**Techno-Economic Analysis Report**

Title: Techno-Economic Analysis of a Packed Bed Adsorption Chromatography Process for Enzyme Production

# 1. Introduction

This report presents a techno-economic analysis of a bioprocess for the production and purification of an enzyme, referred to as POI (Protein of Interest). The process involves two continuous fermentation steps, one for biomass production and another for POI production, followed by packed bed adsorption (PBA) chromatography for purification. A small-scale shake flask fermentation precedes the main biomass fermentation, likely serving as a seed train. The process operates in batch mode with an annual operating time of 8,160 hours and one campaign per year. Individual batch cycle time is 28.14 hours, with 3.6 hours of slack time.

The process is divided into several key procedures: media preparation, sterilization, shake flask seed train, biomass and POI production fermentation, centrifugation, tangential flow filtration (TFF), buffer preparation, chromatography, and various storage and fluid transfer operations. The chromatography procedure comprises equilibration, loading, washing, elution, stripping and cleaning-in-place (CIP). Compressed air is filtered and split to supply both fermenters. CIP operations are employed throughout the process, utilizing distinct caustic and acid solutions for cleaning various equipment units. The final product stream, containing the purified POI, is designated "Product to polishing," implying further downstream processing steps may follow beyond the scope of this analysis. Data gathered emphasizes the scale of the various unit operation and focuses on the material balances and input-output streams for each step.

# 2. Methods

This techno-economic assessment considers key process components, operating conditions, and economic factors to evaluate the viability of the POI production process.

## 2.1 Chemical Components

The primary chemical components involved in the process include:

\* \*\*Growth Media Components:\*\* Glucose, Arabinose, Ammonium Sulfate, Calcium Chloride, Magnesium Sulfate, Sodium Hydrogen Phosphate, Sodium di-Hydrogen Phosphate, Trisodium Citrate.

\* \*\*Inducing agent:\*\* IPTG (Isopropyl β-D-1-thiogalactopyranoside).

\* \*\*Chromatography Buffers:\*\* Sodium Chloride, Sodium Phosphate, Sodium Hydroxide

\* \*\*Cleaning Agents:\*\* Phosphoric Acid, water (WFI), Sodium Hydroxide (CIP Chromatic solution and CIP Caustic solution).

\* \*\*Gases:\*\* Oxygen (O2), Nitrogen (N2) (supplied as compressed air)

## 2.2 Operational Data

\* \*\*Fermentation:\*\* Both biomass and POI production fermenters operate continuously at 30°C, with aeration at 0.5 VVM and a constant pressure operation.

\* \*\*Chromatography:\*\* A PBA column (C-102) is used for purification, with defined steps for equilibration, loading, washing, elution, and stripping. A specific resin is employed, exhibiting a binding capacity of 21.99 g/L. An isocratic elution method is employed and collected fractions are forwarded to a storage tank.

\* \*\*Centrifugation\*\* A disk stack centrifuge (DS-101) is also employed in the process for cell removal. This type of centrifuge is expected to separate liquid and solid phases where the solids being collected in the equipment.

\* \*\*Tangential/Dead-End filtration\*\* Dead-end filtration (DE-103) and continuous filtration processes are employed for material flow throughout the process. The former is assumed to be a membrane process with constant transmembrane flux, working as batch operation with a solid fraction in retentate of 50 %. The later is part of a continuous procedure working with a pressure of 1 bar.

\* \*\*Clean-in-Place (CIP):\*\* CIP procedures are implemented throughout the process, using Water For Injection (WFI), caustic and acid solutions. Parameters such as cleaning agent, temperature, and number of recirculation cycles are defined for each CIP operation.

\* \*\*Storage:\*\* Multiple storage tanks are employed for various process streams, operating under different temperature and residence time conditions.

## 2.3 Process Parameters

\* \*\*Biomass Fermentation:\*\* Target biomass concentration of 20 g/L.

\* \*\*POI Fermentation:\*\* Target concentration of 20 g/L assuming equivalent performance as the biomass production step.

\* \*\*Chromatography:\*\* POI recovery of 86.6% in the load operation.

\* \*\*TFF:\*\*Biomass removal at 99 %.

## 2.4 Economic Data

\* \*\*Raw Material Costs:\*\* Purchasing prices are defined for all input streams, including media components, IPTG, and WFI.

\* \*\*Waste Disposal Costs:\*\* A cost of €0.465/kg is assigned to aqueous waste treatment. No cost is attributed to gaseous emissions.

\* \*\*Revenue:\*\* Revenue is based on the flow of POI in the "Product to polishing" stream, with a user-defined pricing strategy. No other revenue streams are considered.

## 2.5 Labor and Resource Requirements

\* \*\*Labor:\*\* Labor requirements are specified as labor-hours per hour for each operation within each procedure, categorized by operator type. Additional auxiliary power requirements were reported from diverse fluid flow procedures, and utilities such as steam and cooling water are needed for processes such as sterilization and cleaning-in-place.

\* \*\*Resources:\*\* Steam and cooling water are used as auxiliary heating and cooling agents, respectively, with flow rates as specified for each process step. Electricity consumption is outlined for specific equipment, including pumps and compressors.

# 3. Results

## 3.1 Capital Investment Analysis

The total capital investment (TCI) for the POI production facility is estimated at €43,102,000. This includes the Direct Fixed Capital (DFC) of €40,669,000 and indirect costs, such as engineering, procurement, and construction management. A detailed breakdown of the DFC is presented below:

\* \*\*Media & Buffers Preparation:\*\* €17,835,000 This substantial cost likely reflects the need for sophisticated mixing, storage, and sterilization equipment for large-scale media and buffer production.

\* \*\*Fermentation:\*\* €9,806,000 This cost encompasses the two main fermenters (FR-101 and FR-102), the associated compressed air system (G-101, AF-101), sterilization equipment (ST-101), and various smaller tanks and pumps.

\* \*\*Downstream Processing:\*\* €12,978,000 This includes the chromatography system (five C-102 columns), centrifugation (DS-101), filtration (DE-103), associated buffer tanks, and storage vessels.

Equipment specifications and FOB costs are itemized in Table 2 of the provided data. Notably, the five chromatography columns represent a significant investment (€1,000,000), highlighting the importance of this purification stage in the overall process economics. The fermentation equipment also constitutes a large portion of the capital expenditure. Facility requirements, though not explicitly detailed in the provided data, are implicitly incorporated within the DFC costs and likely include building construction, cleanroom facilities, utilities infrastructure, and waste management systems.

## 3.2 Operating Costs

The total annual operating cost (AOC) is estimated at €12,317,000. A breakdown of the AOC is as follows:

\* \*\*Raw Materials:\*\* €2,417,000 (19.62% of AOC) IPTG, Glucose, and WFI constitute the largest raw material expenses, accounting for 20.77%, 19.32%, and 19.54% of the total raw material costs, respectively. This suggests optimization opportunities in media formulation or alternative induction strategies to reduce costs.

\* \*\*Labor:\*\* €846,000 (6.87% of AOC) This cost arises from 18,838 annual operating hours at a unit cost of €44.92/hour.

\* \*\*Facility-Dependent Costs:\*\* €7,478,000 (60.71% of AOC). This major cost driver requires further breakdown to understand contributing factors. Likely elements include depreciation, maintenance, insurance, and facility overhead.

\* \*\*Utilities:\*\* €124,000 (1.01% AOC) Electricity, steam, cooling water, chilled water and CaCl2 brine together form the total cost of these resources.

\* \*\*Waste Treatment:\*\* €819,000 (6.65% AOC) The dominant cost within this category stems from aqueous waste treatment (€790,233), with a unit cost of €0.465/kg. The relatively low cost of waste treatment compared to other expenses suggests efficient waste minimization strategies are in place.

\* \*\*Consumables:\*\* €506,000 (4.11% of AOC) The main cost driver is the chromatography resin (€489,668), which is expected given the significance of this stage.

## 3.3 Key Performance Indicators

\* \*\*Unit Production Cost (UPC):\*\* €10,264.52/kg of purified POI (UPRF)

\* \*\*Net Unit Production Cost:\*\* €10,264.52/kg of UPRF (identical to UPC due to zero revenue)

\* \*\*Unit Production Revenue:\*\* €0.00/kg of UPRF (indicating a missing or placeholder value in the provided data)

\* \*\*Gross Margin:\*\* -100% (reflecting the lack of revenue)

\* \*\*Return on Investment (ROI):\*\* -19.61%

\* \*\*Annual Production Capacity:\*\* 1,200 kg UPRF/year

# 4. Discussion

The economic viability of this POI production process is currently unfavorable, primarily due to the absence of product revenue data. The negative gross margin and ROI indicate that the current cost structure exceeds any potential income. The most significant cost drivers include Facility-Dependent Costs and Raw Materials, especially IPTG, Glucose and WFI. Optimisation strategies should focus on these specific materials. The Facility-Dependent Costs warrant a detailed investigation to understand their contributing factors.

Optimization opportunities include:

\* \*\*Media Optimization:\*\* Explore alternative, less expensive carbon sources and induction agents to replace Glucose and IPTG. Further evaluation is required on the media and buffer sterilization requirements. Efficient media and buffers supply systems will be critical to the economics of the process.

\* \*\*Chromatography Resin:\*\* Investigate lower-cost resins or resin reuse strategies, given the high contribution of resin replacement to consumables costs. Perhaps explore alternative purification strategies altogether.

\* \*\*Facility-Dependent Costs:\*\* Analyze contributing factors within facility-dependent costs and explore cost reduction strategies. Optimization of fluid dynamics and energy requirements for all operations will be key to minimizing operating costs.

\* \*\*Process Intensification:\*\* Explore options to streamline or intensify processes, such as continuous chromatography or integrated downstream processing steps, to reduce overall processing time and potentially capital investment.

Comparison with industry benchmarks is hindered by the lack of product revenue. Once realistic revenue projections are available, a comprehensive benchmark analysis can be performed to assess the competitiveness of this process. Given the potential scalability of the process involving fermentation, a critical analysis of the upstream operation's impact on product titers will also translate to improvements on overall downstream operating costs.

# 5. Conclusions and Recommendations

This techno-economic analysis reveals that the POI production process, while technically feasible, requires significant economic reevaluation due to the absence of revenue data. The unit production cost of €10,264.52/kg is currently meaningless without a corresponding selling price.

\*\*Recommendations:\*\*

1. \*\*Establish Product Pricing:\*\* Define a realistic selling price for the POI based on market analysis and competitor pricing. This is critical to assessing process viability.

2. \*\*Optimize Raw Material Costs:\*\* Evaluate alternative carbon sources, induction methods, and explore bulk purchasing agreements to reduce material expenses.

3. \*\*Detailed Analysis of Facility-Dependent Costs:\*\* Perform a granular assessment of facility-related costs to pinpoint major contributors and identify potential savings.

4. \*\*Chromatography Resin Optimization:\*\* Investigate cheaper alternatives, and evaluate the feasibility of resin reuse with different CIP procedures.

5. \*\*Process Intensification:\*\* Explore continuous processing options for chromatography and other downstream unit operations for process improvement.

The economic feasibility of this process remains undetermined until accurate revenue projections are incorporated. Addressing the outlined recommendations will enable a more comprehensive economic assessment and inform strategic decisions regarding process optimization and commercial viability.