Scaling up Industrial Biotechnology

7th Life Science Symposium – Bioenergy
May 10th 2016

Jeff Lievense
Senior Engineering Fellow
Outline – Scaling up Industrial Biotechnology

- A fermentation guy
- Genomatica
- What is scale-up?
- Why does it matter?
- Three basic principles
- Examples – 1,3-PDO, 1,4-BDO, astaxanthin
- Scale-up reference material

Uses of a Barrel of Crude Oil (by percentage)

- Diesel: 24%
- Jet Fuel: 8%
- Gasoline: 42%
- Other: 25%

Source: Energy Information Administration; data for 2011; image by BCS, Inc.
### A fermentation guy – mostly chemicals, R&D to production

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Product</th>
<th>Ferm Size</th>
<th>Feedstock</th>
<th>Other Unit Ops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus sp.</td>
<td>diagnostic enzymes</td>
<td>1500L (ST)</td>
<td>Dextrose</td>
<td>Sterilization</td>
</tr>
<tr>
<td>Flavobacterium sp.</td>
<td>diagnostic enzyme</td>
<td>1500L (ST)</td>
<td>Dextrose</td>
<td>Batch</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (GM)</td>
<td>aromatics (trp, indigo et al), lys, thr, met cys, ser, glycerol, PDO</td>
<td>600,000L (BC)</td>
<td>Dextrose, starch hydrolysate</td>
<td>HTST continuous Validation</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (GM)</td>
<td>tryptophan</td>
<td>10L (ST)</td>
<td>Dextrose</td>
<td>Fermentation</td>
</tr>
<tr>
<td><em>Pseudomonas</em> (GM)</td>
<td>tryptophan, indigo</td>
<td>10L (ST)</td>
<td>Dextrose</td>
<td>Batch</td>
</tr>
<tr>
<td><em>Corynebacteria</em> (GM)</td>
<td>ribavirin, lysine</td>
<td>10L</td>
<td>Dextrose, molasses</td>
<td>Fed-batch</td>
</tr>
<tr>
<td><em>Xanthomonas campestris</em></td>
<td>xanthan gum</td>
<td>200,000L (BC)</td>
<td>Dextrose</td>
<td>Semi-continuous</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> (GM)</td>
<td>citric acid</td>
<td>200,000L (BC)</td>
<td>Dextrose, sugar, molasses</td>
<td>Continuous</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> (GM)</td>
<td>organic acids (citric, ascorbic, lactic, pyruvic, malic, succinic), xylitol, glycerol, ethanol, hydrocarbons (terpenes)</td>
<td>200,000L (BC) 600,000L (ST)</td>
<td>Dextrose, starch hydrol, sugar, cane syrup, molasses</td>
<td>Filtration</td>
</tr>
<tr>
<td><em>Pichia pastoris</em> (GM)</td>
<td>phytase</td>
<td>1500L (ST)</td>
<td>Dextrose</td>
<td>Membranes (m/f, u/f, n/f)</td>
</tr>
<tr>
<td><em>Yarrowia lipolytica</em> (GM)</td>
<td>citric acid, carotenoids</td>
<td>10L (ST)</td>
<td>Dextrose, Sugar</td>
<td>Rotary vacuum drum</td>
</tr>
<tr>
<td><em>Zygosaccharomyces bailii</em> (GM)</td>
<td>lactic acid</td>
<td>10L (ST)</td>
<td>Dextrose</td>
<td>Horizontal belt</td>
</tr>
<tr>
<td><em>K. lactis and marxianus</em> (GM)</td>
<td>lactic acid</td>
<td>10L (ST)</td>
<td>Dextrose</td>
<td>Centrifugation</td>
</tr>
<tr>
<td><em>Phaffia rhodozyma</em></td>
<td>Astaxanthin</td>
<td>200,000L (ST)</td>
<td>Dextrose</td>
<td>Purification</td>
</tr>
</tbody>
</table>

**GM** = genetically modified  
**ST** = stirred  
**BC** = bubble column

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7th Life Science Symposium - Bioenergy  
Jeff Lievense  
May 10, 2016
Genomatica: proven platform to “industrialize” biotechnology
Bioengineering competencies across computation, experimentation, & bioprocessing, all tightly integrated

Optimal pathway bank
Robust biobased processes and solutions

Visualize & Validate
Large-scale Outcome

Enabling Science
& Industrialization

Scalable Engineering
Designs & Development

Commercial
Realization

in silico pathway, process
design & prototyping

engineering enzyme component

optimizing cell factories

integrated process
development & engineering

guiding with techno-economics

high-fidelity scaling
to commercial

7th Life Science Symposium - Bioenergy
Jeff Lievense
May 10, 2016
What is scale-up?

- Dictionary: an increase in size, quantity, or activity according to a fixed scale or proportion
- Technical: the migration of a process from the lab-scale to the pilot plant-scale or commercial scale

<table>
<thead>
<tr>
<th>Scaling Factor (typical capacity)</th>
<th>Traditional CPI Gas-Liquid Process</th>
<th>Bioenergy Process with Solid Biomass Handling (dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench/Lab</td>
<td>0.001 - 0.1&lt;br&gt;1 – 10 ml/min</td>
<td>0.01 - 0.1&lt;br&gt;1 – 10 g/hr</td>
</tr>
<tr>
<td>Pilot</td>
<td>1&lt;br&gt;1 – 5 l/hr</td>
<td>1&lt;br&gt;1 – 5 kg/hr</td>
</tr>
<tr>
<td>Demonstration</td>
<td>100 – 1000&lt;br&gt;5 – 100 bbl/day</td>
<td>10 – 100&lt;br&gt;1 – 5 t/hr</td>
</tr>
<tr>
<td>Commercial</td>
<td>10000 – 30000&lt;br&gt;30,000 – 100,000 bbl/d</td>
<td>1000 – 5000&lt;br&gt;200 – 1000+ t/d</td>
</tr>
</tbody>
</table>

Solids handling
- Data, quantitative rules
- Experience
- Risk
  - Technology
  - Supply Chain
  - Construction
  - Infrastructure
  - Operational
  - Geographical
  - Market

Typical scaling factors for bioenergy and biofuels projects alongside the scaling factors often used for more traditional CPI gas-liquid processes.
Why does scale-up matter? There’s a lot at stake in cellulosic ethanol!

<table>
<thead>
<tr>
<th>U.S. Cellulosic Ethanol Producer</th>
<th>Capacity (ton/yr)</th>
<th>Cost (mil US$)</th>
<th>Start-up (Year)</th>
<th>Feedstock</th>
</tr>
</thead>
<tbody>
<tr>
<td>INEOS Bio (FL)</td>
<td>24,000</td>
<td>130+</td>
<td>2012</td>
<td>Waste Biomass</td>
</tr>
<tr>
<td>Quad County (IA)</td>
<td>6,100</td>
<td>9</td>
<td>2014</td>
<td>Corn Fiber</td>
</tr>
<tr>
<td>POET-DSM (IA)</td>
<td>76,000</td>
<td>275</td>
<td>2014</td>
<td>Corn Stover</td>
</tr>
<tr>
<td>Abengoa (KS)</td>
<td>76,000</td>
<td>500</td>
<td>2014</td>
<td>Stover, Straw,…</td>
</tr>
<tr>
<td>DuPont (IA)</td>
<td>91,000</td>
<td>225</td>
<td>2016</td>
<td>Corn Stover</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>273,000</strong></td>
<td><strong>1,139</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Current Rate**: 16,000 (6% of total, 15% of operating)
Why does scale-up matter? Either make money or bust!

“Pilot/demo operation is expensive and takes time. Let’s skip those steps, and go direct from the lab to full-scale production. We’ll make a lot more money and faster!”

POET Dry Mill Ethanol Plant, Chancellor, South Dakota
~ US$210 million investment

1,000,000 ton/yr corn
330,000 ton/yr ethanol
300,000 ton/yr cattle feed

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Derived from Iowa State University Ethanol Profitability Model

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<th>Target</th>
<th>Small Miss</th>
<th>Bad Miss</th>
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<tr>
<td>Ethanol revenue</td>
<td>220</td>
<td>187</td>
<td>88</td>
</tr>
<tr>
<td>Feed revenue</td>
<td>50</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>Total revenue</td>
<td>270</td>
<td>232</td>
<td>118</td>
</tr>
<tr>
<td>Corn cost</td>
<td>150</td>
<td>135</td>
<td>80</td>
</tr>
<tr>
<td>Other variable costs</td>
<td>51</td>
<td>49</td>
<td>35</td>
</tr>
<tr>
<td>Fixed costs</td>
<td>11</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Total cash cost</td>
<td>212</td>
<td>195</td>
<td>135</td>
</tr>
<tr>
<td>Depreciation</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Gross margin</td>
<td>37</td>
<td>16</td>
<td>(38)</td>
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<td>Simple payback, years</td>
<td>5.7</td>
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Derived from Iowa State University Ethanol Profitability Model
Three basic scale-up principles

*No oversights, no shortcuts, no problems,… easier said than done*

**Begin with the end in mind**

**Diligent in the 1000s of details**

**Prepare for the unexpected**

**NOT THIS…**

**THIS!**

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**Begin with the end in mind**

- 1985 Yugo GV

**Diligent in the 1000s of details**

- 2014 Honda Fit

**Prepare for the unexpected**

- Use lab fermentors in the plant to quickly isolate root causes

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Photo credits: Table at NY at Flickr, CC BY-SA 2.0
Begin with the end in mind – Work backwards from large-scale

*Whole process centric. Not microbe, process, or business case centric.*

**Design Large Scale, Then Scale Down**

- Susceptibility to contamination
- Microbe approvals, containment, disposal, coproduct
- *(Fed)*batch or (semi)continuous
- Size and number
- Materials of construction, compatibility
- Raw materials and sterilization
- Broth properties (viscosity, foaming, composition, DSP effects)
- Oxygen intensity, cooling mode
- Gas pressures
- Process controls (pH, temp, DO, nutrients, foaming, VOCs)
- Mass and heat transfer
- Heterogeneous environment
- **Capital and operating costs**
1,3-Propanediol example – a biobased polymer feedstock

Highly engineered E. coli, > 40 defined genetic changes

1,3-Propanediol – 1st of a kind, 12 years from invention to start-up

Demanding cost target, product quality, market development, 50:1 process scale-up, site integration

Loudon, Tennessee
64,000 ton/yr capacity
1,3-Propanediol – pilot process validation by proof-of-process (POP)

Use POP data as the design basis for the full-scale plant (50:1 scale up)

- Techno-economic modeling (measure progress)
- 13 m³ scale pilot plant operated by future full-scale team
- Commercial cost targets achieved (no extrapolation)
- 10 batches under fixed conditions
- Product validated in end-use (fiber-grade)
- POP data used as design basis for full-scale plant
1,4-Butanediol example – another biobased polymer feedstock

Highly engineered E. coli, > 40 defined genetic changes

1,4-Butanediol: 5 years from concept to commercial (0.5X 1,3-PDO)

Process and product validation at commercial scale following extensive piloting

- 50 fermentations run at commercial scale with integrated continuous downstream processing
- **No surprises** - consistent performance, all scales
- 1st mass production and sale of an established bulk industrial petrochemical by fermentation
- 2013 Kirkpatrick Chemical Engineering Achievement Award

### Process and Product Validation at Commercial Scale

- **5MM lbs 5 Week Campaign**
- **600,000L**

<table>
<thead>
<tr>
<th>Scale-Up Partners:</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lab</strong></td>
<td>30L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pilot</strong></td>
<td>3,000L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Demo</strong></td>
<td>13,000L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DuPont Tate & Lyle BioProducts
1,3-Propanediol Plant

Tate & Lyle Corn Processing Plant
Loudon TN

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1,4-Butanediol commercial scale performance

Process robust, performed as planned, few surprises, performance upside

Consistent Scale-up to Commercial

- ~ 50x scale-up
- 13,000L

Robust Performance at Commercial-Scale

- Fermentation performance at commercial-scale equivalent to demonstration-scale
- Low variability in fermentation performance indicates process robustness and predictability
- Top 5 commercial-scale fermentations indicate continuous improvement opportunity

Why Did it Work?

- Safety as a priority
- Regulatory approvals
- Waste disposal addressed
- Detailed project management
- Communications and teamwork across three companies
- Rigorous technology transfer
- Formal risk analysis and mitigations
- 100.0% reliable plant infrastructure
- Aseptic fermentation systems
- Experienced production team
- Validated process & product
- Validated local raw materials
- On-site R&D support
- Logistics and sales
Novamont: biobased 1,4-butanediol plant allows vertical integration

World’s first GENO BDO™ plant in Italy, an existing bulk petrochemical becomes biobased

...first licensee, back integrating

- BDO for Mater-Bi product line
- fully funded by Novamont
- $100M in capital
- 30kta facility (expanded from 18kta)
- H2’16 start up

family of biodegradable & compostable bioplastics

BDO Product Storage
Pipe Racks
Distillation
Evaporation

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Astaxanthin product was successfully produced at a Mexico toll manufacturing plant for more than two years. Fermentation time is about 8-10 days, and yeast concentration reaches 70 g dry weight/L. Most cell growth occurs in the first 3 days and most astaxanthin accumulation (inside the cells) over the remaining days. Fermentations at the Mexico plant were generally consistent except for some variability in product concentration. In preparation for transferring technology to a larger plant in the U.K., the fermentation was replicated at a U.S. lab with somewhat better results than in Mexico, although again with variability in product concentration. Subsequent results at the U.K. plant were poor. Yeast growth was similar to the other sites, but astaxanthin concentration was much lower. U.K. results were confounded by frequent contamination of fermentor batches by foreign bacteria and/or yeasts. These contaminations usually developed after the primary growth phase of the *Phaffia* production yeast.

<table>
<thead>
<tr>
<th>Site</th>
<th>Fermentor Scale</th>
<th>Astaxanthin Broth Conc</th>
<th>Microbial Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexico</td>
<td>50,000L</td>
<td>450-550 mg/L</td>
<td>No</td>
</tr>
<tr>
<td>U.S.</td>
<td>10L</td>
<td>500-700 mg/L</td>
<td>No</td>
</tr>
<tr>
<td>U.K.</td>
<td>220,000L</td>
<td>100-300 mg/L</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Astaxanthin – how to diagnose and solve the U.K. problem?

The plant is losing lots of money running at 40% of capacity….

What could be wrong?

### Possible Problems/Explanations

- Bad starter cultures in the U.K.
- Contaminating microbes steal nutrients needed for astaxanthin biosynthesis and/or produce toxins
- Fermentor mixing differences
- Bad process water in the U.K.
- > 100 possible root causes…..

### Corresponding Tests/Solutions

- Shipped to U.S. and validated
- U.K. plant succeeded in reducing extent of contamination with a small improvement in production
- Recruited expert to study mixing
- Switched to RO water; production actually got worse
- Test them all???
A faster, better in-plant scale-up tool

Parallel lab fermentors, indispensable for validation and troubleshooting

2 x 2 lab fermentor study quickly isolates root causes

<table>
<thead>
<tr>
<th>Lab</th>
<th>Prod</th>
<th>Lab</th>
<th>Prod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>Ctrl</td>
<td>Prod</td>
<td>AI</td>
</tr>
<tr>
<td>Protocol/Medium</td>
<td>Prod</td>
<td>Inoculum</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Failure</th>
<th>L</th>
<th>L</th>
<th>P</th>
<th>L</th>
<th>P</th>
<th>F</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-to-lab transfer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Production media prep</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Production inoculum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Full-scale environment</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Scale-up reference material: whole process thinking

Begin with end in mind; diligent in the 1000s of details; prepare for the unexpected

Print publications

- Design and Operating Principles for Bioprocess Chemical Engineering, November 2015
- Technology Challenges and Opportunities in Commercializing Industrial Biotechnology Chemical Engineering Progress, June 2016

On-line publications

- Successfully scaling up industrial fermentations of chemicals/fuels 2014 BIO World Congress on Industrial Biotechnology (May 2014, Philadelphia) http://www.biofuelsdigest.com/bdigest/2014/05/20/no-shortcuts-to-the-top-a-digest-special-report-on-scale-up-in-industrial-biotechnology