Partial purification of endo-1,4-β-glucanase I of *Trichoderma reesei* and use in small-scale enzymatic hydrolysis of cellulose

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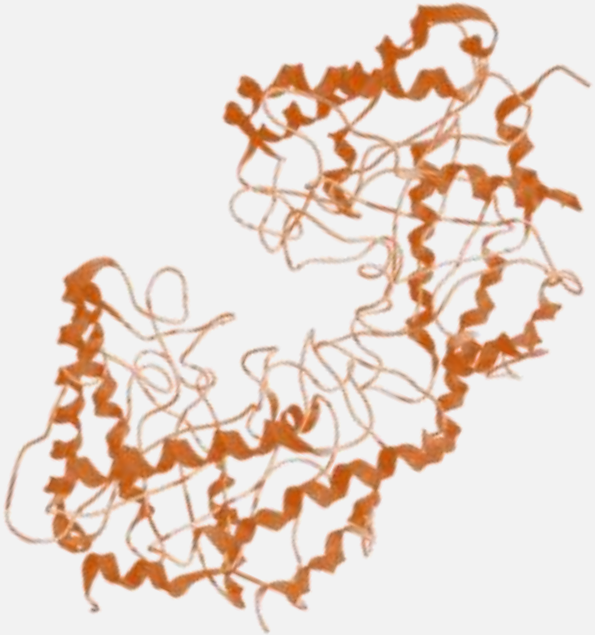
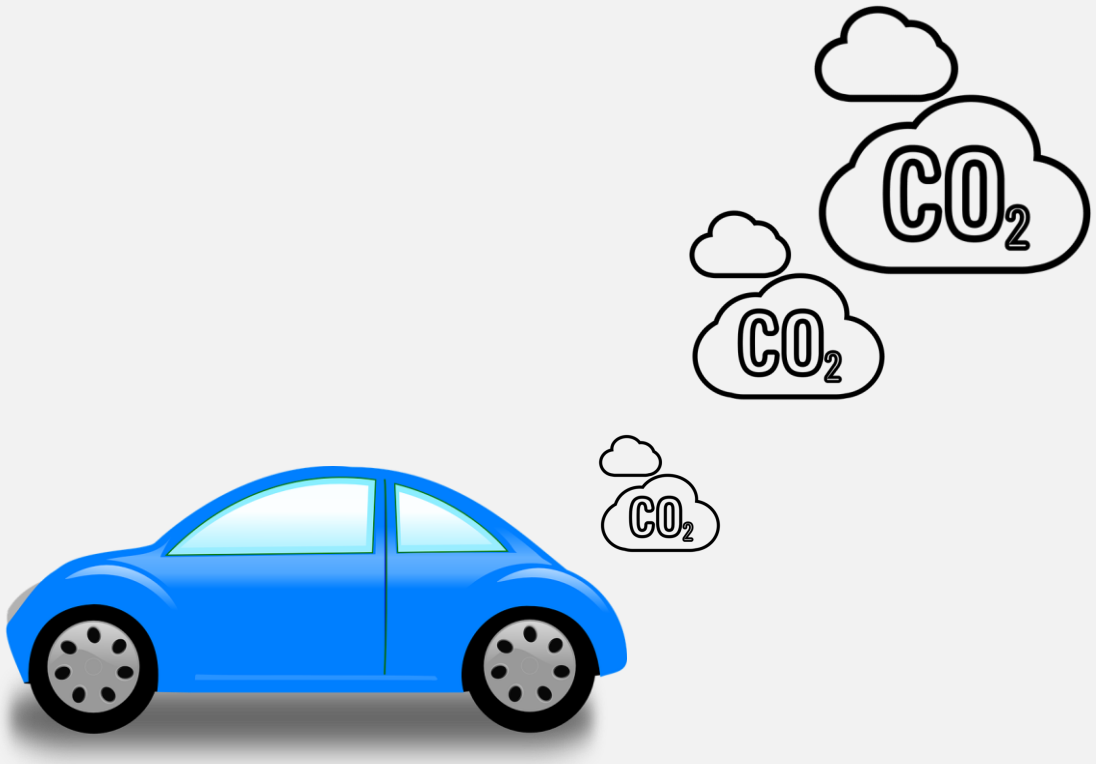
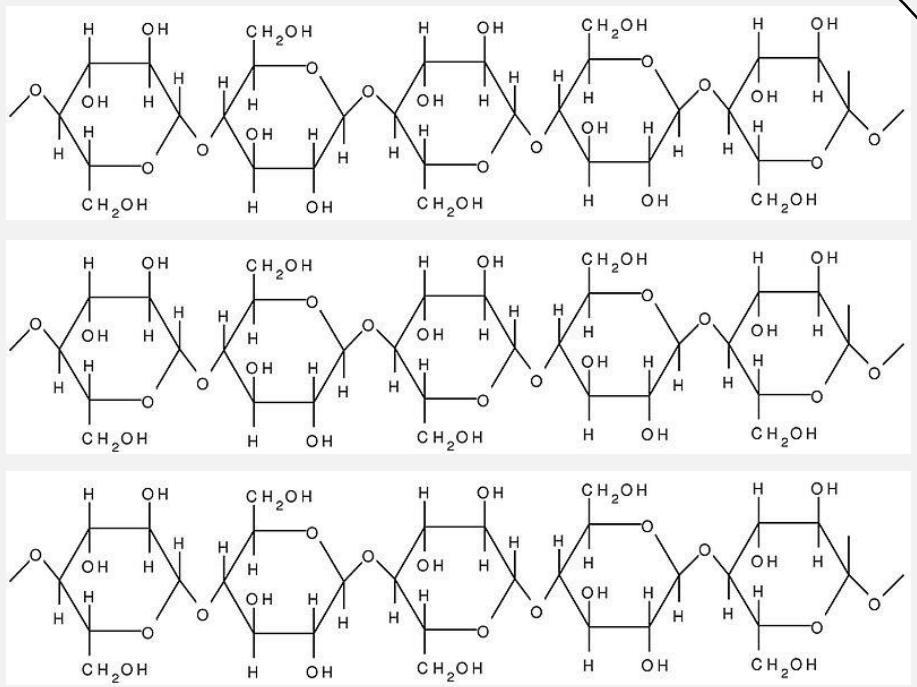


Growing concern of link between human pollution and global warming



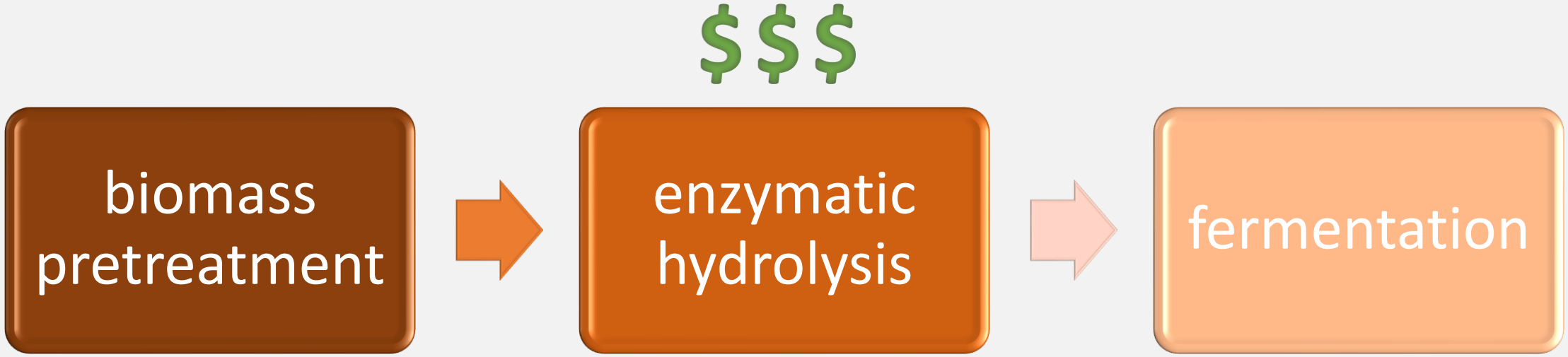
Lignocellulosic ethanol is a potential renewable fuel

Eaton, 2013

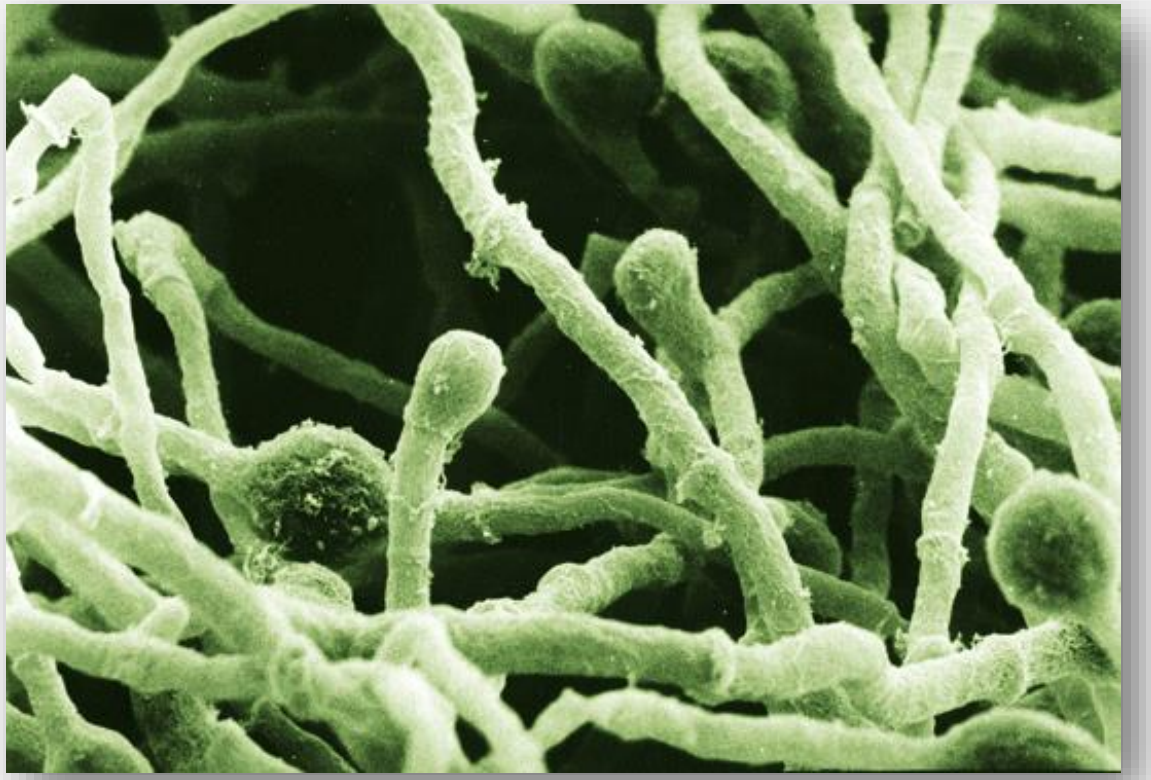


Cellulose

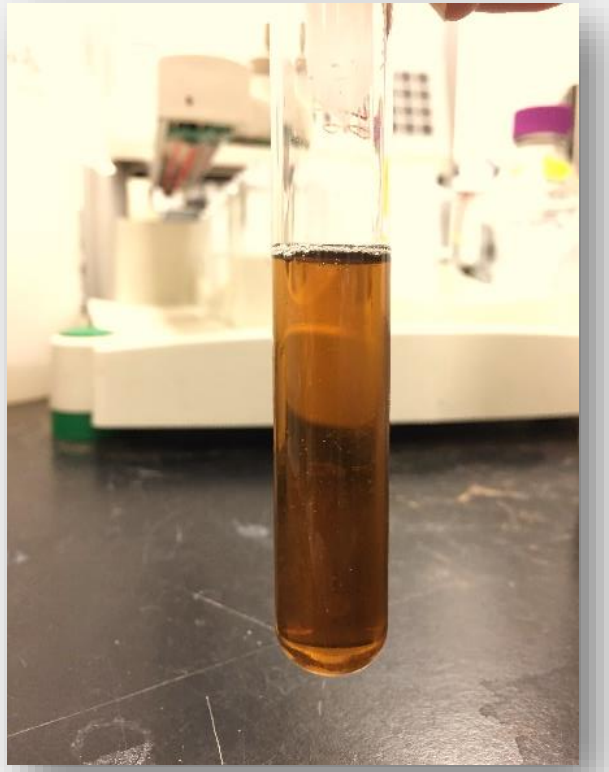
Lignocellulosic ethanol is a potential renewable fuel



Trichoderma reesei is a common type of microbe to produce industrial cellulases.

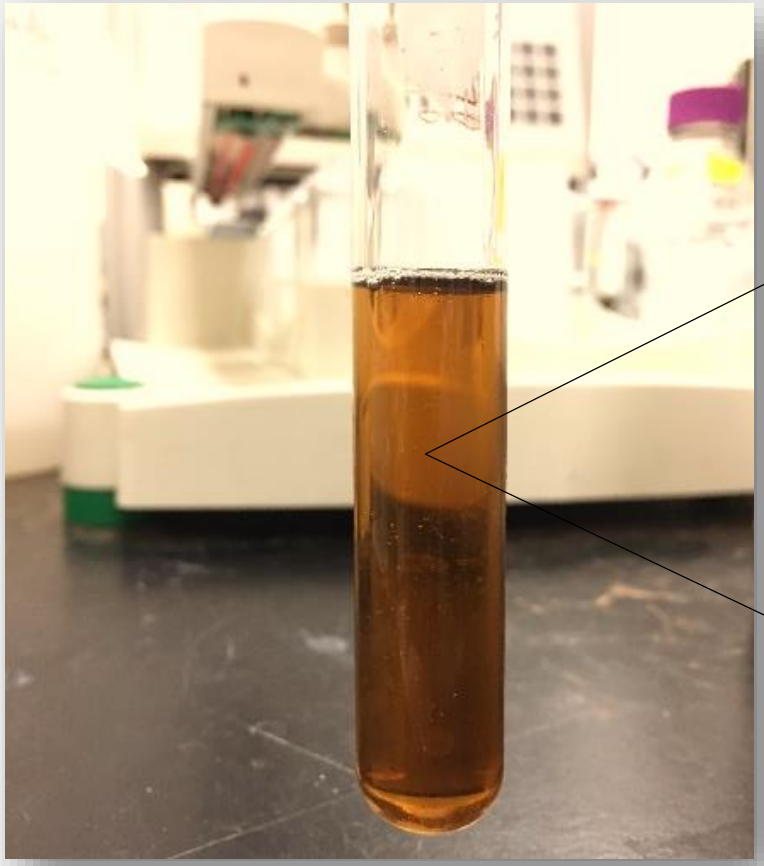


Rinaldi, 2018

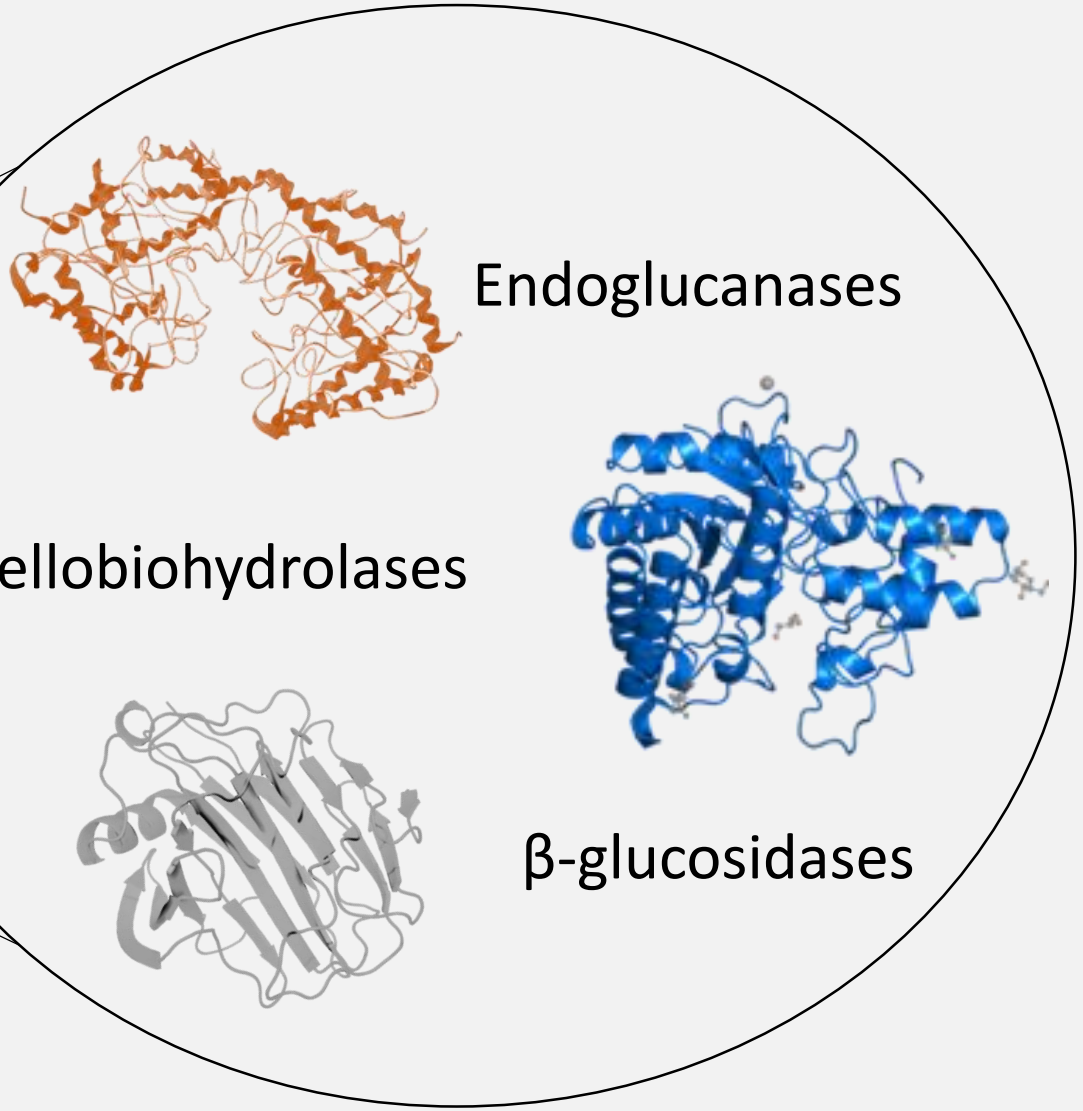


Celluclast, Novozymes

There are three main families of cellulase enzymes



Celluclast, Novozymes



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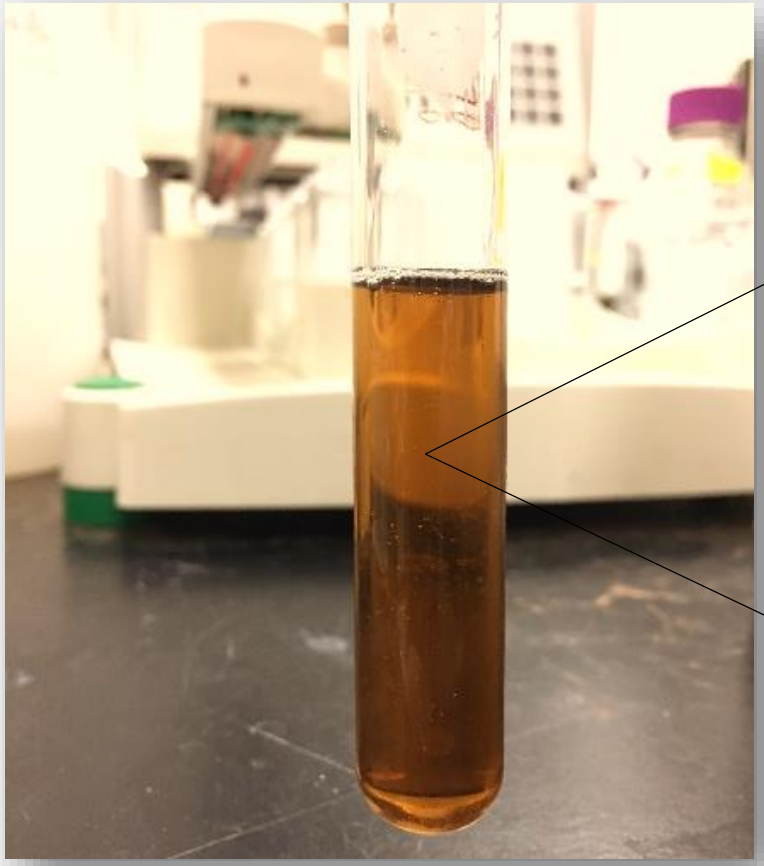

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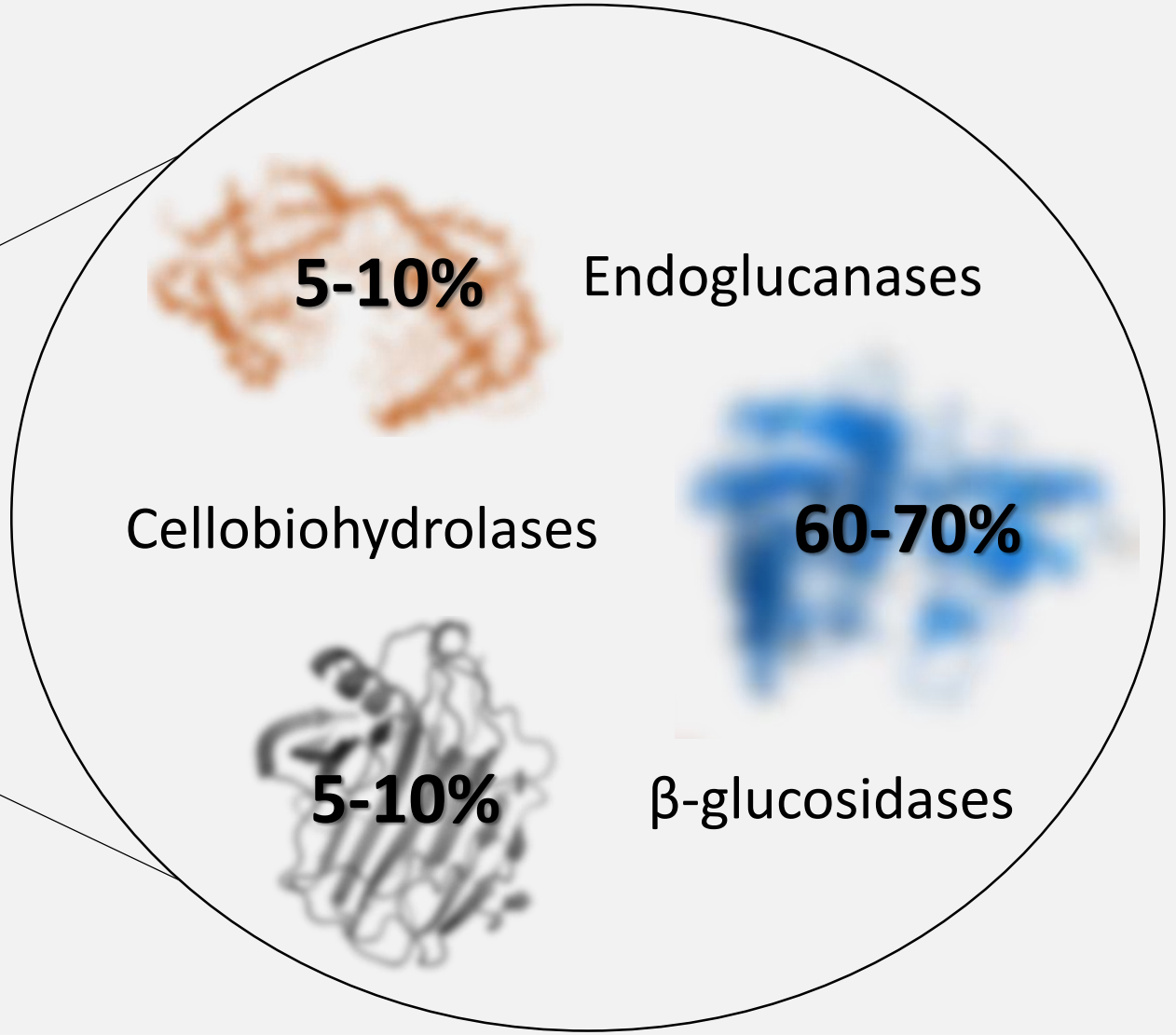
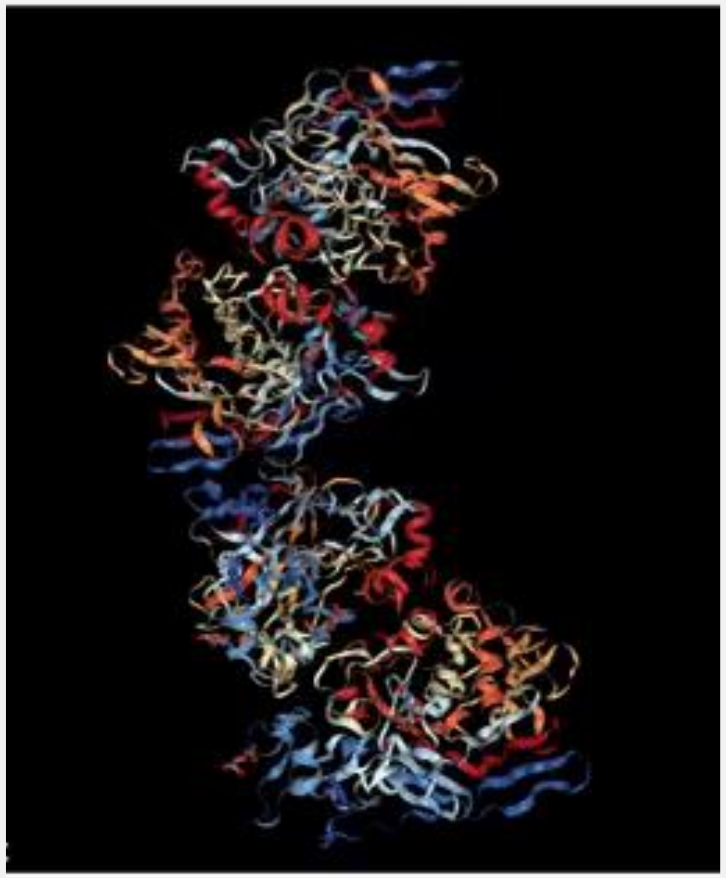
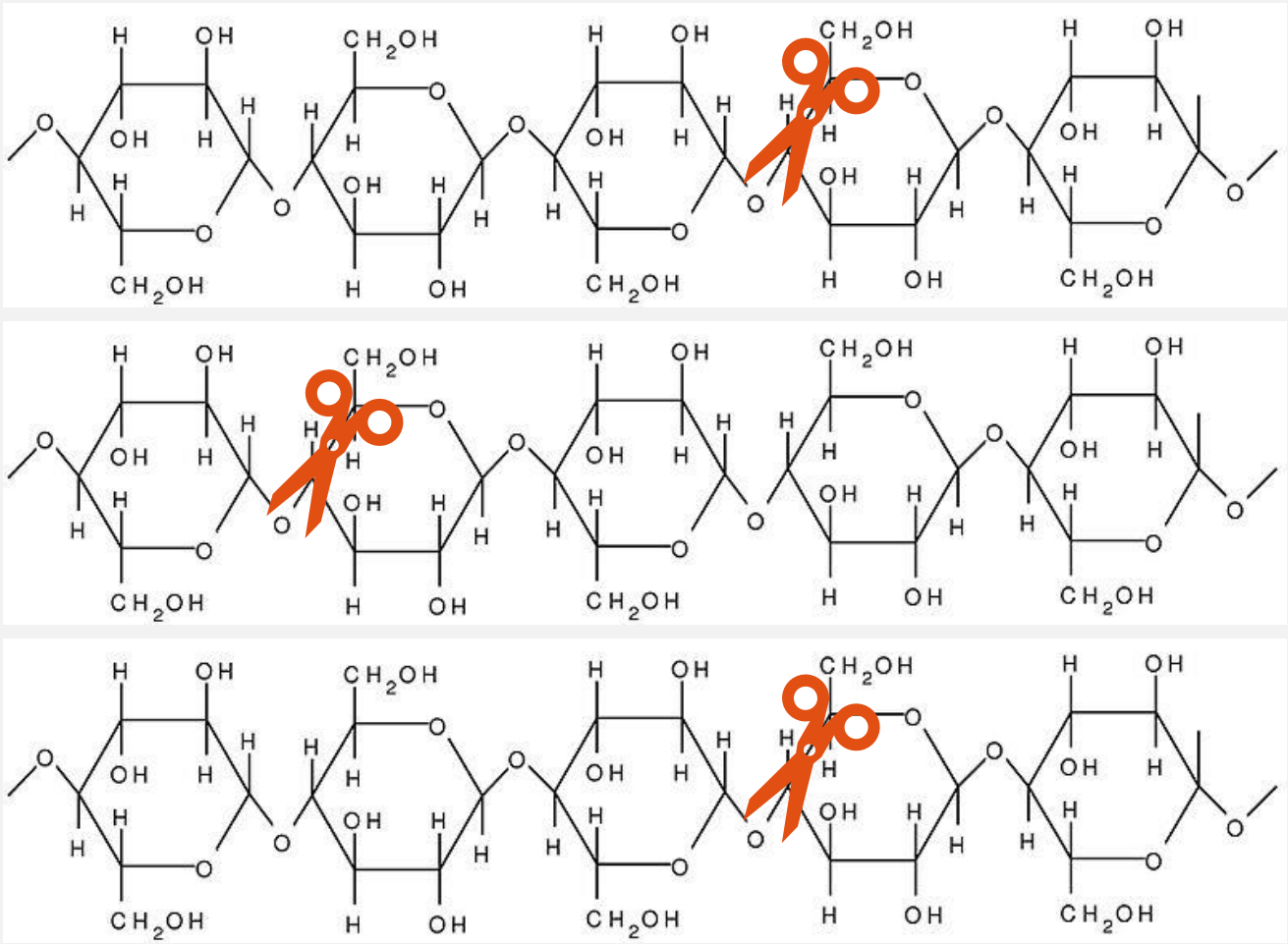


Each enzyme plays a specific role in the hydrolysis of cellulose



Endo-1,4-β-glucanase I
(EG I)



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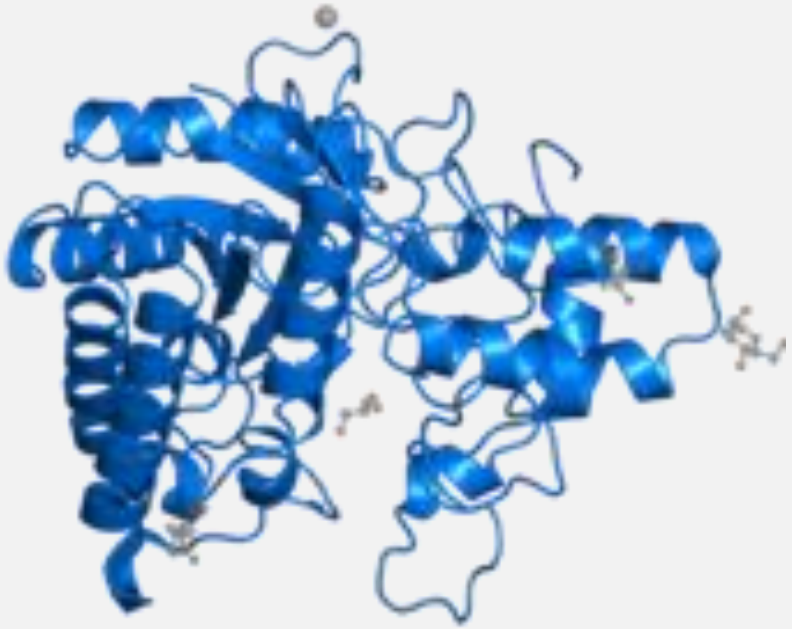
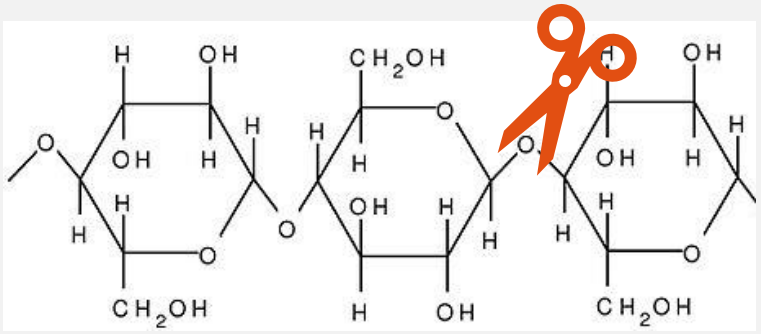
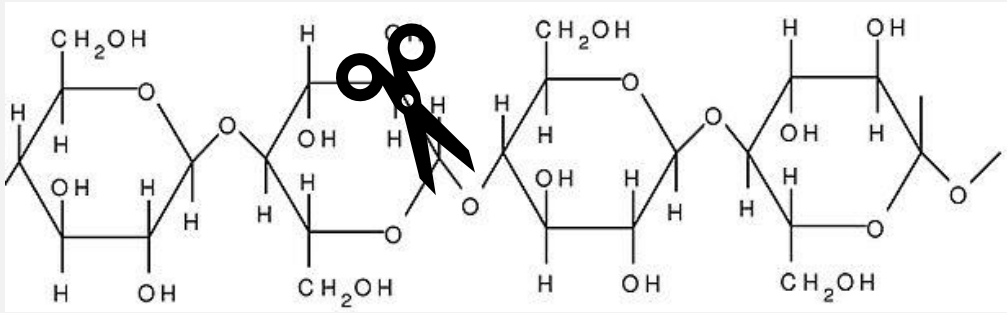
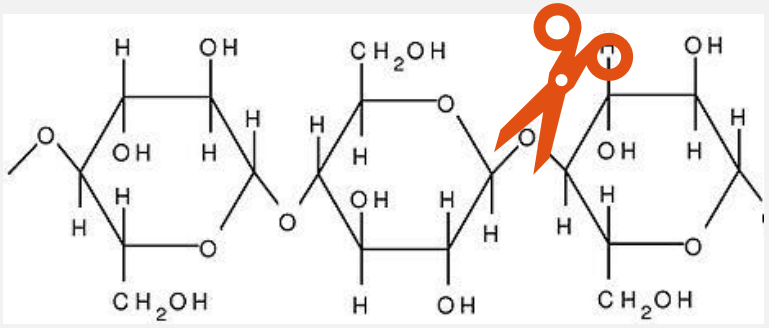


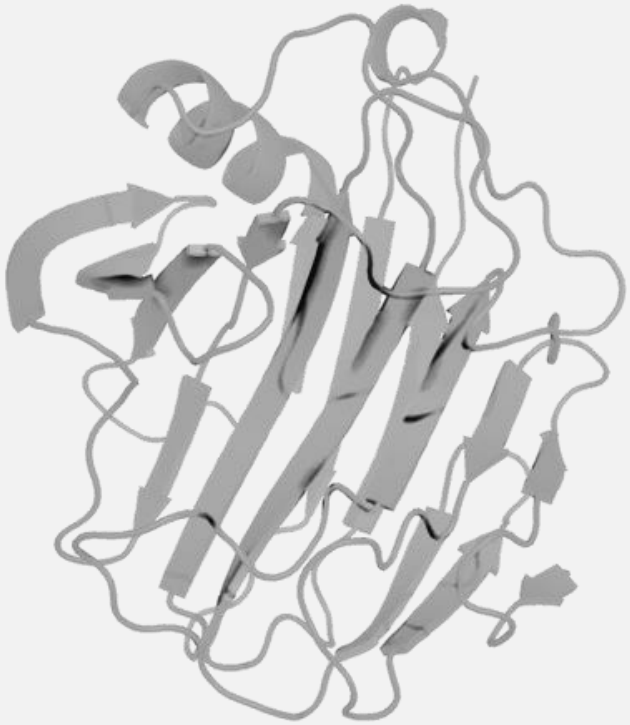
Cellobiohydrolase I & II
(CBH I & II)



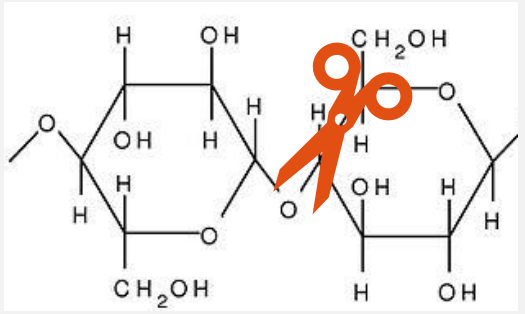


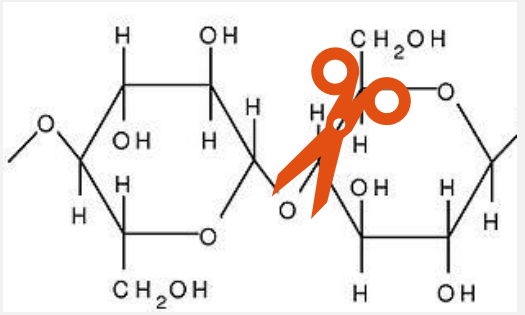


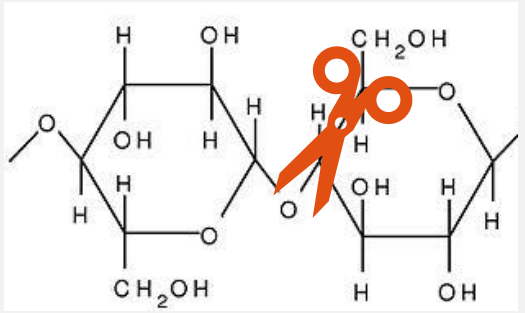
Each enzyme plays a specific role in the hydrolysis of cellulose



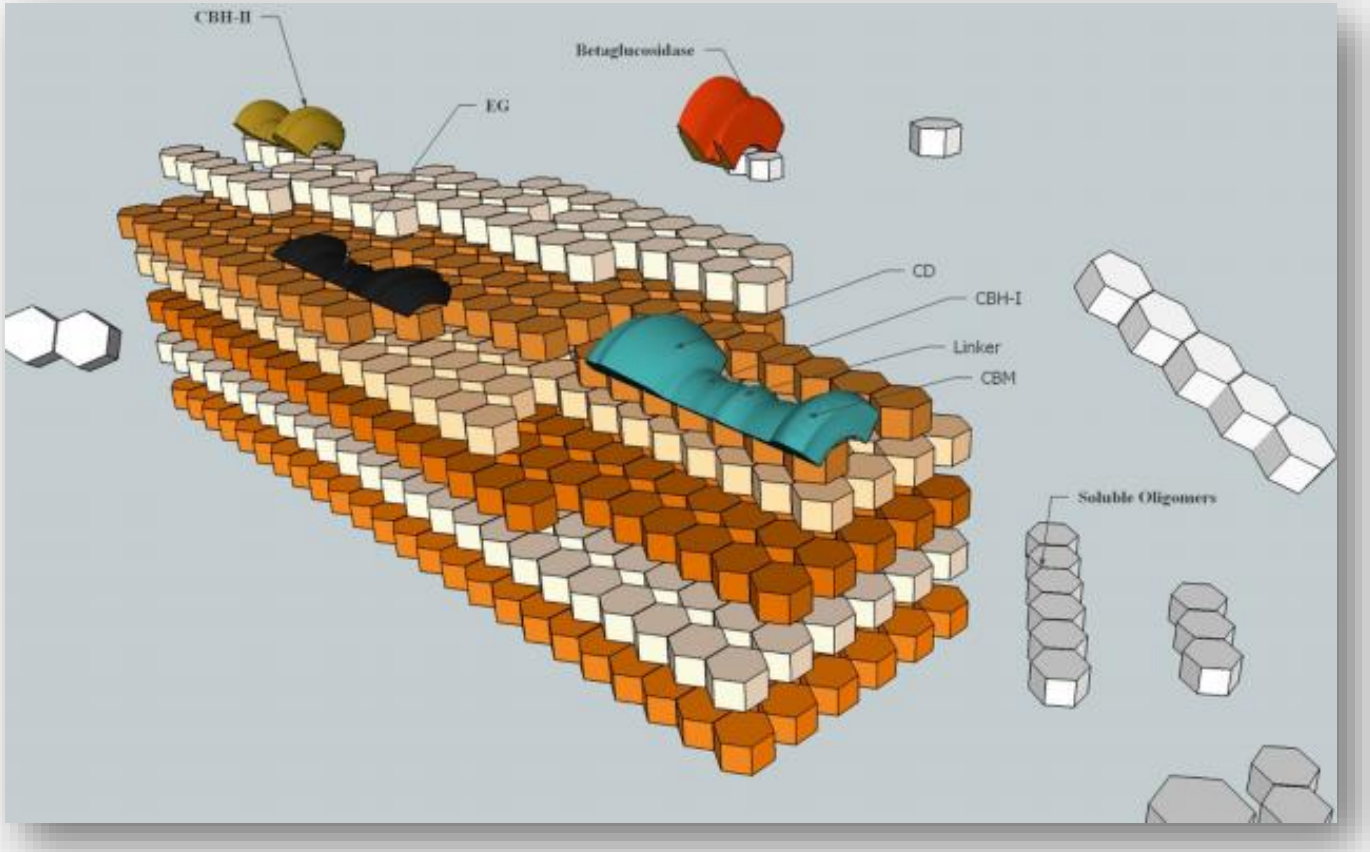
β-glucosidase
(BG)







A predictive model could lead to a reduction in the cost of production



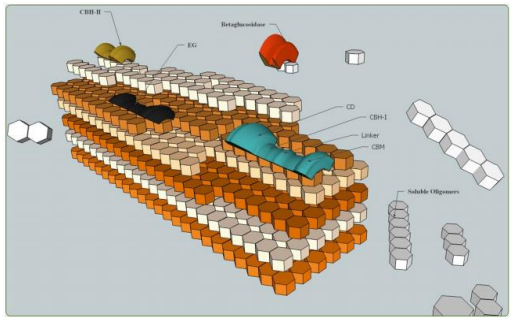
Biomass Composition Parameters

- crystallinity
- degree of polymerization
- surface accessibility
- dynamic morphological changes in structure of cellulose

Enzymes Parameters

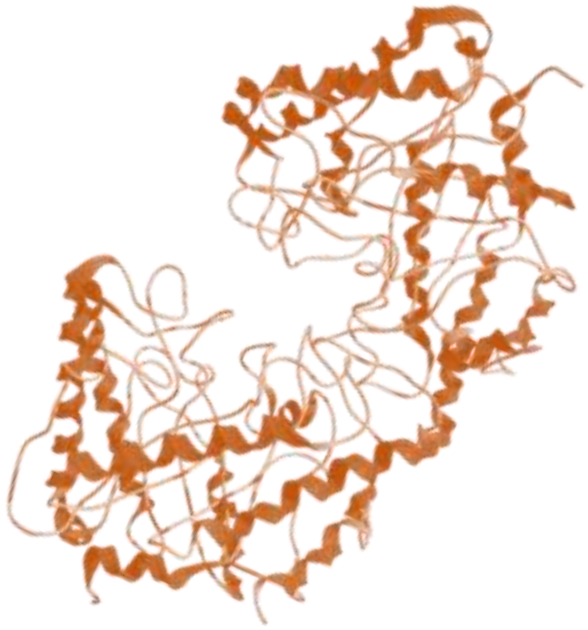
- mode of action
- binding and surface blockage
- inhibition
- synergism

Kumar and Murthy, 2013



Project Objective:

- Purify endo-1,4-β-glucanase I from Celluclast
- Perform enzymatic hydrolysis experiments with individually purified enzymes.



Materials & Methods

Fast Protein Liquid Chromatography

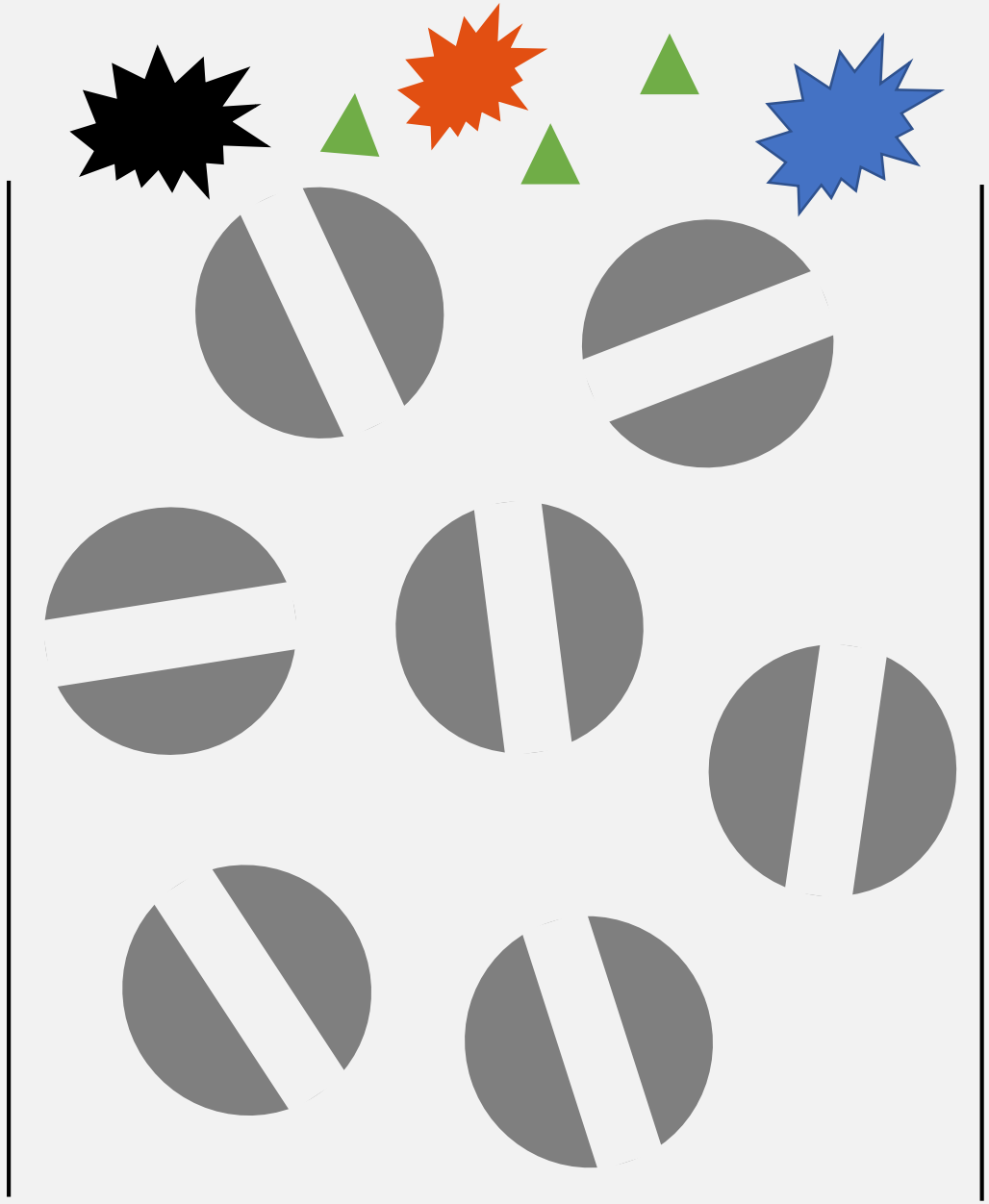
Size Exclusion Chromatography

PURPOSE:

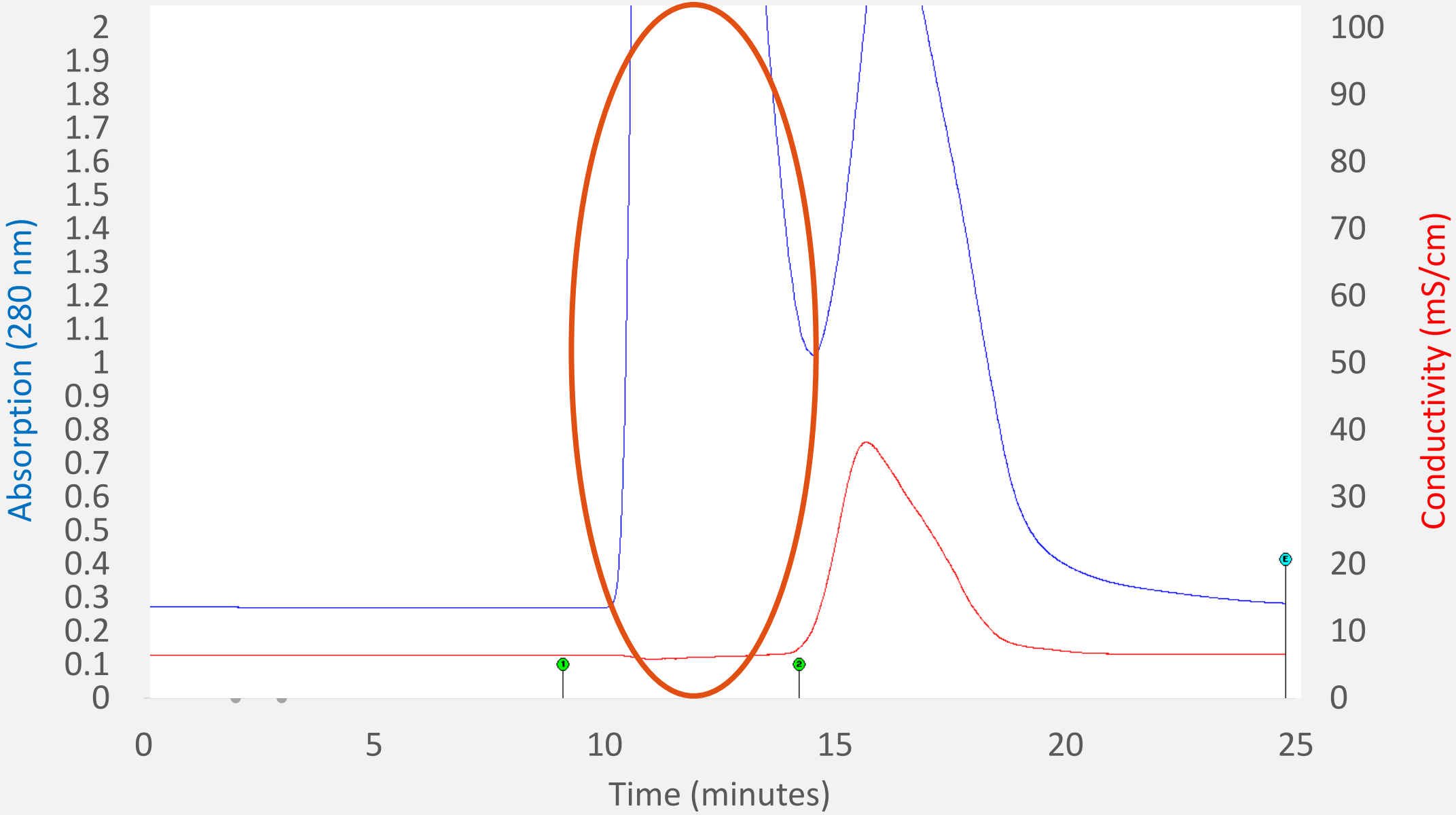
desalting and rebuffering

RUNNING BUFFER:

50 mM Tris-HCl, pH 7.0



Size Exclusion Chromatography

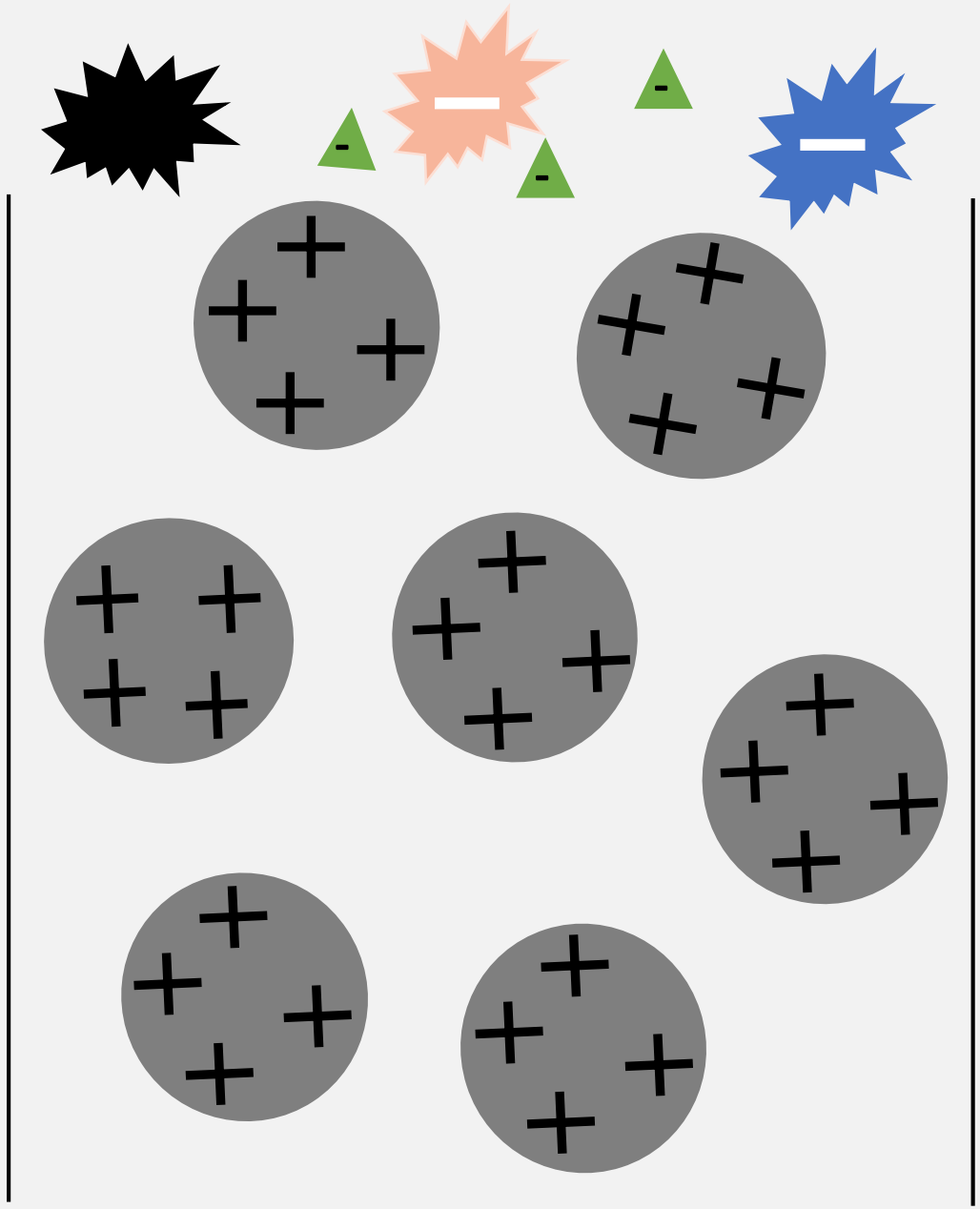


Anion Exchange Chromatography

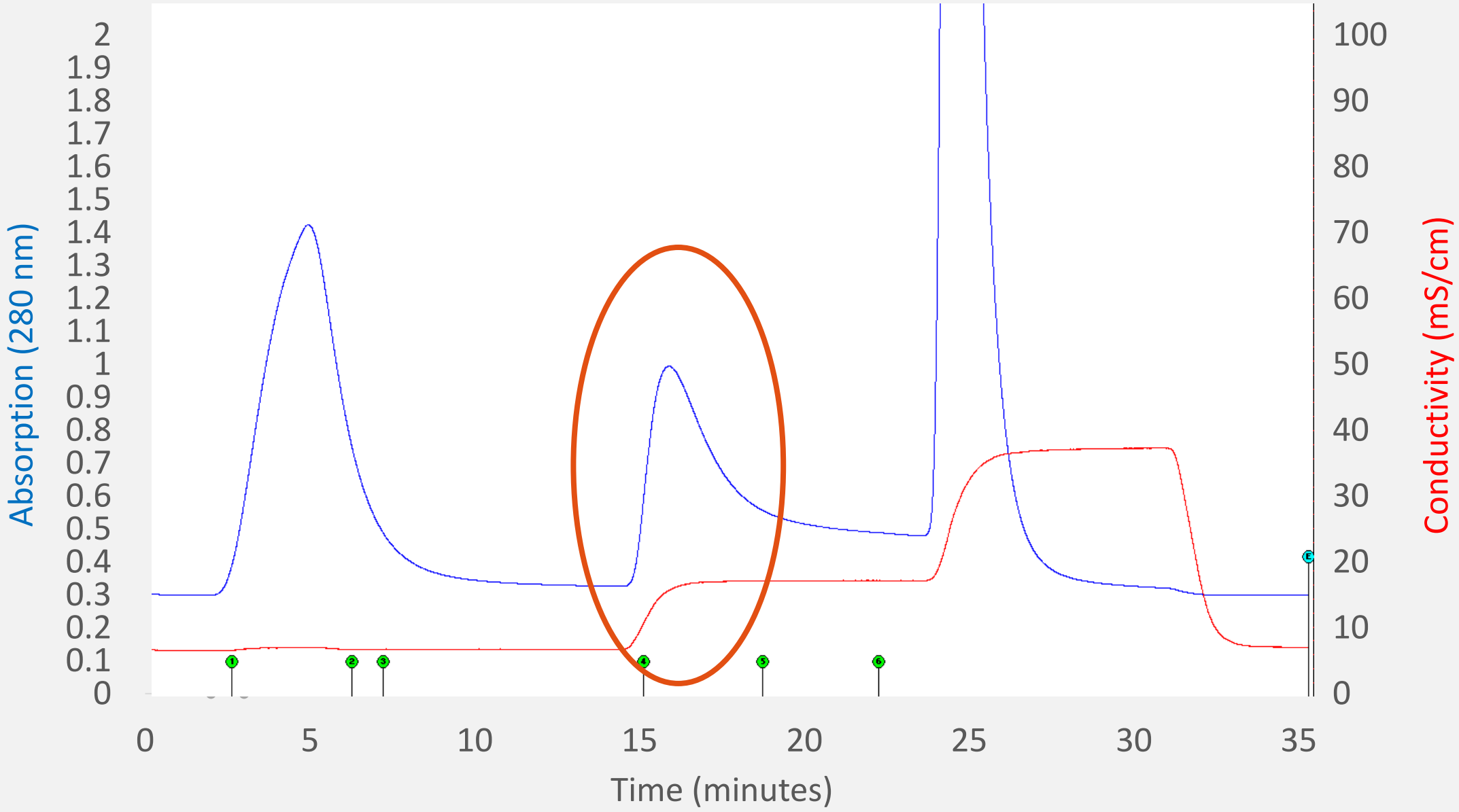
PURPOSE:
intermediate purification of endo-1,4-β-glucanase I

RUNNING BUFFER:
50 mM Tris-HCl, pH 7.0

ELUTION BUFFER:
50 mM Tris-HCl, **0.2 M NaCl**, pH 7.0



Anion Exchange Chromatography



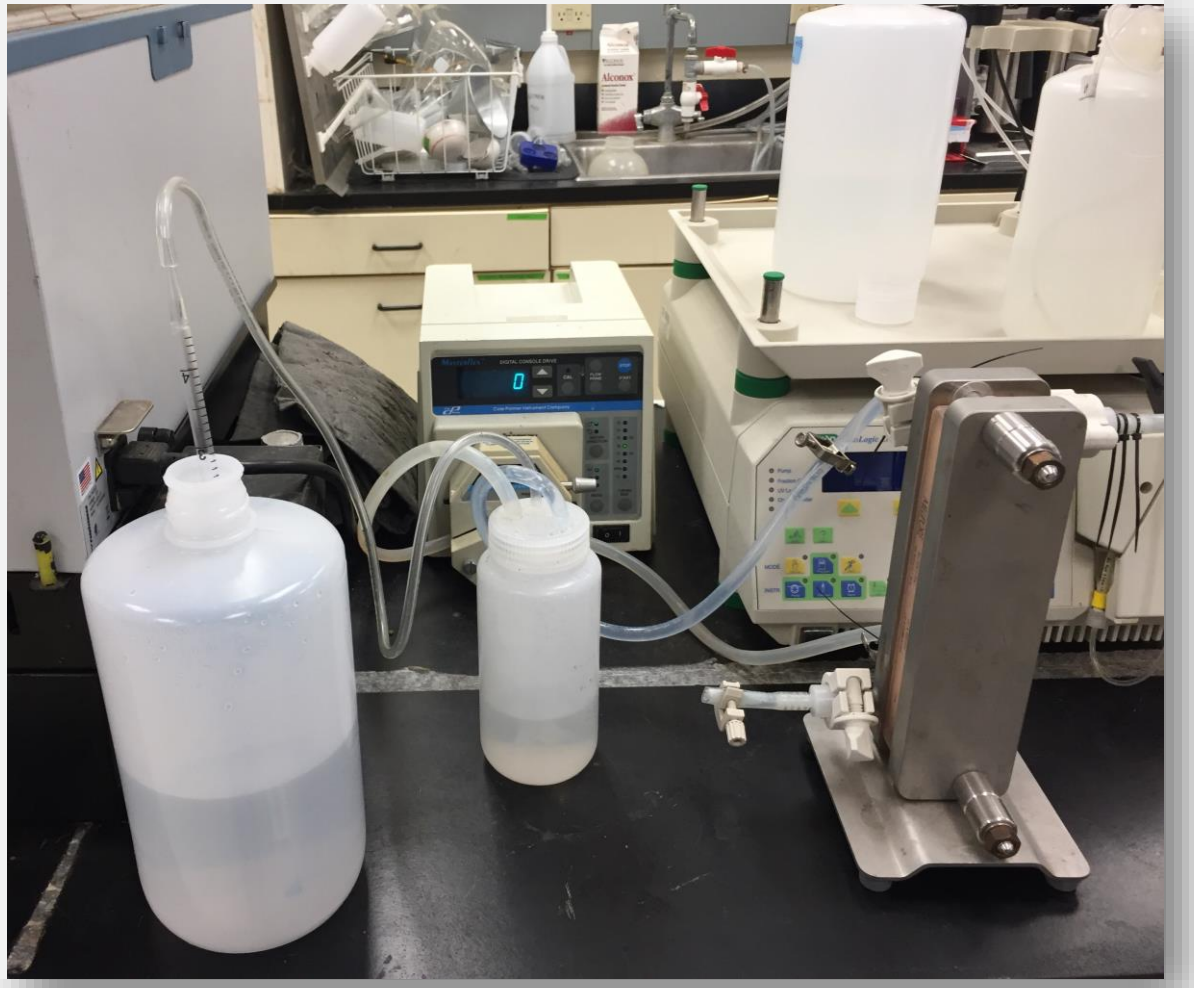
Ultrafiltration/Diafiltration

PURPOSE:

concentration and rebuffering

NEW BUFFER:

25 mM Sodium Acetate, pH 3.5



Cation Exchange Chromatography

PURPOSE:

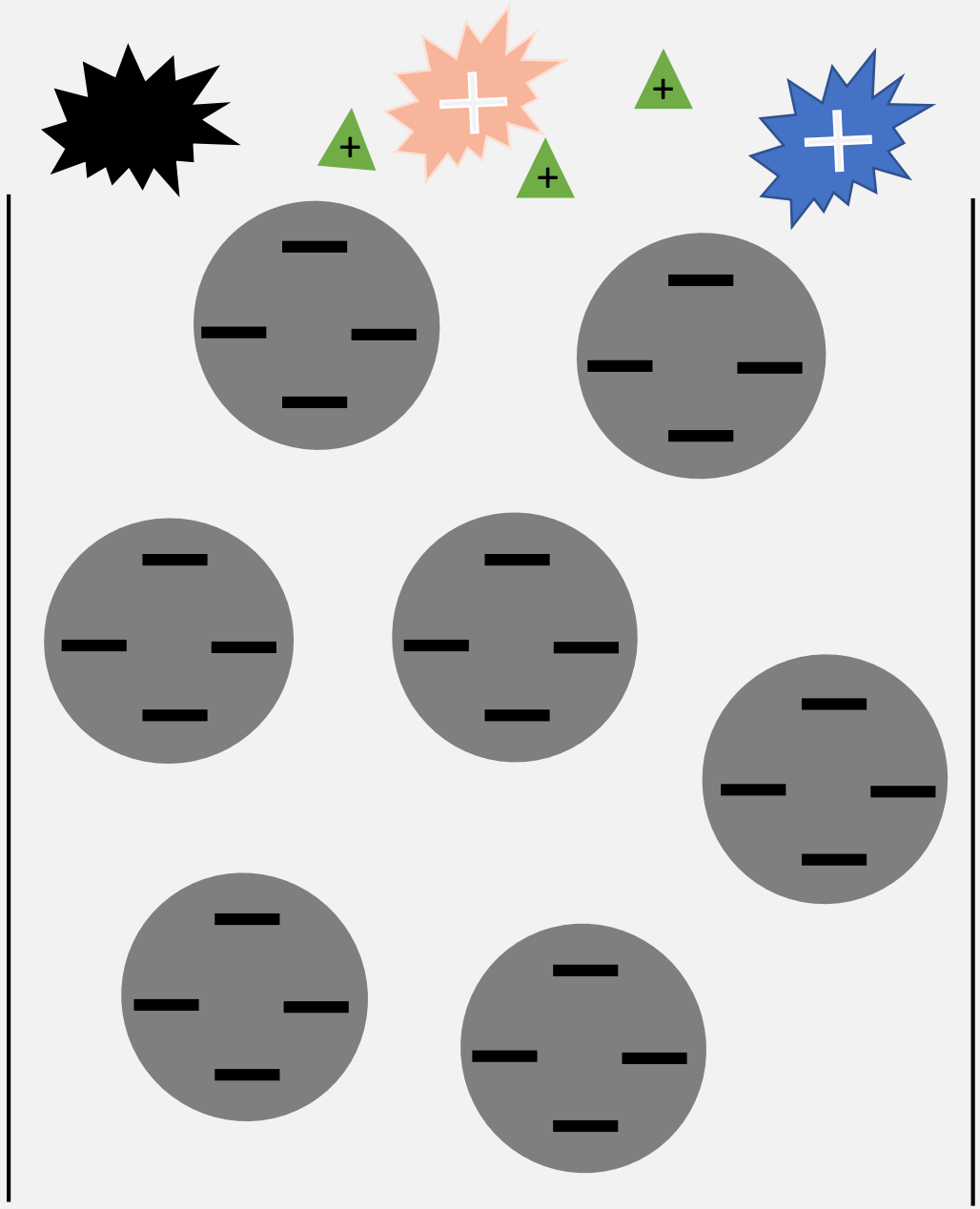
purification of endo-1,4-β-glucanase I

RUNNING BUFFER:

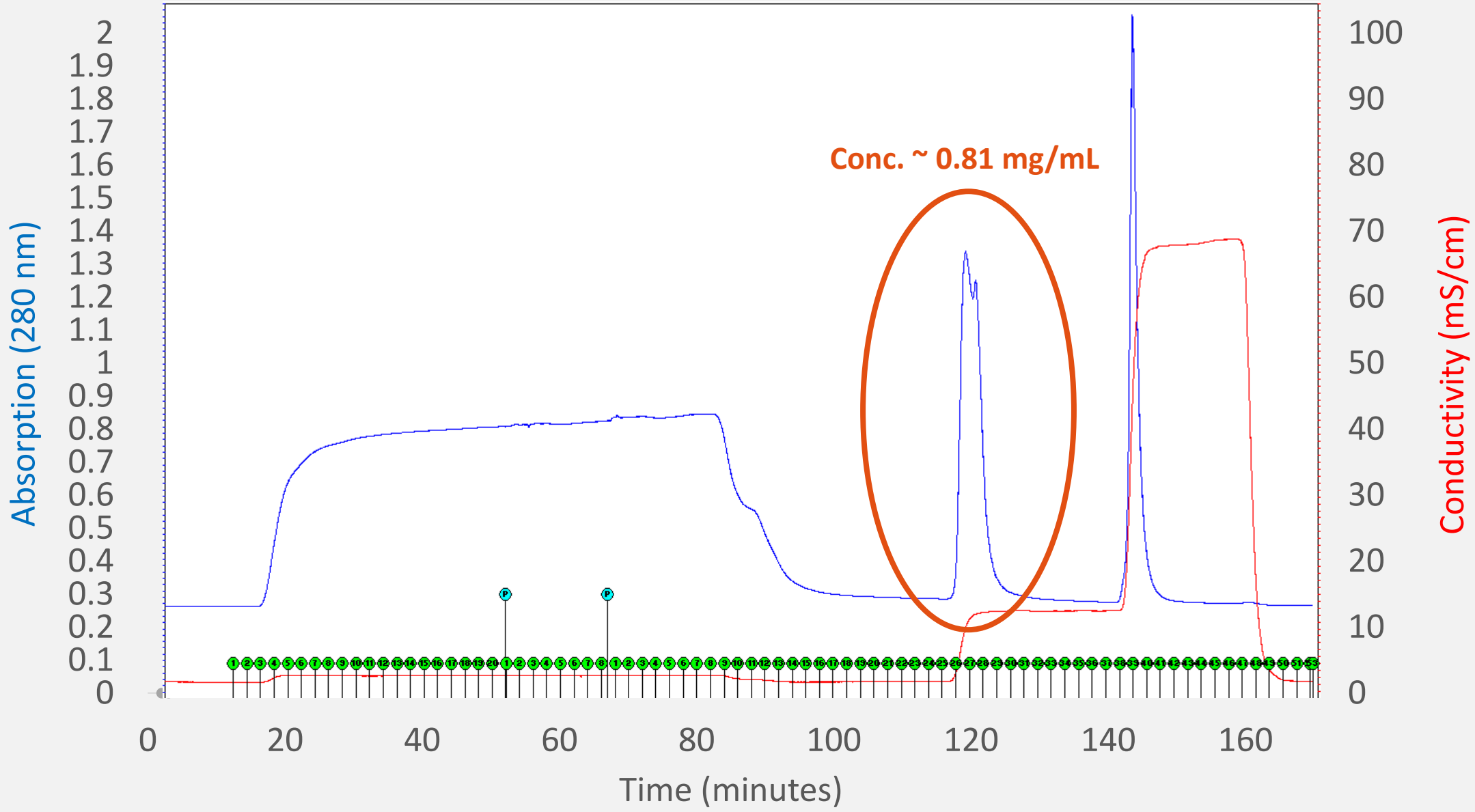
25 mM Sodium Acetate, pH 3.5

ELUTION BUFFER:

25 mM Sodium Acetate, pH 3.5, **1 M NaCl**, pH 3.5



Cation Exchange Chromatography



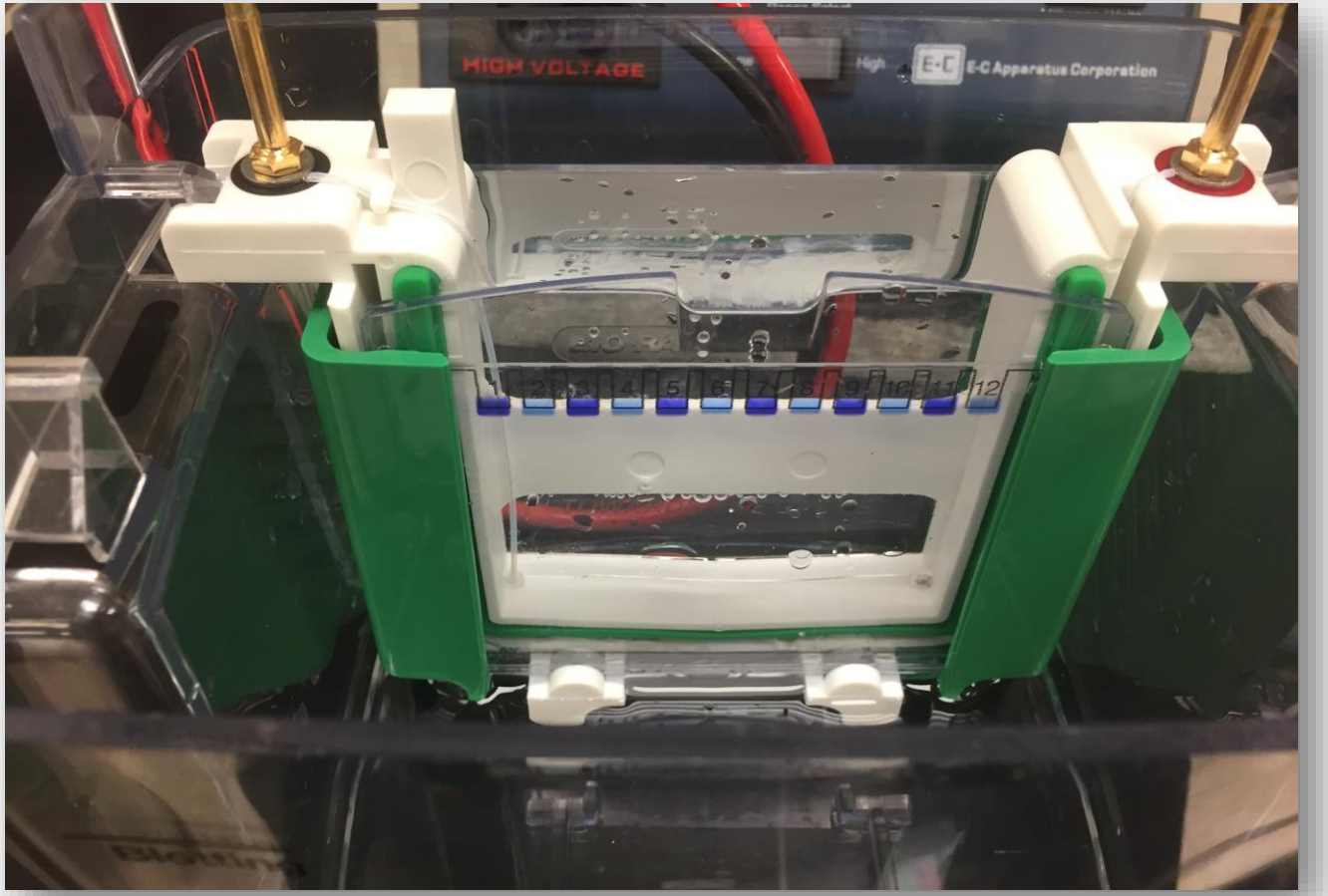
Results & Discussion

Fast Protein Liquid Chromatography

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

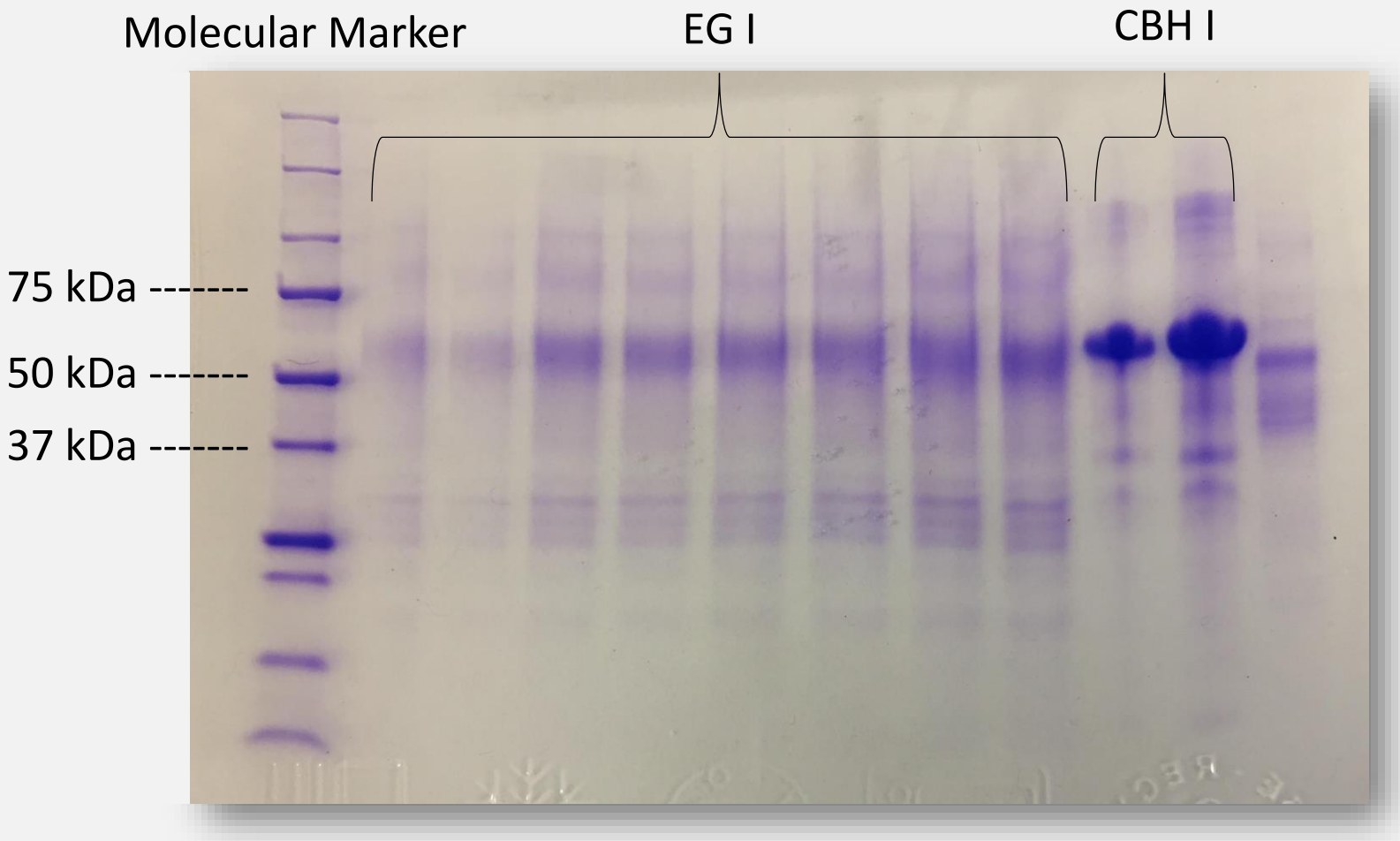
PURPOSE:

determine purity and identity of samples by estimating difference in size



Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

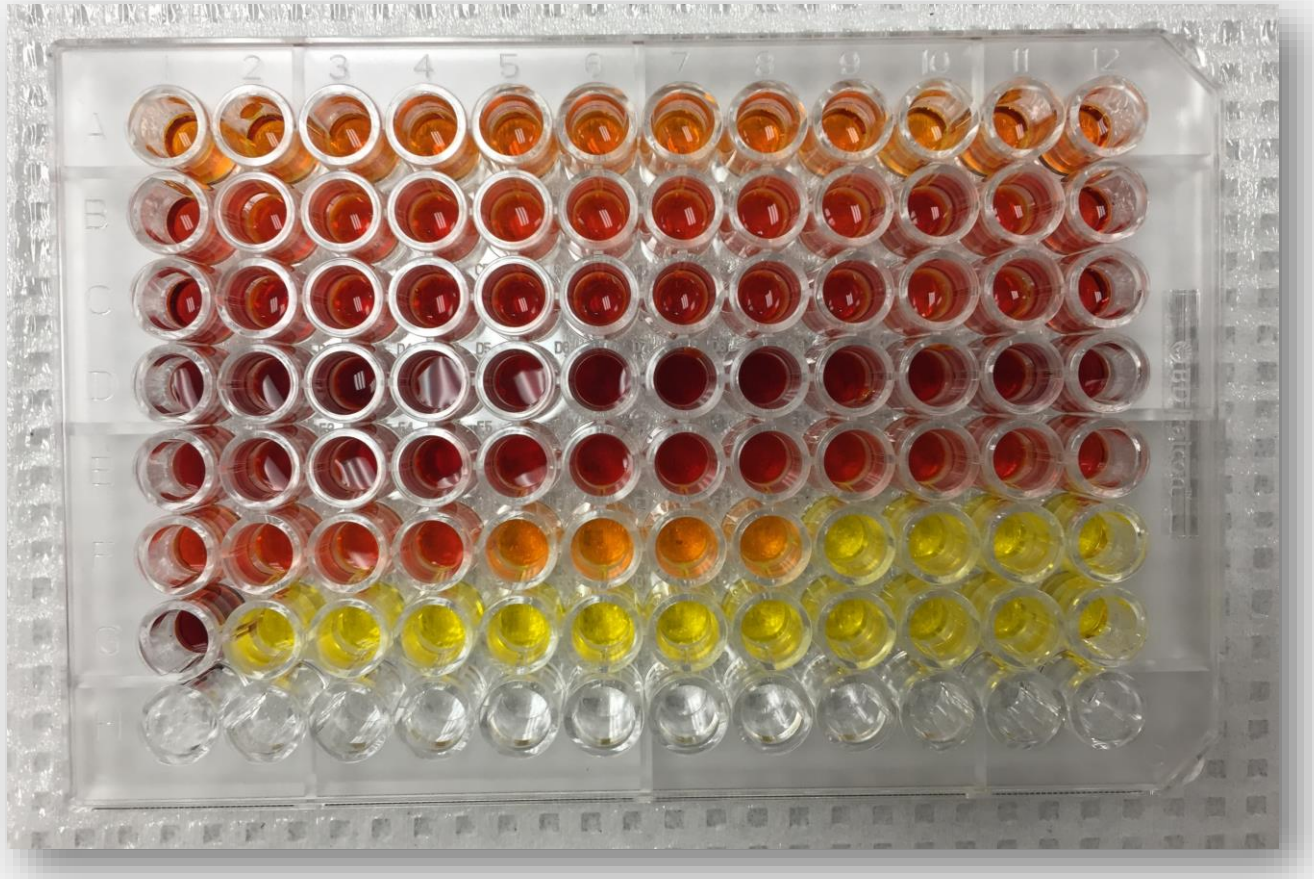
RESULTS:



3,5-Dinitrosalicylic Acid (DNS) Assay

PURPOSE:

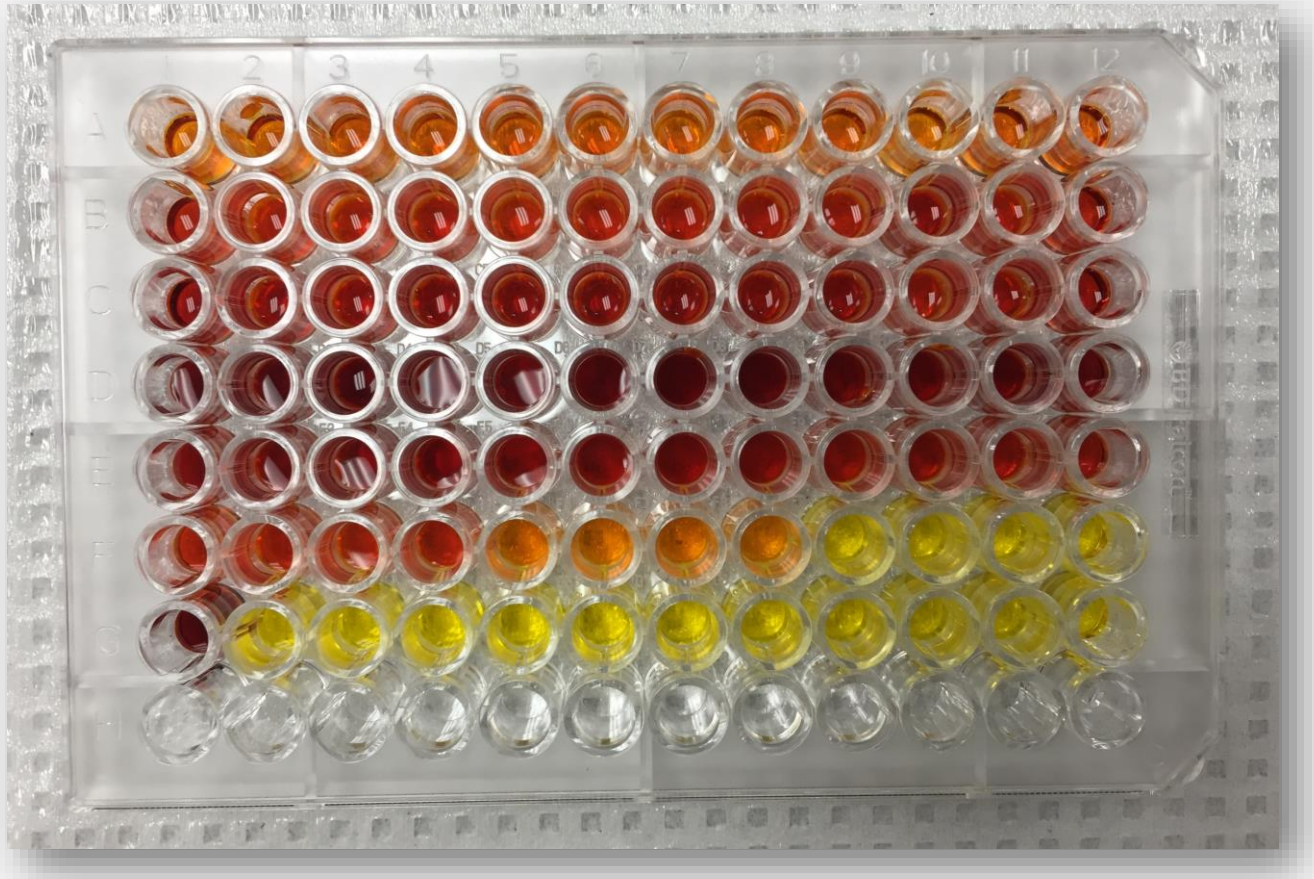
determine the identity and purity of sample by measuring specific activity on cellulose



3,5-Dinitrosalicylic Acid (DNS) Assay

RESULTS:

	Carboxymethyl Cellulose (U/mg protein)	Avicel (U/mg protein)
EG I	0.32	0.11
CBH I	N/A	0.05



Materials & Methods

Enzymatic Hydrolysis

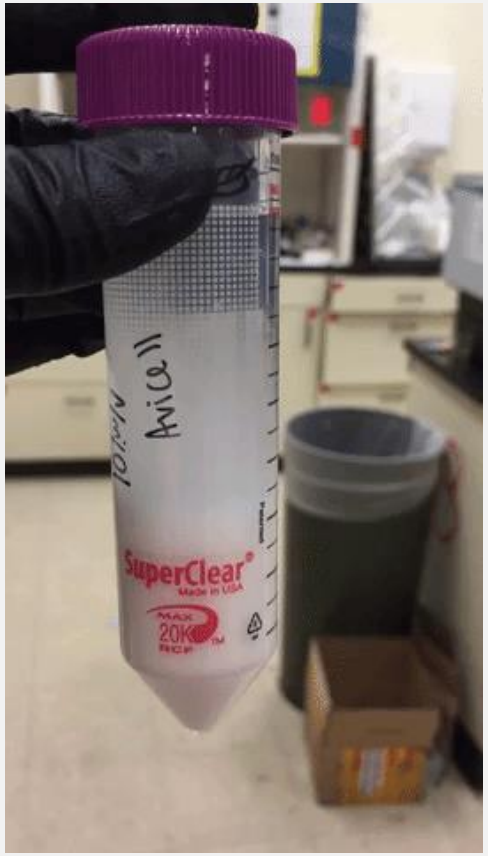
Enzymatic Hydrolysis: Treatments

Treatment	EG I %	CBH I %	CBH II %	BG %
1	3.75	52.5	18.75	25
2	7.5	52.5	15	25
3	15	45	15	25

- 20 mg protein/g glucan
- Cellulose 10% w/v

Enzymatic Hydrolysis: Cellulose Types

Carboxymethyl

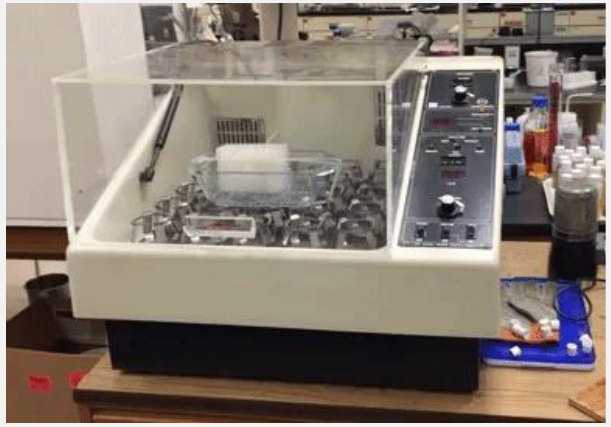


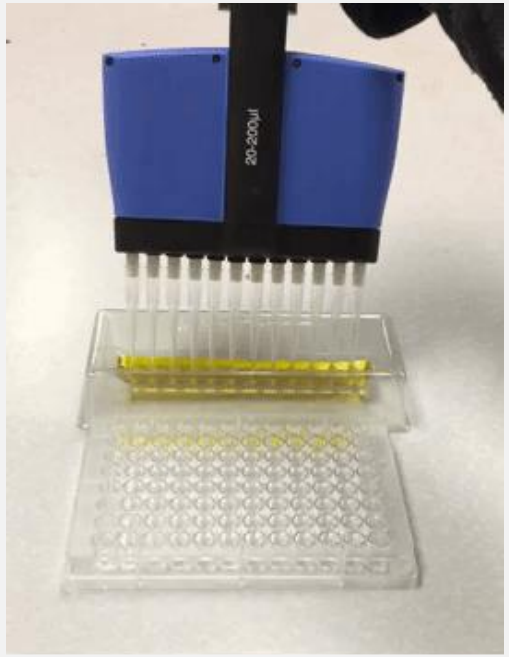
Avicel

Bacterial

Enzymatic Hydrolysis: Reaction Steps

1. Incubated at 50°C for **72 hours**
2. Added 60 µL of DNS reagent and 60 µL of sample into a 96-well plate
3. Heated at 95°C for 5 minutes
4. Ice Bath for 5 minutes
5. Measured at 540 ηm with UV-vis spectrophotometer

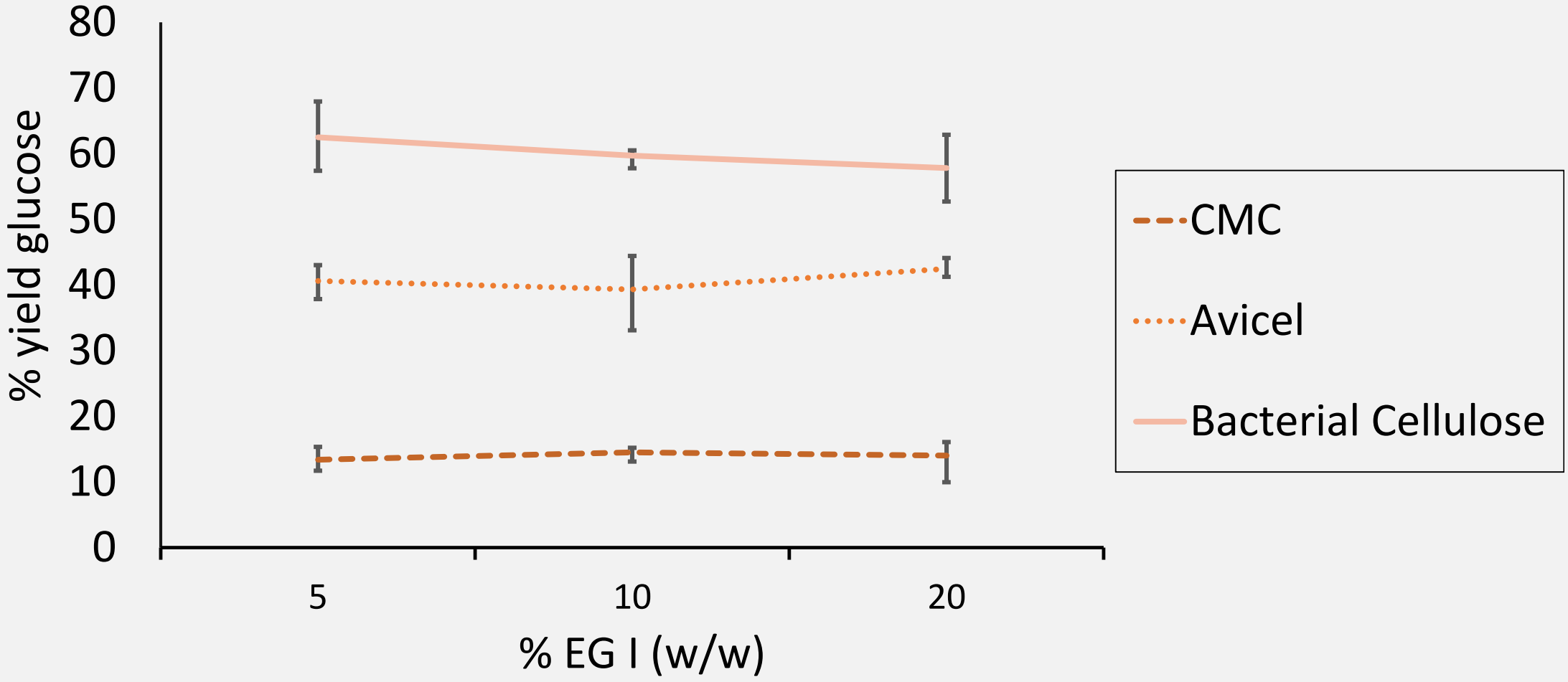




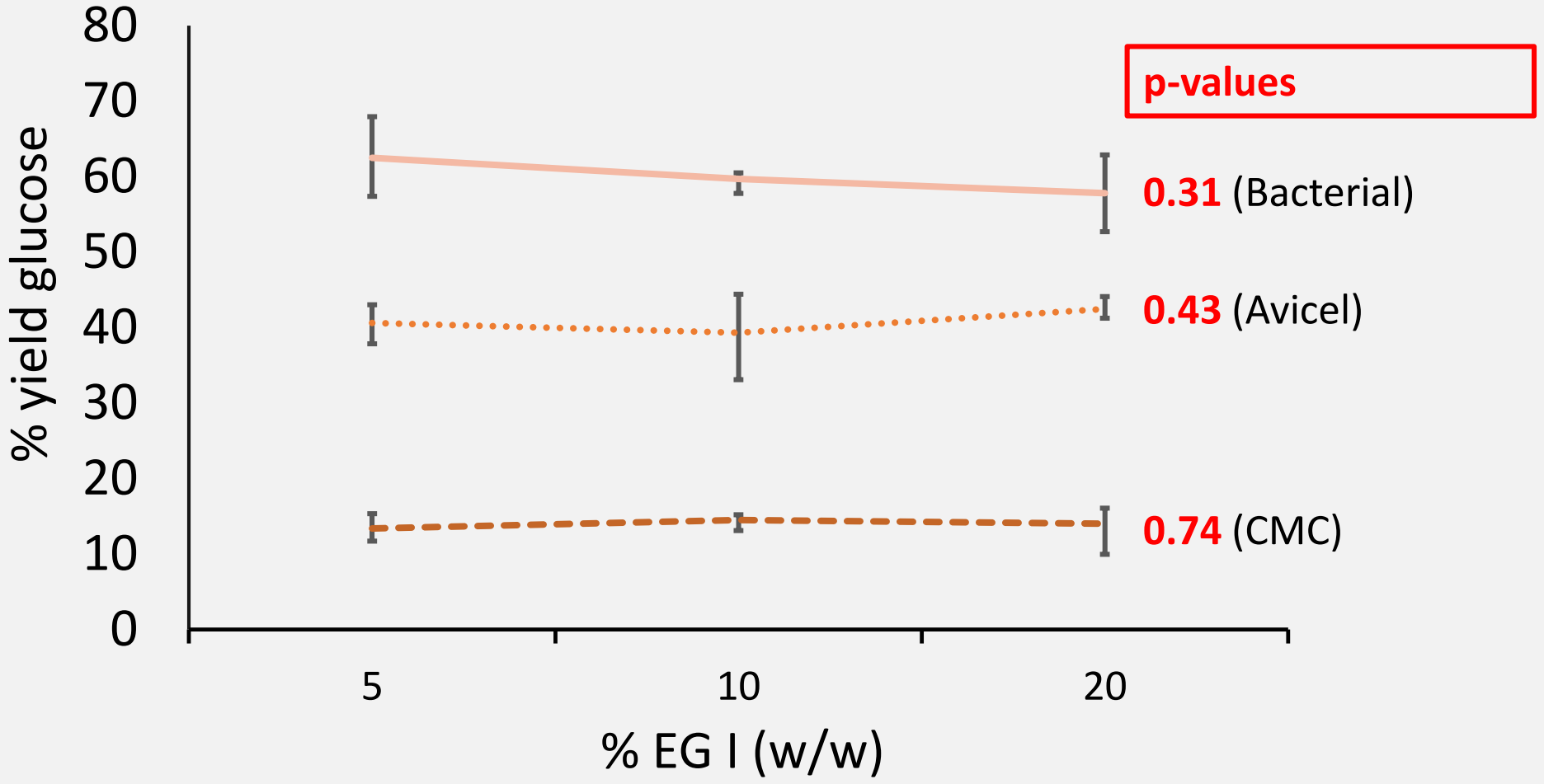
Results & Discussion

Enzymatic Hydrolysis

Enzymatic Hydrolysis: Results



Enzymatic Hydrolysis: Results



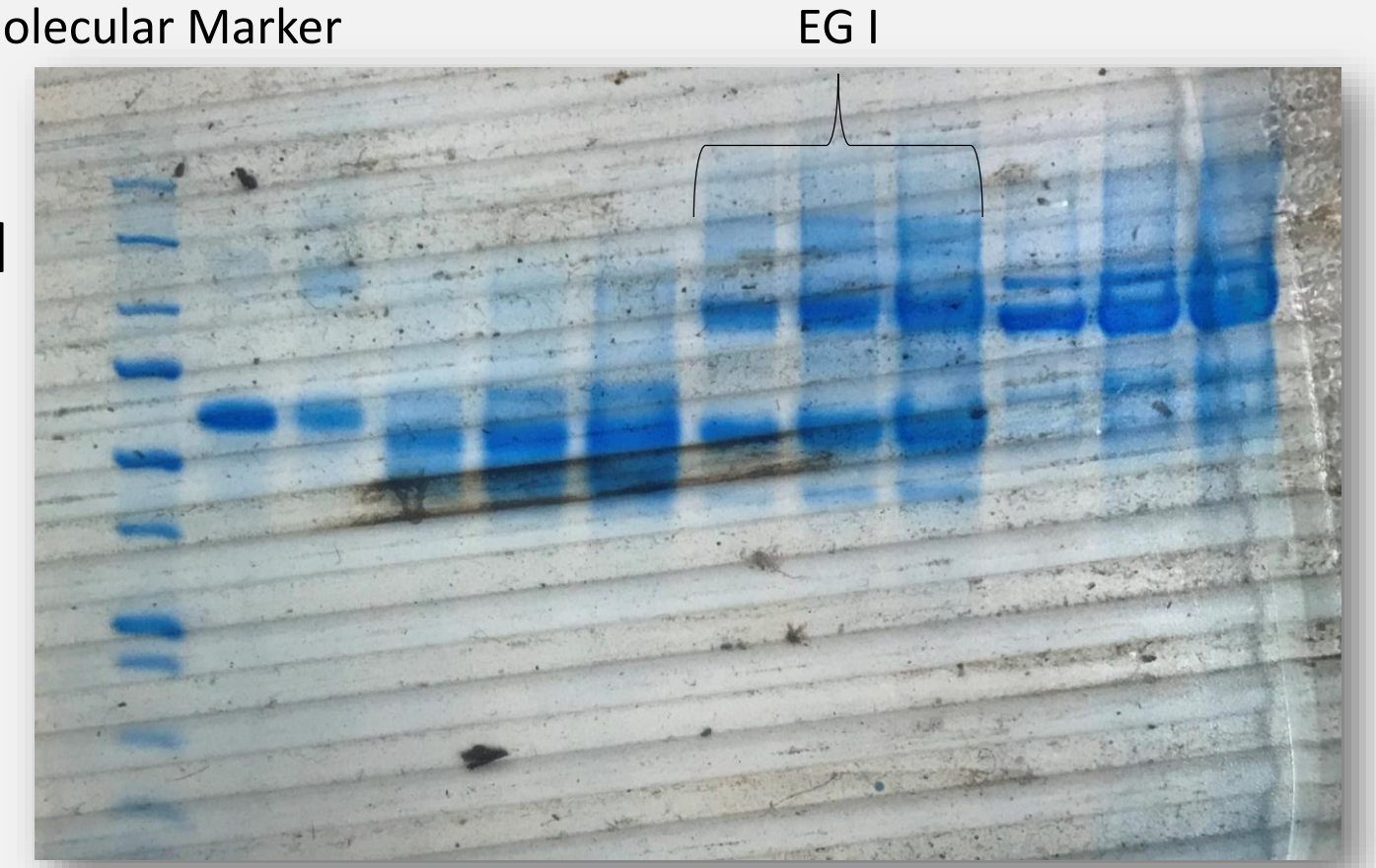
Conclusion

- Endo-1,4-β-glucanase I was partially purified using three steps of fast protein liquid chromatography, confirmed via SDS-PAGE, and a specific activity assay.

- There were no significant differences recorded between the three enzyme treatments on any of the biomasses.

Future Research

- Changing the buffer used in the last purification step, cation exchange, improved SDS-PAGE results.



Acknowledgements

The Sustainable Technologies Laboratory