Vitamin B12 Bioprocess Design: Part II. Reactor, downstream and control

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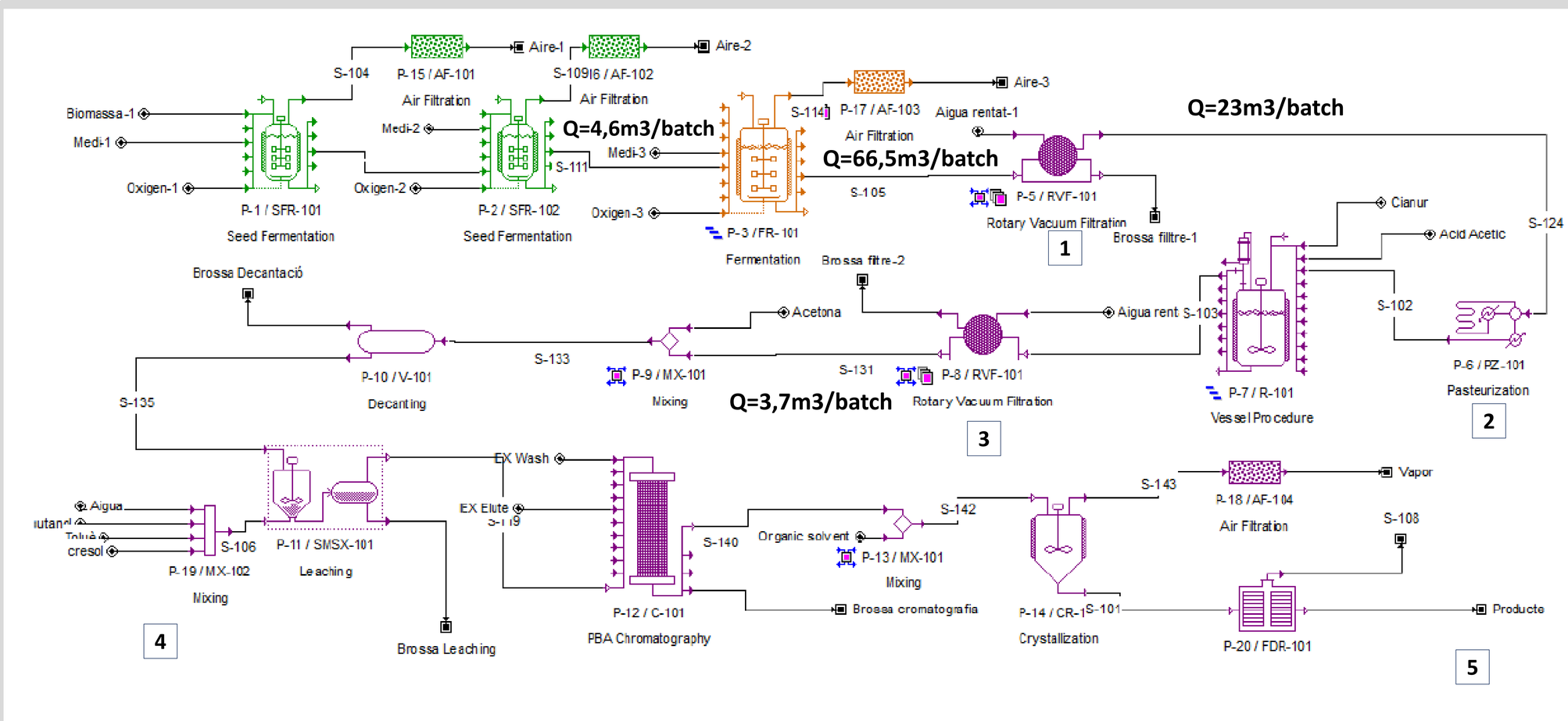
Introduction & Objectives

Vitamin B12, also called cobalamin is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system.
The main objective is to design an efficient purification process for vitamin B12 in a crystalline form. This vitamin will be earmarked for human consumption, which will define its purity (>90%). The aim is to supply the 30% market demand, about 10.000 kg of each vitamin.
The project is also focused on providing lifecycle adaptation that maintain process control and high product quality via *Quality by Design* (QbD) concept.

Flow Diagram



Upstream:
- *Seed Reactor 1*
- *Seed Reactor 2*

Reactor:
- Batch
- 142 kg de vit b12 unpurified/ batch
- Volume: 120 m3

Downstream:
- 11 steps
- Yield: 81%
- Acid pH (4,5-6)
- 115 kg vit B12 pure/batch
- Operating time : 162h
- 88 batch/ year
- Total B12 production: 9.867 kg

Figure 1. *Flow diagram of vitamin B12 production in Pseudomonas denitrificans. In green color represent the upstream, in orange the reactor and in violet the Downstream process. Flow diagram created with SuperPro Designer.*

1. Solid-liquid separation. Volume reduction of 66% throw a rotatory vacuum filtration

2. Cellular disruption. *P. Denitrificans* produces intracellular. KCN addition, cianocobalamin formation.

3 . Solid-liquid separation. Discarding solid phase via rotatory vacuum filtration.

4. Vitamin purity 80%. Acetone precipitation + leaching. Enogh purity to be sold for animal consumption.

5. Vitamin purity ≤ 90%. Ready for selling.

Growing Strategy

P. Denitrificans growing in two steps:
- *P. denitrificans* growth. High DO in bioreactor.
- Vitamin B12 production. Low DO.

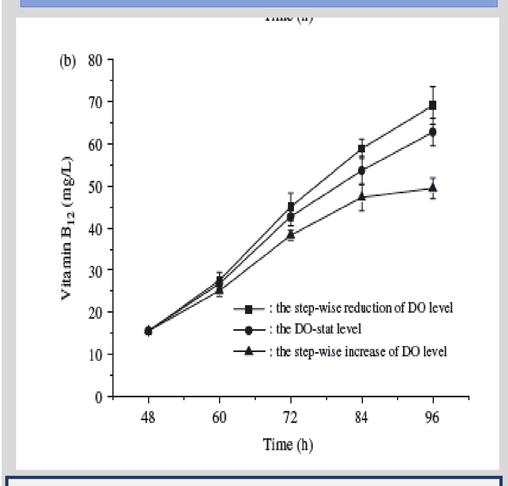


Figure 2*. Time courses of vitamin B12 production under the DO-stat and DO step-wise control strategies.*

↑[O2] inhibits vitamin B12 production because of intracellular medium oxidation. It is used a DO step-wise reduction strategy.

CO2 control during 2nd phase to maximize B12 productivity.

Downstream Alternatives

1. Solid-líquid separation
   - **Centrifugation + filtration**
   - Charcoal column adsorption
2. Cellular disruption
   - **Pasteuritzation**
   - Enzimàtica o forces de cisalla
3. Liquid separation from disruption impurities
   - **Filtration**
   - Charcoal column adsorption
4. B12 purity ~ 80%
   - **Filtration + precipitation + decantation**
   - Evaporation
5. B12 purity ≤90%
   - **Leaching+ EIC+ crystallization**
   - Evaporation + crystallization

Critical control points

Instrumentation for process control is installed as a vehicle to set up a Quality-by-Design (QbD) program. Details of BC bioreactor control and instrumentation:

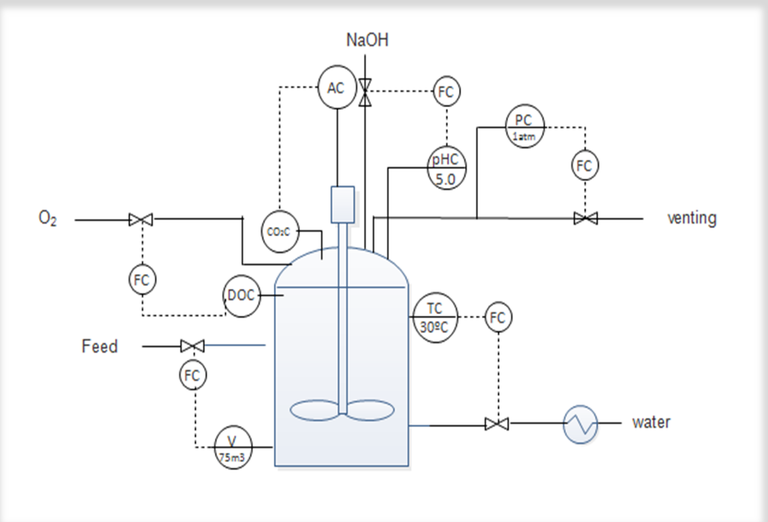


Figure 3. *Bioconversion bioreactor control diagram created with Edraw.*

Conclusions

The annual vitamin B12 production before downstream processing is 12.193kg (in 88 batches). With this total product amount, the product recovery yield should not be lower than 81% in order to supply the 30% of the expected market for human consumption. The process is controlled following the principles of Quality by Design (QbD), showing special interest in bioreactor control.

References

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[2] Ze-Jian Wang, H.-Y. W.-m.-P.-L. (2014). Enhance Vitamin B12 Production by Online CO2 Concentration Control Optimization in 120m3 Fermentation. *J Bioproces Biotechniques*, 4-14.
[3] Ze-Jian Wang, P. W.-L. (2011). Optimization of nutrictional requeriments and ammonium feeding strategies for improving vitamin B12 production by Pseudomnas denitrificans. *African Journal of Biotechnology*, 10551-10561.