Butanol Fermentation from Low-Value Sugar-Based Feedstocks by *Clostridia*

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Current Status of Bio-butanol in China

- Market
- Factories
- Research

Studies on ABE fermentation in Our Lab

- Continuous Fermentation by *Clostridium saccharobutylicum*
During the period of 2005–2011, around 50% of total annual butanol consumption was imported, and butanol consumption increased rapidly at an average rate of 6-8% in China. (Data sources: http://chem.chem99.com/)
Butanol Price Trend in China

Butanol Price (Jilin Petrochemical)

Market: butanol price downturn continuation …
# Factories

## ABE Factories in China (data by 2012.8)

<table>
<thead>
<tr>
<th>Factory</th>
<th>Current Capacity (t/y)</th>
<th>Starting date</th>
<th>Current Status</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laihe Rockley Bio-Chemicals Ltd</td>
<td>150,000</td>
<td>2007.12.5</td>
<td>–</td>
<td>Songyuan, Jilin</td>
</tr>
<tr>
<td>Jilin Cathay Biotechnology Co. Ltd</td>
<td>100,000</td>
<td>2008.3.20</td>
<td>–</td>
<td>Jilin, Jilin</td>
</tr>
<tr>
<td>Guangxi Guiping JinYuan Alcohol Co. Ltd</td>
<td>30,000</td>
<td>2007.8</td>
<td>–</td>
<td>Guiping, Guangxi</td>
</tr>
<tr>
<td>Jimao yuan Biochemical Co. Ltd</td>
<td>40,000</td>
<td>2008.3.2</td>
<td>producing ethanol</td>
<td>Lianyungang, Jiangsu</td>
</tr>
<tr>
<td>Lianhai Biological Co. Ltd</td>
<td>50,000</td>
<td>2008.10</td>
<td>–</td>
<td>Haimen, Jiangsu</td>
</tr>
<tr>
<td>Lianyungang Union of Chemicals Co. Ltd</td>
<td>40,000</td>
<td>2010.4</td>
<td>producing ethanol</td>
<td>Lianyungang, Jiangsu</td>
</tr>
</tbody>
</table>

All ABE factories stopped production or switch to ethanol ...
Choice of Feedstocks for ABE Fermentation

**Starchy materials:**
- corn, cassava, wheat

**Molasses:**
- cane and beet molasses

**Cellulosic biomasses:**
- crop straw hydrolysate

**Energy crops:**
- sweet sorghum, jerusalem artichoke

**Food materials**

**Substitutes**
- Low-value sugar-based feedstocks

**Cost issue**
- “Food versus fuel” debate
Corn material

Profit from corn utilization in ABE fermentation in China

Utilization of corn components (per ton corn)

<table>
<thead>
<tr>
<th>Corn components</th>
<th>Output (Kg)</th>
<th>Price (Yuan/t)</th>
<th>Profits (Yuan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch (10% water)</td>
<td>600</td>
<td>2860</td>
<td>1700</td>
</tr>
<tr>
<td>Fiber</td>
<td>115~120</td>
<td>1350</td>
<td>160~200</td>
</tr>
<tr>
<td>Embryo</td>
<td>50~60</td>
<td>4500</td>
<td>270</td>
</tr>
<tr>
<td>Protein</td>
<td>60</td>
<td>4300</td>
<td>250</td>
</tr>
<tr>
<td>CSL (45% water)</td>
<td>350</td>
<td>2420</td>
<td></td>
</tr>
</tbody>
</table>

*CSL: 10% solid content (3% protein), its profit was not considered.

- In 2012.2, corn price in Jilin is 2,030 yuan/t (14% water), including shipping;
- 4.5~4.7 t corn/t solvent, equal to a material cost of 10,080 yuan/t solvent;
- After utilization of corn component: 3,200 yuan/t solvent, the material cost: 7,000 yuan.
Corn Stover material

Corn Stover Butanol Biorefinery

Corn Cob/stover
  ↓
  Milling
  ↓
  Diluted acid pretreatment

Hydrolysates
  ↓
  Furfural removal
  ↓
  Acids removal
  ↓
  Preparation of cultural medium
  ↓
  Fermentation
  ↓
  Distillation
  ↓
  Biogas etc.

Solids residues
  ↓
  Lignin separation
  ↓
  Lignin solid residues
  ↓
  Cellulose
  ↓
  Butanol
  ↓
  Acetone
  ↓
  Ethanol
Corn Stover Butanol Biorefinery

Corn stover cost when construction: 200 yuan/t; when production: 500–700 yuan/t
Studies on ABE in Our Lab

Continuous Butanol Fermentation from Low-Value Sugar-Based Feedstocks by Clostridium saccharobutylicum DSM13864
Sugars utilization of various Clostridial strains in ABE fermentation

**C. Beijerinckii** DSM 6422, DSM 1739

**C. Saccharobutylicum** DSM 13864

**C. Saccharoperbutylacetonicum** DSM 2152

**C. saccharobutylicum** DSM 13864 could be used for ABE fermentation from cane molasses.

**C. saccharobutylicum** DSM 13864 could be used for ABE fermentation from lignocellulosic hydrolysates.
Scheme of 4-Stage Continuous ABE fermentation

Feeding tank  Blending vessel  Bioreactor 1  Bioreactor 2  Bioreactor 3  Bioreactor 4  Collection tank
Substrate: glucose

Strain: DSM 13864
Batch fermentation in 3-L bioreactor

Total solvent: 18.20 g/L (butanol 11.53 g/L)
Productivity: 0.379 g/L/h
Yield: 0.314 g ABE/g sugar
Fermentation time: 48 h
Substrate: glucose

Strain: DSM 13864
4-Stage continuous fermentation (D=0.03, 0.05, 0.1 h\(^{-1}\))

At D = 0.05 h\(^{-1}\):
Total solvent: 11.57 g/L (butanol 7.29 g/L); Productivity: 0.145 g/L/h

At D = 0.1 h\(^{-1}\):
Total solvent: 8.99 g/L (butanol 6.14 g/L); Productivity: 0.225 g/L/h
**Substrate:** cane molasses

**Strain:** DSM 13864  
**Batch fermentation in 5-L bioreactor**

**Total solvent:** 17.88 g/L (butanol 11.86 g/L)  
**Productivity:** 0.50 g/L/h  
**Yield:** 0.33 g ABE/g sugar  
**Fermentation time:** 36 h
Substrate: cane molasses

Strain: DSM 13864
4-Stage continuous fermentation

At D = 0.1 h⁻¹ (30–130 h)
Total solvent: 11.74 g/L (butanol 7.18 g/L); Productivity: 0.294 g/L/h
Fermentation time: 221 h

At D = 0.15 h⁻¹ (30–100 h)
Total solvent: 13.75 g/L (butanol 8.37 g/L); Productivity: 0.439 g/L/h
Fermentation time: 170 h
### Pretreatment conditions of various straw

<table>
<thead>
<tr>
<th>Straw types</th>
<th>g straw/L liquid</th>
<th>Glucose g/L</th>
<th>Xylose g/L</th>
<th>Arabinose g/L</th>
<th>Total sugar g/L</th>
<th>Enzymolysis ratio %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn straw</td>
<td>60</td>
<td>33.34</td>
<td>11.16</td>
<td>1.04</td>
<td>45.54</td>
<td>72.41</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>42.88</td>
<td>14.32</td>
<td>1.33</td>
<td>58.52</td>
<td>80.51</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>48.52</td>
<td>16.18</td>
<td>1.59</td>
<td>66.29</td>
<td>80.54</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>57.19</td>
<td>18.84</td>
<td>2.03</td>
<td>78.06</td>
<td>85.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>37.09</td>
<td>11.70</td>
<td>49.43</td>
<td>78.59</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>44.00</td>
<td>13.63</td>
<td>0.68</td>
<td>58.30</td>
<td>80.21</td>
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<tr>
<td>Rice straw</td>
<td>80</td>
<td>49.53</td>
<td>14.71</td>
<td>0.59</td>
<td>64.84</td>
<td>78.78</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>53.83</td>
<td>17.15</td>
<td>1.59</td>
<td>72.56</td>
<td>79.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>38.34</td>
<td>12.50</td>
<td>51.27</td>
<td>81.52</td>
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<tr>
<td></td>
<td>70</td>
<td>45.43</td>
<td>15.74</td>
<td>0.78</td>
<td>61.96</td>
<td>85.23</td>
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<tr>
<td>Wheat straw</td>
<td>80</td>
<td>42.02</td>
<td>14.74</td>
<td>0.83</td>
<td>57.58</td>
<td>69.96</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>53.16</td>
<td>18.79</td>
<td>1.17</td>
<td>73.12</td>
<td>79.70</td>
</tr>
</tbody>
</table>

- **Alkaline Pretreatment**: 1% NaOH, 120 °C for 2 h
- **Cellulase hydrolysis**: 20–30 FPIU/g straw, pH 4.8 and 50 °C for 40 h
Substrate: corn stover hydrolyzate

Strain: DSM 13864
Batch fermentation in 3-L bioreactor

Total solvent: 16.1 g/L (butanol 10.59 g/L)
Productivity: 0.40 g/L/h
Yield: 0.33 g ABE/g sugar
Fermentation time: 40 h
Substrate: corn stover hydrolyzate

5-stage temperature-shifting continuous fermentation in 500-mL tanks
(Stage 1, 2: 37 °C, Stage 3–5: 30 °C)

Total solvent: 12.28 g/L (butanol 8.50 g/L) after 80 h
Productivity: 0.25 g/L/h
Yield: 0.33 g ABE/g sugar
Fermentation time: 270 h (D=0.1 h⁻¹)
Substrate: corn stover hydrolyzate

Strain: DSM 13864
4-Stage Continuous fermentation

At $D = 0.1 \, h^{-1}$
Total solvent: 13.44 g/L (butanol 9.29 g/L); Productivity: 0.336 g/L/h
Fermentation time: 150 h

At $D = 0.15 \, h^{-1}$:
Total solvent: 11.43 g/L (butanol 7.81 g/L); Productivity: 0.429 g/L/h
Fermentation time: 220 h
Substrate: cassava

Strain: *C. acetobutylicum* A
Batch fermentation in 3-L bioreactor

Total solvent: 20.9 g/L (butanol 12.0 g/L)
Productivity: 0.211 g/L/h
Yield: 0.33 g ABE/g sugar
Fermentation time: 96 h
Substrate: cassava

Strain: *C. acetobutylicum* A

4-Stage continuous fermentation in 3-L bioreactor

Total solvent: 22.8 g/L (butanol 12.38 g/L)
Productivity: 0.57 g/L/h
Yield: 0.33 g ABE/g sugar
Fermentation time: 96 h (D=0.1 h⁻¹)
## Batch and Continuous ABE fermentation

<table>
<thead>
<tr>
<th>Operation mode</th>
<th>Strain</th>
<th>Substrate</th>
<th>Dilution rate (h(^{-1}))</th>
<th>Productivity (g/L/h)</th>
<th>Total solvent (g/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Batch Fermentation</strong></td>
<td><strong>C. beijerinckii P260</strong></td>
<td>Corn stover</td>
<td>0.31</td>
<td>26.27</td>
<td></td>
<td>Qureshi et al. 2010</td>
</tr>
<tr>
<td></td>
<td><strong>C. acetobutylicum 2N</strong></td>
<td>Beet molasses</td>
<td>0.34</td>
<td>16.32</td>
<td></td>
<td>Fan et al. 2010</td>
</tr>
<tr>
<td></td>
<td><strong>C. saccharobutylicum</strong></td>
<td>Cane molasses</td>
<td>0.50</td>
<td>17.88</td>
<td></td>
<td>Ni et al. 2012</td>
</tr>
<tr>
<td></td>
<td><strong>C. acetobutylicum XY16</strong></td>
<td>Glucose</td>
<td>0.63</td>
<td>20.30</td>
<td></td>
<td>Guo et al. 2012</td>
</tr>
<tr>
<td><strong>Continuous fermentation</strong></td>
<td><strong>C. beijerinckii BA101</strong></td>
<td>Glucose</td>
<td>2.0</td>
<td>15.80</td>
<td>7.9</td>
<td>Qureshi et al. 2000</td>
</tr>
<tr>
<td><strong>Immobilized cell</strong></td>
<td><strong>C. beijerinckii ATCC 55025</strong></td>
<td>Glucose</td>
<td>0.2</td>
<td>1.76</td>
<td>8.99</td>
<td>Zhang et al. 2009</td>
</tr>
<tr>
<td><strong>Continuous fermentation</strong></td>
<td><strong>C. pasteurianum ATCC 6103</strong></td>
<td>Glycerol</td>
<td>0.9</td>
<td>8.3</td>
<td>9.2</td>
<td>Malaviya et al. 2012</td>
</tr>
<tr>
<td><strong>Membrane cell bioreactor</strong></td>
<td><strong>C. saccharoperbutylacetonicum N1-4</strong></td>
<td>Glucose</td>
<td>0.11</td>
<td>7.55</td>
<td>8.58</td>
<td>Tashiro et al. 2005</td>
</tr>
<tr>
<td><strong>Continuous fermentation</strong></td>
<td><strong>C. saccharobutylicum DSM 13864</strong></td>
<td>Gelatinised sago starch</td>
<td>0.05</td>
<td>0.46</td>
<td>9.10</td>
<td>Liew et al. 2006</td>
</tr>
<tr>
<td><strong>Free cell</strong></td>
<td><strong>C. acetobutylicum BCRC 10639</strong></td>
<td>Glucose</td>
<td>0.054</td>
<td>0.37</td>
<td>6.85</td>
<td>Yen et al. 2011</td>
</tr>
<tr>
<td></td>
<td><strong>C. saccharobutylicum DSM 13864</strong></td>
<td>Corn stover hydrolysate</td>
<td>0.1</td>
<td>0.336</td>
<td>13.44</td>
<td>Ni et al. Unpublished</td>
</tr>
</tbody>
</table>
Conclusions

- Low-value sugar-based feedstock was utilized in batch and continuous ABE fermentation, including: cane molasses, corn stover hydrolyzate, etc.

- Using cane molasses, the ABE fermentation could be steadily operated for over 100 h at D=0.15 h⁻¹, the average total solvent of 13.75 g/L (butanol 8.37 g/L); and productivity of 0.439 g/L/h were obtained in a 4-stage continuous fermentation.

- Corn stover hydrolysate was prepared using alkaline pretreatment and enzyme hydrolysis. In a 4-stage continuous fermentation, the process was operated for 220 h at D=0.15 h⁻¹, the average solvent was 11.43 g/L (butanol 7.81 g/L), and the average solvent productivity was 0.429 g/L/h.
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