Production of Fuel Ethanol and Industrial Chemicals from Sorghum

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Sorghum as a Feedstock for Biobased Products

- Adaptable to almost all temperate and tropical climates as annual or short perennial crop
- Drought resistant
- Starch contents of grains are equivalent to corn
- Fermentable sugars in juice only slightly lower than in sugarcane
- Additional fermentable sugars can be obtained from residual straw
- Requires less fertilizers compared to sugarcane
Grain Sorghum as a Feedstock for Ethanol Production

- Production in the US ranks third among cereal crops
- 9.9 million metric tons (389 million bushels) produced in the US in 2013
- 90% used for animal feed and 10% used for ethanol production
- Among 210 ethanol plants in the US, only 10 plants use sorghum (in addition to corn) for ethanol production
- Main reason for limited use of sorghum for ethanol production could be its reduced starch digestibility and lower ethanol yield compared with corn
### Chemical Composition of Sorghum Grains

<table>
<thead>
<tr>
<th>Components</th>
<th>Range (%, dry basis)</th>
<th>Average (%, dry basis)(^{(1)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>64.3-73.8</td>
<td>70.1 ± 2.10</td>
</tr>
<tr>
<td>Protein</td>
<td>8.19-14.02</td>
<td>11.19 ± 1.42</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.41-2.55</td>
<td>1.82 ± 0.24</td>
</tr>
<tr>
<td>Lipids</td>
<td>2.28-4.98</td>
<td>3.54 ± 0.57</td>
</tr>
<tr>
<td>Ash</td>
<td>1.46-2.32</td>
<td>1.80 ± 0.19</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Average of 70 samples

Effect of Tannin Contents on Fermentation Efficiency

- High tannin contents cause high viscosity and reduce fermentation efficiency
- Tannins are located mainly in the pericarp and pigmented testa of the grains
- Removal of pericarp and testa (decortication) could reduce tannin content to negligible levels (<0.1%) and improve fermentation efficiency
- Decortication also causes loss of starch

Sweet Sorghum as a Feedstock for Biobased Products

- Juice typically contains 12% - 15% fermentable sugars
- Additional fermentable sugars can be obtained from residual straw
- Total sugar (TS) content is positively correlated with dry matter (DM): \( TS = (0.62 \times DM) - 3.17 \) \( (r^2 = 0.90) \)
- High yield crop: Theoretical ethanol production estimated at 97 kg/ton (32 gal/ton) (dry basis) or 5,820 kg/ha (1922 gal/ha) – 45% from juice and 65% from residual bagasse

(1) Bennett and Anex, Transactions of the ASABE (2008), 51:603-613
Juice Extractability and Total Sugars from Sweet Sorghum Varieties Using Different Post-harvest Treatments

<table>
<thead>
<tr>
<th>Variety</th>
<th>Post treatment</th>
<th>Juice extractability (%)</th>
<th>Total sugars in juice (g/L)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M81</td>
<td>W</td>
<td>35 ± 2</td>
<td>130 ± 21</td>
</tr>
<tr>
<td></td>
<td>WP</td>
<td>43 ± 2</td>
<td>123 ± 10</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>48 ± 2</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>Topper</td>
<td>W</td>
<td>40 ± 1</td>
<td>148 ± 17</td>
</tr>
<tr>
<td></td>
<td>WP</td>
<td>37 ± 1</td>
<td>184 ± 4</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>39 ± 2</td>
<td>191 ± 13</td>
</tr>
<tr>
<td>Theis</td>
<td>W</td>
<td>35 ± 3</td>
<td>135 ± 5</td>
</tr>
<tr>
<td></td>
<td>WP</td>
<td>43 ± 2</td>
<td>147 ± 18</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>47 ± 1</td>
<td>138 ± 7</td>
</tr>
</tbody>
</table>

(1) W: the whole plant; WP: the plant without panicle; S: the stalk (plant without panicle and leaves)
(2) Grams of juice recovered per 100 g material milled

Ref: Guigou et al (2011), Biomass and Bioenergy 35:3058-3062
Total Sugar Concentration vs. °Brix in Sweet Sorghum Juice

HPLC can be used for determination of individual sugars

Ref: Guigou et al, Biomass and Bioenergy 35: 3058-3062 (2011)
Characteristics of Sweet Sorghum Juice (KKU40 from Mitr Phu Viang Sugar Mill, Thailand)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.9</td>
</tr>
<tr>
<td>Total soluble solids (°Brix)</td>
<td>18</td>
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<tr>
<td>Total sugar (g/L)</td>
<td>173.02</td>
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<tr>
<td>Glucose (g/L)</td>
<td>20.85</td>
</tr>
<tr>
<td>Fructose (g/L)</td>
<td>16.8</td>
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<tr>
<td>Sucrose (g/L)</td>
<td>124.05</td>
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</table>

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Contents</th>
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<tbody>
<tr>
<td>NH₄⁺ - N (ppm)</td>
<td>21.4</td>
</tr>
<tr>
<td>NO₃⁻ - N (ppm)</td>
<td>4.4</td>
</tr>
<tr>
<td>Total P (ppm)</td>
<td>20</td>
</tr>
<tr>
<td>Total K (ppm)</td>
<td>1790</td>
</tr>
<tr>
<td>Total Na (ppm)</td>
<td>170</td>
</tr>
<tr>
<td>Total S (ppm)</td>
<td>120</td>
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<tr>
<td>Total Ca (ppm)</td>
<td>166</td>
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<tr>
<td>Total Mg (ppm)</td>
<td>194</td>
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<tr>
<td>Total Fe (ppm)</td>
<td>2</td>
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<tr>
<td>Total Mn (ppm)</td>
<td>3</td>
</tr>
<tr>
<td>Total Cu (ppm)</td>
<td>0.3</td>
</tr>
<tr>
<td>Total Zn (ppm)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Ref: Laopaiboon et al (2009), Bioresource Technol. 100:4176-4182
Sweet Sorghum Juice as Feedstock for Ethanol Production

- All sugars (sucrose, glucose, fructose) can be fermented by the yeast *Saccharomyces cerevisiae*
- Relatively low final ethanol concentrations but still sufficient for recovery by distillation
  - Theoretical ethanol yield is 0.51 kg/kg sugar so 150 g/L sugar juice will produce 76.5 g/L ethanol solution
- Low N contents but sufficient for complete fermentation of all sugars
- If sugars are added to raise final ethanol concentrations, additional nutrients may be needed

Sweet Sorghum Juice as Feedstock for Production of Industrial Chemicals

- The major sugar in sweet sorghum juice, i.e. sucrose, may not be efficiently utilized, or even cannot be metabolized at all by several industrial microorganisms.
- Sucrose can be “inverted” by either acid or enzymatic (invertase) hydrolysis to glucose and fructose, which can be metabolized by virtually all industrial microorganisms through glycolysis.
- Heat sterilization also causes inversion of sucrose but for complete conversion to glucose and fructose invertase is needed.
- High total fermentable sugars (>100 g/L) may cause substrate inhibition. Not all microorganisms can tolerate high sugars like yeast:
  - Dilution of the juice to alleviate the substrate inhibition problem
  - Concentration of the juice for use as a feed solution in a fed-batch process.
- Addition of nutrients is needed due to low levels in the juice.
Examples of Industrial Chemicals that Can Be Produced from Sweet Sorghum Juice

- Lactic acid

- DHA (docosahexaenoic acid, a ω-3 fatty acid)
  - Liang et al (2010), Bioresource Technol. 101:3623-3627

- Poly-β-hydroxybutyric acid (PHB)
  - Tanamool et al (2009), The 3rd International Conference on Fermentation Technology for Value Added Agricultural Products

- Butanol

- Hydrogen

- Succinic acid
  - Eastern Regional Research Center, ARS, USDA
Succinic Acid and Products

- Succinic acid
- γ-Butyrolactone
- Tetrahydrofuran
- 2-Pyrolidone
- 1,4-Butanediol
- Succindiamide
- 1,4-Diaminobutane
- Succinate Deicers
- NMP
- Poly-Succinate Esters
- Succinate Dimethyl Ester
Biological Production of Succinic Acid

- Several microbial strains have been developed for production of succinic acid
  - *Anaerobiospirillum succiniciproducens* and *Actinobacillus succinogenes* (MBI International)
  - *Corynebacterium glutamicum* (Japan)
  - *Mannheimia succiniciproducens* (South Korea)
  - *Escherichia coli* (US DOE)
  - Others

- *E. coli* strains were developed at the U.S. DOE’s National Labs under the Alternative Feedstocks Program
  - AFP111
    - Capable of glucose utilization
  - AFP184
    - Higher succinic acid yields from glucose compared to AFP111
    - Capable of glucose and xylose utilization
Succinic Acid Production from Sweet Sorghum Juice (ERRC)

- Fermentation medium
  - Sweet sorghum juice (Topper, Delta Biorenewables LLC)
  - (NH₄)₂SO₄ 3.3 g/L
  - K₂H PO₄ 1.4 g/L
  - KH₂PO₄ 0.6 g/L
  - MgSO₄.7H₂O 0.4 g/L
  - Corn steep liquor (Sigma) 15 g/L
  - Antifoam A-204 (Sigma) 1 mL/L

- Microorganism: *E. coli AFP184*

- 500-mL fermentor with pH and temperature control

- **Dual-phase batch process**
  - Aerobic growth phase (6.5-7 hours)
  - Anaerobic succinic acid production phase

- Temperature maintained at 37°C

- pH maintained at 6.5 with 1.5 M Na₂CO₃
Succinic Acid Production from Sweet Sorghum Juice (ERRC)

Overall yield: 0.52 g SA/g total sugar consumed

Yield during production phase: 0.63 g SA/g total sugar consumed
Sweet Sorghum Bagasse as Feedstock for Biobased Products

- Composition of sweet sorghum bagasse (Clemson University’s Pee Dee Research and Education Center, Florence, SC)
  - 38.3 wt % glucan
  - 22.4 wt % xylan
  - 1.7 wt % arabinan
  - 23.5 wt % lignin
  - 2.1 wt % ash

- The carbohydrates in the bagasse can be converted to fermentable sugars and used for production of fuels and chemicals
ERRC Cellulosic Biorefinery Process Technology Using SAA Pretreatment

- Pretreatment using Soaking in Aqueous Ammonia (SAA)
- Hydrolysis with commercial xylanases → xylose-rich solution → value-added products by fermentation
  - Xylitol
  - Itaconic acid
  - Lactic acid
  - Butyric acid
  - Astaxanthin
  - Others

- Fermentation of cellulose-enriched residue to ethanol
  - *Saccharomyces cerevisiae* is used for ethanol fermentation
  - Non-recombinant microorganism
  - Most efficient ethanol-producing organism used commercially
  - High ethanol yields
  - High ethanol tolerance

- Hydrolysis of cellulose-enriched residue with commercial cellulases → glucose-rich solution → valued-added products by fermentation
Production of Ethanol and Coproducts from Sweet Sorghum

SS straw → SAA Pretreatment
- 15 wt % NH₄OH
- 1:10 Solid:Liquid ratio, 65°C and 15 hours

Washing & S/L Separation
- Solids
- Waste liquid

Enzyme Hydrolysis
- Accellerase® XY (xylanase) at 50 μL/g solids, 10 wt % solid loading, pH 5, 50°C, 72-h

S/L Separation
- Solids

SS stalks → Extraction
- SS straw

SS juice → Enzyme Hydrolysis
- Xylose-rich solution (8.0 g/l xylose, 0.2 g/l glucose)

Ethanol Fermentation
- Saccharomyces cerevisiae and Accellerase 1500® (cellulase) at 50 μL/g solid, 7 wt % solid loading, 32°C, Batch SSF

Value-added Products
Sweet Sorghum Bagasse

SAA Pretreatment

Pretreated Solids

DI Water

1310.4 g

Xylanase Hydrolysis

S/L Separation

Xylose-rich Hydrolysate

Cellulose-enriched Solids

131.3 g (dry basis)
70.9 g glucan (54.0 ± 1.3 wt %)
25.9 g xylan (19.7 ± 0.2 wt %)
2.5 g arabinan (1.9 ± 0.03wt %)
Lignin sample was lost

210 g (dry basis)
80.4 g glucan (38.3 ± 0.01 wt %)
47.0 g xylan (22.4 ± 0.03 wt %)
3.6 g arabinan (1.7 ± 0.02 wt %)
49.4 g lignin (23.5 ± 0.6 wt %)
4.4 g ash (2.1 ± 0.05 wt %)
Total mass: 88.0 wt %

145.6 g (dry basis) Recovery (%)
75.1 g glucan (51.6 ± 0.7 wt %) Glucan 93.4
36.8 g xylan (25.3 ± 0.4 wt %) Xylan 78.3
3.3 g arabinan (2.3 ± 0.00 wt %) Arabinan 91.7
22.4 g lignin (15.4 ± 1.4 wt%) Lignin 45.3
2.2 g ash (1.5 ± 0.00 wt %) Ash 50.0
Total mass: 96.1 wt %

520.0 ml Recovery (g)
0.19 g/l glucose Glucose 0.1
8.0 g/l xylose Xylose 4.2
Arabinose was below detection limit

Total Recovery in Combined Hydrolysate and Cellulose-enriched Solids (% of Untreated Material)

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Glucan</td>
<td>88.3</td>
<td></td>
</tr>
<tr>
<td>Xylan</td>
<td>63.0</td>
<td></td>
</tr>
<tr>
<td>Arabinan</td>
<td>69.4</td>
<td></td>
</tr>
</tbody>
</table>
Conventional Yeast Fermentation of SS Juice and SS Juice plus Cellulose-enriched Residue Made from SS Bagasse

![Graph showing ethanol concentration over fermentation time for SS Juice and SS Juice plus cellulose-enriched residue.](image)

Note: Ethanol concentrations were calculated from CO₂ weight loss data.
Future Work

- Low moisture anhydrous ammonia (LMAA) pretreatment process (collaboration with Dr. Tae-Hyun Kim, Kongju National University, Korea)

- Sorghum-based biorefining for production of fuels and industrial chemicals
Low Moisture Anhydrous Ammonia (LMAA) - An Improved Pretreatment Process

- Washing of pretreated biomass is eliminated
- Tremendous reduction of water usage
- Potential of being performed as a continuous process
- Low ammonia consumption, ~0.1-0.2 kg/kg biomass (dry basis)
Acknowledgements

- Sweet sorghum juice was provided by Delta Renewables LLC

- Sweet sorghum bagasse was provided by the Clemson University’s Pee Dee Research and Education Center in Florence, SC

- Accellerase® XY and Accellerase® 1500 were provided by DuPont Industrial Biosciences

- Gerry Senske, Justin Montanti and Jennifer Thomas assisted in the experiments and sample analysis
Thank You!

QUESTIONS?