

TECHNICAL CHARACTERISTICS OF THE PROCESS



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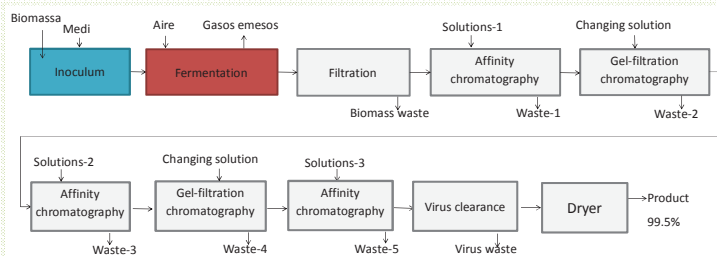
INTRODUCTION: The main objective of this project is to design a biotechnological industrial plant to produce recombinant tissue plasminogen activator (r-tPA), known commercially as Tenecteplase (TNK). With the goal of covering 5% of the tPA sales in China, the production will be of 30 kg TNK/year. The use of simulators to create different diagrams will help in defining the units of the process.

PROCESS

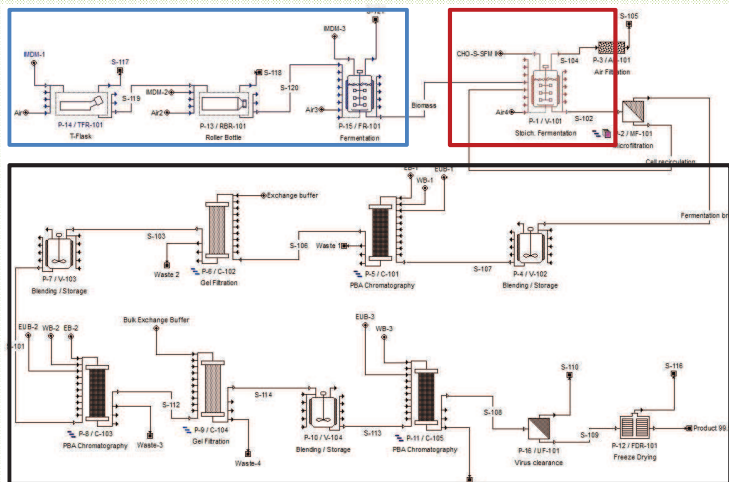
- Product: Tenecteplase
- Operation mode: Perfusion with immobilized cells
- Cell line: Chinese Hamster Ovarian cells (CHO) DUKxB11
- Production: 30 kg TNK /year

The process is constituted by three essential parts:

- In the **upstream** section, cells are cultured in order to achieve the required concentration for the TNK production.
- In the **reaction** step, cells synthesize TNK and release it to the medium.
- In the **downstream** section, TNK will be isolated with a minimum purity of 99.5% for its further use in the pharmaceutical industry.

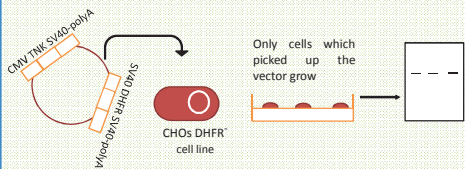


SuperPro Designer is a simulator that facilitates the modeling, evaluation and optimization of end-of-pipe processes. When the equipment necessary for the TNK production have been studied in detail, together with the reactions that take place inside them and the raw materials used, SuperPro Designer will enable us to represent the process flow diagram.



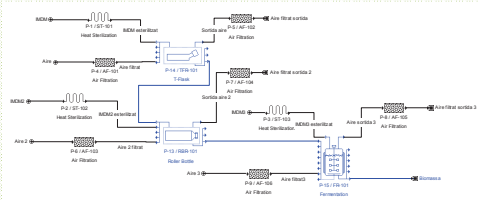
UPSTREAM

Recombinant CHO DUKxB11 cell line obtention



Inoculum preparation

When the most productive cell line has been isolated, from an initial CHO cells culture we do serial amplifications in flasks, roller bottles and a reactor. This part of the process operates in batch and lasts 10 days.



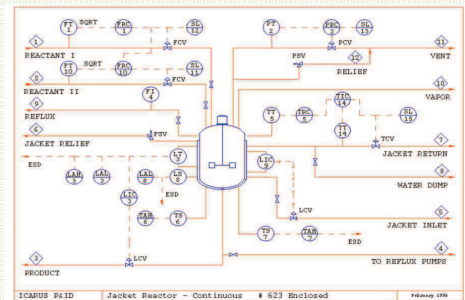
TECNIC CHARACTERISTICS	EQUIPMENT	Volume (L)	Nº units
Initial culture	10 ⁶ cells		
Final culture	6.4 · 10 ¹⁴ cells		
Medium	IMDM		
Duration	10 days		
	T-Flask	0,1	6
	Roller bottle	2,2	3
	Reactor	120,22	1

REACTION

The bioreactor is fed with the biomass inoculum coming from the upstream section, CHO-SFM-II medium (defined and without FBS) and air.

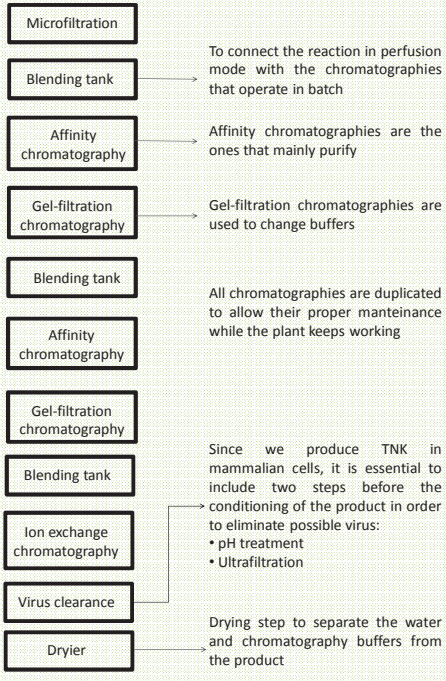
The reaction takes place in perfusion with the cells immobilized on a matrix and in a 650L packed reactor.

The following figure shows the Piping and Instrumentation diagram of the reactor, in which controllers and sensors of different parameters are indicated.



PARAMETER OF THE REACTOR	VALUE
Temperature	33,5 °C
pH	7,2-7,4
Pressure	1 bar
Aeration rate	0,5 vvm
Volumetric entry flow (medium)	500 L/h
Glucose concentration	0,3-1,5 mg/L
Working volume	598 L
Residence time	1,2 hours

DOWNSTREAM



* According to patent WO 2012/085933 A1

AUXILIARY EQUIPMENT

Other equipment, apart from the represented in the process flow diagram, will be necessary in our plant to allow the desired production of TNK:

- Pumps to make liquids flow at the correct rate and pressure
- Compressors to allow the correct air circulation
- Filters to guarantee an exit of sterilized air
- Filters to sterilize the medium
- CIP and SIP processes integrated in the equipment

REFERENCES

- Genova biopharmaceuticals. (2012). WO 2012/085933 A1. World Intellectual Property Organization.
- Davydov, L, Cheng, J. **Tenecteplase a Review**. Clinical therapeutics, 23: 7 (2001), pp. 982-977
- **Products: SuperPro Designer**. Intelligen, Inc. 2013. Available at: http://www.intelligen.com/superpro_overview.html. Visited on 18/05/2013
- **Piping and Instrumentation Drawings V7.1**. AspenTech. 2009. Available at: <http://archivos.labcontrol.cl/DINAMICA/icarusPID.pdf> Visited on 15/05/2013
- **Catàleg INVITROGEN**. Available at: <https://products.invitrogen.com/ivgn/product/R71807?CID=search-product>. Visited on 20/04/2013