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

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INTRODUCTION

This project consists on designing a **whole new process** for the development of **recombinant t-PA** molecule expressed in **Escherichia coli** strains in order to supply the demand due to the expected growth of cardiovascular disease in China. The project is divided in three posters which are focused on the key aspects of the bioprocess and they are explained in the following order:

RTPAECOL-1
- Objectives
- Layout
- Operation strategies
- Refolding

RTPAECOL-2
- Process design
- Upstream
- Downstream

RTPAECOL-3
- Process analysis
- Economic analysis
- Sustainable analysis

OBJECTIVES

The project objectives pretend to supply the **40% of the treatable patients** in 2030 according to the previsions **(1)**. The plant will be installed in China because it is a country with an emerging economy where there has recently been an increase in the population that could be treated with our medicine. Each treatment consists in **two doses of 18.1 mg of rt-PA** each **(2)**. So, **46.3kg** of rt-PA must be produced every year. It has been planned to carry out **300 batches/year** which means a production of **155 g/batch**. Considering all design parameters we will be able to determine the **viability** of the project.

LAYOUT

Physical places are distributed according to the needs of the plant. Besides the reactor, upstream and downstream we should consider where to store the materials, carry out the R+D, where will be the offices or where are going to park the car the employees. The plant is divided in four different sections: **Warehouse and storing** (storage of substrates and raw materials); **Main Process** (Upstream, bioreaction and downstream); **Laboratories** (Quality control and R&D); **Offices** (direction and administration of the plant). This layout was design using ***GOOGLE SKETCHUP***.

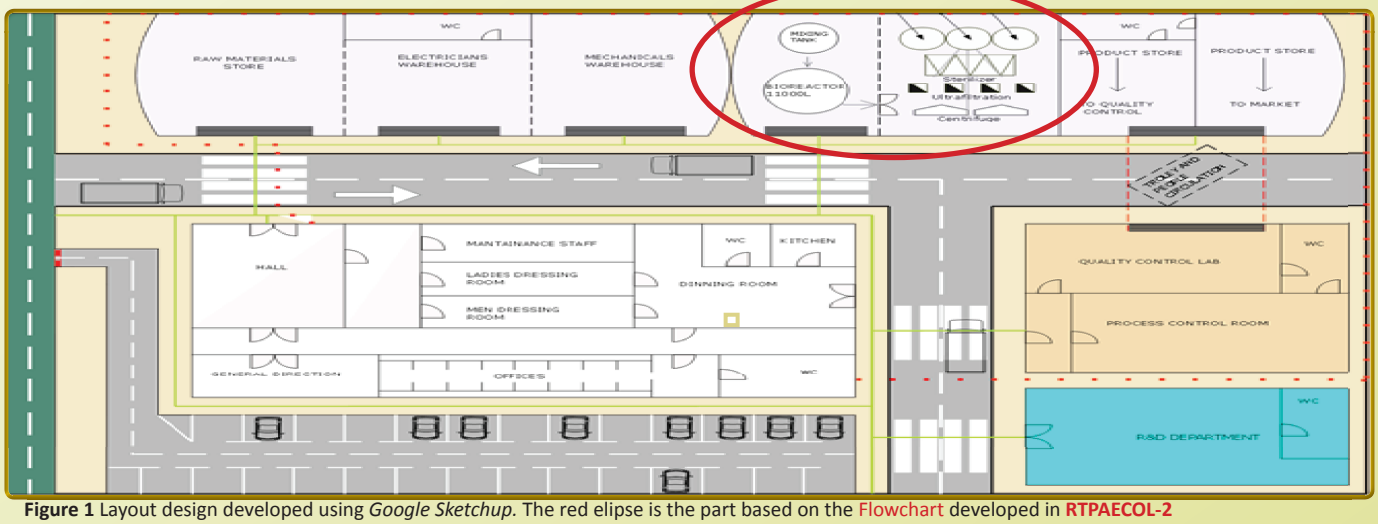

Figure 1 Layout design developed using *Google Sketchup*. The red elipse is the part based on the Flowchart developed in **RTPAECOL-2**

OPERATION STRATEGIES

Our premise: **Developing a biotech molecule by recombinant technology in order to cover a part of the potential thrombolytic market in China.** It must be considered the following process aspects:

- **Biochemical and bioengineering design** *(RTPAECOL-1,2)*
- **Establishment of separation and purification systems** *(RTPAECOL-1,2)*
- **Flowchart design*** *(RTPAECOL-2)*
- **Sustainability analysis** *(RTPAECOL-3)*
- **Layout design**

*Using SuperPro Designer we were able to develope an accurate approximation of a real process flowchart

In order to develop the project these are the production conditions taken

- **Discontinuous** operation due to its **simplicity**
- ***E. coli*** utilization due to the great **knowledge** at molecular and metabolism level
- Nearly the **60% of rt-PA** produced is expressed as **inclusion bodies**. Protein **refolding** is essential to obtain the properly molecule estructure

PROTEIN REFOLDING

The protein refolding is a key aspect in the process. Most of our protein is retained in inclusion bodies as agregates proteins **(3)**. We need to **denature** those agregates in order to **solubilize** them. Once is solubilized we **refold** the proteins with protein disufide isomerase **(PDI)**.

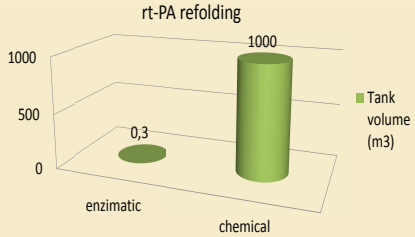

Figure 2 Tank Volume with enzimatic and chemical treatment

As we can see in Fig. 2, enzimatic treatment implies a **lower volume** and **less reducing agents** than chemical treatment. Enzimatic treatment is more specific and more expensive but gives a **yield** much more **higher**.

References

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