Techno-economic Appraisal of Alternative Feed-stocks for Bioconversion to Ethanol:

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Ethanol as a Chemical Feedstock: 

Characteristic Features:

• Bioconversion route vs Chemical Synthesis

• Biphasic demand curve

• Represents highest product concentration among microbial bioconversion products
Chemical vs Biochemical Routes

**Chemical Synthesis:**

\[ \text{CH}_2=\text{CH}_2 + \text{H}_2\text{O} \rightarrow \text{C}_2\text{H}_5\text{OH} \]

- \( K_{eq} = 0.15 \) at 30\(^{0}\)C and 70 atm.
- Pressure
- 100 % carbon conversion (recycling)
- 0.61 kg C\(_2\)H\(_4\)/kg ethanol

**Bioconversion:**

\[ \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2 \]

(Yeast/ Bacteria)

- Carbon conversion : 66.7%
- Fermentation efficiency>85%
- Glucose required / kg ethanol: 1.96 kg
- Raw Materials: molasses, sugar cane juice, corn, agro-residues (lignocellulosics) etc.
- Uses biomass based renewable feed stocks
Bioconversion of Glucose to Ethanol

Glucose $\rightarrow 2\text{NAD}^+ + 2\text{Ethanol}$

$2\text{Pyruvate} + 2\text{NADH} + \text{H}^+ + 2\text{Acetaldehyde}$

$2\text{CO}_2$
Biphasic demand curve

Inelastic (potable alcohol, Human antibiotics)

Elastic (Industrial/fuel alcohol, Animal feed antibiotic)
Potential feedstocks for bio-conversion to ethanol

1. Sugar containing raw materials
   - Sugarcane juice
   - Molasses
   - Mahua flowers
   - Sugar beets

2. Starch based raw materials
   - Cereal grains
     (corn, spoiled wheat / rice/ sorghum)
   - Tubers
     (Low grade potatoes, tapioca)

3. Lignocellulosic Agro-residues:
   Wheat / Rice Straw, Bagasse etc.

Alternative uses (?)
Ethanol Production in India – current scenario

• Total number of distilleries: 356
• Distilleries affiliated to sugar mills: 141
• Installed capacity: 4230 m lit. (based on molasses)
• Current production: 2600 m lit.

• Present consumption: 2000 m lit.
• Availability for blending: 600 m lit.
• Demand (5% blend): 1300 m lit.
• Demand (10% blend): 2600 m lit.
Bioprocess criteria:

- High rate of substrate utilization
- High ethanol productivity
- Ethanol concentration in the brew > 8.5%
- Reduction in the volume and strength of effluent
- Automation and reduction in down time

Raw Material(s)

Biocatalyst

Bioprocess Technology

Distillation

Effluent treatment and water recycling
Ethanol from Saccharine Feedstocks

- **Sugar beet**
  - Diffusion
  - Pulp
  - Concentration
  - Sugar solution (200 g/l)

- **Molasses**
  - Fermentation
  - Distillation
  - Stillage
  - Ethanol

- **Sugar cane**
  - Grinding pressing
  - Concentration
  - Bagasse
  - **1 ton cane**
    - 67 kg EthOH (85 l)
    - 140 kg bagasse

- **1 ton beet**
  - 70 kg EthOH (89 l)
  - 54.5 kg pulp
Ethanol production from substrates containing starch

(Misselhorn, K. 1979 in Dellweg, H (ed), 4th Symposium Technische Microbiologie Berlin)
Energy (MJ/l of pure ethanol) required to produce absolute alcohol… *(Misselhorn, 1979)*

<table>
<thead>
<tr>
<th>Process stage</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beets</td>
</tr>
<tr>
<td>Digestion/Hydrolysis</td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>4-5</td>
</tr>
<tr>
<td>Continuous</td>
<td>-</td>
</tr>
<tr>
<td>Cane Mill</td>
<td>-</td>
</tr>
<tr>
<td>Extraction</td>
<td>0.8-1</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td>0.06</td>
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<tr>
<td>Continuous</td>
<td>0.1</td>
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<tr>
<td>Distillation</td>
<td></td>
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<tr>
<td>Single-staged</td>
<td>10-13</td>
</tr>
<tr>
<td>Optimized</td>
<td>5-7</td>
</tr>
<tr>
<td>Process</td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>16</td>
</tr>
<tr>
<td>Optimized</td>
<td>7</td>
</tr>
</tbody>
</table>

*(Misselhorn, K. 1979 in Dellweg, H (ed), 4th Symposium Technische Microbiologie Berlin)*
Bioconversion of different raw materials to ethanol

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Crop yield T/ ha./yr.</th>
<th>Alcohol yield L/T</th>
<th>Productivity L/ha/yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Sugarcane</td>
<td>60-100</td>
<td>60-80</td>
<td>5000-6000</td>
</tr>
<tr>
<td>-Sugar beet</td>
<td>30-60</td>
<td>50-110</td>
<td>3000-4000</td>
</tr>
<tr>
<td>-Molasses</td>
<td>--</td>
<td>225-300</td>
<td>--</td>
</tr>
<tr>
<td>-Potato</td>
<td>15-30</td>
<td>80-120</td>
<td>2000-3000</td>
</tr>
<tr>
<td>-Cassava</td>
<td>10-20</td>
<td>150-200</td>
<td>2000-3000</td>
</tr>
<tr>
<td>-Sorghum (dry)</td>
<td>2-5.5</td>
<td>340-400</td>
<td>1000-2000</td>
</tr>
<tr>
<td>-Corn (dry)</td>
<td>3-7.5</td>
<td>360-400</td>
<td>1500-2000</td>
</tr>
</tbody>
</table>
Bioconversion of Lignocellulosics to Ethanol

- Selection of Appropriate Feedstock
- Pretreatment and Delignification
- Saccharification / Hydrolysis
- Fermentation to Ethanol
- Recovery and Purification
- Process Integration, Techno-economic evaluation and Scale Up
Cellulose to ethanol: process inhibition pattern

\[(C_6)_n \xrightarrow{[A]} n_1 C_6 + n_2 C_{12} \xrightarrow{[C]} 2nCH_3CH_2OH + 2nCO_2 \xrightarrow{[B]} \quad n = n_1 + n_2 \]

[A]: Sugars (Glucose & Cellobiose) inhibit cellulase enzyme (Saccharification)

[B]: Ethanol inhibits alcoholic fermentation

[C]: Ethanol inhibits cellulase enzyme (Saccharification)

[D]: Accumulation of cellobiose reduces the yield of fermentable sugars.
Sweet Sorghum - a potential crop

- An alternative to sugar cane
- Production of high biomass yield
- High percentage of fermentable sugars and organics
- Shorter growth period
- Tolerance to draught stress
- Low fertilizer requirement
- Provides grains for additional ethanol productivity
Sweet Sorghum ( *Sorghum bicolor* L.)

SSG 601      PC 009      PCH 106      PC 121      PC 023

- Released by IARI in 1996; single cut variety; 120-150 days cycle time (mid June – Oct end); rain fed & requires low fertilizers input.
- Total plant biomass yield: 620 Q/ha
- Dry matter: 35% w/w (217 Q/ha)
- Juice recovery: 27900 l/ha
- Total fermentable sugars: 15.8% w/v (4408 kg/ha)
- Sorghum bagasse: 186 Q/ha (dry wt.)
- Sorghum grain: 12.5 Q/ha

*Courtesy Dr. S. Solomon, Dept. of Genetics IARI, New Delhi*
Sweet Sorghum - Sorghum bicolor
Sweet Sorghum:

- Stalks → Juice → Ethanol

- Bagasse → SSF → Ethanol + Residues

- Grains → Liquefaction → Saccharification → Ethanol
Liquefaction

Sorghum grains

\[ \downarrow \]

Milling

\[ \alpha\text{-amylase} \downarrow \text{Ca}^{++}, \text{pH 6.0, 90}^\circ\text{C, 2.5 h} \]

Solubilized starch + Residue
Saccharification of liquified sorghum grain in an IME packed bed reactor

- Enzyme: Amylo 300 L (crude AMG + pullulanase)
- Substrate: 30% (DS) enzyme liquified syrup
- Carrier: Activated bagasse
- pH: 4.5
- Temperature: 50 °C ± 1°C
- Specific activity: 1000 IU/g
- Km (approx.): 3.0x 10^{-4} M
- Packed bed volume: 800 ml
- Residence time: 3.5 hours
Activated sorghum bagasse matrix

-\textit{OH} + \textit{CH}_2-\textit{CH}-\textit{CH}_2 \text{ Cl} \ (\text{epichlorohydrin})

1 N ONaH, 60 °C, 30 min

-\textit{O-} \textit{CH}_2-\textit{CH}_2-\textit{CH}_2 + \text{ HCl}

\text{NH}_2 \ (\text{CH}_2)_6 \text{NH}_2, \text{ pH 11, 60 °C, 2h}
(\text{hexamethylene diamine})

-\textit{O-} \textit{CH}_2-\textit{CH}-\textit{CH}_2

OH \text{ NH} \ (\text{CH}_2)_6

\text{NH}_2

\text{aminohexyl matrix}
Bioconversion of Sorghum juice and saccharified grains

- Ethanol conc. % (v/v)
- Sugar conc. (mg/ml)

Graph showing the change in sugar concentration and ethanol concentration over time.
Delignification of bagasse

Pulp composition

<table>
<thead>
<tr>
<th></th>
<th>Sugarcane</th>
<th>Sorghum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose (% w/w)</td>
<td>43.7</td>
<td>38</td>
</tr>
<tr>
<td>Hemi cellulose (% w/w)</td>
<td>23</td>
<td>27.3</td>
</tr>
<tr>
<td>Lignin (% w/w)</td>
<td>9.8</td>
<td>13.0</td>
</tr>
<tr>
<td>Crude Protein (% w/w)</td>
<td>3.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Sugarcane / Sorghum bagasse (1 kg dry wt.)
Pressure - 4.2 kg/m²
Time - 3 + 1.5 hrs

Pulp
Delignification: 89-90%
Yield: Sugarcane bagasse 42.0% w/w
          Sorghum bagasse 48.5% w/w

NaOH (0.22 kg)
Water (5 Lit.)
Washing
Drying
Enzyme production by solid-state fermentation

Dry air for 6 hrs.
Production of Cellulase by *Trichoderma reesei*

![Graph showing production of cellulase by *Trichoderma reesei*. The graph plots time in hours on the x-axis and FP (IU/ml), B-glucosidase (IU/ml), and SP (mg/ml) on the y-axis. The graph shows the increase in soluble proteins, filter paper activity, and B-glucosidase over time.](image-url)
Production of beta-glucosidase by *Aspergillus wentii* Pt 2804

**Graph:**
- **Title:** Production of beta-glucosidase by *Aspergillus wentii* Pt 2804
- **Y-axis:** Enzyme activity (IU/ml)
- **X-axis:** Time (hr.)
- The graph shows the increase in beta-glucosidase activity over time, peaking at approximately 8 IU/ml.

**Note:**
- The graph indicates a significant increase in enzyme activity between 20 and 30 hours, suggesting an optimal period for enzyme production.
- The activity stabilizes after 30 hours, maintaining a constant level.

**Conclusion:**
- The study highlights the efficient production of beta-glucosidase by *Aspergillus wentii* Pt 2804, with optimal conditions observed between 20-30 hours.

**Further Analysis:**
- Further experiments could explore the environmental factors influencing enzyme activity for improved production.

**Implications:**
- This research could lead to advancements in the production of beta-glucosidase for various applications, such as in biofuel production or biodegradation processes.
A schematic diagram of the reactor for bioconversion of bagasse pulp to ethanol

- Solid/Liquid (1:5)
- Cellulase: 8 IU/g
- Yeast: 50 mg/g
- Temp. :30°C / 35°C
Bioconversion of pulp to ethanol

*S. cerevisae* (IMTECH strain)

Solid / liquid (1:8), FPA: 8 IU/g pulp, β glucosidase: 4 IU/g pulp,
Yeast: 10 mg/g pulp, Temp.: 38 °C, pH: 4.8
**Inputs**

- Sorghum bagasse (1 ton)
- NaOH (44 kg)
- Cellulase ($1.94 \times 10^6$ IU FPA)
- $\beta$-Glucosidase ($2.42 \times 10^5$ IU)
- Yeast (1.94 kg)

**Major Equipment**

- Rotary Digester
- Pulp washer
- Evaporator
- Incinerator
- Battery of reactors (10,000 L, 1,000 L, 100 L, 10 L)
- SSF reactor (3)
- Plate and frame filter
- Storage tanks

**Outputs**

- Spent solid (337.56 kg) (dry weight)
- Fermented Broth (2590 L (4% Ethanol) equivalent to 105.3 L 95% (v/v) Ethanol)
Total Utilization of sweet sorghum for ethanol production
(Basis: 1 Hectare of Sweet Sorghum cultivation)

Sweet sorghum 620 Q

- Stalk 465 Q
  - Milling
    - Juice 27900 lit
    - Hydrolysis
      - Fermentation
        - Distillation
          - Ethanol 4525 lit

- Leaves 62 Q
  - Saccharification (PFR)

- Panicles 93 Q
  - Residues
    - Liquefaction
      - Grain 12.5 Q
      - Bagasse 180 Q
Alternative strategies to enhance the ethanol production

• Secondary cane juice for distilleries affiliated to sugar mills
• Use of low grade potatoes/cereals for scarification & fermentation
• Sorghum juice & grains
• Mixed feed-stocks
Bioconversion of a flexible feedstock system (e.g. pretreated lignocellulosics mixed with presaccharified low cost starchy materials) to ethanol can provide a decentralized and sustainable alternative to meet the growing needs of ethanol as an organic feed stock in a sustainable manner.