The acetone-butanol (ABE) fermentation industries in China

Zhihao Sun & Zhongping Shi

The Key Laboratory of Industrial Biotechnology, Ministry of Education
School of Biotechnology, Southern Yangtze University (SYTU)
Wuxi 214036, China
Scopes of The Presentation

- History of Acetone-Butanol Fermentative Industries in China
- Acetone-Butanol Industrial Fermentation Techniques in China
- The Current Situations & Future Perspective of The Acetone-Butanol Fermentation Industries in China
The History of Acetone-Butanol}

Fermentative Industries in China
### Strains & Fermentation Materials

#### Industrial Fermentation Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Company/Institution (isolated by)</th>
<th>Major Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. acetobutylicum</em></td>
<td>Microbiology Institute of Chinese Science Academy (CSA)</td>
<td></td>
</tr>
<tr>
<td>AS 1.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. acetobutylicum</em></td>
<td>Shanghai Solvent Plant</td>
<td>Various excellent phage-resistant properties</td>
</tr>
<tr>
<td>NA-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. acetobutylicum</em></td>
<td>Shanghai Institute of Plant Physiology, CSA</td>
<td>High ratio of butanol content</td>
</tr>
<tr>
<td>EA 2018</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Fermentation Materials

- Corns;
- Cassava, Sweet Potato, Potato
Foundation of The Chinese ABE Fermentation Industries

Locations & Historic Changes of The Chinese Fermentative Solvent Plants

The Corn Belt in China

1st generation’s plant, 1960-1965

2nd generation’s plant, 1965-1980

3rd generation’s plant, 1980-2000
## The Capacity of The Chinese ABE Fermentation Industries

<table>
<thead>
<tr>
<th>Time &amp; Periods</th>
<th>Total Capacity (Ton) in The Nation</th>
<th>Capacity in Single Ordinary Solvent Plants (Ton)</th>
<th>Capacity in Solvent Plant with Highest Production</th>
<th>Numbers of Total Solvent Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960’s</td>
<td>N/A</td>
<td>1,000</td>
<td>N/A</td>
<td>4-5</td>
</tr>
<tr>
<td>1965-1970</td>
<td>40,000-50,000</td>
<td>3,000</td>
<td>10,000 (Shanghai Solvent Plant)</td>
<td>15</td>
</tr>
<tr>
<td>1980</td>
<td>170,000</td>
<td>5,000-10,000</td>
<td>N/A</td>
<td>28</td>
</tr>
<tr>
<td>1990-2000</td>
<td>0-60,000</td>
<td>N/A</td>
<td>N/A</td>
<td>4-5</td>
</tr>
<tr>
<td>2001-present</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
One of The Major ABE Solvent Companies in China

Huabei Pharmaceutical Co. Ltd., Shijiazhuang, Hebei Province
One of The Major ABE Solvent Companies in China

Major ABE fermentation facilities & Equipment – Fermentation Plant
One of The Major ABE Solvent Companies in China

Seed Tank

Main Fermentors

Major ABE fermentation facilities & Equipment – Fermentors
One of The Major ABE Solvent Companies in China

Corn Deposit Tower
Distillation Tower

Major ABE fermentation facilities & Equipment
The Acetone-Butanol Industrial Fermentation Techniques in China
The High Butanol Ratio’s Production Strain EA 2018

ABE Fermentation with strain EA2018
(Y.Zhang et al., 1996)

A:B:E = 18.2~26.3: 67.6~75.0: 6.4~11.7
versus traditional A:B:E ratio = 3:6:1
The High Butanol Ratio’s Production Strain EA 2018

<table>
<thead>
<tr>
<th>Strain</th>
<th>Batch No.</th>
<th>Acetone g/L</th>
<th>Ethanol g/L</th>
<th>Butanol g/L</th>
<th>Total Solvent g/L</th>
<th>Butanol Ratio %</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA2018</td>
<td>1</td>
<td>4.44</td>
<td>0.53</td>
<td>11.69</td>
<td>16.69</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.47</td>
<td>0.57</td>
<td>11.87</td>
<td>16.90</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.49</td>
<td>0.64</td>
<td>11.86</td>
<td>16.99</td>
<td>69.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.84</td>
<td>0.67</td>
<td>12.77</td>
<td>18.29</td>
<td>69.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.34</td>
<td>0.75</td>
<td>14.24</td>
<td>20.37</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.43</td>
<td>0.73</td>
<td>14.52</td>
<td>20.70</td>
<td>70.2</td>
</tr>
<tr>
<td>EA2019</td>
<td>1</td>
<td>4.42</td>
<td>0.64</td>
<td>11.60</td>
<td>16.66</td>
<td>69.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.72</td>
<td>0.65</td>
<td>12.24</td>
<td>17.61</td>
<td>69.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.37</td>
<td>0.71</td>
<td>13.97</td>
<td>20.06</td>
<td>69.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.14</td>
<td>0.64</td>
<td>13.12</td>
<td>18.90</td>
<td>69.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.65</td>
<td>0.66</td>
<td>12.21</td>
<td>17.51</td>
<td>69.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.99</td>
<td>0.66</td>
<td>13.25</td>
<td>18.89</td>
<td>70.1</td>
</tr>
</tbody>
</table>

Solvent concentrations and butanol ratio in batch fermentation with EA2018 series strains (Y.Zhang et al., 1996)
The High Butanol Ratio’s Production Strain EA 2018

Corn \[\rightarrow\text{fermentation} \rightarrow \text{Cl. acetobutilicum}\]

- Acetone: 20.95% vs 30%
- Butanol: 71.45% vs 58%
- Ethanol: 7.6% vs 12%
- \(H_2\)
- \(CO_2\)
<table>
<thead>
<tr>
<th>Plant Batch No.</th>
<th>Acetone</th>
<th>Butanol</th>
<th>Ethanol</th>
<th>Raw Grain Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ton</td>
<td>Ratio</td>
<td>Ton</td>
<td>Ratio</td>
</tr>
<tr>
<td>94.08.25</td>
<td>26.24</td>
<td>20.7</td>
<td>85.82</td>
<td>67.6</td>
</tr>
<tr>
<td>94.09.25</td>
<td>23.52</td>
<td>26.3</td>
<td>68.21</td>
<td>68.7</td>
</tr>
<tr>
<td>94.10.25</td>
<td>22.24</td>
<td>22.2</td>
<td>69.60</td>
<td>69.5</td>
</tr>
<tr>
<td>94.11.25</td>
<td>22.73</td>
<td>21.4</td>
<td>76.18</td>
<td>71.8</td>
</tr>
<tr>
<td>94.12.25</td>
<td>32.90</td>
<td>20.3</td>
<td>116.95</td>
<td>72.2</td>
</tr>
<tr>
<td>95.01.20</td>
<td>33.20</td>
<td>20.7</td>
<td>117.10</td>
<td>72.9</td>
</tr>
<tr>
<td>95.02.20</td>
<td>36.69</td>
<td>22.4</td>
<td>115.13</td>
<td>70.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95.03.20</td>
<td>26.52</td>
<td>18.9</td>
<td>105.48</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95.04.20</td>
<td>21.52</td>
<td>18.2</td>
<td>88.60</td>
<td>74.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>245.1</td>
<td>20.95</td>
<td>843.10</td>
<td>71.45</td>
</tr>
</tbody>
</table>

(1): corn; (2): broomcorn

Batch data of the industrial fermentation using EA 2018 strain, reported by Jizhong Solvent Plant, Hebei Province (Y.Zhang et al., 1996)
Continuous ABE Fermentation Techniques

The schematic flow chart of continuous ABE fermentation (Z. Sun, 1981)
Continuous ABE Fermentation Techniques

The experimental results of the continuous ABE fermentation (Z. Sun, 1981)
Multiple stages (6-11 tanks in-series) were set for the optimal cells growth & solvent production, maintaining the cell metabolic activities at highest level.

The fresh substrate solution was continuously fed into the first tank together with periodic addition of seed culture broth, assuring strains always at their activated phase.

Productivity increased about 20%, compared with the traditional batch fermentation. Less strain contamination & mutation risks.
Products Recovery & Wastes Utilization Techniques

- Recovery of solvent contained in exhaust gas (a mixture of A:B:E=55:22:23, about 1-2% of the total solvent amount.

- Recovery of the evolved CO₂ and H₂ as the by-products into compressed tanks. 36 m³ CO₂ & 24 m³ H₂ harvested per 100kg starch.

- Average solvent production yield per 100kg starch: Acetone 11kg; Butanol 22.5kg; Ethanol 2.7kg.

- Wastes treatment: to use the waste mash for anaerobic fermentation to get the dried slurry as fertilizer, or for getting DDGS & DDG feedstock.

The overall flow chart of the acetone-butanol fermentation from corns (Sun, Z. & Jiao, R., 2003)
## Repeated Batch ABE Fermentation with Immobilized Cells

A total of 10 batches, 32 days

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Total Solvent (g/L)</th>
<th>Butanol</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Residual Starch %</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.54</td>
<td>6.31</td>
<td>3.21</td>
<td>1.02</td>
<td>0.22</td>
<td>Free Cells</td>
</tr>
<tr>
<td>1</td>
<td>12.03</td>
<td>6.96</td>
<td>4.20</td>
<td>0.87</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.20</td>
<td>7.00</td>
<td>3.57</td>
<td>1.38</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.68</td>
<td>7.45</td>
<td>4.00</td>
<td>1.24</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10.58</td>
<td>6.63</td>
<td>2.52</td>
<td>1.43</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.20</td>
<td>6.55</td>
<td>3.45</td>
<td>1.20</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>11.00</td>
<td>6.58</td>
<td>3.50</td>
<td>0.90</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>13.38</td>
<td>8.37</td>
<td>3.84</td>
<td>1.17</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>11.72</td>
<td>7.30</td>
<td>3.33</td>
<td>1.09</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>11.11</td>
<td>6.89</td>
<td>3.31</td>
<td>0.92</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.87</td>
<td>6.98</td>
<td>3.18</td>
<td>0.71</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

Repeated batch acetone-butanol fermentation with immobilized cells (Z. Sun et al., 1987)
Continuous ABE Fermentation with Immobilized Cells

**Conditions:**

- Immobilization carrier: ceramic rings, \( \rho \): 700g/L, Size: \( \phi 12 \times 12 \) mm;
- Packed column reactor, 3 in-series: \( V_T = 5.18 \) L, \( V_{ef} = 4.66 \) L, carrier packing amount = 3.23 kg, total broth volume = 2.89 L;
- Feeding substrate: 5%-8% fresh corn mash;
- Strain: *C. acetobutylicum* AS 1.70

Cascaded packed column bioreactor (3 in-series) for ABE fermentation (Z. Sun et al., 1988)
Experimental result of the extremely long-term continuous ABE fermentation with immobilized cells (Z. Sun et al., 1988)
Typical Solvents Purification Process, 4 towers fractionation System

1: Mash tower  2: Butanol tower  3: Acetone tower  4: Ethanol tower
The Current Situation & Future Perspective of The Acetone-Butanol Fermentation Industries in China
The Problems & Challenges of ABE Fermentation in China

Problems

- The strong competition from the petrochemical industry, economically;
- The rising grain price (RMB1,200[$150]/Ton-corn, much higher than US domestic corn prices);
- Low solvent concentrations, yield and productivity; unfavorable solvents contents or ratio; high cost for product purification and wastes treatment

Challenges

- Severe shortage in oil resources, large amount of crude oil relying on import ⇒ seeking substitutable resources;
- Energy & material strategies as a country lack of oil, but enriched with biomass, including starches, cellulose, and agricultural/industrial/daily-life wastes;
- Air & environmental pollution control
The Future Perspective of ABE Fermentation Industries in China

- The severe shortage and price rise-up of oil price forced the government officers, scholars, and enterpriser to face and pay more attention on the development of the fermentative acetone-butanol techniques;

- AB are important products closely related with the national economy and people’s daily life. The raw materials for fermentative acetone-butanol production are the renewable biomass resources. The products obtained by green biotechnology and fermentation methods are not only more applicable for the food and pharmaceutical industries, but also playing a key contribution role in relieving the problems of energy shortage & environmental pollution;

- With the development in microbial breeding and fermentation technologies, the traditional ABE fermentation industry still has a strong vitality, and development of ABE fermentative industries in China would have bright future.
The Solutions for Modernization of ABE Fermentation

- Efficient substrates utilization, including using the agricultural products residuals and waste cellulose as the raw fermentation materials;

- Improving strains resistant abilities to metabolites, increasing solvents conc.;

- Modification of fermentation technologies, such as improvement of the performance of the continuous or immobilized cell production processes;

- Improvement of the entire technologies, including comprehensive utilization of by-products, and upgrading waste-water treatment techniques

- Fermentation techniques without downstream distillation
Southern Yangtze University (SYTU), School of Biotechnology
Top-ranked institution in Fermentation Eng. area of the nation, specialized in

- Bio-catalytic conversion
- Enzymatic engineering
- Industrial microbiology
- Bio-energy & Bio-resources
- Bioreactor engineering
Location of Southern Yangtze University

The Yangtze River

Nanjing

Wuxi

Taihu Lake

Suzhou

Hangzhou

Shanghai

Yangtze River Delta

Yellow Sea

Eastern China Sea

Yangtze River Delta
School of Biotechnology
Southern Yangtze University
Thank you, and welcome to visit SYTU

http:www.sytu.edu.cn
Prof. Zhihao Sun

Ph.D Advisor,
Head of Biocatalysis Laboratory,
Southern Yangtze University

- **Tel / Fax**: 86-510-5808498
- **Email**: sunw@public1.wx.js.cn; sunzhihao@sytu.edu.cn