Biomass to Sugar Conversion Mechanism Through Thermochemical Pretreatment and Enzymatic Reactions For Biofuels and Biochemicals Production

Rajesh Gupta

12-Apr 2021

LEE ENTERPRISES CONSULTING, INC.

Expertise: World's largest bioeconomy consulting group - over 150 subject matter experts (SME's)- all areas of the bioeconomy.

Approach: Project interdisciplinary teams to meet exact needs of specific projects.

POC: Handle projects with one agreement and single point of contact.

Cost Advantage: Single POC = lower administrative costs = lower project cost.

Key Messages

- In addition to lignocellulosic feedstock, algal biomass (especially microalgae) is a good source of fermentable sugars.
- Lignocellulosic and algal biomass can be utilized to produce biofuels (bioethanol or biogas) or biochemicals.
- Pretreatment method should be chosen based on type of feedstock and its composition.
- In addition to Cellulase, supplemental enzymes synergistically work with cellulase and helps reduce overall enzyme loading, thus reducing the enzyme cost in lignocellulosic biofuels production.
- To reduce the overall enzyme cost, same pretreated biomass should be utilized for enzyme production which is used as the feedstock.
- Enzyme production should be integrated within the biorefinery process to reduce the overall production cost of biofuels or biochemicals.

Pretreatment/Enzymatic Hydrolysis for a Variety of Feedstocks and Products

Biomass

Lignocellulosic
Algae

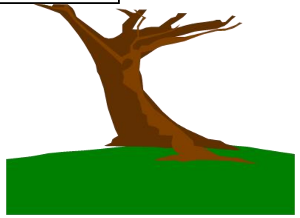
→ Pretreatment

Enzymatic Hydrolysis

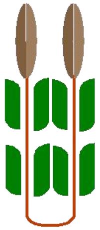
→ Sugar Fermentation

→ Biogas
→ Bioethanol
→ Biochemicals

Agricultural Residues
(Corn stover, Wheat straw etc.)



Woody Substances
(Poplar, Pine etc.)

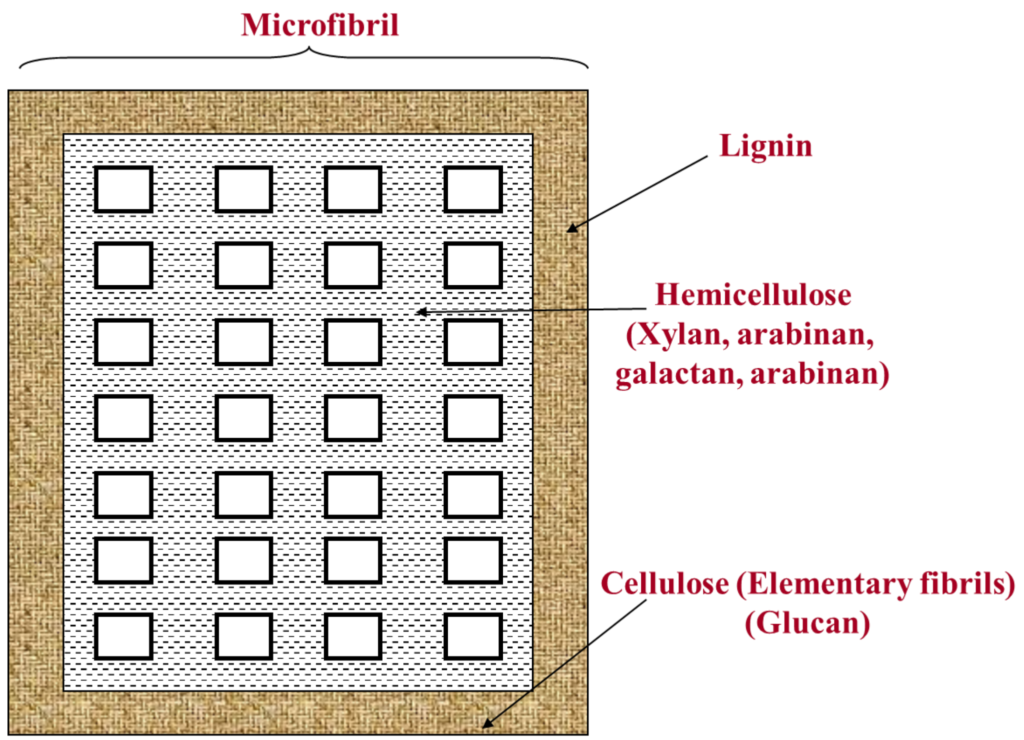


Perennial Crops
(Switch grass, Miscanthus etc.)

Algae

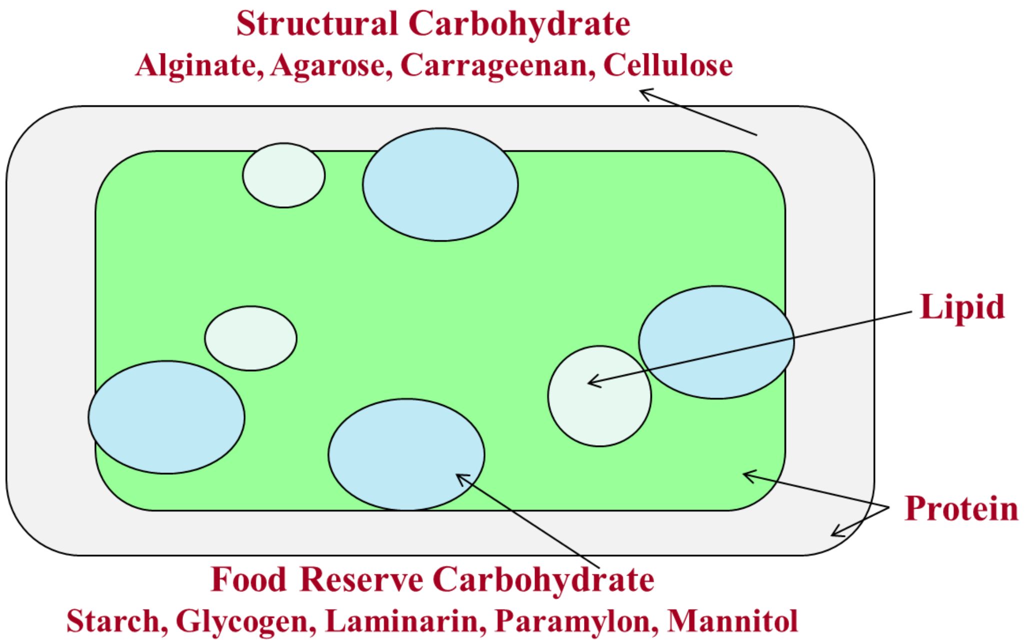
LEE ENTERPRISES CONSULTING, INC.

Schematics For Lignocellulosic And Algal Biomass



Cell Wall Microfibril Schematics in Lignocellulosic Biomass

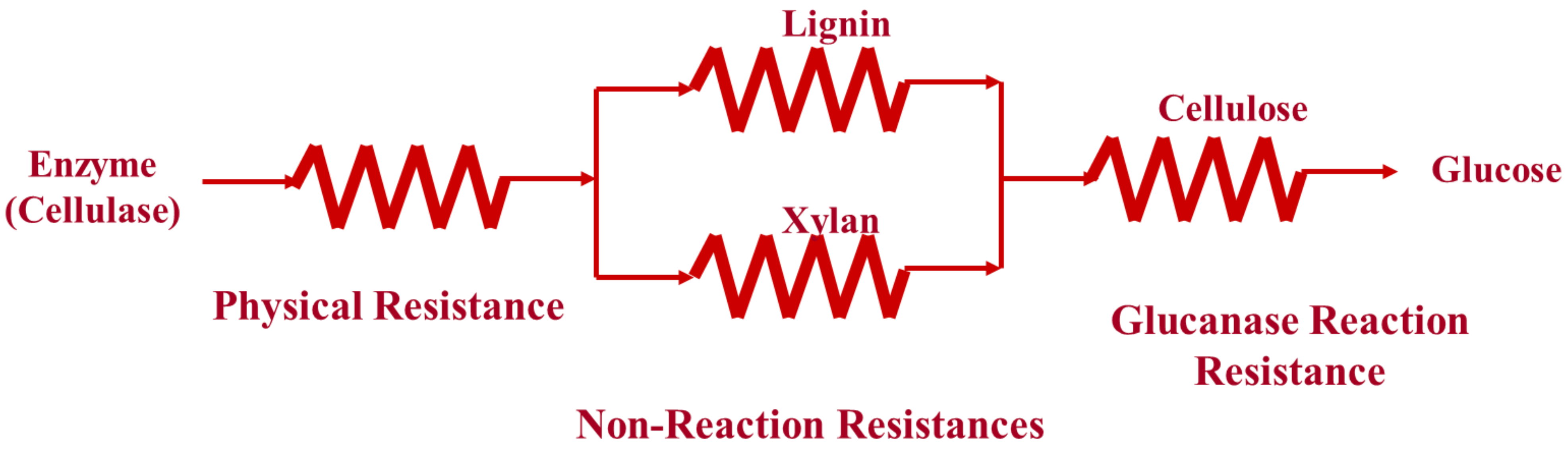
(Zhang et al., 2004)



Algal Cell Schematics

LEE ENTERPRISES CONSULTING, INC.

Resistances For Enzymatic Hydrolysis Of Lignocellulosic Biomass



- Biomass Surface Area
- Enzyme Adsorption Capacity to Biomass

- Limits internal diffusion of cellulase to cellulose

- Crystallinity
- Degree of Polymerization

Pretreatment of biomass reduces these resistances and increases enzymatic digestibility

LEE ENTERPRISES CONSULTING, INC.

Feature Of Some Popular Pretreatments

Pretreatment Method	Pros	Cons
Dilute Acid	High digestibility of pretreated solid Hemicellulose sugars are dissolved as monomer	Neutralization of liquor is costly Inhibitory compounds produced for fermentation
Steam Explosion	Works well with high lignin substrate	Inhibitory compounds produced for fermentation Hemicellulose recovery is low
Steam Explosion with SO2 impregnation	High hemicellulose recovery Low sugar degradation	Hemicellulose recovery in oligomer form will require higher enzymes
Hydrothermal	No chemical cost as only hot water is used for pretreatment	Does not work very well for high lignin substrate
Ammonia based (AFEX or Aqueous ammonia pretreatment)	High retention of hemicellulose	High operating pressure and ammonia handling
Lime pretreatment	Typically done at less severe conditions, hence lower capex and safer operation	Pretreatment time may be much longer than other methods

Cellulosic Biomass Hydrolyzing Enzymes

- **Cellulose**
  - Cellulase: Multi-enzyme system of Endo-glucanase, Exo-glucanase and β-glucosidase
  - β-glucosidase: Converts cellobiose and COS to glucose

- **Hemicellulose**
  - Xylanase: Helps with pretreated biomass which retains xylan
  - β-Xylosidase: Remove inhibitory effect of XOS by converting it to xylose
  - Pectinase: Helps with biomass with high pectin and converts XOS to xylose
  - Feruloyl Esterase: Increases arabinoxylan digestibility and works with xylanase to deconstruct hemicellulose lignin matrix and promotes hydrolysis of XOS
  - Acetyl Xylan Esterase: Helps to improve xylanase activity by breaking acetyl linkage in xylan
  - α-L-arabinofuranosidase: Helps to hydrolyze arabinose substituted XOS
  - Mannanase: Works synergistically with xylanase and cleaves side chains containing mannose and arabinose.

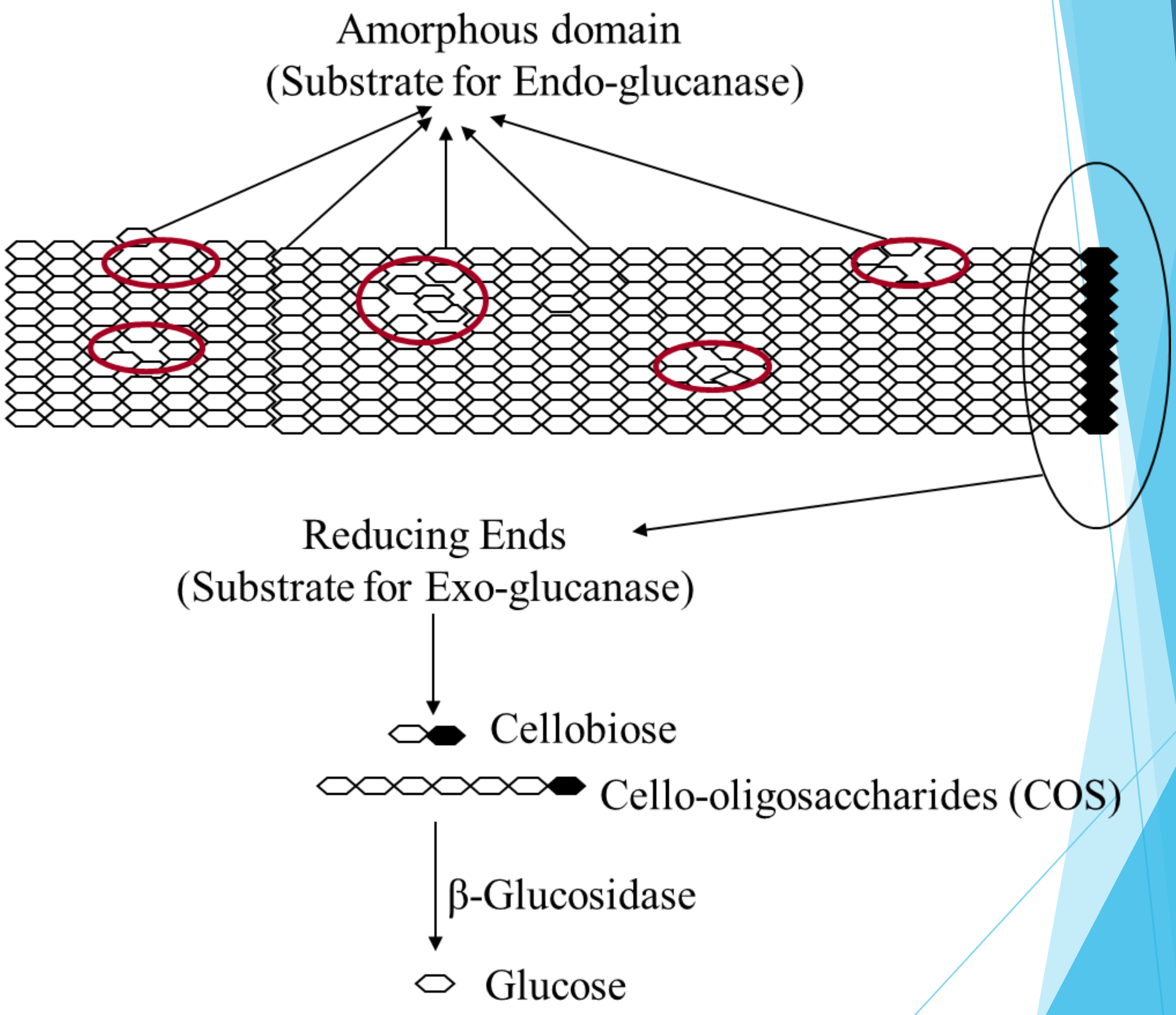
COS: Cello-oligosaccharides
XOS: Xylo-oligosaccharides

- **Lignin**
  - Laccase and lignin peroxidase cleave lignin molecules, improve cellulase activity

Gupta & Lee, 2013

Enzymatic Hydrolysis Of Cellulose By Cellulase

- Hydrolysis rate of cellulose by cellulase enzyme depends primarily on its degree of polymerization and crystallinity.
- Endo-glucanase cleaves cellulose chain at amorphous region while Exo-glucanase hydrolyze the chain from non-reducing end.
- β-Glucosidase converts cellobiose and low DP COS produced from exo-glucanase reaction to glucose.
- Different pretreatments alters DP and crystallinity of cellulose in biomass and will require different activities of these cellulase components.
- Amorphous cellulose tends to produce high DP Cello-oligosaccharides (COS) which remains unconverted and also inhibit enzyme activity.
- Pretreatment severity should be adjusted to prevent formation of high DP COS or else glucose yield will be less after enzymatic hydrolysis.



Gupta & Lee, 2008

Algae Hydrolyzing Enzymes

- **Starch (Amylose and Amylopectin)**
  - Isoamylases and pullulanases
  - α- amylases
  - β-amylases
  - Glucoamylases
- **Floridean/Glycogen**
  - Same enzymes as starch
- **Glycogen**
  - Same enzymes as starch
- **Laminarin/Chrysolaminarin**
  - Glucanases
  - β-glucosidase
- **Alginate**
  - Lyases
- **Agarose**
  - α- agarases
  - β-agarases
  - β-agarobiose hydrolase
  - α-neoagarobiose hydrolase

Abdallah, Nixon & Fortwendel, 2016

A Recipe For Low Enzyme Cost In Biorefinery

- **Choose the biomass pretreatment appropriate as per biomass characteristics such as lignin and hemicellulose ratio and type of their structure and composition.**
- **Severity of pretreatment should be adjusted so it does not cause degradation of monomer sugars during pretreatment.**
- **Another criterion for pretreatment tuning should be possibility to avoid high DP COS formation during enzymatic hydrolysis which remains unreacted during enzymatic saccharification.**
- **High throughput assays and machine learning algorithms can be used to optimize the dosage of supplementary enzymes in addition of cellulase with an objective to lower the total protein loading.**
- **Supplementary enzymes and cellulase producing micro-organisms should be used to express these enzymes in optimized ratio to minimize the production cost of final product. This is the key challenge that a microbiologist needs to work on for a particular feedstock mix.**

Rajesh Gupta
Accredited Member
Lee Enterprises Consulting, Inc.
experts@lee-enterprises.com

Lee Enterprises Consulting
www.lee-enterprises.com
1+ 501 833-8511

LEE ENTERPRISES CONSULTING, INC.