Success Through an Integrated Approach

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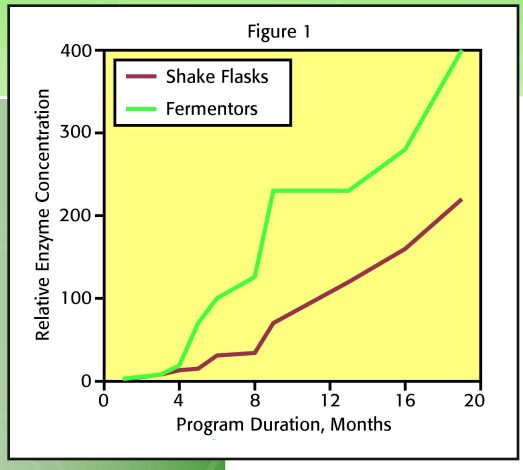
Combining skilled classical and recombinant strain improvement with a solid fermentation development program is the fastest, most cost effective way to a commercial process. Over 56 years of strain improvement success has proved that statement true. BTR programs typically involve a strategy for overproducing a compound or enzyme using classical and/or recombinant approaches. Integrating strain improvement with process development shortens the development time as seen by the following three examples.

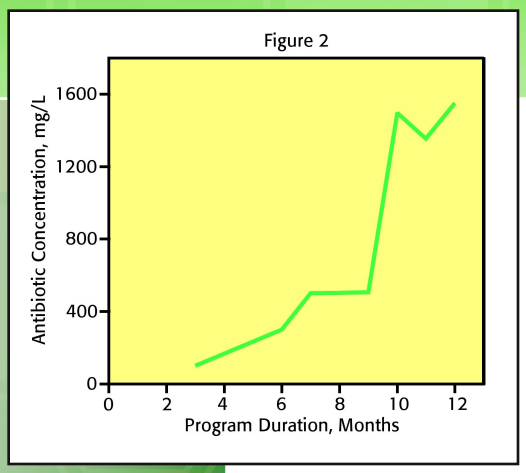


Classical Mutagenesis Combines with Fermentation Development to Yield Commercial Enzyme Process

Figure 1 tracks the timeline of a classical strain improvement program focused on increasing the yield of an enzyme. This program used a primary, agar-based screening method to identify improved mutants. These mutants were obtained by mutagenesis using ultraviolet irradiation or chemical mutagens coupled with a specific screen for increased enzyme activity. Improved strains were confirmed in a shake flask screen, and then subjected to further rounds of mutagenesis.

In this program the fermentation development began one month after shake flask implementation. The fermentation component optimized media formulations and improved process conditions for mutants derived from the strain improvement program. Significant increases in productivity can 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.

By the integration of strain improvement and process development programs, a 400-fold increase was achieved in just 18 months. This enzyme process is now in production at 150,000-liter scale.



Antibiotic Process Now Viable Due to Integrated Approach

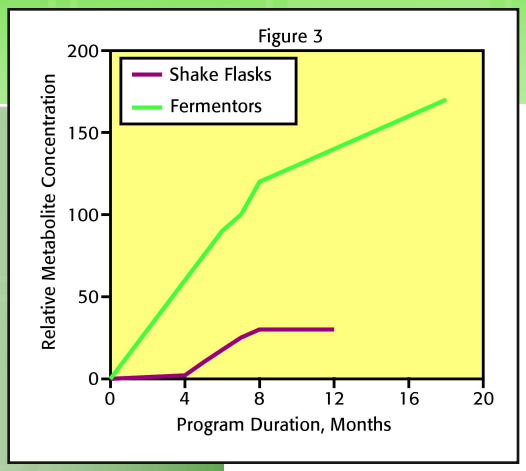
In the second example, BTR's client was interested in developing a proprietary process to produce an antibiotic. Due to existing intellectual property for recombinant approaches, a classical strain and process improvement program was established. A starting strain was purchased from a culture collection with a reported productivity of 100 mg/L of the antibiotic. BTR implemented classical strain improvement strategies including inhibitor and amino acid analogues and zone inhibition assays to select and screen for improved mutants.

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.

Figure 2 is a 12-month timeline showing the titers of these antibiotic-producing strains at the 14-L scale. A 16-fold increase in titer was achieved due to a combination of improved mutants from the strain development program combined with an optimized process. BTR assisted transferring the technology of this strain/process to a toll manufacturing site identified by the client.

Fermentation Program Reveals Success of Strain Improvement

Figure 3



In some cases, a combination of both classical and recombinant strain improvement techniques are used as in the program highlighted in Figure 3. Classical mutagenesis and selection was used to select for blocked mutants that produce an important pathway intermediate. The initial process development was performed in shake flasks, with the fermentation program beginning at 6 months. The levels of production in the fermentors was significantly higher than that seen in shake flasks, due in part to the increased biomass. Further increases in production were achieved by growing these strains under controlled conditions in fermentors.

The strain development focus of the program switched to applying recombinant techniques at about 10 months. Further improvements were obtained by amplifying important early genes in the biosynthetic pathway. One important enzyme, when over-expressed in a strain grown in shake flasks, showed no overproduction of the metabolite, even though the enzyme assays showed increased enzyme activity. This same transformant when grown in a fermentor showed a 30 % increase in metabolite production, again illustrating the importance of using an integrated approach to strain improvement.