Biotechnology final project - Universitat Autònoma de Barcelona

BIOPROCESS DESIGN FOR rHSA PRODUCTION EXPRESSED IN PICHIA PASTORIS

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Introduction

Albumin is the most common blood protein. Its countless applications, which inlude drug transportation and treatment for hypoalbuminemia, have increased its sales during the last years, thus raising the interest of the pharmaceutical companies in its industrial production.

Objective

To design a biotechnological plant producing 12 tons per year by a *Pichia pastoris* recombinant strain. The process should be as cheap, secure, and environmentally and socially friendly as possible. Higher purity has been aimed in order to overcome the present constrains and possibly open a new market niche.

Location

The plant is located in the Lithuanian coast due to its good both ground and maritime communication with its principal market: Europe. Furthermore, Lithuania sums the trustworthiness of a EU member to one of the lowest tax systems of the Eurozone.

Materials and methods

For planing and modeling the biotechnological process SuperPro Design simulator software has been employed.

Critical coloring [1]

rHSA is contaminated with coloring matters characteristic to rHSA, proteins originating in the host cells, polysaccharides, etc. In particular, it is necessary to sufficiently eliminate components originating in the host cells, since they are foreign matters for living organisms including human being and thus can cause a problem of antigenicity .

Recombinant Human Albumin [6]

- Isoelectric point: 4.9
- World sales: 400-500 tons/year [2]
- Hydrophilic and non-glycosylated protein
- Blood concentration: 40 or 50 g/L

Process information

- Fermentation: repeated-batch
- rHSA final reactor concentration: 12 g/L [3]
- Yx/s: 0,575
- Plant production characteristics:11,983 kg/year
- Plant operating time: 7,843.09 h
- Downstream efficiency: 64% rHSA recovered
- Methanol-induced AOX promoter. [3]

Main Items [1]

1.Media. A media with glycerol is used in the first 24 hours, during which the biomass grows without promoter activation.
When a cellular concentration is raised to 20g/L, a media with methanol, without glycerol, triggers rHSA production through promoter induction.

2.Fermentation tank. The reaction is a repeated fed-batch composed by 4 cycles of 264 h each. The fermentation conditions include a pH of 5.85, temperature of 25ºC, $pO_2$of 20% and agitation of 800 rpm.

3.Heat treatment. The temperature is raised to 68ºC in order to denaturalize proteases.

4.Microfiltration. Its aim is to eliminate the biomass.

5.EBA (Expanded bed adsorption). It is among the most important downstream steps. In this step most of impurities are removed, but it is also where most of rHSA is lost.
The rHSA is adsorbed to the resin and then eluted with a pH 9 buffer stream.

6.Heat treatment. The temperature is increased to 60ºC to solve the coloration problems caused by the EBA resin. The turbidity (coloring problems) is caused by hydrodynamic friction.

7.HIC (hydrophobic interaction chromatography). This second purification step is used to eliminate impurities from the stream.
A pH of 6.8 is applied.

8.IEC (ionic exchange chromatography). This third purification step contributes to get rid of rHSA coloring compounds.

9.Chelate resin treatment. Most of the coloring matters are absorbed in this process according to the linking resin capacities.

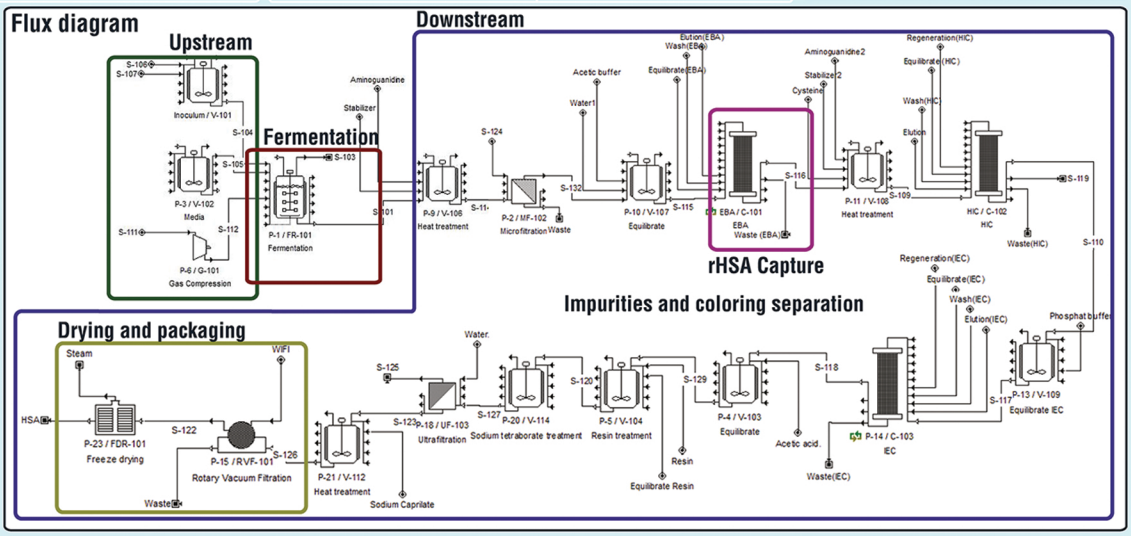
10.Sodium tetraborate treatment. A basic solution with a pH of 4.5 is used to precipitate the remaining impurities.

11.Ultrafiltration. Albumin is separated from the precipitate.

12.Rotary Vacuum filtration. It is used to separate the solid product from the solvent.

13.Freeze drying. At the end of the process a rHSA of 93.33% purity is obtained.

Flux diagram



Economic analysis

Economical data

Total Capital Investment ($)	Operating Cost($/yr)	Revenues ($/yr)	Unit Production Revenue ($/kg MP)
110,919,000	22,626,000	41,939,000	3,500.00

ROI	Payback time(year)	IRR	NPV($)	Unit Production Cost ($/kg MP)
16,48%	6.07	9.77%	20,467,000	1,888.22

Interest %	7.00	9.00	11.00
NPV ($)	20,467,000	5,429,000	-6,958,000

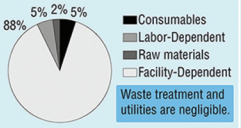
The table indicates that when the interests are higher than 9,77%, the project is not viable.

Sensitivity

ROI	Payback time(year)	IRR	NPV($)	Unit Production Cost ($/kg MP)
16,46%	6.07	9.77%	20,310,000	1,891.22

Even if the price of energy increases by 300% the economical impact in the viability of the project is practically zero, because the main cost of the product is the investment and amortization.

Operational process cost

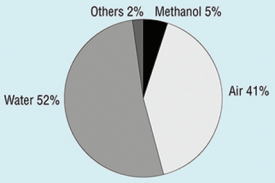


Waste treatment and utilities are negligible.

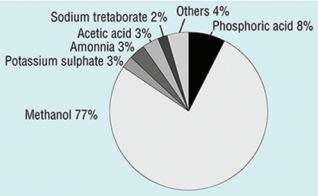
The main process cost is attributed to facilities due to the high number of units.

Environmental analysis [4]

MASS INDEX

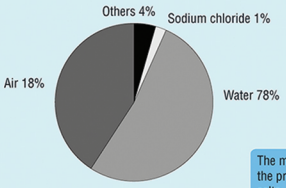


ENVIRONMENTAL INDEX

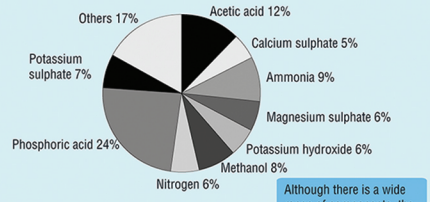


INPUTS

OUTPUTS

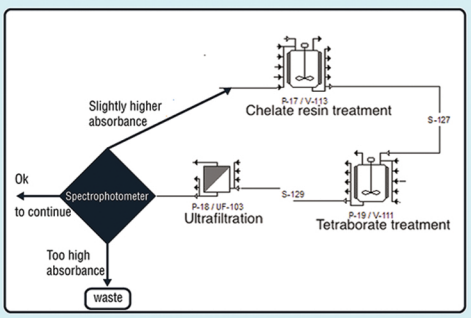


The main resources used in the process are water and salts, so the wastes are mainly water with acid and salts dissolved.



Although there is a wide range of components, the total mass of them is low.

Coloring control [1]



After the stream undergoes the ultracentrifugation step it is expected to have an albumin coloring inferior to 0.015 absorbance units, calculated as an average absorbance at 350nm and 280nm. At the point where a sample is sent to spectrophotometer, in the case that the coloring parameter is higher to 0.015 units, measures have to be taken:

- If the value of coloring is too high the batch is discarded.
- If the coloring value is slightly higher than 0.015 the stream is recirculated to the resine tank.

Further improvements:

The process could be further improved through different approaches, including:

- Development of a low cost resin with better affinity.
- Development of a recombinant strain with low level of proteases capable of albumin degradation, through a knock-out of the his4, pep4 i prb1 genes. [5]
- Producing a different final product --> injectable serum albumin.
- Study a new plant design, based on disposable single use units.

Conclusions:

- High purification is achieved.
- High economical benefits are obtained.
- Social responsibility with the country and consumer is taken into account.
- The environmental impact is low.
- The process should be tested.

References:

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