Abstract

Cellobiose Dehydrogenase (CDH) is produced by many white-rot fungi in response to growth on cellulosic biomass. Although its role in biomass degradation is unclear, it is well known to transfer electrons from cellobiose and short cello-oligomers to various acceptors, such as metal ions and organic cofactors. This electron transfer is mediated through both a heme group and a flavin group bound to the enzyme. Glucose is not oxidized and oxygen cannot be used as an acceptor. Additionally, CDH can transfer electrons to several artificial acceptors, including Dichlorophenolindophenol (DCIP). This dye changes color from blue to clear when reduced, allowing CDH activity to be measured directly. We have utilized CDH purified and characterized from *Phanerochaete chrysosporium* to quantify cellobiose equivalents produced during hydrolysis of cellulosic biomass with cellulase enzymes. This technique allows direct measurement of cellulase activity on real world substrates without the requirements of reducing sugar determination by HPLC or traditional colorimetric methods.

Introduction

Cellobiose dehydrogenase (CDH), despite its nomenclature, is not directly involved in cellulose degradation. Its main function seems to be oxidation of cellobiose to cellobionic acid with the generation of two electrons. The role of CDH in biomass degradation is not well understood, and several theories have been proposed. What is known is that CDH is produced mainly by white-rot fungi (lignin degraders) during biomass degradation. It is a rare example of a secreted fungal protein containing both a heme and a flavin group. It uses a variety of electron acceptors, including Fe^{+3}, Cu^{+2}, and some organic compounds, but it does not use molecular oxygen. It generates free radicals during cellobiose oxidation and these may be involved in the chemical hydrolysis of cellulose, hemicellulose, and/or lignin. CDH is produced early in cellulase induction, but production drops off with increasing cellobiose levels. It is prone to substrate (cellobiose) inhibition, and this may serve as a regulatory mechanism to keep CDH from converting too much cellobiose to cellobionic acid, which would result in an energy loss for the fungus. CDH clearly plays a role in mediation of electron transfer between cellobiose and an acceptor. This acceptor could potentially be lignin and CDH may have a role in dissociating cellulose from lignin. It may also have a role in decrystallizing recalcitrant cellulose through radical formation. Enzymatic lignin degradation by peroxidases and laccases has been looked at extensively and although we are currently exploring the action of a putative lignin depolymerase enzyme, there has not been an in-depth study on the role of CDH as an accessory enzyme in biomass conversion.

Materials

Reagents- Dichloroindophenol (Sigma-Aldrich) and used as an electron acceptor from CDH at a stock concentration of 0.42 mM in distilled water.

62 mM glycine/citrate/phosphate buffer was used to assay the pH range of the enzyme.

Cellulose digestion assays were carried out in 100 mM acetate buffer, pH 5.0.

Filter Paper Assays were carried out in 50 mM Citrate buffer, pH 4.8

Cellobiose was ACS reagent grade from Sigma-Aldrich

Enzymes- E1 Endoglucanase was purified by standard methods from a recombinant *E. coli* strain producing the catalytic domain only. Stock concentration was 280 μg/mL

CBHI Cellobiohydrolase was purified by standard methods from a commercial *Trichoderma reesei* cellulase preparation. Stock concentration was 400 μg/mL

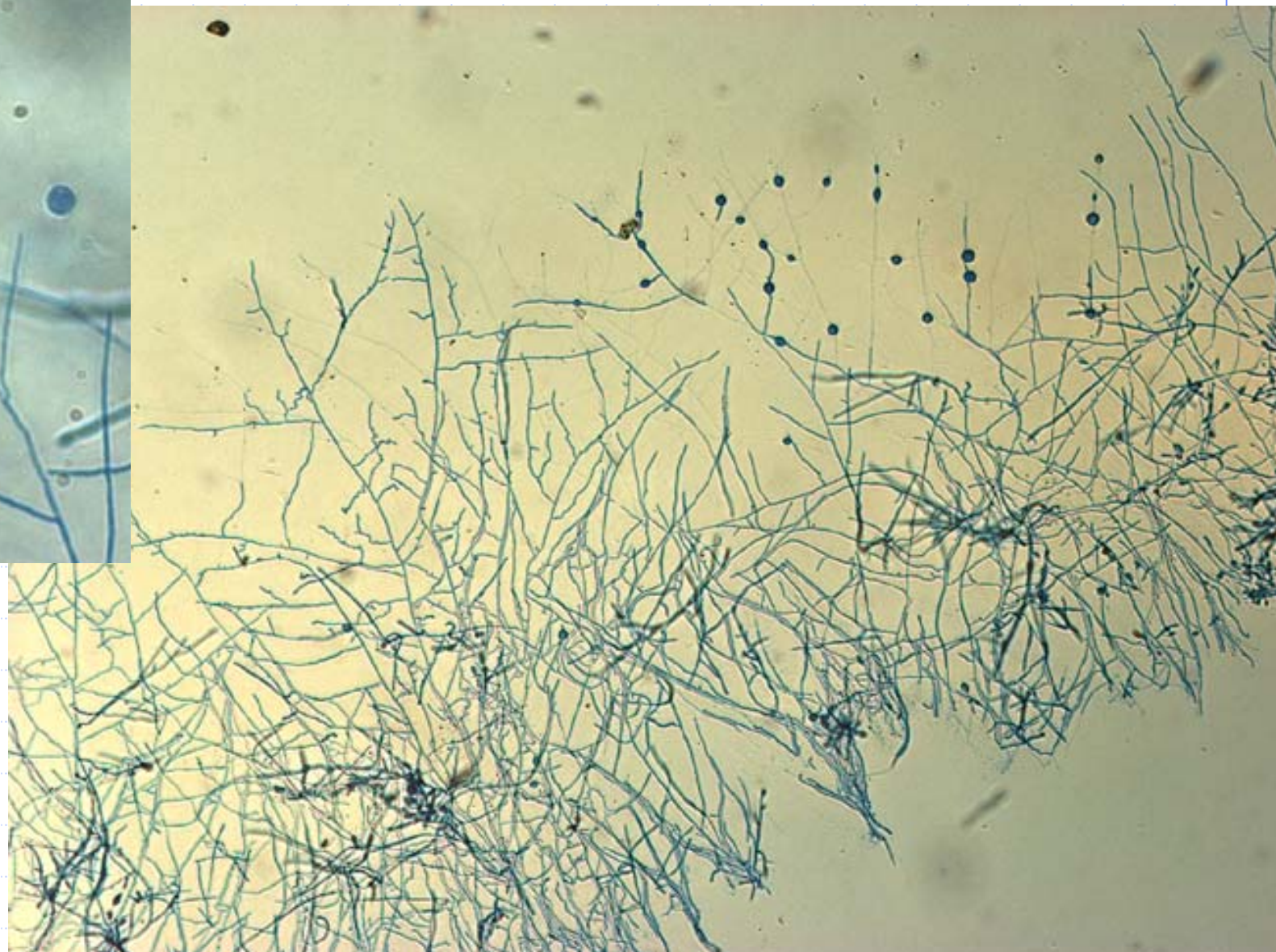
Cellobiose Dehydrogenase was purified from a 10L culture of *Phanerochaete chrysosporium* Sc26 and used at a stock concentration of 310 μg/mL

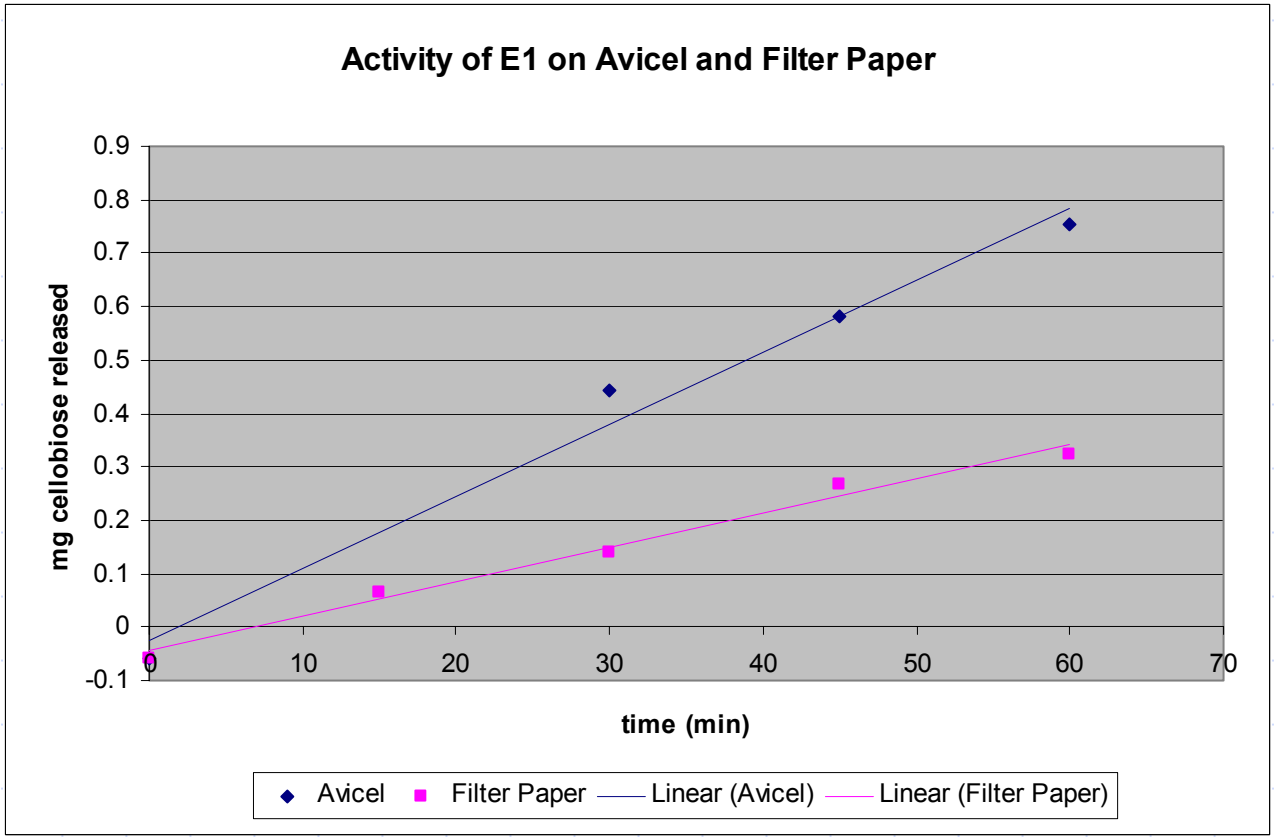
Spezyme (Genencor, Inc.) was desalted and used at a stock activity of 4.25 FPU/mL

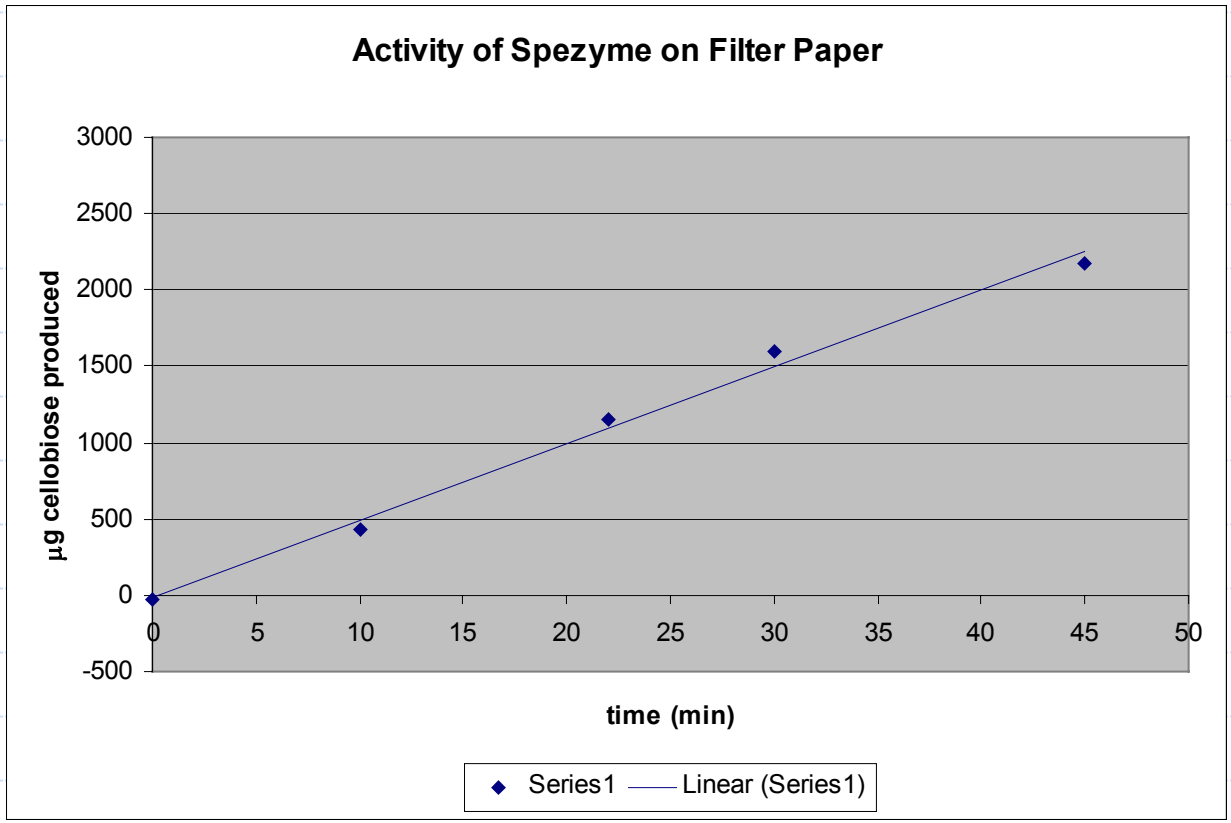
Methods

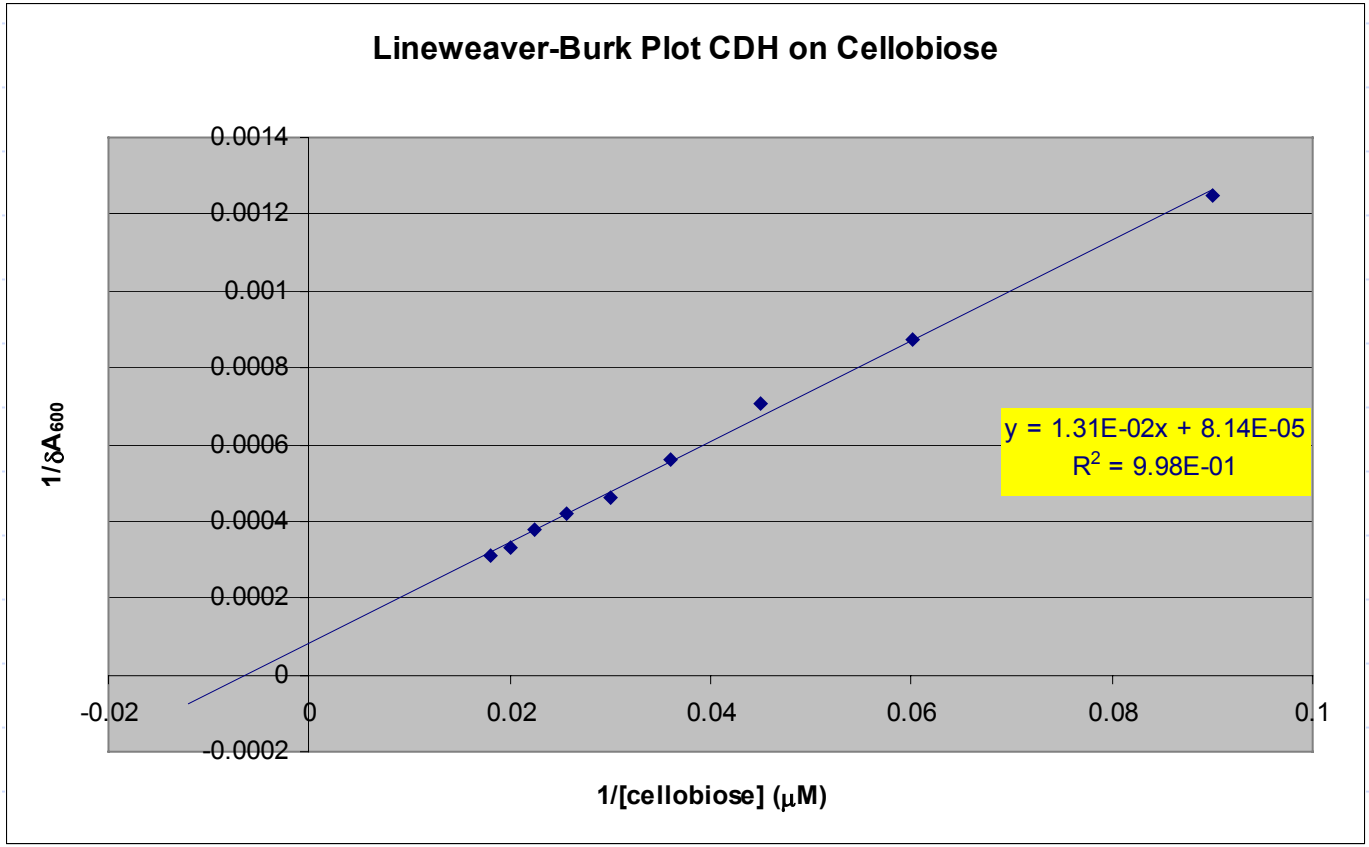
Purification of Cellobiose Dehydrogenase- Phanerochaete chrysosporium Sc26 was grown on 1% Solka Floc in Complete Medium in a 10L New Brunswick BioFlo3000 fermenter at 30°C, 300rpm, 20% dO_2, pH 6.5. Mycelia were harvested at 24 hours and the filtered broth was concentrated 34-fold into 20 mM Bis-Tris buffer, pH 5.8 using an Amicon DC-30 diafiltration concentrator with a 10kDa MWCO cartridge. The diafiltered sample was passed over a 6 mL Resource Q ion exchange column (Pharmacia). The flow through was collected, brought to pH 8.0, reloaded onto the RS-Q column, and eluted with a 0.0 to 0.5M NaCl gradient in 20mM Tris, pH 8.0. The active fractions were pooled, brought to 1.0M $(NH_4)_2SO_4$, loaded onto a 20mL phenyl-sepharose HIC column (Pharmacia), and eluted with a 1.0 to 0.0M $(NH_4)_2SO_4$ gradient. The active peak was pooled and concentrated and loaded onto a SuperDex200 16/60 SEC column (Pharmacia).

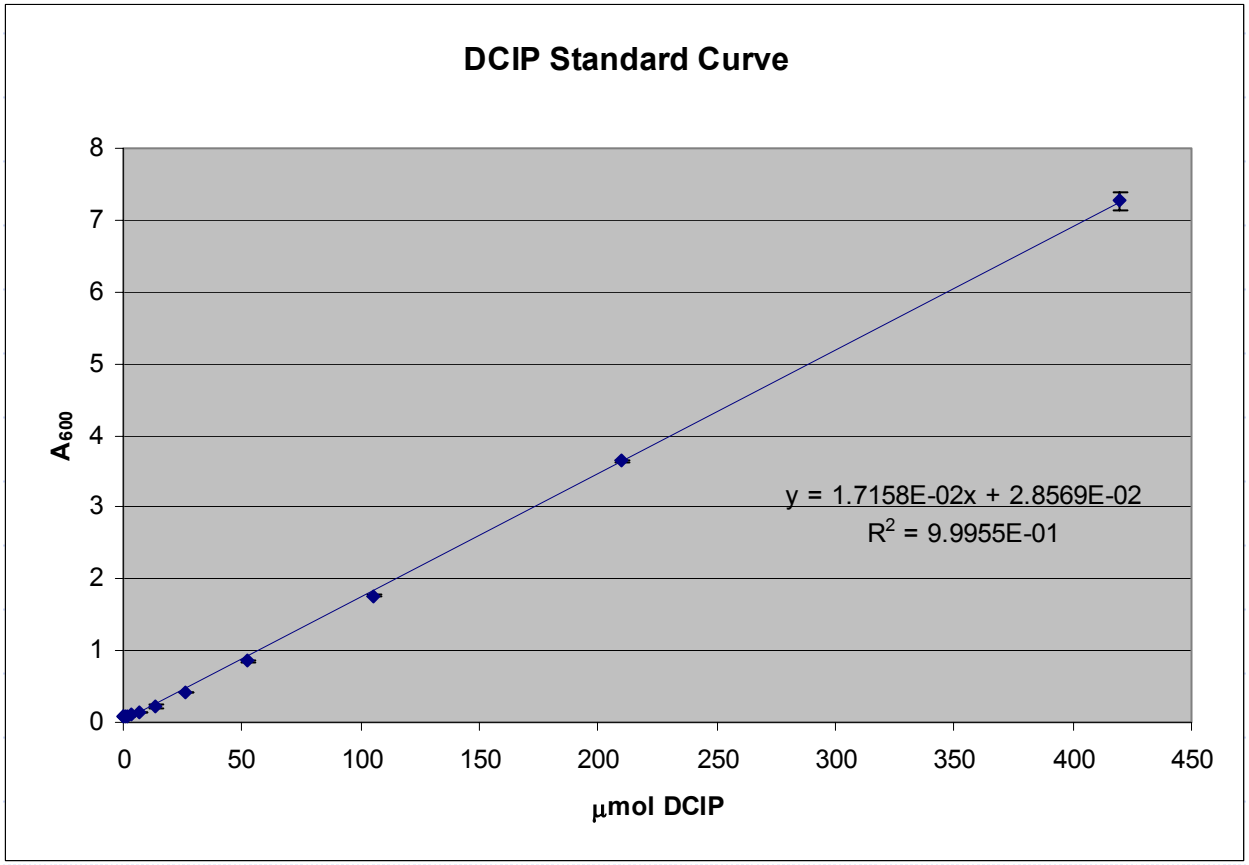
Phanerochaete chrysosporium Cellobiose Dehydrogenase

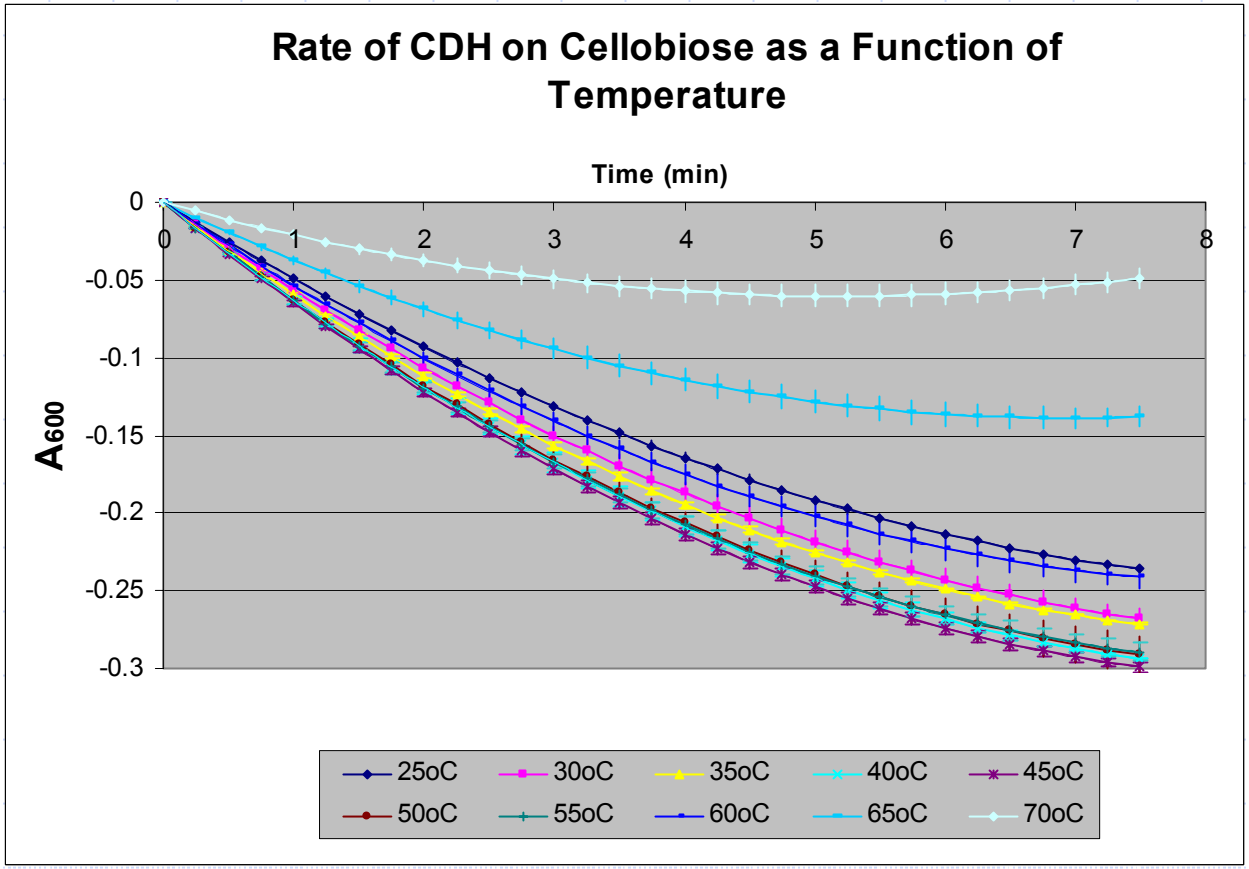


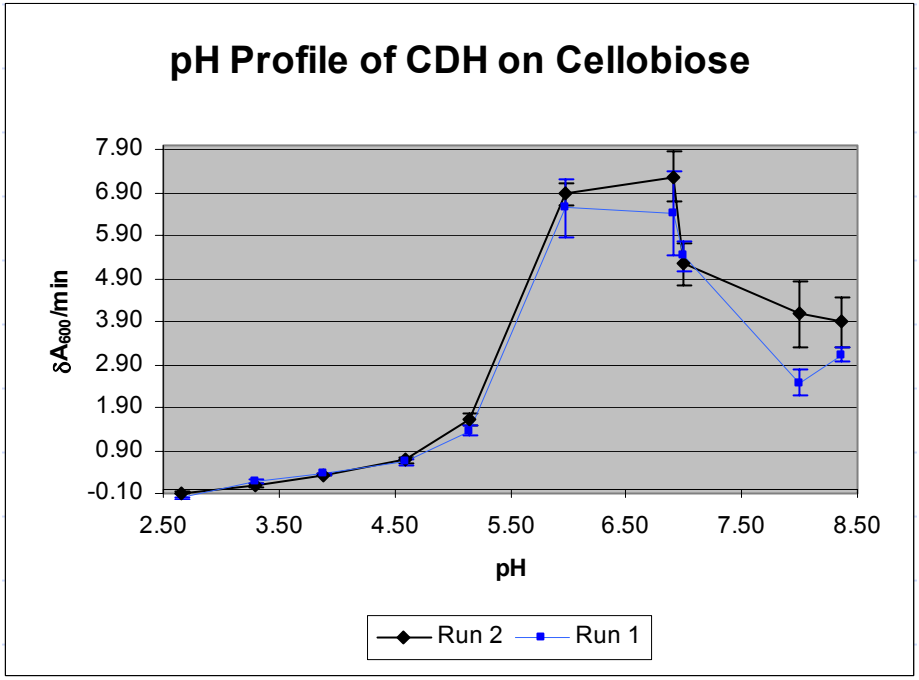

Activity of E1 on Avicel and Filter Paper
mg cellobiose released
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
10
20
30
40
50
60
70
time (min)
Avicel
Filter Paper
Linear (Avicel)
Linear (Filter Paper)


Activity of Spezyme on Filter Paper
µg cellobiose produced
3000
2500
2000
1500
1000
500
0
-500
0
5
10
15
20
25
30
35
40
45
50
time (min)
Series1
Linear (Series1)


Lineweaver-Burk Plot CDH on Cellobiose
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
-0.0002
-0.02
0
0.02
0.04
0.06
0.08
0.1
1/δA600
1/[cellobiose] (μM)
y = 1.31E-02x + 8.14E-05
R2 = 9.98E-01


DCIP Standard Curve
A600
8
7
6
5
4
3
2
1
0
0
50
100
150
200
250
300
350
400
450
µmol DCIP
y = 1.7158E-02x + 2.8569E-02
R2 = 9.9955E-01


Rate of CDH on Cellobiose as a Function of Temperature
Time (min)
A600
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
0
1
2
3
4
5
6
7
8
25oC
30oC
35oC
40oC
45oC
50oC
55oC
60oC
65oC
70oC


pH Profile of CDH on Cellobiose
7.90
6.90
5.90
4.90
3.90
2.90
1.90
0.90
-0.10
δA600/min
2.50
3.50
4.50
5.50
6.50
7.50
8.50
pH
Run 2
Run 1

Acknowledgements

This work was funded by the Biochemical Conversion Element of the Biofuels Program of the U.S. Department of Energy.

References

Ghose, T.K. 1987. "Measurement of cellulase activities". *Pure and Appl. Chem*. 59:257-268.