Recombinant t-PA production process expressed in *E. coli*

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PROCESS DESIGN

A complete and detailed bioprocess for the recombinant t-PA production using *Escherichia coli* is explained in this project. It is focused on the technical parts of the production process focusing on the upstream, bioreaction and downstream parts and their main steps. It is reproduced a process chart designed by the project team using the simulation *software* SuperPro Designer.

UPSTREAM (RED)

V-102 is the storage tank where medium components (water and salts) are mixed. The step before the fermenter is S-102 which is a sterilizer. BR-101 represents a DTR with a capacity of 11,8 m^3. Fermentation conditions are: 37ºC, 100 rpm and a cell growth until DO_{550}nm is 0.4 (1). At this moment IPTG is added. Production lasts 4 hours with a final rtPA concentration of 465 mg/l (2) (60% inclusion bodies, 40% soluble protein).Other chemical agents such as glucose and ammonia are introduced in the bioreactor previously filtered.

$$C_6H_{12}O_6 + 2{,}33\ O_2 + 0{,}9\ NH_4^+ \longrightarrow 3{,}8\ CH_{1.77}O_{0.49}N_{0.24} + 4{,}36\ H_2O + 2{,}18\ CO_2$$

DOWNSTREAM (BLUE)

Downstream is a highly more complex and expensive process compared to upstream. CF-101, HG-101 and CF-102 are used to extract medium, degrade cells and isolate inclusion bodies respectively before starting the refolding process. The refolding process (**GREEN**) consists in 3 steps: resolubilization in V-103, sulfonation in V-104 and renaturalization. Enzymatic refolding using protein disulphide isomerase **(PDI) permits a volume reduction of 3000 times** in regard to the chemical refolding process. The following steps consist in a volume reduction by ultrafiltration equipment (UF-102, 103, 104), specific isolation of rt-PA by affinity chromatography (C-101) and specific isolation of well folded rt-PA by gel filtration chromatography (C-102). FDR-101 is a freeze dryer that permits to get solid rt-PA.

Table 1: Downstream yield and time analysis

Process	Yield (%)	Time (hr)
Buffer tank 1	100	0.14
Centrifugation 1	90	0.33
Cell disruption	100	6.42
Centrifugation 2	90	0.33
Solubilitzation	80	3.33
Ultrafiltration 1	90	4
Sulfonation	100	7.08
Refolding (3)	20	4.17
Ultrafiltration 2	80	4
Cromatography 1	75	0.89
Ultrafiltration 3	90	4
Buffer tank 2	100	4.01
Cromatography 2	54	1.21
Ultrafiltration 4	90	4
Sterilization	91	5
Liofilization	100	3.28
Downstream	**2.8**	**56.3**

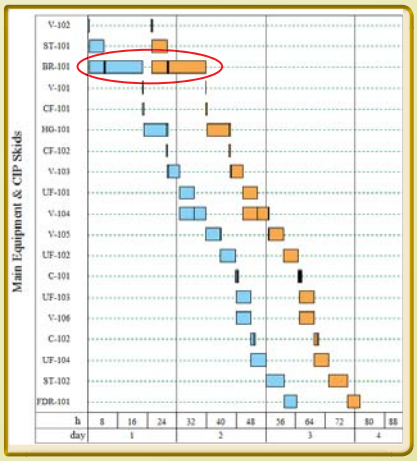

Chart 1: Gantt chart designed with *SuperPro Designer*

Table 2: Receipe scheduling summary

Process	Value
Batch Time	56.3 hr
Cycle time	17 hr
Batches/yr	300

SuperPro Designer Process chart

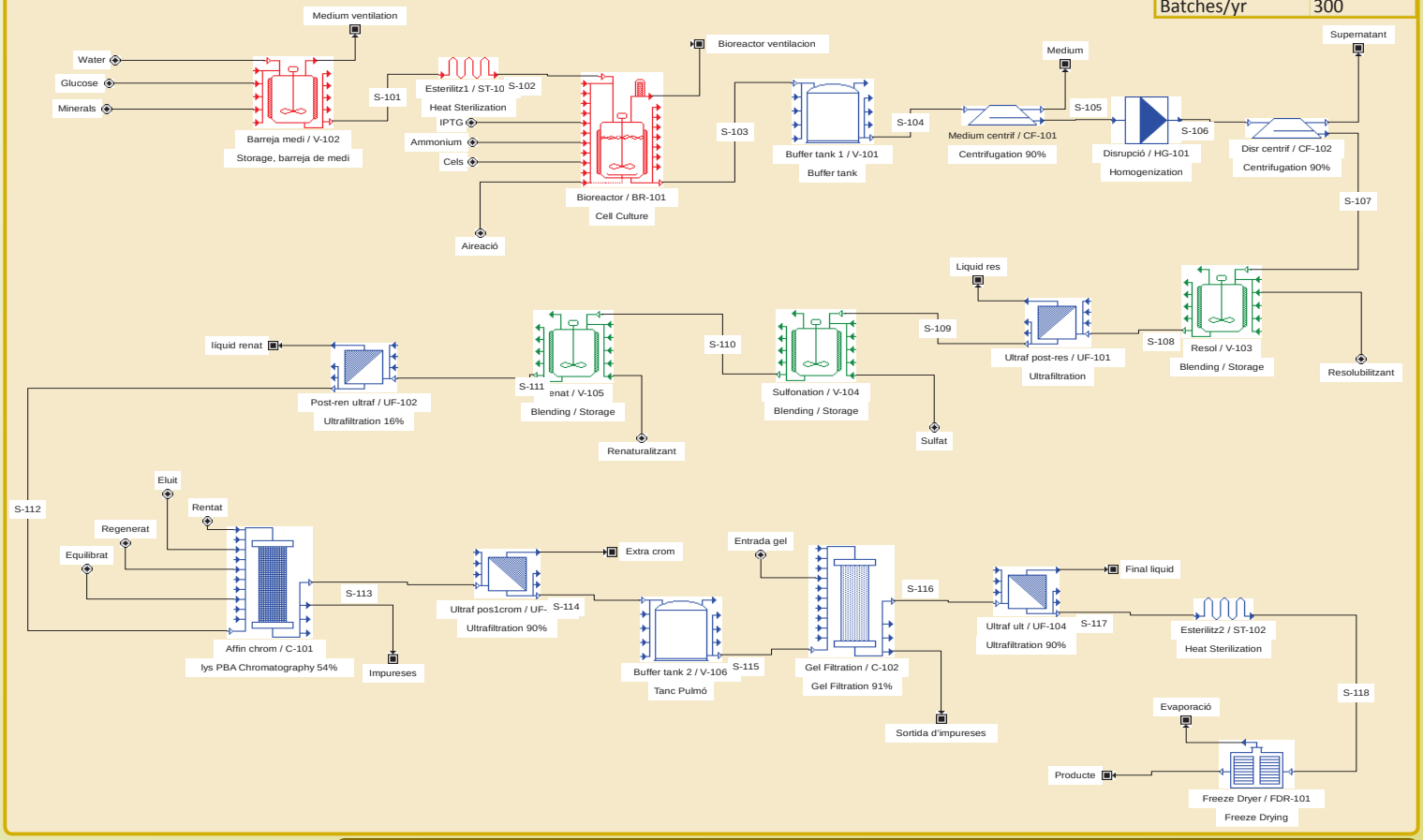

Chart 2: Process chart design. Based on data from (1) and (2)

REFERENCES

(1) Mir Mohammad Sadeghi, H., Rabbani, M., Rismani, E., Moazen, F., Khodabakhsh, F., Dormiani, K. i Khazaei, Y.: Optimization of the Expressions of Reteplase in Escherichia coli. *Research in Pharmaceutical Science* **6**(2):87-92 (2011)
(2) Datar, R. V., Cartwright, T., Rosen, C.: Process Economics of Animall Cell and Bacterial Fermentations: A Case Study Analysis of Tissue Plasminogen Activator. *BIO/TECHNOLOGY* **11**:349-357 (1993)
(3) Youchun, Z., Ge W., Yang K., Yanbing W., Changkai Z., Chao C., Bufeng L.: Increase Renaturation Yield of Reteplase Using the Recombinant Human Protein Disulfide Isomerase. *High Technology Letters* **10**(1):49-51 (2004)