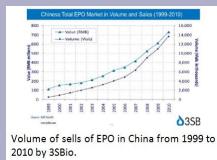




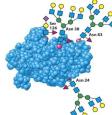
EPOTECH is an emerging biotechnological company centered on the production of biopharmaceutical products. This project proposes the construction of a new plant in Sai Mai, 20 Km far from Bangkok (Thailand) for the production of recombinant human erythropoietin (rhEPO). The market to be exploited is China because it is an industrially emergent country that is adopting Western habits and it makes it a potentially pharmaceutical new market. Concretely, it is estimated that there are 119.5 million of CKD (Chronical Kidney Disease) patients that need rhEPO to treat anemia. 3SBio is the company that currently distributes this product, so Epotech is planning to sell them rhEPO as an API (Active Pharmaceutical Ingredient) for a lower price than its current suppliers.



OBJECTIVE

Design a production plant of rhEPO expressed on CHO cells by using a batch production system.

Determine the volume of production necessary to treat the 5.6% of CKD patients in China (62M people), which means producing 462 Kg of rhEPO per year.

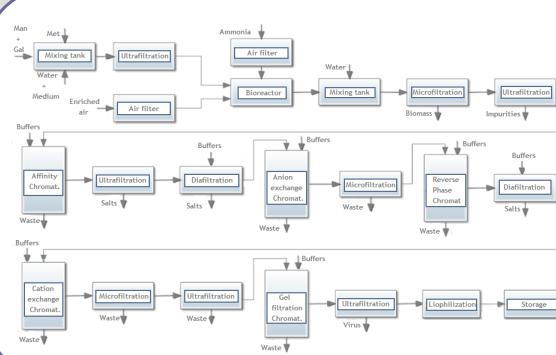


Erythropoietin (EPO) is a glycoprotein of hormonal system that regulates erythropoiesis: it stimulates the proliferation and differentiation of precursor cells of erythrocytes placed in the bone marrow.

At a molecular level, human EPO is a glycoprotein of 165 amino acids and a molecular weight of 35 KDa approximately depending on the pattern of glycosylation. It has been observed that EPO isoforms rich in sialic acids (negatively charged) at the last sugar of N-glycosylation present higher half lives.

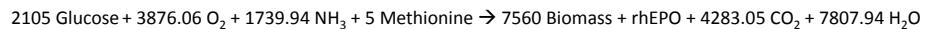
The production cell line is **rhEPO-CHO.K1** (Chinese Hamster Ovary). It was obtained from the Cell Cultures Laboratory of the Biochemistry and Biological Sciences Faculty at the Universidad Nacional del Litoral (Santa Fe, Argentina).

The operational system used is a **batch**, which eases the entrance to a new market.



BLOCKS DIAGRAM

STOCHIOMETRIC REACTION:



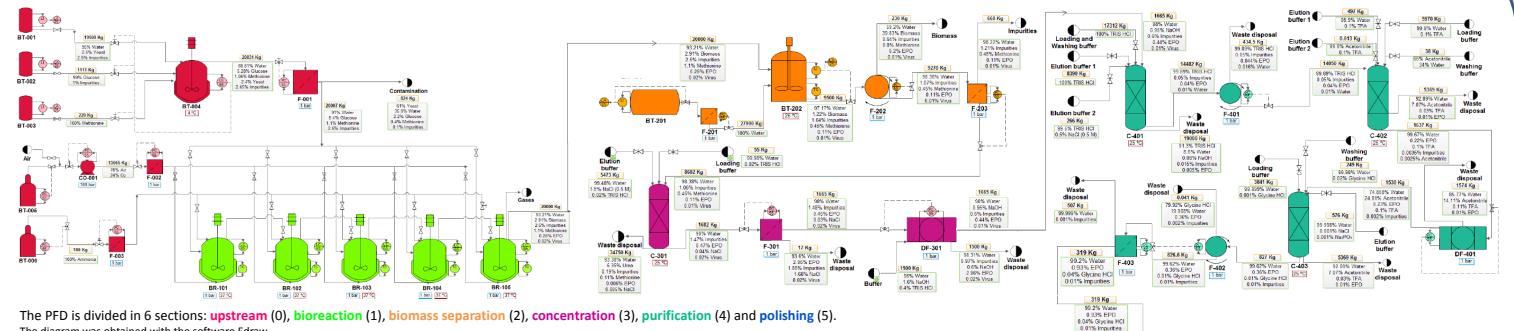
Fermentation time=7 days
Volume of the reactor= 20.000 L
 $[\text{rhEPO}]$ =2.4 mg/L
DS efficiency=21%
Production/batch=11 Kg (5 downstream cycles)
Batch/year=42
7.45 mg rhEPO/year-CDK patient (in average)

These data were obtained by performing simulations of the process using the software SuperProDesigner.

FOR EACH BATCH (11 Kg rhEPO):

Inputs:	Waste:
125 tones Water	51.3 Kg Biomass
1100 Kg Glucose	1034 Kg CO ₂
220 Kg Methionine	26000 Kg TRIS HCl
13000 Kg Enriched air	511 Kg Impurities
100 Kg Ammonia	83 Kg NaCl
	602 Kg Acetonitrile
	24 Kg NaOH
	8 Kg TFA
	2.4 Kg Na ₃ PO ₄

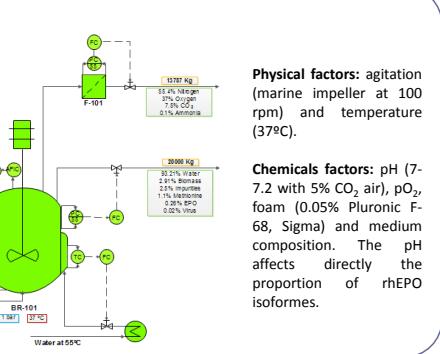
PROCESS FLOW DIAGRAM



The PFD is divided in 6 sections: **upstream** (0), **bioreaction** (1), **biomass separation** (2), **concentration** (3), **purification** (4) and **polishing** (5).

The diagram was obtained with the software Edraw.

CONTROL OF THE BIOREACTOR



Physical factors: agitation (marine impeller at 100 rpm) and temperature (37°C).

Chemicals factors: pH (7-7.2 with 5% CO₂ air), pO₂, foam (0.05% Pluronic F-68, Sigma) and medium composition. The pH affects directly the proportion of rhEPO isoforms.

The nomenclature used is international ISA-5.1-1984 (R1992), *Instrumentation Symbols and Identification*, which is recommended by ISA (Instrument Society of America).

The diagram contains all the equipments and the physical parameters:
 BT (Blending Tank), F (Filtration), CO (compressor), BR (Bioreactor), C (Chromatography), DF (Diafiltration), and FDR (Lyophilisation). The first number refers to the section of the process and the last number refers to the number of equipment at the same section.
 The control parameters are L (Level), T (Temperature) and P (Pressure).

CONCLUSIONS

In a production process based on mammalian cells and containing a complex downstream processing like the process above, the critical parameters must be controlled through an accurate system for not altering the quality of the final product. The properties of rhEPO must be reproducible batch to batch. The lack of coherence between the two bibliographic sources complicated the process calculations.

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