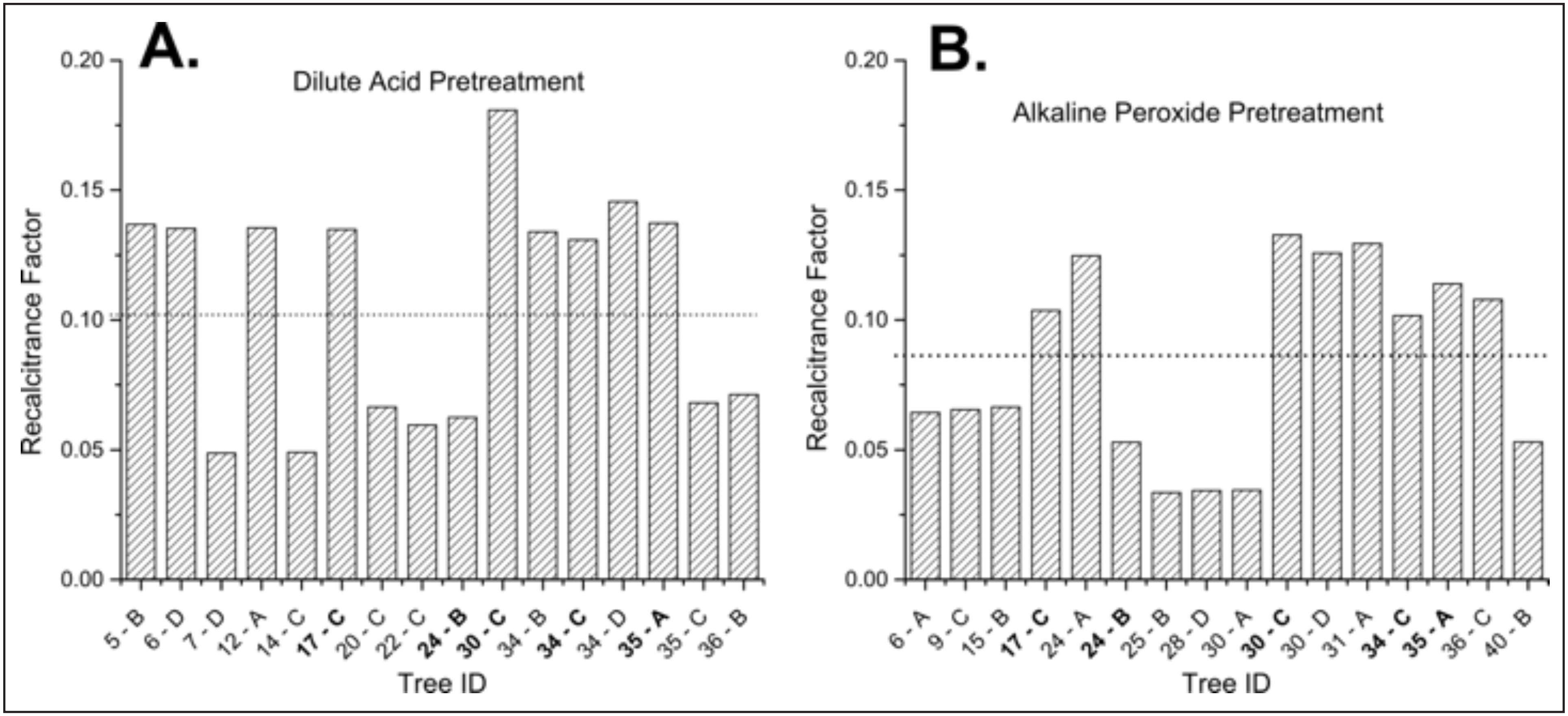

Figure FSD-1.1. Recalcitrance factors of the most and least recalcitrant trees using dilute acid (A) and alkaline peroxide (B) pretreatments. ID numbers of trees present in both graphs are highlighted in bold.

TASK 2: DETERMINE AN ESTIMATE OF THE GENETIC PARAMETERS AND POSSIBILITY FOR IMPROVEMENT OF THE BIOMASS RECALCITRANCE OF DOUGLAS-FIR FEEDSTOCK

OBJECTIVE

This test aims to further quantify variations in Douglas-fir trees and estimate the genetic parameters associated with traits associated with recalcitrance. This goal is intended to lay groundwork for the potential improvement of Douglas-fir feedstock through the application of tree improvement methods.

METHODOLOGY

A second population of over 300 trees was tested using the methods established in Task 1. A statistical analysis approach was utilized to estimate the heritability, genetic gain, and genetic correlation coefficients for the recalcitrance traits observed.

RESULTS

The heritability estimates (narrow-sense), along with the associated variance components, calculated are shown in Table FSD-1.1. It is clear that the pretreatment yield appears the most heritable trait observed in this study, while the heritability of pretreated holocellulose shows the least considerable effect. The hydrolysis yield shows the greatest influence on the recalcitrance factor, and as such, the heritability estimates are closely linked and similar. The heritability estimate for the hydrolysis yield is roughly 0.5, leading to a similar heritability for the recalcitrance factor of 0.443.

Genetic gains estimated in this study were also good. The highest predicted gain for the recalcitrance factor is 34.72%, a promising result. This value is associated with a predicted gain in hydrolysis yield of 40.63%.

Table FSD-1.1. Heritability estimates generated in Task 2

Trait	Heritability	Error
Density	0.3145166	0.2188238
Pretreatment Yield	0.7667844	0.1803037
Pretreated Holocellulose	0.1853049	0.1903503
Hydrolysis Yield	0.4957738	0.1420026
Recalcitrance Factor	0.4428915	0.1356903

The heritability of the recalcitrance factor is lower than that of the hydrolysis yield, which appears to arise due to the negative correlation between pretreatment and hydrolysis yield. This is somewhat expected, however, as traits leading to higher susceptibility to pretreatment (resulting in lower solids yield) result in a greater degree of biomass deconstruction and higher susceptibility to the hydrolysis process. Since the variation of the hydrolysis yield outweighs that of the pretreatment yield, and thus has a higher influence on the resulting recalcitrance factor, the correlation coefficient shown between hydrolysis yield and recalcitrance factor is high as expected. For the purpose of tree breeding, this indicates that it is more important to select trees with greater yield from hydrolysis than those with high yield from pretreatment; thus, despite the higher heritability of pretreatment yield, the considerably higher potential gains from hydrolysis should be preferentially utilized in order to maximize the improvement of recalcitrance factor.

CONCLUSIONS/DISCUSSION

The presence of significant heritability shows good promise for using tree breeding techniques for improving the biomass recalcitrance of forestry resources. Considering the amount of variation, heritability, and the predicted gains shown for all three parameters representative of recalcitrance tested here, it is clear that efforts to improve potential yield of carbohydrates from bioconversion should focus on improving the overall recalcitrance factor, primarily through improvement of the hydrolyzability of pretreated substrate. These metrics represent the greatest overall utility for a program aimed at improving biochemical conversion potential due to their high variability and genetic gains.

In addition, these correlations also provide some insight into the underlying factors that drive biomass recalcitrance, a subject that has seen little agreement between studies in the past (Foston, 2012). The genetic correlation coefficients observed in this study imply a relationship between the density of biomass and the yield of sugars from hydrolysis, but opposite of what would be expected based on conventional explanations of recalcitrance. In addition, we found that the holocellulose of samples after pretreatment showed no significant heritability, unlike that of the raw biomass. Both of these observations lead to interesting questions regarding the nature of the recalcitrance of softwoods, and will provide a good foundation for future research investigating the nature of how biomass recalcitrance varies.

NARA OUTPUTS

PUBLICATIONS

Geleynse, S., Alvarez-Vasco, C., Garcia, K., Jayawickrama, K., Trappe, M., & Zhang, X. (2014). A multi-level analysis approach to measuring variations in biomass recalcitrance of Douglas fir tree samples. BioEnerg. Res., 7, 1411-1420. doi:10.1007/s12155-014-9483-z

PRESENTATIONS

Quantifying Variations in the Biomass Recalcitrance of Douglas Fir. American Institute of Chemical Engineers, 2014 Annual Meeting: Biomass Characterization, Pretreatment, and Fractionation Session. November 17, 2014

Investigations into the Chemical and Structural Factors Contributing to Large Differences in the Biomass Recalcitrance of Douglas Fir Trees. American Institute of Chemical Engineers, 2014 Annual Meeting: Recalcitrance of Woody Biomass Session. November 17, 2014

NARA OUTCOMES

While the variation and genetic parameters associated with the properties of Douglas-Fir has been explored extensively in the past (Howe, 2006), this research is the first to explore traits associated with recalcitrance of biomass. This knowledge helps pave the way to the potential development of a tree improvement program that could help the forest products industry generate more useful feedstocks for the generation of biofuels and bioproducts.

FUTURE DEVELOPMENT

Provided this groundwork regarding the genetic parameters that control biomass recalcitrance of Douglas-Fir, a tree improvement program or cooperative could further utilize the methods and data generated here to begin to improve biomass recalcitrance of their trees. While some additional screening and analysis would be required before complete implementation of the concept, the data we generated demonstrates that the process is feasible and provides direction for carrying it out.

These data also provide some important insights into the nature of the variations in recalcitrance of Douglas-fir trees. The search for a more complete and fundamental understanding of biomass recalcitrance is a considerable research effort, which is aided by further understanding of how traits relevant to recalcitrance vary and how they are controlled. Further research extending from this work will be carried out in order to better understand the underlying factors behind biomass recalcitrance and the results we observed.

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