Bioprocess Design for Human Hemoglobin Production in *Saccharomyces cerevisiae* Part II – Process Development

Bachelor's Degree Final Project – Biotechnology
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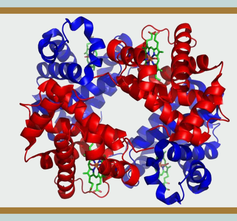
INTRODUCTION

The main goal of this project is to build an industrial production plant of hemoglobin which could be used as a replacement for blood transfusions in a close future. This way, we would completely end with the donation-dependent actual system and the transfusions would become much easier. In this part of the project we treat the plant design aspects: first of all, how to produce the microorganism previously designed, in this case, S*accharomyces cerevisiae*, then how to synthesize the hemoglobin with this microorganism and last of all, how to separate and purify hemoglobin.

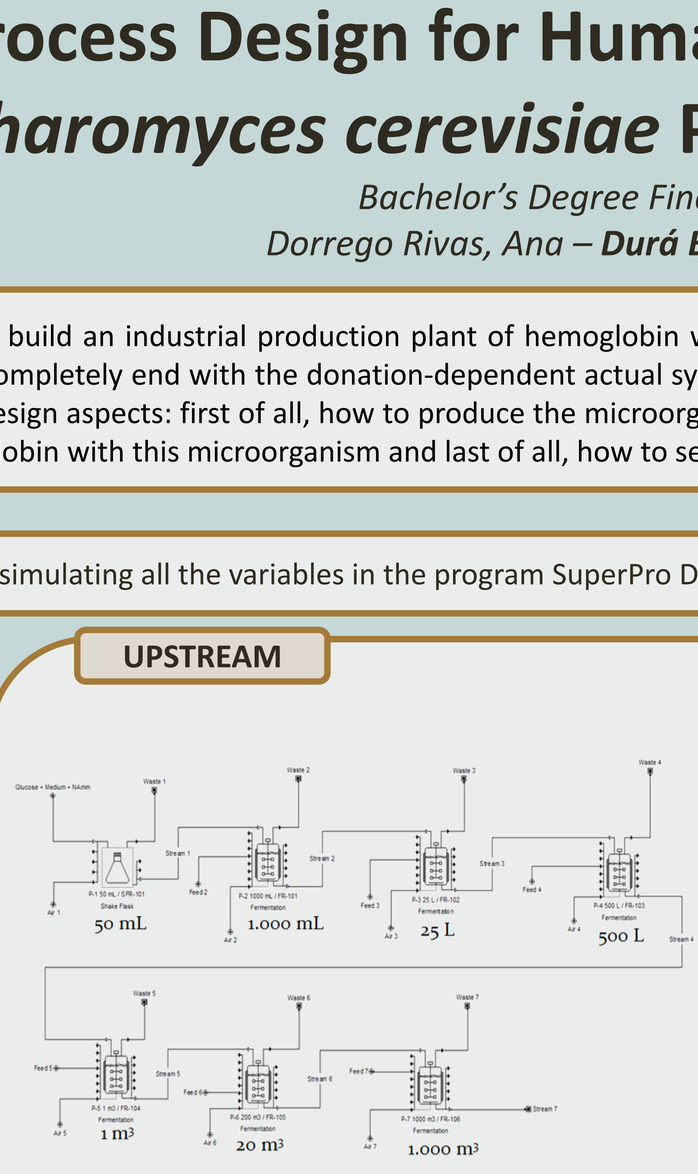


DESIGN

Process design is carried out simulating all the variables in the program SuperPro Designer. This helps us to get a realistic process without necessary experimental results.

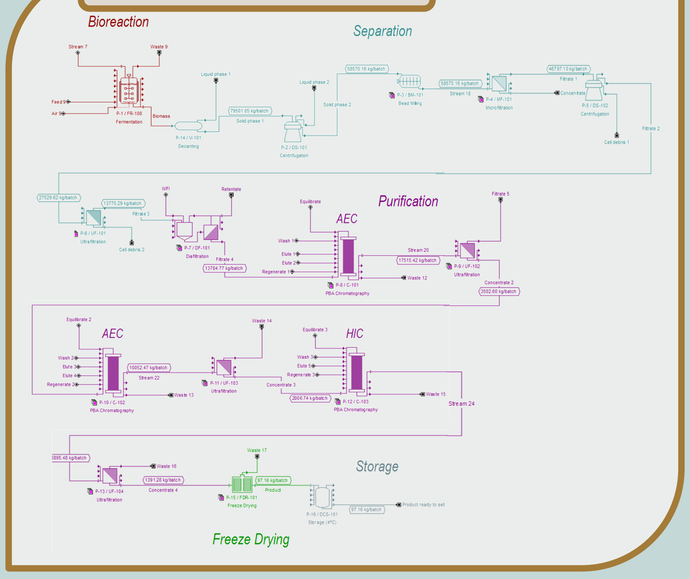
Getting a big amount of biomass in order to produce the hemoglobin is the first important part of the process. To do so, we use a number of discontinuous batches in which we grow *Saccharomyces*. The volume of the reactors will become greater in each step until we get to 1000m^3. To achieve this, we use glucose as a substrate for the growth, which allows us to get the greatest growth rate possible.

UPSTREAM

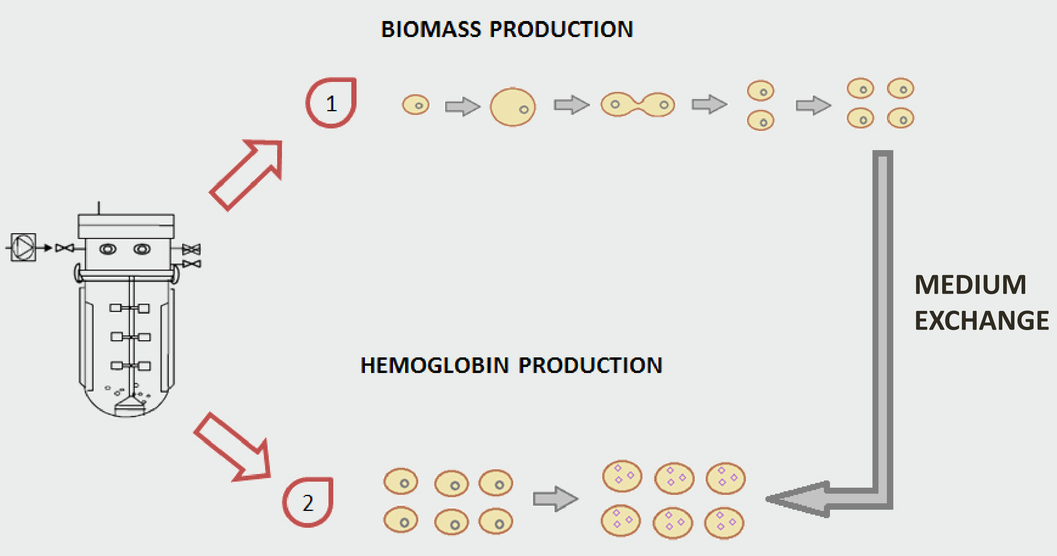


Once we have the necessary amount of biomass, we will induce the hemoglobin production in the same last bioreactor[1]. Later, the hemoglobin will be purified following first of all, volume reduction steps like centrifugation and microfiltration, and then purifying steps (mainly chromatographies). After all these phases, we obtain hemoglobin as a product made following GMP.

DOWNSTREAM



BIOREACTION



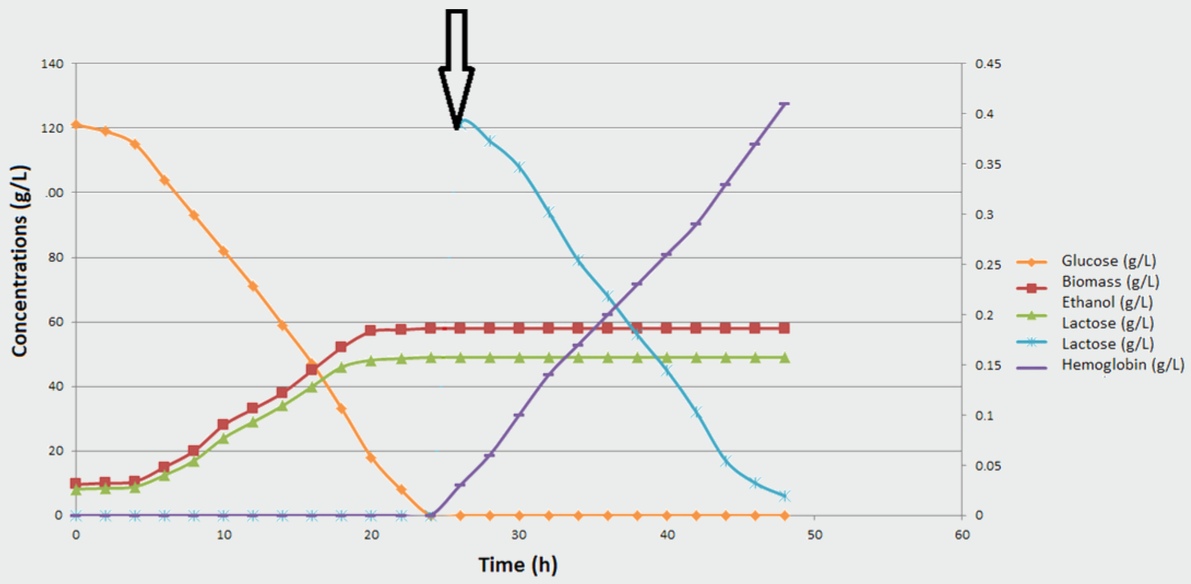
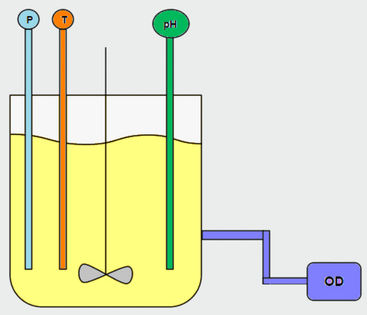


Figure 1. Evolution of the principle compounds of the reaction.

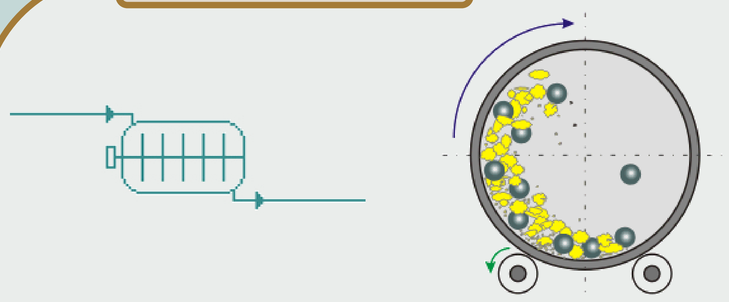
1. *S. cerevisiae* has a typical growth curve when it is cultured, with 3 phases: lag, exponential growth and stationary phase. We use yeast in exponential phase in order to inoculate each reactor. In presence of medium with all of the necessary compounds and glucose as a substrate, the biomass keeps increasing in every step. However, during the growth, *S. cerevisiae* produces ethanol has a by-product that can inhibit the growing process. The concentration of this by-product should, then, be controlled.
2. Hemoglobin's production in the modified yeast is under the control of the promoter of the lac operon so we need to add lactose so that the product is generated. Once the biomass has grown , the medium containing the remaining glucose will be removed from the reactor. Then, new medium containing lactose will be added. It is then that the stationary phase can begin and the hemoglobin's production will be induced due to the lac-operon control. Later, we will need to purify this product.

CONTROL



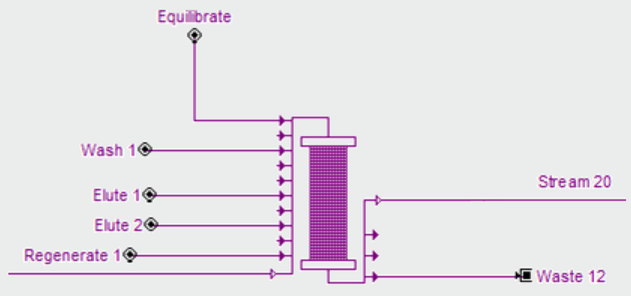
During the process of the bioreaction there are several variables that need to be controlled in order to get the best growth rate for *S. cerevisiae*. The temperature, pH, pO_2, and OD are critical for the reaction. The process has been designed based in the Quality by Design method. This allows us to watch over each variable almost in real time and correct possible risks before it is too late for the product. This can be done due to the actual PAT technologies.

DISRUPTION



The fact that the hemoglobin produced isn't secreted to the extracellular medium is an advantage. This allows us to reduce the volume of the solution easily, since the hemoglobin is protected in the intracellular environment. However, the product needs to be separated from the rest of the cellular compounds and the first step is breaking the cellular membrane using a mechanical method of disruption, in this case, a bead milling. It consists on a cylinder with balls of different sizes –beads– that move when the rotor spins and impact against the cells breaking their membranes.

CROMATOGRAPHY



Several steps of purification are performed to obtain GMP qualified hemoglobin. However, the most important is the chromatography. There are three of them in this process (two of them based in anionic exchange and one of them based in hydrophobic interaction). These chromatographies use the particular hemoglobin properties in order to differentiate it from the rest of the proteins in the cell and separate it[2].

CONCLUSIONS

The method here presented is an alternative for the production of recombinant hemoglobin in *Saccharomyces cerevisiae.* This process allows us to generate hemoglobin with a very high purity (99.98%) which could be used for blood transfusions in the future once it is encapsulated in liposomes. However, the purifying steps could be improved since there are many losses of hemoglobin during the process.

BIBLIOGRAPHY

[1] Palomares LA, Estrada-Mondaca S, Ramírez OT. Production of recombinant proteins: challenges and solutions. Methods Mol Biol. 2004;267:34-5.
[2] Nakajou K, Hoashi Y, Kai, T, Uno T, Otagiri M., inventors; Nipro Corporation, assignee. Production of recombinant human hemoglobin using pichia yeast. United States patent US 2009/0098607. 2009 Apr 16.
Figure 1. SuperPro Designer simulation.