Characterization and Pretreatment of Milled Hemp (*Cannabis sativa* L.) Fibers from High-Cannabinoid Type Crops for Biochemical Conversion

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Hanah T. Rheay & Catherine E. Brewer

College of Engineering | Department of Chemical and Materials Engineering

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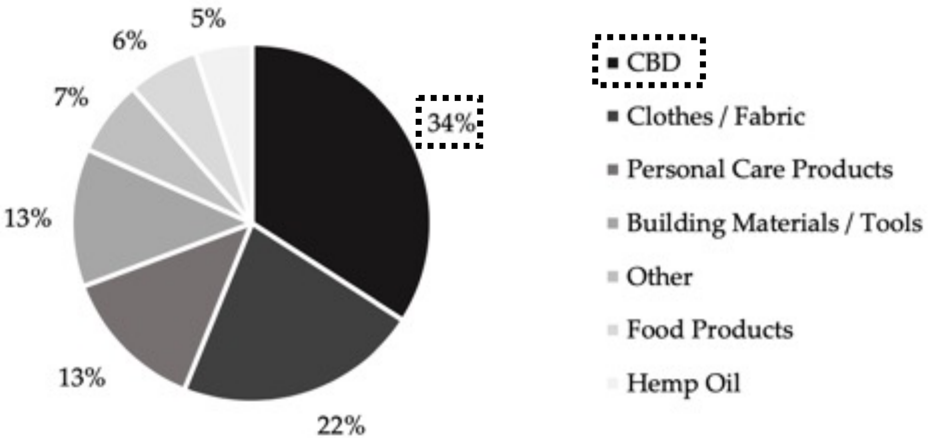
Types of Hemp

- Types of hemp: CBD/essential oil; grain; fiber; dual-purpose
- **Different morphology, physiology, and chemical profile between types**
- Current US market is dominated by CBD products

Different hemp types: (left) CBD-type; (right) industrial-types



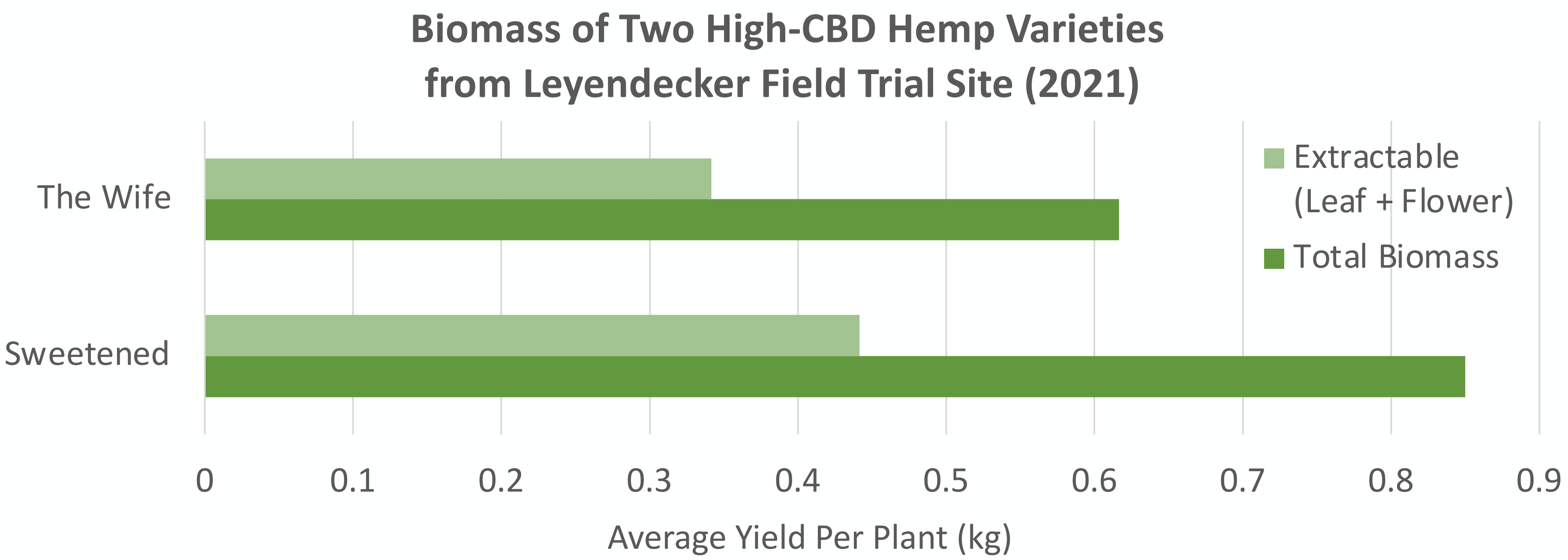
Percentage of total instances of hemp use by product category (Kolodinsky et al., 2020)

Kolodinsky, J., Lacasse, H., & Gallagher, K. (2020). Making hemp choices: Evidence from Vermont. *Sustainability*, *12*(15), 1–15. https://doi.org/10.3390/SU12156287.

BACKGROUND

Total vs. Extractable Biomass

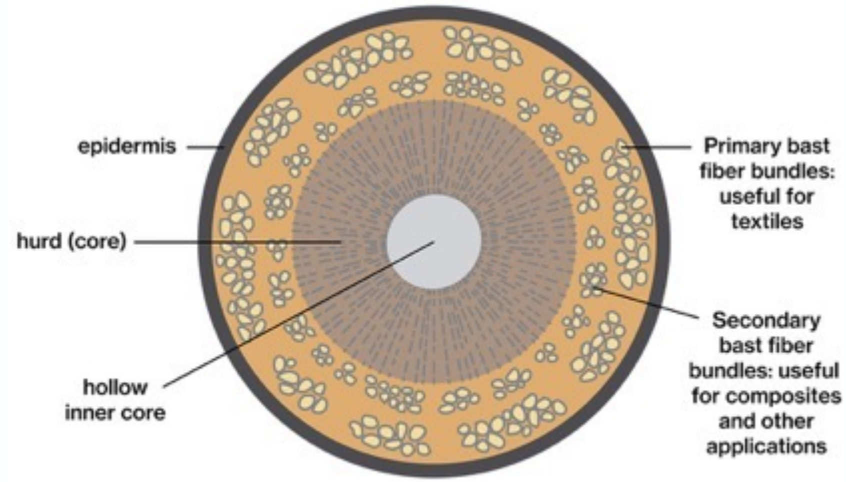
- Considerable amounts of residual lignocellulosic biomass (stalks/stems) are left from floral hemp production
- **30-51% of total crop weight was fiber across all NMSU sites/varieties**



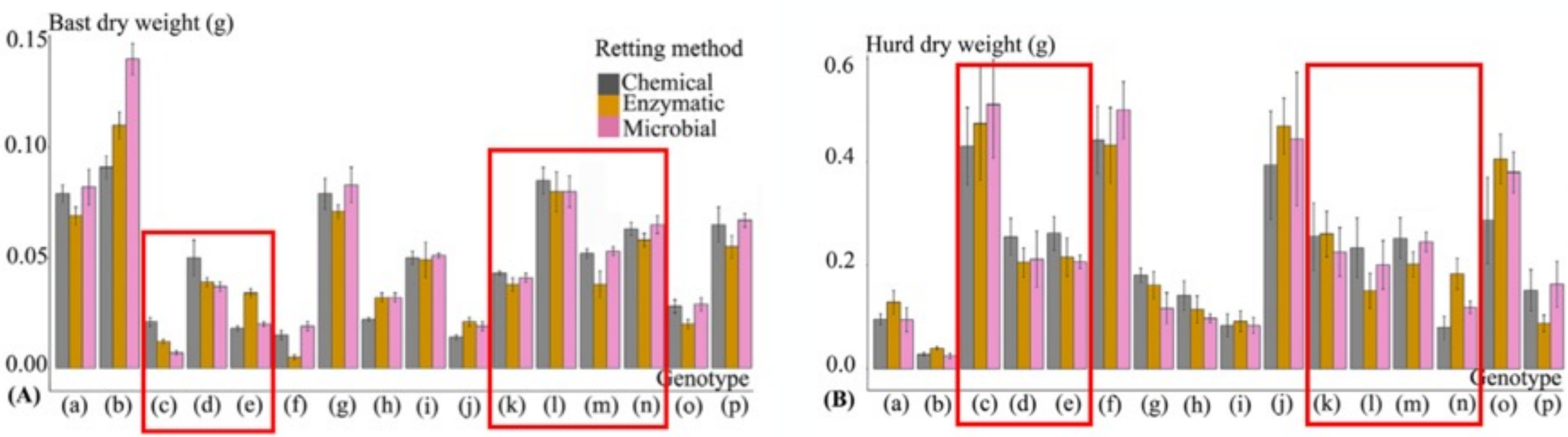
BACKGROUND

Hemp Fibers

- Traditional processing requires a decorticator to separate bast/hurd
- Minimal information is available on fibers of high-cannabinoid varieties
- **Genotype variation plays a major role in fiber yields across hemp types**



Hemp stalk cross-section (Fibershed, 2020)



Graphs representing mean dry weights (kg) of bast fibres (A) and mean dry weights of hurd fibers (B), under different retting treatments of hemp genotypes (Amarasinghe et al., 2022)

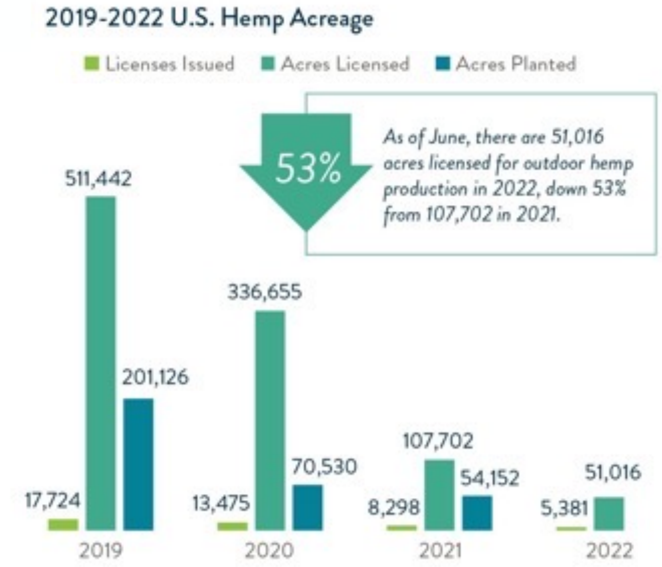
Amarasinghe, P., Pierre, C., Moussavi, M., Geremew, A., Woldesenbet, S., & Weerasooriya, A. (2022). The morphological and anatomical variability of the stems of an industrial hemp collection and the properties of its fibres. Heliyon, 8, e09276. https://doi.org/10.1016/j.heliyon.2022.e09276

Harvesting Hemp: Reflecting on Opportunities with the One Acre Exchange. (2020). Fibershed. https://fibershed.org/2020/09/16/harvesting-hemp-reflecting-on-opportunities-with-the-one-acre-exchange/

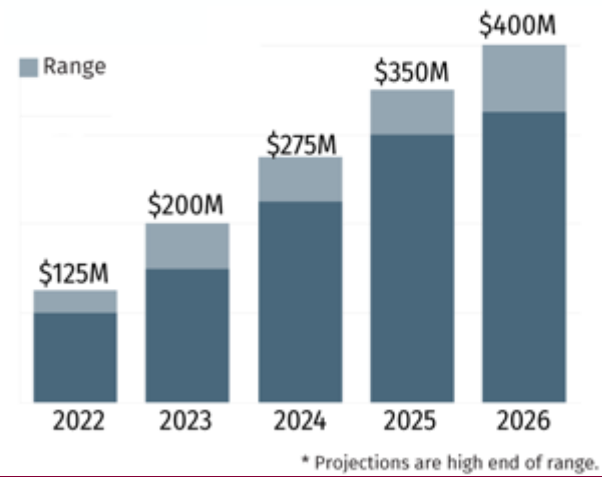
BACKGROUND

Hemp's Future in New Mexico

- Industrial hemp production unlikel
  - Difficulty growing grain/fiber varieties at low latitudes
- NM recreational *Cannabis* sales went live April 1, 2022
  - Adds to the existing medicinal program
  - 326 *active* Producer/Micro-producer licenses issued *(as of 7/21/22)*
- **Many hemp growers have switched to recreational production**



Projected revenues from adult-use recreational *Cannabis* in NM (MJBiz©, 2022)



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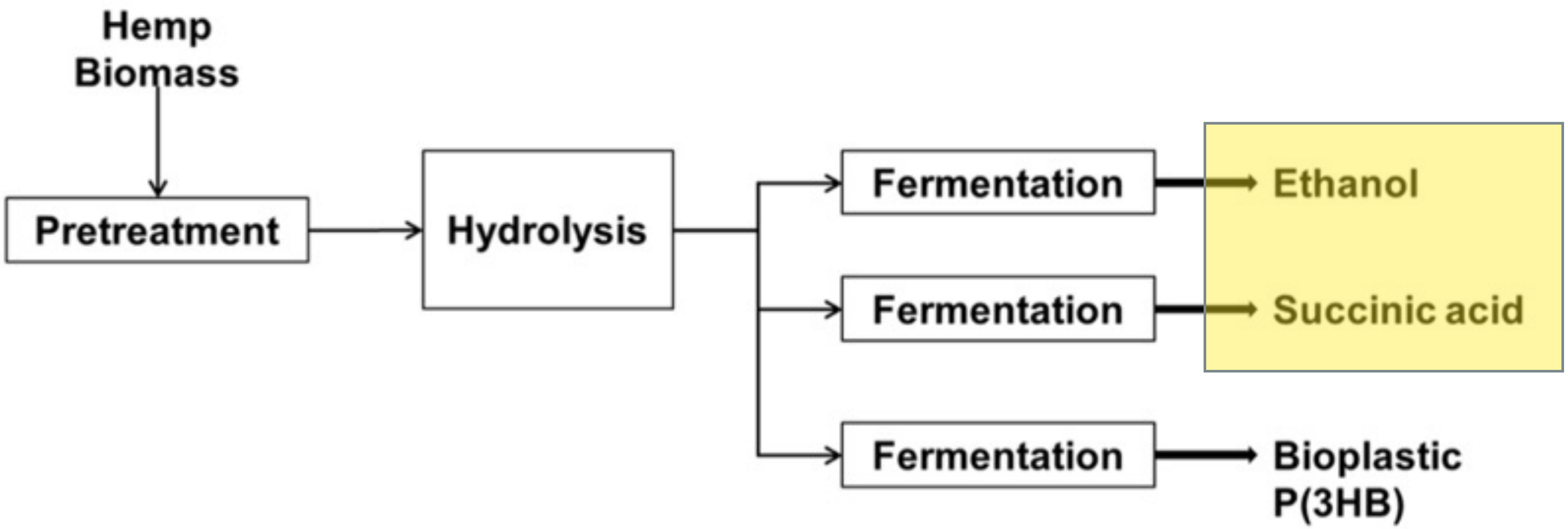
Singular, E. (2022). Midterm Review: A 2022 U.S. Hemp Production Outlook. New Frontier Data. https://newfrontierdata.com/cannabis-insights/midterm-review-a-2022-u-s-hemp-production-outlook/

Smith, J. (2022). New Mexico set to launch $400 million adult-use marijuana market likely to attract Texans. *MJBiz Daily*. https://mjbizdaily.com/new-mexico-set-to-launch-400-million-adult-use-marijuana-market-likely-to-attract-texans/

Saccharification and Fermentation to Bio-Based Chemicals

- Major products from the hydrolysis/fermentation pathway: bioenergy (ethanol), bio-based chemicals (succinic acid), bioplastic polymers (P3HB)
- **Only industrial hemp (grain/fiber) varieties have been evaluated**

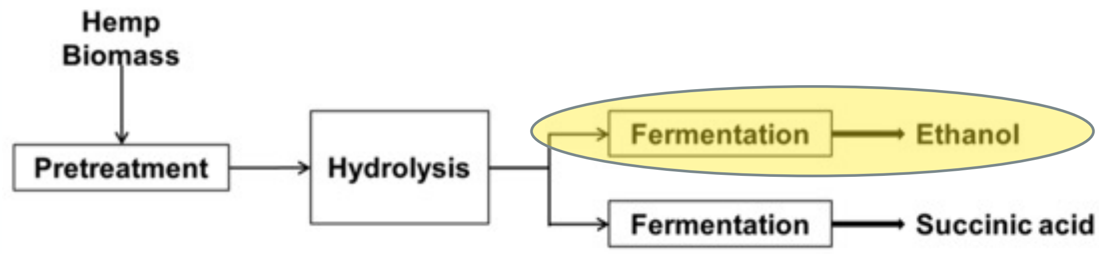


Reported bioprocessing routes for industrial hemp (Ji et al., 2021)

Ji, A., Jia, L., Kumar, D., & Yoo, C. G. (2021). Recent advancements in biological conversion of industrial hemp for biofuel and value-added products. *Fermentation*, *7*(1). https://doi.org/10.3390/fermentation7010006

LITERATURE REVIEW

Fermentation to Ethanol



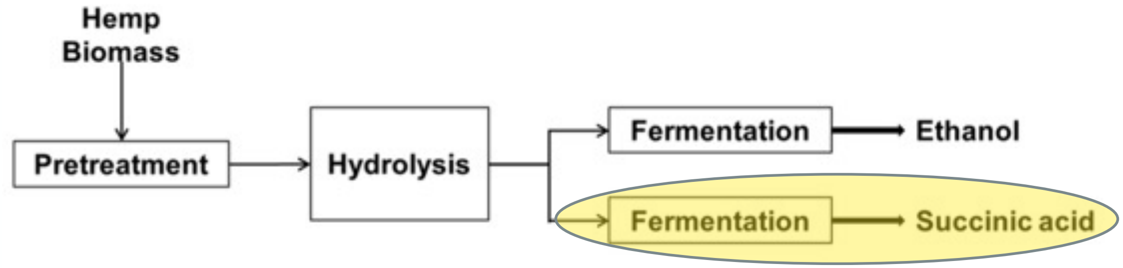
- Focus have been on optimizing pretreatments
- **Majority of literature is in relation to ethanol production**

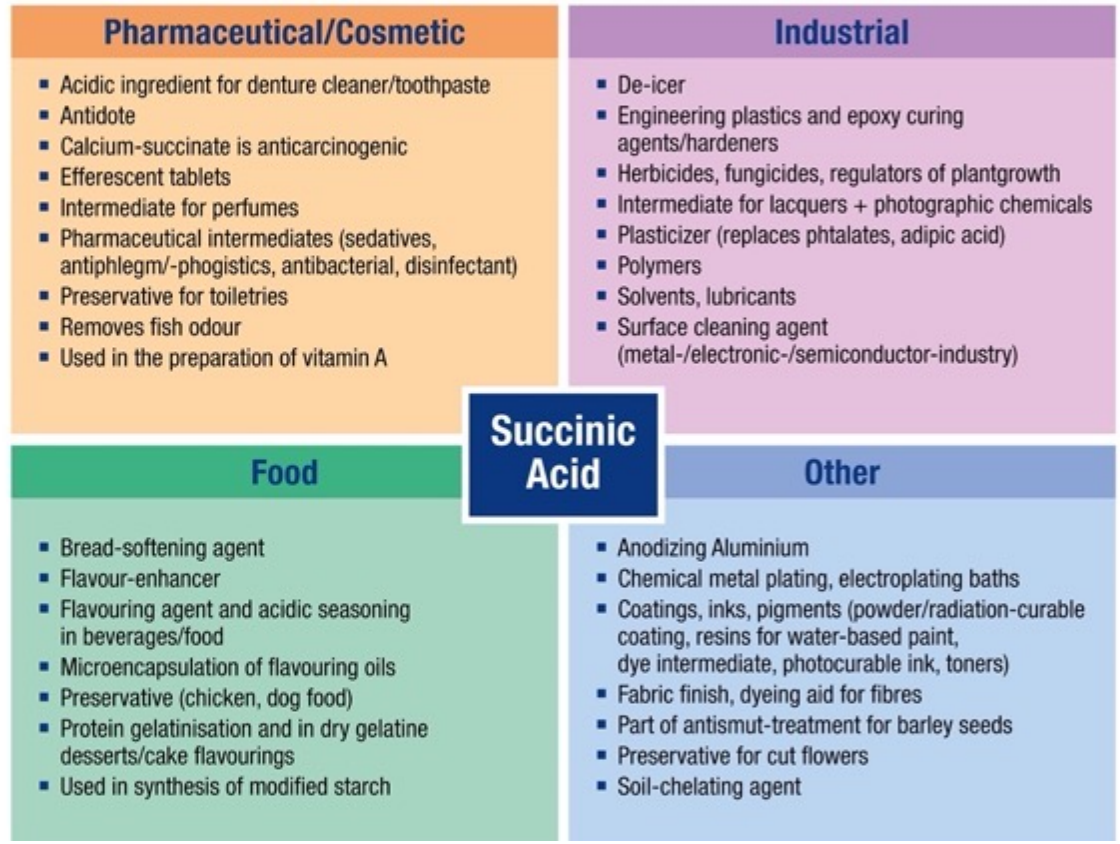
Literature Reports for Pretreatments, Hydrolysis Conversion, and Ethanol Yield from Hemp

Feedstock/Variety	*Pretreatment*	*Hydrolysis Solids [w/v, %]*	*Cellulose Conversion [%]*	*Ethanol (EtOH) Yields*	*Ref.*
4 industrial varieties: Helena, SS Beta, Tygra, Eletta Campana	Liquid hot water	5	53.9-71.7	9.2-10.9 g/L	[5]
	Acid (H_2SO_4)		41.7-58.7	11.9-13.8 g/L	
	Alkali (NaOH)		59.1-88.9	18.2-20.3 g/L	
Felina 32	Untreated	5	29.6	2.9 g/L	[6]
	Acid (H_2SO_4) + steam		37.7-73.6	4.6-10 g/L	
Futura 75	Acid (H_2SO_4) + steam	2	58.3-89.3	15.4-21.3 g/L	[7]
Industrial hemp (Fedora 17)	Untreated	7.5	30.3	7.2 g EtOH/ 100 g	[8]
	Acid (H_2SO_4) + steam		69-72.4	14.9-15.5 g EtOH/ 100 g	
	Alkaline (NaOH) + oxidative (H_2O_2)		72-80	16.6-17.5 g EtOH/ 100 g	
Hemp hurds	Steam	5	62-83	8.5-14.1 g EtOH/ 100 g	[9]

Bio-Based Succinic Acid

- Interest sparked in 2014
  - "Top 12 value-added chemicals produced from biomass" (Werpy & Petersen, 2004)
  - High oil prices
- Research interest declined with lower oil prices
- **Renewed urgency due to fluctuating petroleum prices and ongoing supply chain issues**

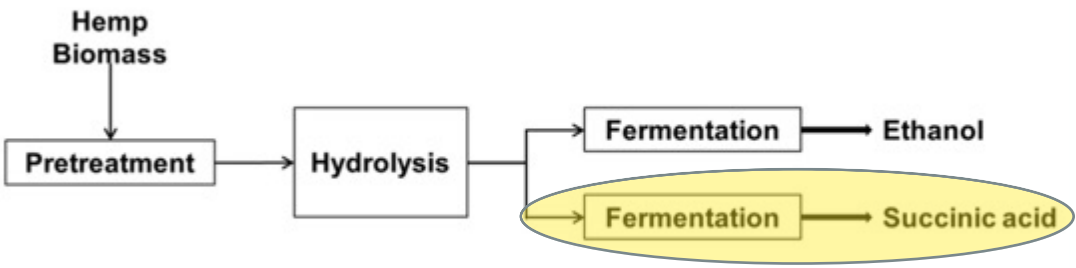




Uses of succinic acid (Renewable Carbon News, 2019)

Levulinic acid and succinic acid – A realistic look at the present and future of two versatile bio-based platform chemicals and their market development. (2019). *Renewable Carbon News*. https://renewable-carbon.eu/news/levulinic-acid-and-succinic-acid-a-realistic-look-at-the-present-and-future-of-two-versatile-bio-based-platform-chemicals-and-their-market-development/

Fermentation to Succinic Acid



- Ethanol and succinic acid production from hemp in a biorefinery concept (Gunnarsson et al., 2015; Kuglarz et al., 2016)
  - Optimal pretreatment for succinic acid yield: 121 °C and addition of 3% H_2O_2
  - Optimal pretreatment for combined ethanol/succinic acid: 1.5% H_2SO_4

Limitations of this work

- Only utilized industrial-type (fiber/grain) hemp feedstock
- Focused on chemical pretreatment conditions
- Used low solids loading content in hydrolysis tests

Expanding on this work

- **Utilize high-cannabinoid hemp as feedstock**
- **Focus on influence of mechanical pretreatment (particle size) on effectiveness of chemical pretreatments**
- **Investigate particle size / solids loading on hydrolysis yield**

Gunnarsson, I. B., Kuglarz, M., Karakashev, D., & Angelidaki, I. (2015). Thermochemical pretreatments for enhancing succinic acid production from industrial hemp (Cannabis sativa L.). Bioresource Technology, 182, 58–66. https://doi.org/10.1016/J.BIORTECH.2015.01.126

Kuglarz, M., Alvarado-Morales, M., Karakashev, D., & Angelidaki, I. (2016). Integrated production of cellulosic bioethanol and succinic acid from industrial hemp in a biorefinery concept. Bioresource Technology, 200, 639–647. https://doi.org/10.1016/J.BIORTECH.2015.10.081

Basic Analysis

- Particle Size
  - Distribution
  - Density
  - Surface Area
  - Uniformity
- Moisture
- Ash/Volatiles
- Extractives (organic/inorganic)
- Elemental Analysis (organic/inorganic)

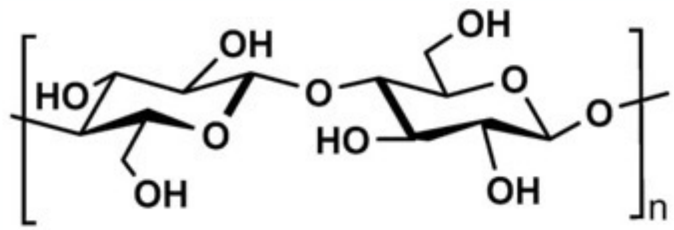
Milled hemp fiber (0.2 mm) from 2021 crop

Retsch® SM 300 Cutting Mill

https://profilab24.com/en/laboratory/mills/retsch-cutting-mill-sm-300

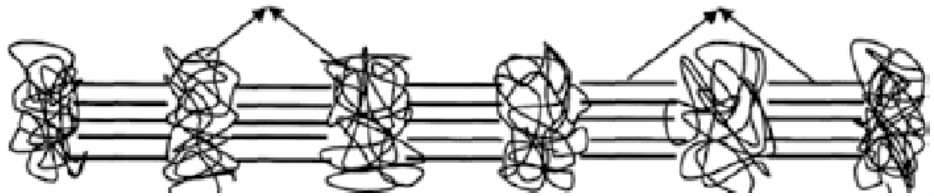
Degree of Polymerization (DP) and Crystallinity Index (CI)

- DP describes the number of monomer units in a polymer
  - Size exclusion / gel permeation chromatography
- CI indicates the fraction of cellulose in crystalline form
  - X-ray powder diffraction analysis (XRD)
- **Materials with lower DP and CI values are more readily hydrolyzed**



$$\text{Degree of Polymerization} = \frac{\text{Molecular weight of polymer}}{\text{Molecular weight of monomer}}$$

Monomer unit of cellulose (Nešić et al., 2020)



$$\text{Crystallinity Index} = \frac{\text{Area of all the crystalline peaks}}{\text{Area of all the crystalline and amorphous peaks}}$$

Representation of crystalline and amorphous regions in cellulose (Ilyas et al., 2018)

Ilyas, R. A., Sapuan, S. M., Ishak, M. R., Zainudin, E. S., & Atikah, M. S. N. (2018). Characterization of Sugar Palm Nanocellulose and Its Potential for Reinforcement with a Starch-Based Composite. In Sugar Palm Biofibers, Biopolymers, and Biocomposites (pp. 189–220). CRC Press. https://doi.org/10.1201/9780429443923-10

Nešić, A., Cabrera-Barjas, G., Dimitrijević-Branković, S., Davidović, S., Radovanović, N., & Delattre, C. (2019). Prospect of Polysaccharide-Based Materials as Advanced Food Packaging. *Molecules 2020, Vol. 25, Page 135*, *25*(1), 135. https://doi.org/10.3390/MOLECULES25010135

Lignin and Structural Sugars

Two-step Hydrolysis and HPLC-UV Quantification

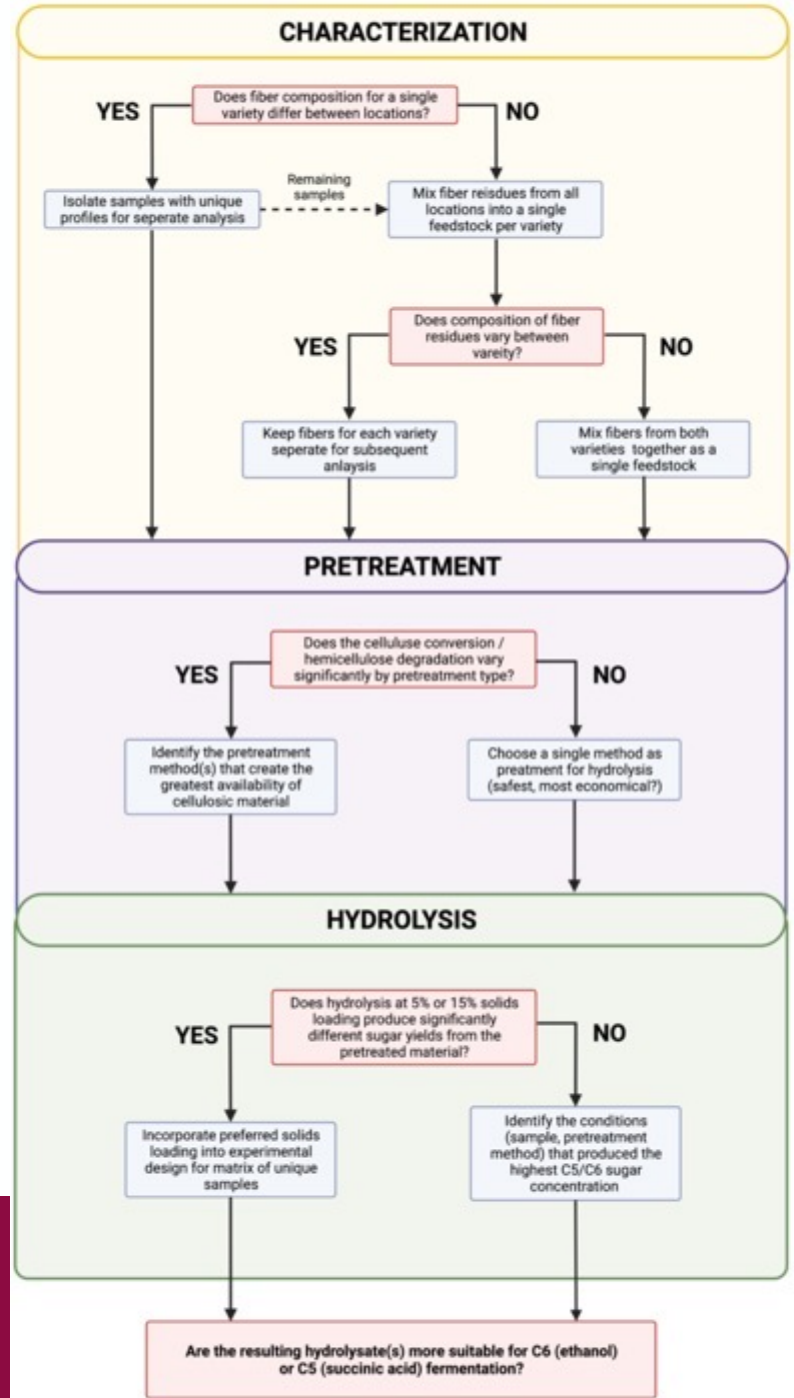
$$\text{Cellulose, Hemicellulose} \xrightarrow{30^{\circ}\text{C, 72\% } H_2SO_4} \text{Released Polysaccharides}$$

$$\text{Released Polysaccharides} \xrightarrow{121^{\circ}\text{C, 4\% } H_2SO_4} \text{Glucose + Xylose + Galactose + Arabinose + Mannose}$$

- Literature often only evaluates glucose and xylose when determining total sugar yield after hydrolysis
- Concentration of minor monosaccharaides in high-cannabinoid genotypes cannot be assumed negligible
- Hydrolysis of hemp biomass can produce galactose, arabinose, and mannose
- Minor monosaccharides can be fermented to succinic acid by *Actinobacillus succinogenes*

Characterization, Pretreatment, and Hydrolysis

- Does fiber composition vary by variety and/or location?
  - 2 varieties x 3 locations
- Does cellulose conversion / hemicellulose degradation vary by pretreatment type?
  - 4 pretreatments: steam, acid, alkali, oxidative
- Does sugar yields from pretreated material change with solids loading?
  - 2 solids loadings: low (5%) and high (15%)
- **Are the resulting hydrolysates more suitable for C6 (ethanol) or C5 (succinic acid) fermentation?**



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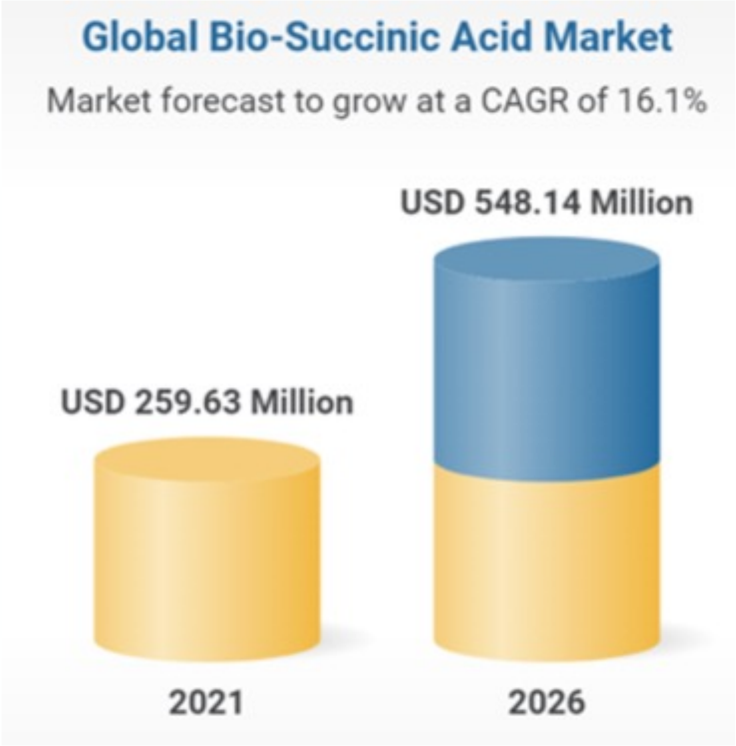
Considerations for Techno-economic Analysis

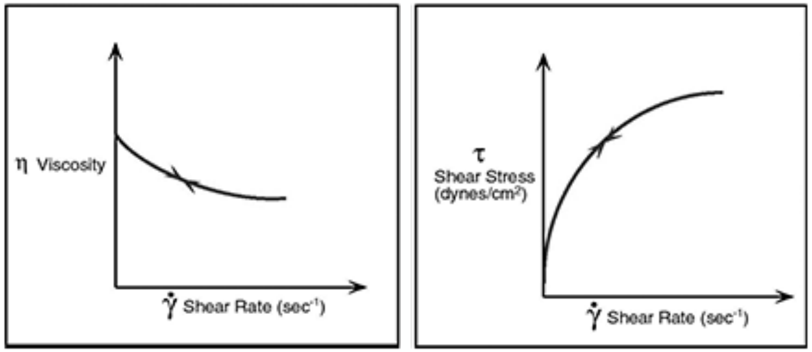
- Impacts of recreational and medicinal *Cannabis* markets
  - How much residual fiber material is available from high-cannabinoid production?
- Costs association with processing steps
  - What combination of hydrolysis experimental conditions result in the highest sugar yield?
- Economic outlook of succinic acid
  - **What is the potential value of succinic acid given the yields and feedstock availability?**



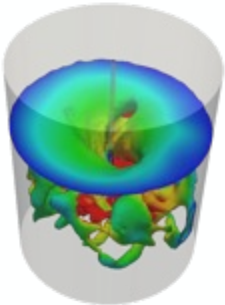
Infogence Global Research. (2021). *Global Bio-Succinic Acid Market (2021-2026)*. https://www.researchandmarkets.com/reports/5544100/global-bio-succinic-acid-market-2021-2026-by?utm_source=GNOM&utm_medium=PressRelease&utm_code=8j4lnw&utm_campaign=1691483+-+The+Worldwide+Bio-succinic+Acid+Industry+is+Expected+to+Reach+%24548+Million+by+2026&utm_exec=jamu273prd

Future Paths of Research - Hydrolysis

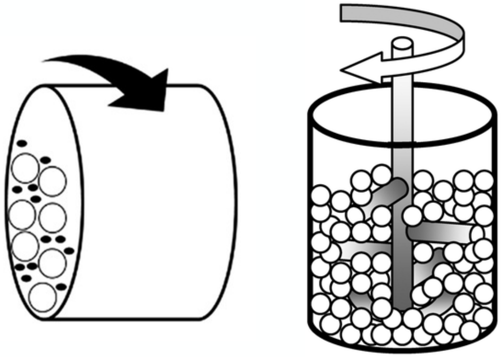
- Rheological studies
  - Influence of particle size / solids loading on hydrolysis
  - CFD models to evaluate mixing homogeneity; stress parameters
- Modified reactor design to increase yield without chemical pretreatment
  - Ball-mill reactor
  - Agitator shape



Pseudo-plastic behavior (Brookfield Ametek, Inc.)



CFD model of mixing in CSTR (SimScale, 2017)



Different orientations of ball-mill reactors (Piras et al., 2019)

Brookfield Ametek, Inc. (n.d.). *What is viscosity?* https://www.brookfieldengineering.com/brookfield-university/learning-center/learn-about-viscosity/what-is-viscosity

Piras, C. C., Fernández-Prieto, S., & De Borggraeve, W. M. (2019). Ball milling: a green technology for the preparation and functionalisation of nanocellulose derivatives. *Nanoscale Advances*, *1*(3), 937–947. https://doi.org/10.1039/C8NA00238J

vaibhav_s. (2020). CFD Simulation of CSTR – Mixer. SimScale. https://www.simscale.com/projects/vaibhav_s/mixer/

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Questions?

Contact Information

Hanah Rheay

New Mexico State University

Department of Chemical and Materials Engineering

handsr@nmsu.edu