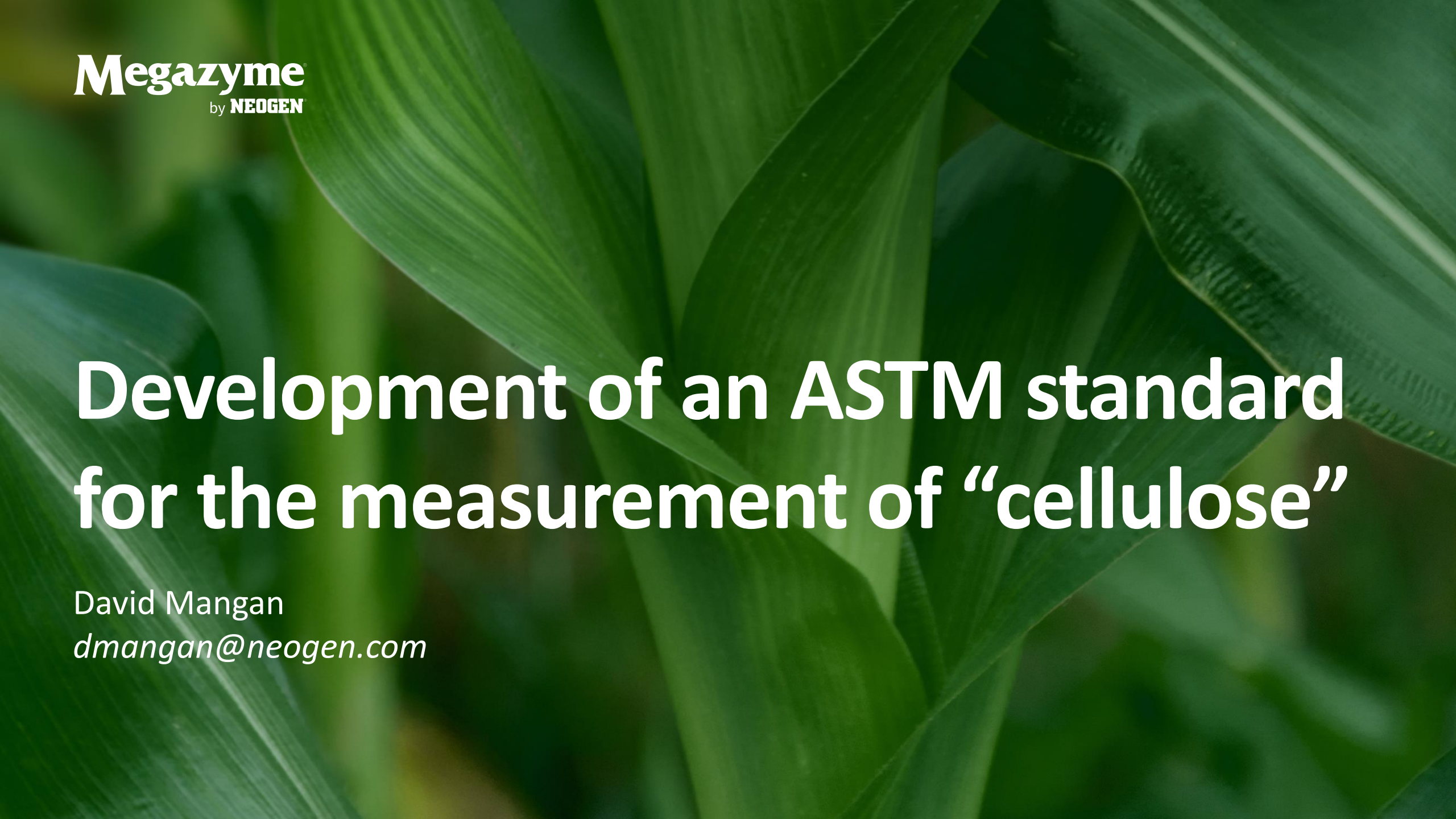

Megazyme by NEOGEN

Development of an ASTM standard for the measurement of “cellulose”

David Mangan
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Overview

01 CKF Conversion – Meeting Industry Need

02 E-3417: The Ultimate Collaborative Project

03 Unlocking D3 RINs

04 Conclusions and Future Perspectives

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01

CFK Conversion – Meeting Industry Need

The Need for A Method

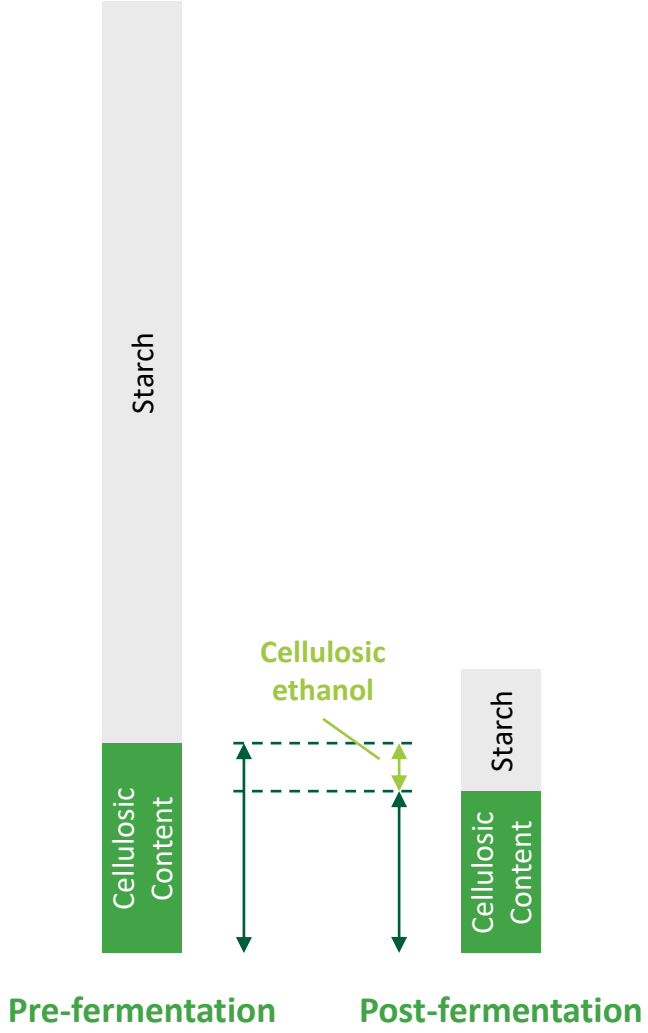
Designation: E3181 – 20

Standard Practice for
Determination of the Converted Fraction of Starch and Cellulosic Content From a Fuel Ethanol Production Facility

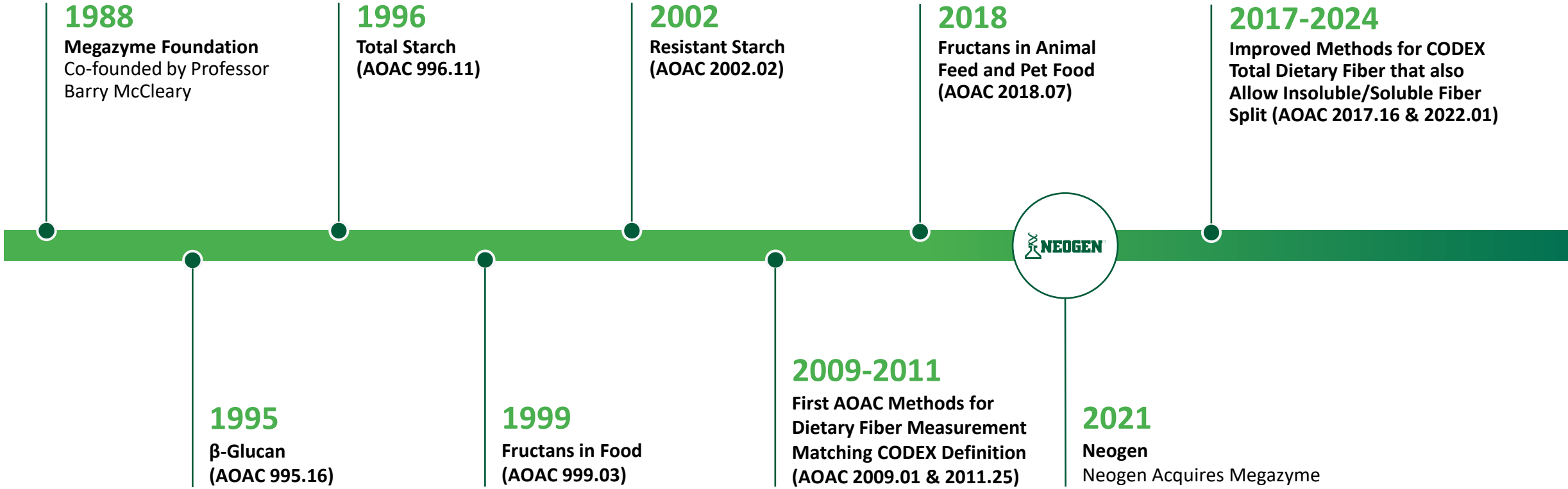
$$Ash_{ratio} = (Ash_{AC} / Ash_{BC}) \qquad (X5.3)$$

$$CF_c = 1 - ((Cellulosic\ \ Content_{AC} / Ash_{ratio}) / Cellulosic\ \ Content_{BC})$$

Note:
EPA has defined “cellulosic content” as the sum of cellulose, hemicellulose and lignin



Megazyme and Polysaccharide Assay Expertise



02

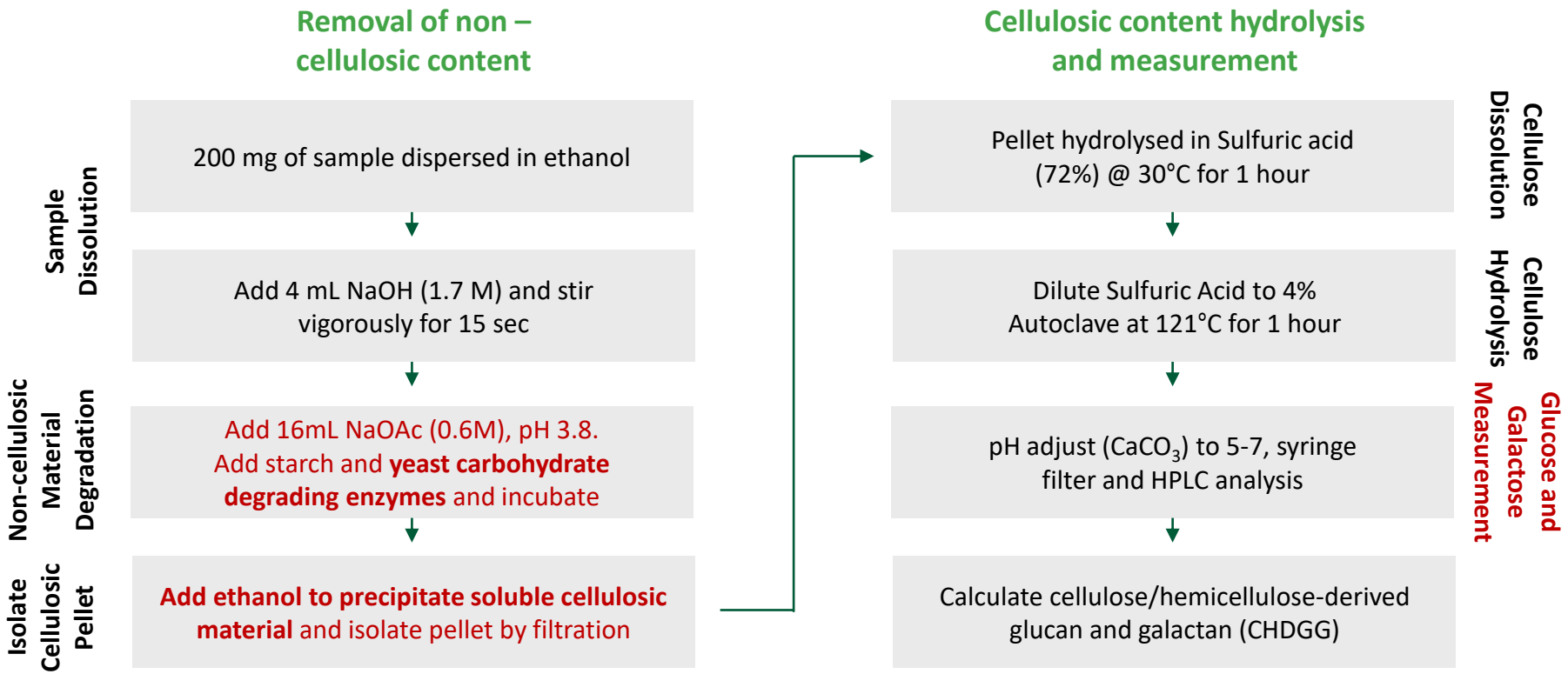
E-3417: The Ultimate Collaborative Project

Neogen (Modified NREL) Assay (2023)

NREL Transforming ENERGY

Cellulose (2021) 28:1989–2002
https://doi.org/10.1007/s10570-020-03652-2

ORIGINAL RESEARCH



CHDGG = Cellulose/Hemicellulose-Derived Glucan and Galactan

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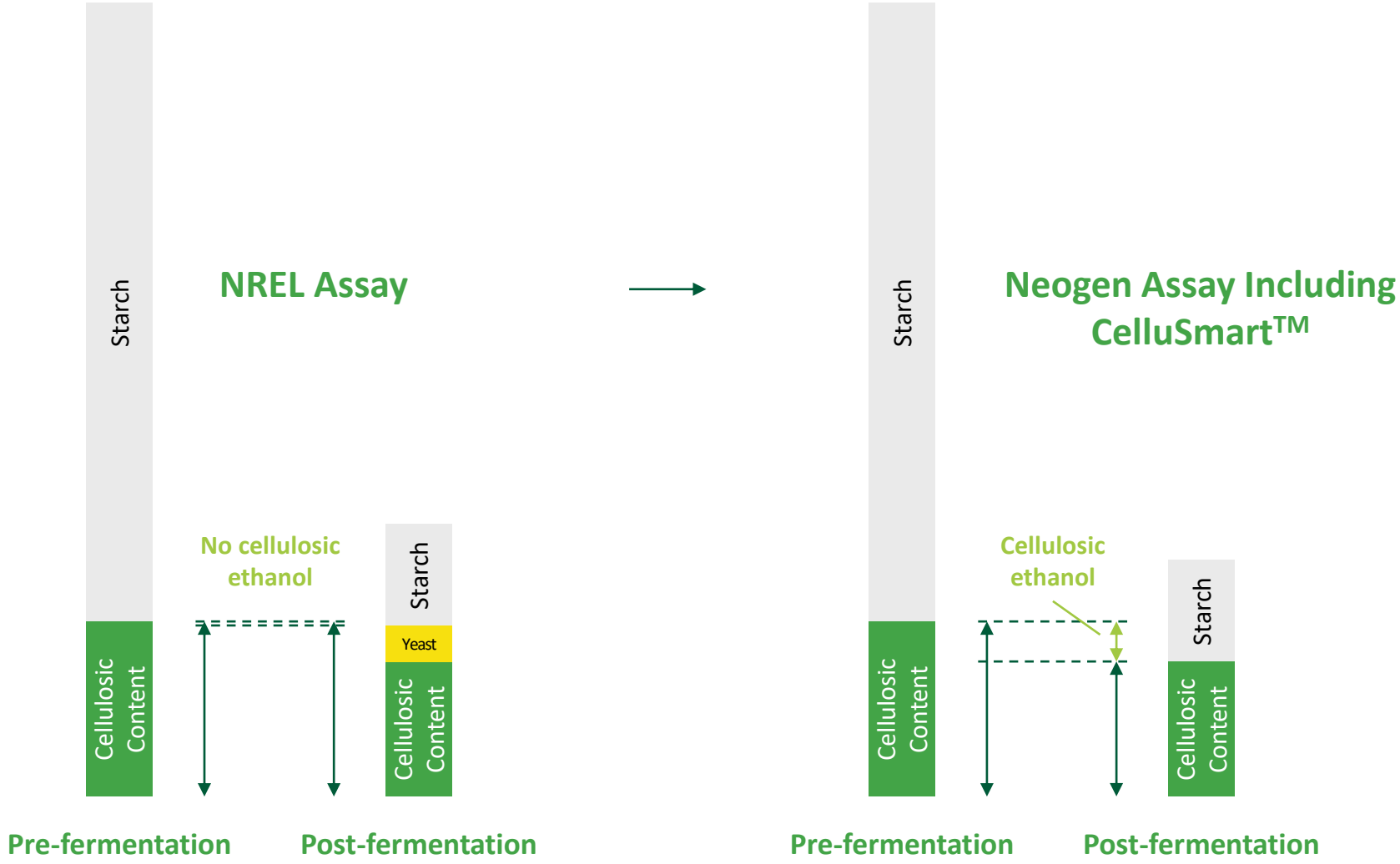
Bias 1 – Effect of Yeast in Pre- and Post-fermentation Samples



Bias 2 – Loss of Cellulosic Content Due to NaOH Treatment

NREL LAP

Determination of Cellulosic Glucan Content in Starch Containing Feedstocks

200 mg + 10 mg sample

Addition: 0.4 mL 190 proof EtOH

Adapted from Megazyme RTS-NaOH 2019

Ice Bath
4 mL, 1.7 M NaOH
15 minutes

16 mL, 600 mM NaOAc

50 °C Water Bath
0.2 mL α-amylase,
0.2 mL glucoamylase
60 minutes

Method Continues

Proposal for Evaluation

200 mg + 10 mg sample

Addition: 0.4 mL 190 proof EtOH

Adapted from AOAC Official Method 996.11

Boiling Water
4 mL, DMSO
6 minutes

0.2 mL, α-amylase
6 minutes

50 °C Water Bath
0.2 mL glucoamylase
60 minutes

Method Continues

Neogen

200 mg + 10 mg sample

Addition: 0.4 mL 190 proof EtOH

Ice Bath
4 mL, 1.7 M NaOH
15 minutes

16 mL, 600 mM NaOAc

50 °C Water Bath
0.2 mL α-amylase,
0.2 mL glucoamylase
60 minutes

Ethanolic Precipitation

Method Continues

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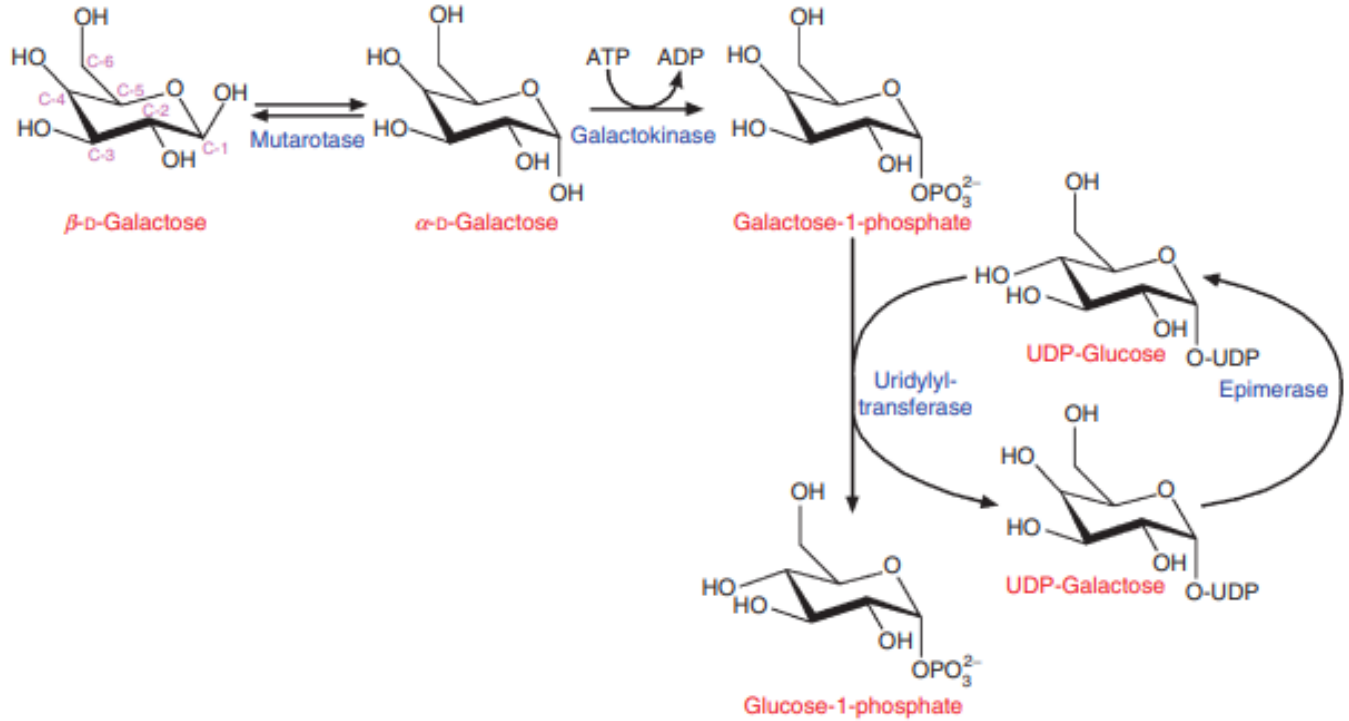
NREL Transforming ENERGY novonesis POET

Bias 3 – Exclusion of Galactose in the Analyte Determined

novonesis



ELSEVIER

International Review of Cell and Molecular Biology

Volume 269, 2008, Pages 111-150

Chapter 3 Galactose Metabolism in Yeast—Structure and Regulation of the Leloir Pathway Enzymes and the Genes Encoding Them

Christopher A. Sellick, Robert N. Campbell, Richard J. Reece

BIOTECHNOLOGY and BIOENGINEERING

Article

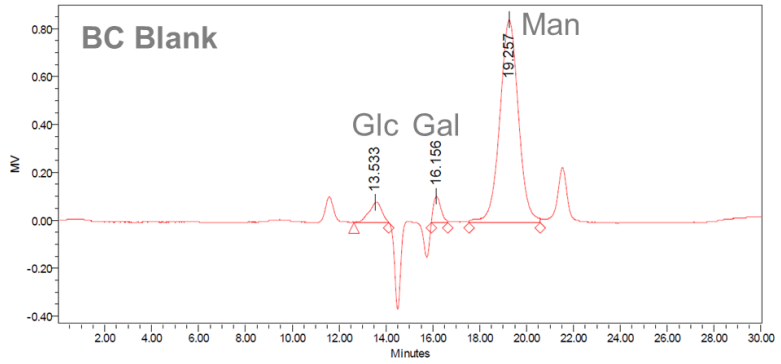
Physiological studies in aerobic batch cultivations of *Saccharomyces cerevisiae* strains harboring the *MEL1* gene

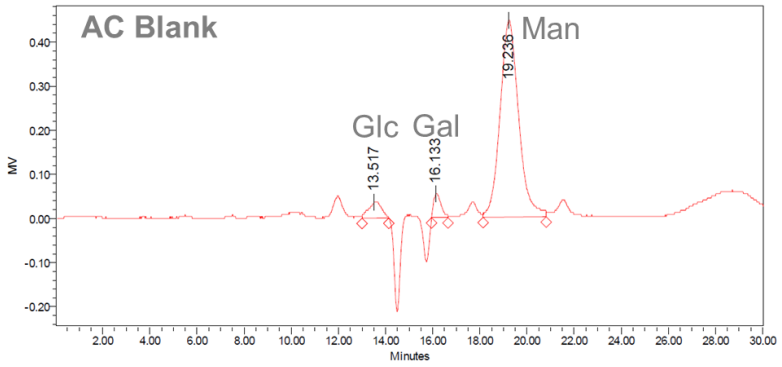
Simon Ostergaard, Christophe Roca, Birgitte Rønnow, Jens Nielsen, Lisbeth Olsson

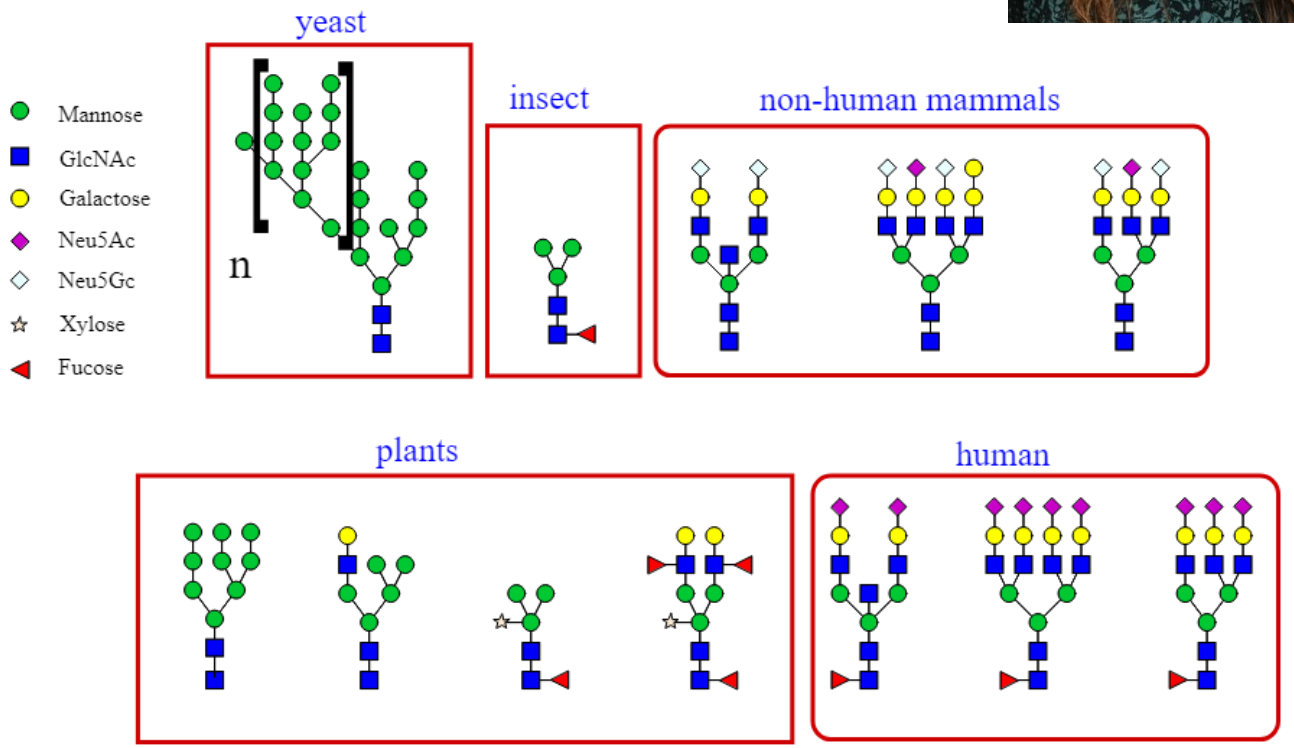
First published: 31 March 2000 |
https://doi.org/10.1002/(SICI)1097-0290(20000505)68:3<252::AID-BIT3>3.0.CO;2-K | Citations: 45

Reagent Black Requirement

soliton







Evolution of NREL assay to ASTM 3417

- ✓ Bias 1 – yeast glucan removed through CelluSmart™
- ✓ Bias 2 – solubilized cellulosic precipitation using ethanol
- ✓ Bias 3 – inclusion of galactan, identification of CHDGG as target analyte

(Plus addition of reagent blank)

	NREL Method	ASTM 3417
Sample	% Cellulosic Ethanol	
Conventional	-0.64	-0.13
CKF Process	0.11	0.88

03 Unlocking D3 RINs

The Pathway to EPA Approval

3 x Subcommittee Ballots
3 x Main Committee Ballots

2021
E48.05 December Meeting
Reintroduction of Cellulosic Content Method Discussion

2022
E48.05 December Meeting
Novozymes Presentation on DFO Method Investigation

2023
July

2023
November

2024
7 x New Facilities Approved by EPA for the Generation D3 RINs from In-suite CKF Conversion Processes in March

EPA

2022
E48.05 June Meeting
Starch Methodology Presentation and Cellulose Working Group Formation

Working Group Meets Virtually Six Times

2023
E48.05 June Meeting
Agreement to Move Neogen Method to Ballot

FELC 2023
FUEL ETHANOL LABORATORY CONFERENCE

ASTM INTERNATIONAL

2024
E-3417 Approved in January

FELC
FUEL ETHANOL
LABORATORY CONFERENCE

2024
Today – 65 x Bioethanol Facilities Approved for D3 Generation

~$2.5M/facility
~$160M annually

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How Does It Work?

Phase 1

Producer objective: Prepare for facility registration by EPA

CelluSmart™; (E-RDYDC)

Tasks:

- Purchase CelluSmart™; (E-RDYDC) under supply agreement and perform in-house analysis or outsource to your analytical partner
- Gather % cellulosic ethanol data from multiple fermentations
- Plant fermentation process development (if required)
- Predict % cellulosic ethanol

https://www.epa.gov/fuels-registration-reporting-and-compliance-help/how-register-new-renewable-fuel-producer-renewable

Phase 2

Producer objective: Generate D3 RINs

CelluSmart™; (E-YDC)

Tasks:

- Order CelluSmart™ (E-YDC; $FOC) under supply agreement and perform in-house analysis or outsource to your analytical partner
- Generate % cellulosic ethanol data from multiple fermentations
- Complete 3rd party engineering audit and submit to EPA for facility registration
- Re-submit data to EPA every 500K gallons for re-certification

https://www.govinfo.gov/content/pkg/CFR-2022-title40-vol19/pdf/CFR-2022-title40-vol19-sec80-1451.pdf - see page 4

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04

Conclusions and Future Perspectives

Summary

A highly collaborative effort among multiple industry stakeholders has led to the creation of a novel method for measuring the relevant portion of cellulosic content as defined by the EPA

Key milestones:

- ASTM approval as standard E3417
- Endorsement by the EPA
- Rapid uptake by 65 bioethanol facilities as of end September 2024

Future developments:

- An update to E3417 is currently being balloted in ASTM to expand market application to corn-sorghum blended feedstocks and improve overall usability

Edeniq

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Acknowledgements

Core Neogen (Megazyme) R&D Team
- Ruth Ivory
- Anna Draga
- Tadas Kargelis
- Amaya O'Cochláin
- Lucie Charmier
- Artur Rogowski
- Entire Megazyme site manufacturing and quality control teams

NREL
- Justin Sluiter
- Katie Michel

POET
- Bart Plocher
- Melissa Tille

Novonesis
- David Gogerty
- Geoff Moxley

TCD
- John O'Brien
- Manuel Ruether

CCRC
- Paristoo Azadi

ASTM standard collaborators
- Too many to list!

Matt Nichols

cellusmart@neogen.com

For more information on the commercial aspects of accessing the analytical technology

David Mangan

dmangan@neogen.com

For more information on how to join ASTM and help support the next steps in the story


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