DESIGN PROJECT FOR

PRODUCTION OF IFN-ALPHA

IPRO 345
Chemical Engineering Design IPRO
Objectives

• Design a process for production of the biotherapeutic compound IFN-Alpha from Chinese Hamster ovaries

• Assess whether the production design of this biotherapeutic compound is economically feasible and profitable
Background to Interferons

- Appear early after viral infection locally and systematically to limit spread of viral infection

- Inhibit viral activity by preventing RNA replication of the invading virus and certain other types of antigens and mark out tumor cells to be destroyed.

- Three naturally occurring forms: alpha, beta and gamma.
Background of Interferon-Alpha

- B-lymphocytes are the cellular producers of INF-alpha
- IFN-alpha is a multifunctional immunomodulatory cytokine
- IFN-alpha was approved by the Federal and Drug Administration (FDA) on February 25, 1991 to treat hepatitis C
Uses of Interferon-Alpha

• IFN-alpha remains the most frequently used IFN for both research and clinical applications

• Anti-viral applications such as chronic Hepatitis B and C now make up the bulk of IFN sales
Outline of IFN-alpha production

- Isolate IFN-alpha gene
- Ligate into Histag-vector
- Transfect into CHO cells
- Grow in batch reactor system

- Freeze-drying
- Ultrafiltration
- Centrifuge
- Elute IFN-alpha in Ni^{2+} affinity column
Cell Preparation

- Stimulation of T-cells by activator
- T-cells are collected and undergo RNA isolation
- Real Time RT-PCR to amplify the IFN-alpha gene
- PCR Amplification of IFN-alpha gene with restriction enzyme sites encoded on each end
- Cloning of the IFN-alpha insert into an appropriate cloning vector to verify correct PCR amplification
• IFN-alpha will be excised from the cloning vector and incorporated into the pSecTag2/Hygro vector to eventually allow for purification of Histidine-tagged proteins

• The IFN-alpha/pSecTag2/Hygro vector can be appropriately transfected into CHO cells to allow for the secretion of IFN-alpha

• A batch reactor system will be used for production
Histidine-tagged protein purification

• “His-tag” is the most commonly used tag for the facilitation of the purification of expressed recombinant proteins by affinity chromatography.

• A protein containing a histidine-tag is selectively separated based on affinity to a metal-ion charged medium.
Nickel-affinity column

- A gravity flow purification system which allows for separation of histidine-tagged proteins.

- Histidine-tagged proteins will be bound to the resin based on their Ni^{2+} affinity once a binding buffer is passed through the column.

- An elution buffer can be used to separate only the successfully histidine-tagged IFN-alpha proteins.
Powder Mixture

1.91E-06 g/mL
1150000 mL/each
2.20 g of N/each
136.24 g/yr

Reactor 1
1150 L
304 gal

Reactor 2
1150 L
304 gal

Oxygen Supply

DELAY:  
Exp Growth Per Reactor:
1.2 days for Growth; 3.22 * 10^12 cells; 194 g of Product; 2.8 * 10^6 cells/mL; 1150 L

Water

Note: Units/Cycle

Buffer after Delay

Column

Centrifuge

Ultra Filtration

Freeze Dry

95% waste, water

waste, water

95% product

water

product

waste
Cells + Nutrients $\rightarrow$ More cells + Products
Batch Reactor Cell Growth Equations

\[ \frac{dC_c}{dt} = r_g - r_a \]

\[ C_c = C_{c0} (e^{\mu t}) \]

\[ C_c = C_{c0} e^{\frac{\mu}{K_{max}} \left( \frac{S}{C^* + K_s} \right)} \]

Based on constraint of given variables and specified production output, two 1150 L batch reactors in parallel were designed

388 g of IFN-Alpha/cycle

30 cycles/year

Production Rate : 10 kg/yr
Separation

Ni-Affinity Absorption Column

Resin attaches to IFN-Alpha and after water is removed buffer is added for detachment
Separation Stages

**Centrifuge**: Uses rotation around fixed axis so centrifugal force is used for separating materials based on densities

**Ultra filtration**: Uses pressure through a semi-permeable membrane with pores sized to retain solids and pass water

**Freeze Drying**: Removes water from the food matrix by sublimation and is useful for sensitive and high-valued fluids
GANTT CHART

A chart that depicts progress in relation to time, often used in planning and tracking a project.
Cost Estimate

- Total bare module cost for fabricated equipment, CTFE: $186,000.

- Total direct permanent Investment CDPI: $416,600.

- Total Depreciable capital CTDC: $561,100.

- Total permanent investment: $639,700.
Approximate Profitability Analysis

- Minimum Proposed price of IFN-alpha: 1mg = 40 cents.
- Sales of 10kg of IFN-alpha: $4 million.
- Total production cost: $3.5 million.
- Pre-tax Earnings: $463,000.
- Net Earnings: $292,000.
- Process is Profitable.
Approximate Profitability Analysis

• 30 % return on Investment.

• Payback Period: 1.667yr

• Process is still profitable at subsidized price.
Rigorous Profitability Analysis

- Investor’s return rate: 23.76%.
- Emphasizes profitability.

Discounted Annual Cash Flow Over 15 yrs
Humanitarian Consideration

• High Price of product is a deterrent to patients.

• A lot of people need this product.

• Give out certain amount of free products.

• Sell at subsidized price.

• Suggested Selling price: 1mg = 40 cents.
Conclusion

• Large scale production of IFN-alpha would increase supply to meet its demand.

• Positive effects on patients.

• More Competitive industry.

• Reduce high price of product.
Questions
For more information, visit our website:
www.iit.edu/~ipro345s06