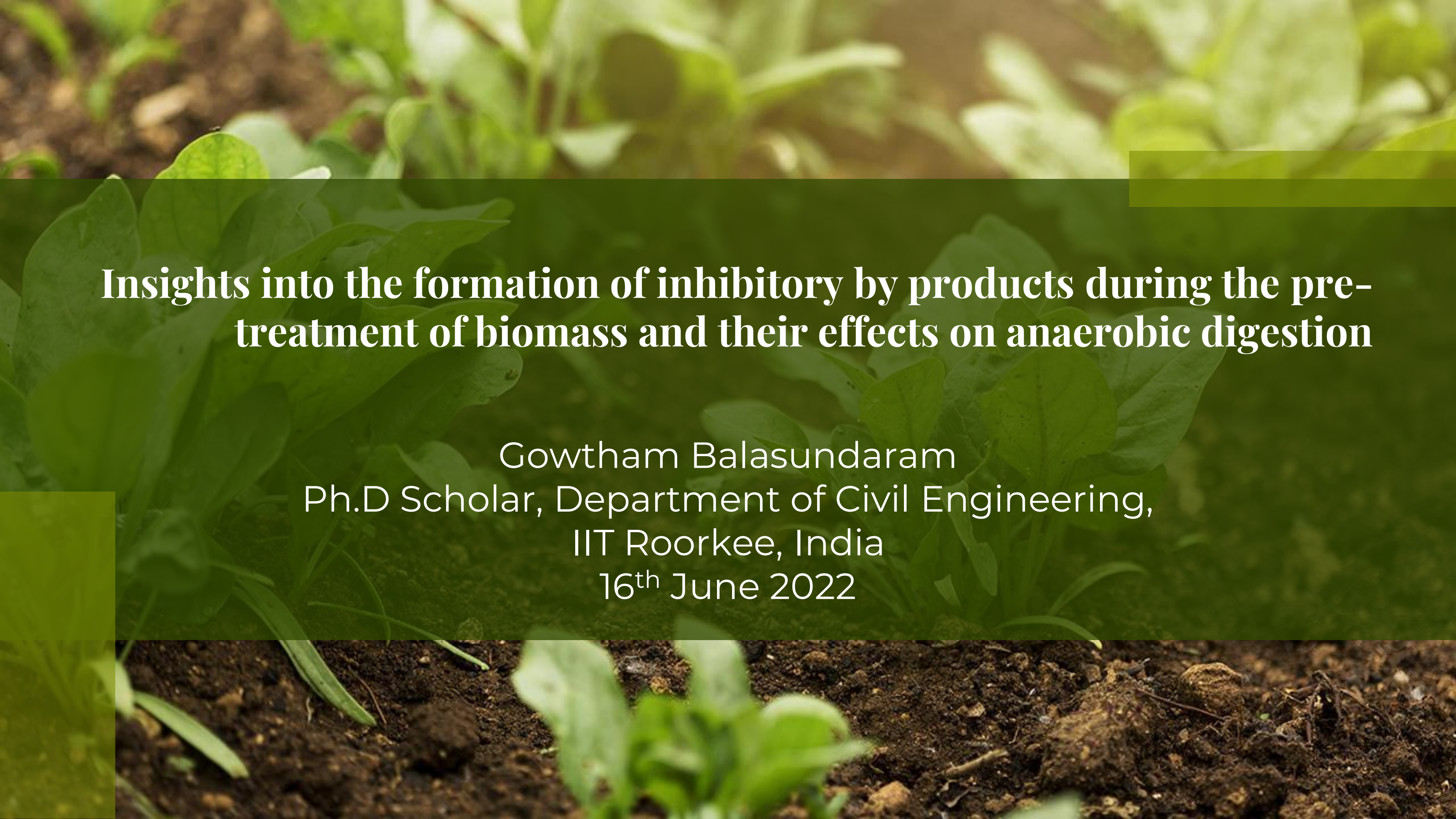

# Insights into the formation of inhibitory by products during the pre-treatment of biomass and their effects on anaerobic digestion

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- Ion exchange process
- Biological methods

Detoxified feedstock goes for Biomethanation

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# Introduction

- ✓ Anaerobic digestion (AD) -most commonly used - organic waste stabilization worldwide due to low energy input requirement, energy-rich methane generation, and reduced waste volume (Tyagi and Lo, 2011)
- ✓ Limitations of AD- Low biodegradability and Longer retention time.
- ✓ Hydrolysis – Rate Limiting step (Gonzalez et al., 2018).
- ✓ 20–30% of the lignocellulosic biomass is converted into biogas if feedstock pretreatment is not employed (Kucharska et al., 2018; Lee et al., 2019; Oleszek et al., 2014 )
- ✓ Pretreatment methods- physical (thermal, ultrasonication, high-pressure homogenizer), chemical (acid, alkali, peroxidation), and biological methods (bacterial and fungal strains).
- ✓ Severe pretreatment conditions- phenolic compounds, soluble derivatives, and soluble organics including acetate, furan aldehydes such as furfural, hydroxymethylfurfural and phenolics that can inhibit microbial activities during fermentation and enzymatic degradation (Cao et al., 2013 )
- ✓ To enhance the biodegradability of the pretreated biomass, it is crucial to understand the mechanism of recalcitrant production.

# Pre-treatment methods

- Chemical
  - Alkali
  - Acid
  - Oxidative
  - Organosolvents
- Physical
  - Mechanical
  - Thermal
  - Ultrasound
  - Microwave
- Biological
  - Enzymatic
  - Fungal
  - Bacterial
- Combined
  - Steam explosion
  - Thermochemical

Recalcitrant/ Inhibition by products ( Sugars, Furan derivatives, phenolic compounds, weak acids etc)

# Recalcitrant- Types and formation

- Physical and chemical pretreatments have been proven to show higher efficiency; however, they produce recalcitrant organic compounds under harsh treatment conditions.
- Recalcitrant compounds that cause inhibition effects can be classified into furans derivatives, phenols, and short-chain organic acids (Palmqvist and Hahn-Hagerdal, 2000)
- Based on the chemical functional groups, Jayakody et al. (2016) classified the recalcitrant into ketones, organic acids, aldehydes, and phenols
- Based on the chemical properties and origin of the inhibitor, Sjulander and Kikas (2020) grouped the compounds into four groups, mainly sugar derived aldehydes (Glycolaldehyde, furfural, hydroxymethylfurfural, coniferyl aldehyde, vanillin, syringaldehyde), short-chain organic acids (Formic acid, acetic acid, lactic acid, levulinic acid) and aldehydes, aromatic compounds (4-hydroxycoumaric acid, syringic acid, vanillic acid, salicylic acid, ferulic acid, 4-hydroxy-benzoic acid, catechol, guaiacol, Benzyl alcohol, cinnamaldehyde, benzoic acid, cinnamic acid, para- and *ortho*-toluic acid, 3,4-dimethoxy-cinnamic acid, *p*-Benzoquinone 2, 6-Dimethoxybenzoquinone), and others (Monomeric and oligomeric pentoses, xylose, copper, iron nickel, chromium).

# Furan derivatives

- Furfural and Hydroxymethyl furfural (referred to as HMF hereafter) are the furan derivatives found in lignocellulose hydrolysates .
- Dehydration of pentose and hexose sugars- furan derivatives. Xylose, mannose, galactose, acetic acid, and glucose are liberated because of hemicellulose degradation.
- Xylose is further degraded to furfural under high temperature and pressure. Hexose degrades to form HMF. HMF and furfural further break down to form formic acid. HMF degradation also gives rise to the formation of levulinic acid (Palmqvist and Hahn-Hagerdal, 2000)
- Maillard and caramelization reactions are more likely to occur in the pretreatment steps during the thermal pretreatment. Maillard reaction (Maillard, 1912) occurs between reducing sugar and amino group at higher temperatures resulting in dark brown colored, UV quenching, and hardly biodegradable polymers (Zhang et al., 2020). Meanwhile, another non-enzymatic browning reaction at low water activity is called caramelization (Barber, 2016; Gonzalez et al., 2018). These reactions could form inhibitory compounds like HMF, furfural, caramelans, and melanoidins (Bolado-Rodríguez et al., 2016; Monlau et al., 2012)

# Furan derivatives

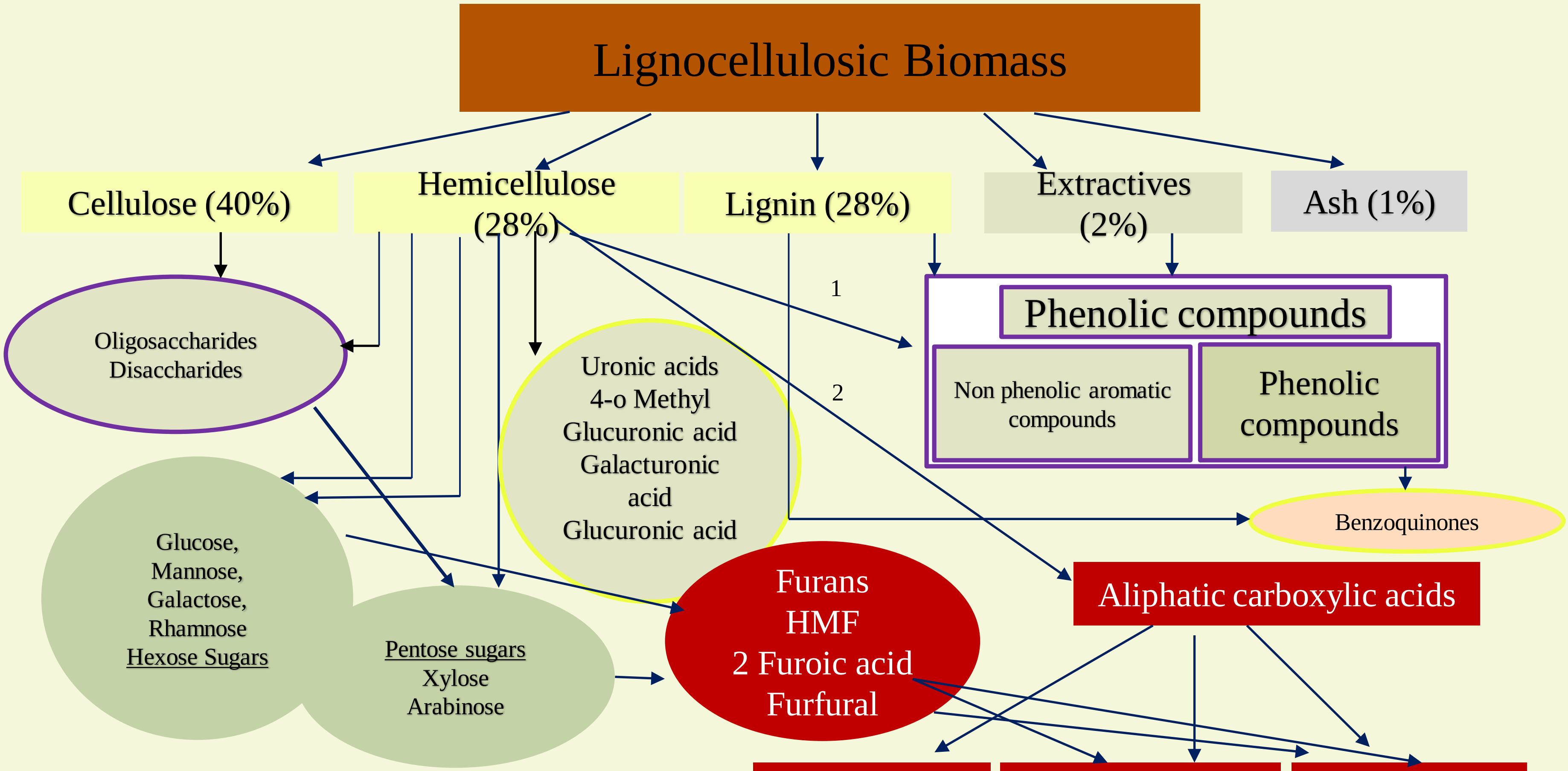
- The thermal pretreatment at 180 ◦C showed a significant increase in methane yield; however, above 200 ◦C, HMF and furfural concentrations were significantly increased above 300 mg/L and 3000 mg/L, respectively.
- Partial conversion of lignin polysaccharides and lignin to heterocyclic or phenolic compounds like furfural, vanillin, vanillic alcohol, and HMF has occurred at temperatures above 160 ◦C.
- Mild thermal pretreatment conditions (<160 ◦C) may prevent the formation of these inhibitory compounds (Barakat et al., 2012; Zieminski and Kowalska-Wentel, 2017).

# Phenolics

- Acid-catalyzed hydrolysis can cause the formation of phenolic compounds.
- Phenolics interfere with the cell membrane by changing its protein to lipid ratio and influencing its function (Jonsson et al., 2013)
- Pretreatment of lignin causes depolymerization and repolymerization leading to the formation of degradation byproducts, which are mainly phenols. Depolymerization is caused due to hydrolysis, and repolymerization is caused by the reactions between carbonium ions and nucleophiles (Li et al., 2007).
- For example, Microwave pretreatment of switchgrass resulted in the production of phenolic compounds (Jackowiak et al., 2011).
- Because of their low molecular weight (MW), phenolic compounds could penetrate cell membranes and damage their internal structures making them more toxic for anaerobes when compared to the other recalcitrant (Nguyen et al., 1999).

# Weak organic acids

- Degradation of sugar results in levulinic and formic acids. Uronic and acetic acids are released from hemicellulose. HMF degrades to form levulinic acid and formic acid under severe pretreatment conditions, i.e., longer reaction times, higher temperature, and acid concentration.
- Carbohydrate degradation occurs during alkaline pretreatment leading to the formation of carboxylic acids. Saponification of the acetyl groups forms acetic acid (Jonsson et al., 2013; Jonsson and Martín, 2016). The inflow of undissociated acid into the cytosol causes inhibition effects on the microorganisms.
- The acetic acid released as the wood component when eucalyptus wood chips exposed to high-temperature steam, which further catalyzes the hydrolytic reactions of the constituent polymers (Martin-Sampedro et al., 2011).
- Phuttaro et al. (2019) reported a significant increase in acetic acid production with increasing pretreatment temperatures. When the pretreatment temperature was increased from 125 ◦C to 200 ◦C, acetic acid production increased from 294.9 ± 3.2 to 3058.5 ± 13.6 mg/L. When rice straw and rice husk were hydrothermally pretreated (210◦C, 160 min) formation of ferulic acid, diferulic acids, p–OH–benzoic acids, p–OH–phenylacetic acid, truxillic acid, p–OH–benzaldehyde, protocatechuic aldehyde, vanillin, and vanillic acid were reported (Wu et al., 2018).



Lignocellulosic Biomass

Cellulose (40%)

Hemicellulose (28%)

Lignin (28%)

Extractives (2%)

Ash (1%)

Oligosaccharides
Disaccharides

Uronic acids
4-o Methyl
Glucuronic acid
Galacturonic acid
Glucuronic acid

Glucose,
Mannose,
Galactose,
Rhamnose
Hexose Sugars

Pentose sugars
Xylose
Arabinose

1

2

Phenolic compounds

Non phenolic aromatic compounds

Phenolic compounds

Benzoquinones

Furans
HMF
2 Furoic acid
Furfural

Aliphatic carboxylic acids

Acetic acid

Levulinic acid

Formic acid

1. Hydrolysis of esterified phenols
2. Hydrolysis of acetyl groups

*Source : Jonsson et al., 2013*

# Effects of Recalcitrants on AD

- Furan derivatives - high molecular weight, are difficult to degrade by anaerobes, and cause inhibition to the biogas yield (Ortega-Martínez et al., 2021)
- Furfural >>inhibitory to microbial processes than HMF because it affects the enzymatic activity during glycolysis and TCA (tricarboxylic acid) cycle. Furfural also damages the membranes of mitochondria, nuclear chromatin, vacuoles, and actin cytoskeleton by causing the accumulation of Reactive oxygen species (ROS). However, HMF may reduce cell growth and extend the lag phase of the biological process. HMF persists much more prolonged than furfural since the conversion rate of furfural is four times faster than HMF, leading to the biological process lasting longer (Kim, 2018).

# Effects of Recalcitrants on AD

- Phenolics- DNA breakdown and hence inhibits the RNA and synthesis of proteins. Phenolics with high carbonyl content have been reported to interact strongly with essential amino acids (Sammond et al., 2014).
- Even in low concentrations, phenolics such as syringaldehyde, syringic acid, hydroxybenzaldehyde, coniferyl aldehyde, and vanillin have proven to show strong inhibition effects on fermentation (Clark and Mackie, 1984)

# Effects of Recalcitrants on AD

- Weak Organic acids: Internal acidification and direct interference with the acid are the main reasons for inhibition (Pampulha and Loureiro-Dias, 1989). The undissociated parts of the weak acids cause the influx of protons from the medium to cytosol by diffusion. After entering the cell, these undissociated acids dissociate into protons and anions because of neutral cytosolic pH. To maintain the internal pH near neutrality, and prevent dissipation of the proton motive force, protons must be pumped out via the plasma membrane requiring the hydrolysis of ATP. With the increase in the concentration of organic acids, more ATP has to be hydrolyzed, which demands more energy. When the influx of protons exceed the capacity of the ATPase, acidification of the cytosol will occur, followed by cell death. (Liu, 2011)

# Mitigation with Recalcitrant toxicity

- Recalcitrant formed during the pretreatment include furan derivates, phenols, sugars, weak acids, etc., Alkali treatment for inhibitors precipitation, the addition of sulfide for toxicity reduction, phenol-oxidase (laccase) for degradation of phenolic compounds, biological detoxification, adsorption using activated carbon, ion exchange, calcium-oxide based pH adjustment are some of the previously studied techniques for mitigating the recalcitrant toxicity (Gurram et al., 2011)
- Use of specific microorganisms or microbial enzymes has been reported to reduce the inhibitory effects of phenolic compounds, weak acids, and furan aldehydes.
- The effective removal of inhibitory compounds in the acid pretreated lignocellulosic hydrosylates has been achieved using microorganisms such as *Paecilomyces variotii, Coniochaeta ligniaria, Ureibaciilus thermosphaericus, S. cerevisiae*, and *T. reesei* mutants (Cao et al., 2013)

# Mitigation with Recalcitrant toxicity

- Technologies such as overliming and the use of activated carbon have been reported to remove inhibitors effectively. Overliming removed 75.6% of furan derivatives, whereas activated carbon resulted in 78% removal. The phenolic reduction was reported between 49-100% and 1–75% using activated carbon and overliming. The combination of overliming followed by the use of the activated carbon resulted in efficient removal of most inhibitory compounds (Zhang et al., 2018).
- Various conductive materials such as biochar, carbon fiber, granular activated carbon (GAC), magnetite, hematite, Carbon cloth, have been used by the researchers to enhance anaerobic digestion (Gahlot et al., 2020 )

# Conclusions

- High temperature thermal pretreatment (>165 ◦C) alone or in conjugation with chemical pretreatment (highly acidic or alkaline) produced recalcitrant their management is needed to evaluate the effects of recalcitrant formed during different pretreatment processes since they could have synergistic or additive effects on the enzymes or microorganism involved in the processes
- Optimization of the pretreatment conditions can minimize the formation of recalcitrant.
- Detoxification step for removal of recalcitrant can be considered before anaerobic digestion
- Biological detoxification, adsorption using activated carbon, ion exchange, and calcium-oxide based pH adjustment methods shows promising results in effective removal of recalcitrant from hydrolysate .

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# Thankyou

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