Fermentation Process Development Examples

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Example 1. Lactose-Induced Production of Glucosamine and *N*-Acetylglucosamine in *E. coli*

- **BTR internal R&D program:** develop a robust commercial process for glucosamine production using metabolically engineered *E. coli* strains
- **Program highlights:**
  - Glucosamine process is prone to acetate accumulation. Trace metal addition was optimized to control growth and product formation.
  - Glucosamine titer reached 18 g/L
  - With further metabolically engineered strains, a biphasic process was developed for *N*-acetylglucosamine production
  - A single addition of lactose at a low level provided an efficient induction for gene expression and product formation
  - *N*-acetylglucosamine produced over 110 g/L within 60 hrs
  - Process successfully scaled up to 250-liter scale
- For more details, please see PowerPoint slide show "Fermentation Process Development for Glucosamine and *N*-Acetylglucosamine Production"

Example 2. Fermentation Optimization for Expression of a Soluble Protein

- **BTR internal R&D program:** produce and partially purify 50 grams of linoleate isomerase protein using recombinant *E. coli* for bioconversion of linoleic acid

- **Program highlights:**
  - Recombinant isomerase (55 kDa) expressed at high level in *E. coli*, but almost all sequestered in inclusion bodies (IB) as inactive enzyme under standard conditions
  - Developed a defined mineral salts-based medium supplemented with N-Z amine
  - Level of active enzyme improved by ~370 fold compared to LB medium-based process

- For more details, please see PowerPoint slide show "*E. coli* Fermentation Process Development for a Soluble Recombinant Protein"

Example 3. Fermentation Process Development for Protein Expression and Secretion in *Bacillus subtilis*

- **Client needs**
  - Develop and optimize fermentation process to produce proteins for pharmaceutical applications
  - Scale up and demonstrate the process
- **Development Program**
  - Optimized fermentation medium and operation conditions
  - Substantially increased product titer
  - Process scaled up to 14-liter and 60-liter scales
  - Developed a scalable purification process
  - Prepared several grams of purified protein to enable Client's clinic trials

Example 4. Pharmaceutical Biomass Product

- **Client needs**
  - A proprietary fermentation process, based on non-animal ingredients, to produce an anaerobic microorganism
  - Develop a recovery protocol
- **Development program**
  - Bottle studies to define appropriate culture medium
  - Fermentation process development and demonstration at

    1-L scale

    - Final fermentation process $\geq 10^9$ viable cells/mL
  - Developed partial purification process
  - Biomass short and long-term stability verification
- **Development time: 9 months (in three phases)**

Example 5. *E. coli* Fermentation Process Development and Product Recovery

- **Client needs**
  - Define and optimize a fermentation process for production of an active recombinant protein (requiring external cofactor) in *E. coli* for pharmaceutical applications
  - Scale up and demonstrate the process at 250-liter scale
- **Development Program**
  - Developed a defined medium free of animal ingredients, using readily available, consistent raw materials
  - Optimized medium, induction, co-factor feeding and operations to maximize productivity
  - Substantially improved titer
  - Process scaled up to 14-liter scale
  - Successfully demonstration at 250-liter scale
  - Prepared a package for technology transfer to the manufacturing site

Additional Examples of Fermentation Process Development

- Increased the fermentation yield of a therapeutic protein while identifying a mutation and correcting the gene sequence

- Led the technology transfer of a generic antibiotic process, doubled the yield through process development, and successfully scaled the new process in our client's production facilities

- Increased the yield of an enzyme by 600% and assisted in identifying three new market opportunities

- Conducted a complete technical and economic assessment of an enzyme transformation which was used to determine feasibility, set technical goals, establish a budget, and develop time frames and work schedules for commercialization