Integrated Strain and Bioprocess Development Examples

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Success for Commercial Bioprocess Development

- Integrated strain and process development allows for the most efficient, data-based design and testing of strains and process

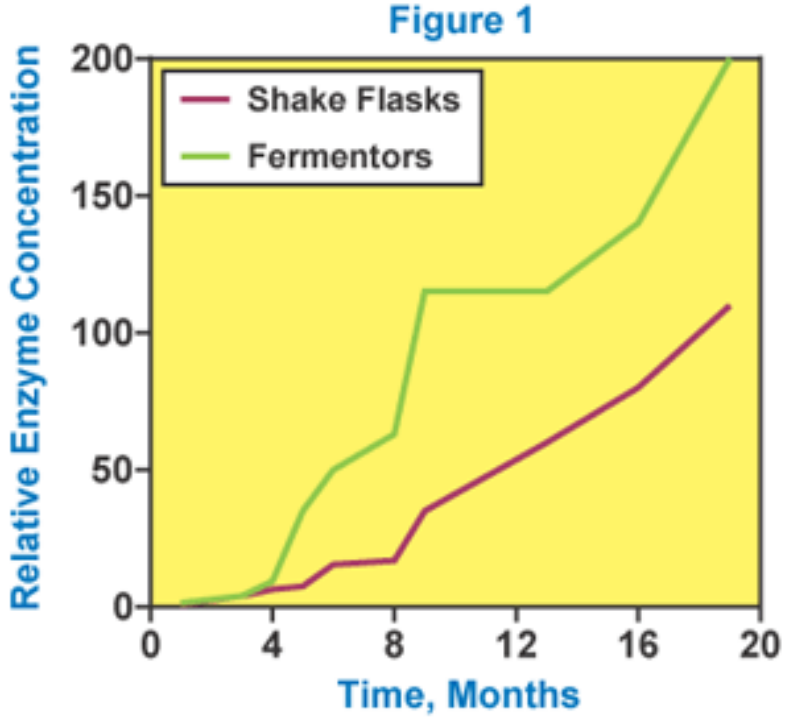
- Integrated program is often required to overcome complex challenges in developing robust commercial processes

- Each project is unique. BTR has the experience to develop a process using the right combination of techniques for success.

Example 1. Classical Mutagenesis Combines with Fermentation Development to Yield Commercial Process

- **Client needs:** increase the yield of an enzyme through classical mutagenesis, screening and fermentation process development
- **Development program:**
  - An agar-based method was used for primary screening to identify improved mutants (generated by UV mutagenesis or using chemical mutagens)
  - Improved strains were confirmed in a secondary shake flask screening, and then subjected to further rounds of mutagenesis
  - Fermentation development began one month after shake flask implementation, focusing on medium formulations and process conditions for mutants derived from the strain improvement program
  - Significant increases in productivity could be directly attributed to process improvement done in fermentors, since early experiments in fermentors led to better screening methodology and more rapid identification of improved, scalable mutants

Example 1. Classical Mutagenesis Combines with Fermentation Development to Yield Commercial Process (continued)

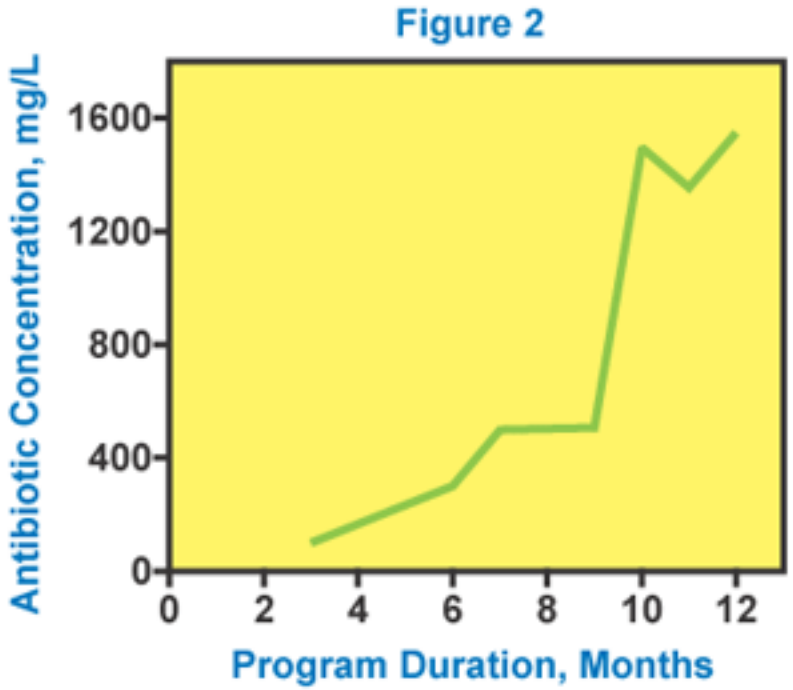


- Figure 1 tracks the timeline of a classical strain improvement program focused on increasing the yield of an enzyme
- By integrating strain improvement and process development, a 400-fold increase was achieved in 18 months. The enzyme process is now in production at 150,000-L scale.

Example 2. Antibiotic Process Viable Due to Integrated Approach

- **Client needs:** develop a proprietary process to produce an antibiotic, through a classical strain development and process improvement program
- **Development program:**
  - Starting strain has a reported productivity of 100 mg/L of the antibiotic.
  - BTR implemented classical strain improvement strategies to generate and screen mutants:
    - Inhibitors
    - Amino acid analogues
    - Zone inhibition assays
  - While the classical strain improvement techniques were being implemented, a team of fermentation scientists began looking at the fermentation process with the wild-type organism
  - At about six months when improved mutants were identified, these were compared in 1-L fermentors. Additional media and process development experiments were also concurrently being run at the 14-L scale

Example 2. Antibiotic Process Viable Due to Integrated Approach (continued)

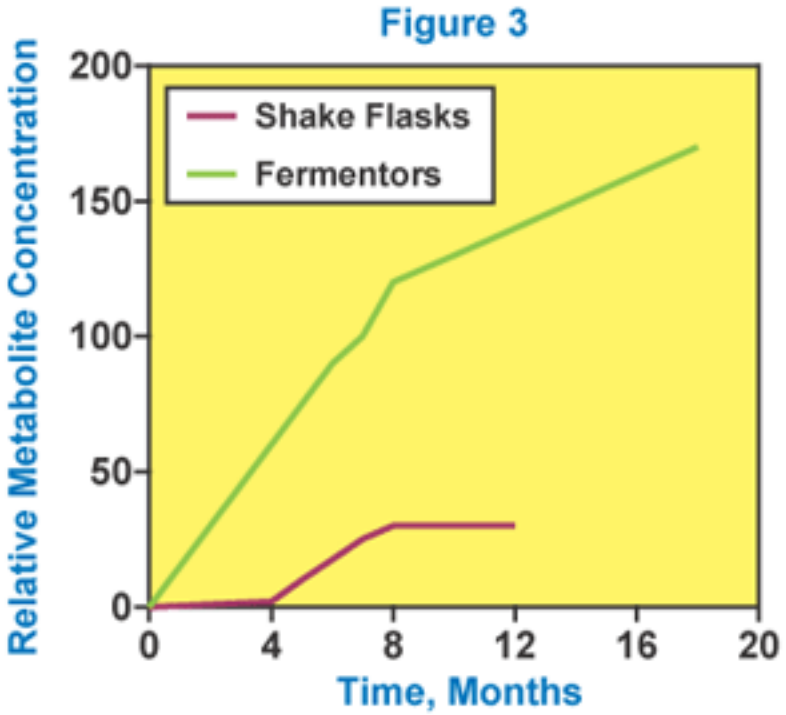


- Figure 2 is a 12-month timeline showing the titers of antibiotic-producing strains at the 14-L scale
- A 16-fold increase in titer was achieved due to a combination of improved mutants and an optimized process
- Strain and process technology transferred to a toll manufacturing site identified by the client

Example 3. Fermentation Program Reveals Success of Strain Improvement

- **Client needs:** improve strains for production of a pathway intermediate
- **Development program:**
  - Classical mutagenesis and selection were used to select for blocked mutants that accumulated the intermediate
  - Initial process development was performed in shake flasks,
  - Fermentation program started at six months. The level of production in fermentors was significantly higher than in shake flasks, due in part to the increased biomass
  - Further increases in production were achieved by growing improved strains under controlled conditions in fermentors
  - The focus of the program switched to applying recombinant techniques at about 10 months. Further improvements were obtained by amplifying genes involved in upstream steps of the biosynthetic pathway
- **Highlights:** one important enzyme was overexpressed:
  - Showed no overproduction of the metabolite when grown in shake flasks, even though the enzyme assays showed higher activity
  - Showed a 30% increase in metabolite production when grown in fermentors
  - Illustrating the importance of using an integrated approach to strain improvement.

Example 3. Fermentation Program Reveals Success of Strain Improvement (continued)



- Figure 3 shows improvement of the titer of a pathway intermediate in shake flasks and fermentors
- Both classical and recombinant strain improvement techniques were used to reach the target