Vitamin B12 Bioprocess Design: Part III. Analysis and Future Improvements

Lopez Galvan, M., Heredia Arroyo, S., **Rodriguez Calado, S.**

Universitat Autònoma de Barcelona

Introduction & Objectives

Growing concern about preventive care in large urban areas has a great impact in the demand of vitamin B12 and makes its biotechnological process attractive for an investment.

The main objective of this work is to analyze the process in order to determine weather it is sustainable or not. This further analysis is performed under economic, environmental, and social point of view, obtaining all the necessary parameters for the final decision.

A plant layout is proposed giving a complete view about the final project implementation as an industry, locating its different operational areas and determining the dimensions.

Finally, as a global industry, it has to be competitive with other worldwide companies, looking for plausible improvements for the overall design.

Sustainability Assessment

Economic Analysis

Total economic data

Plant location in Brasil provides affordable prices for raw materials and allows the aperture of a new market in America, taking advantage of a growing market in a growing country.

Table 1. *Comparison between using 1 or 5 fermenters in plant costs and revenues*

	1 fermenter	5 fermenters
Equipment Cost ($)	9.380.000	17.920.000
Initial Investment ($)	58.942.000	112.722.000
Annual Operational Costs ($)	12.784.000	30.986.000
Annual Production (kg/year)	2.065	9.867
Unitary Production Cost ($/kg)	6.190	3.140
Unitary Selling Price ($/kg)	5.000	5.000
Gross Margin (%)	-23,80	37,19
Payback time (years)	20,94	5,35
Return of Inversion (ROI) (%)	4,77	18,7
Net Present Value at 7% ($)	-31.211.544	37.596.000

Cash flow analysis

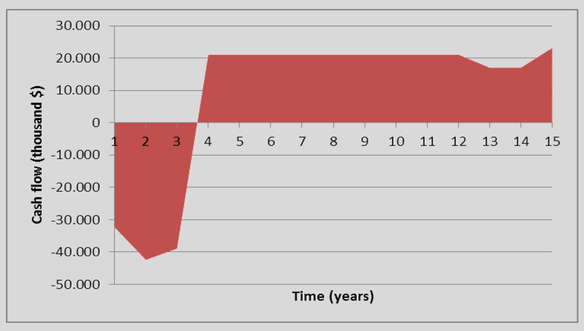

Figure 1. *Cash flow of the process for 15 years. The plant starts to operate on the fourth year and there is a positive cash flow since then until the end of the project.*

Sensibility Analysis

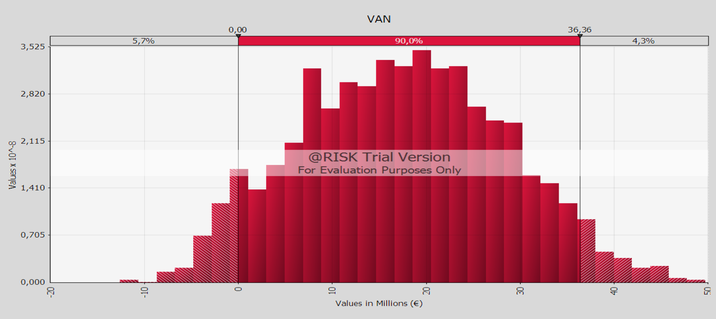

Figure 2. *Stability analysis of the project, considering different scenarios regarding initial investment variations, selling forecast and taxes changes. The probability of success is 94.3%; and the mean of net present value equal to approximately $18M.*

To sum up

- The overall process has a positive NPV
- The use of five fermenters is necessary for a positive gross margin.
- The process has a relative low payback time and high final revenues.
- Stability analysis shows a 95.6% of probabilities of a favorable process, making it robust.

Environmental Analysis

Dangerous chemicals treatment

Proper waste separation using methods as activated carbon or zeolite treatment are implemented.

Assessment results

Table 2*. Environmental assessment results for the process*

Assessment metric	Input	Output
Mass Index MI (kg/kg P)	2184.9	857.07
Number of A-components	5	7
Environmental Index EI (point/kg P)	1.97	2.31
General effect Index GEI (0-1)	0.0014	

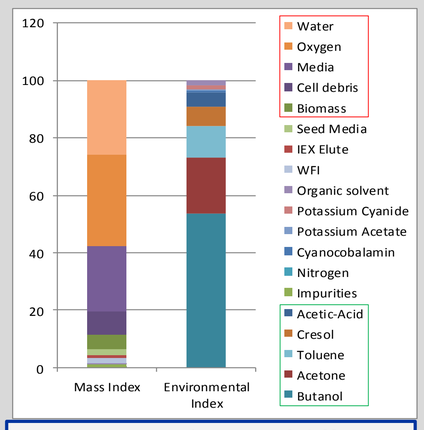

Figure 3*. Contribution of different components to MI and EI. Red: main contributors to MI. Green: main contributors to EI*

To sum up

- Although the number of A-components is high, they have a low final EI.
- The General Effect Index reflects the low impact of hazardous components due to their low MI.

Social Analysis

Green Chemistry's Postulates	
Prevention	Catalysis
Atom economy	Reduce derivates
Less hazardous chemical synthesis	Use of renewable feed-stocks
Designing safer chemicals	Design for degradation
Safer solvent	Real-time analysis
Design for energy efficiency	Inherently safer chemistry

To sum up

- Green Process
- Several jobs opportunities
- 16 operators will be needed plus the personnel in charge and the R+D researchers.
- A proper salary according with Brazilian laws and specific formation will be provided

Plant layout

The proposed design considers the necessities of the plant and is divided in six different areas: **Upstream&Bioreaction** (with its storage and available plant expansion land), **Downstream&Packaging** (and storage) , **Waste Treatment, Laboratories** (Quality, Control and Utilities), **Workshop** and **Offices.**

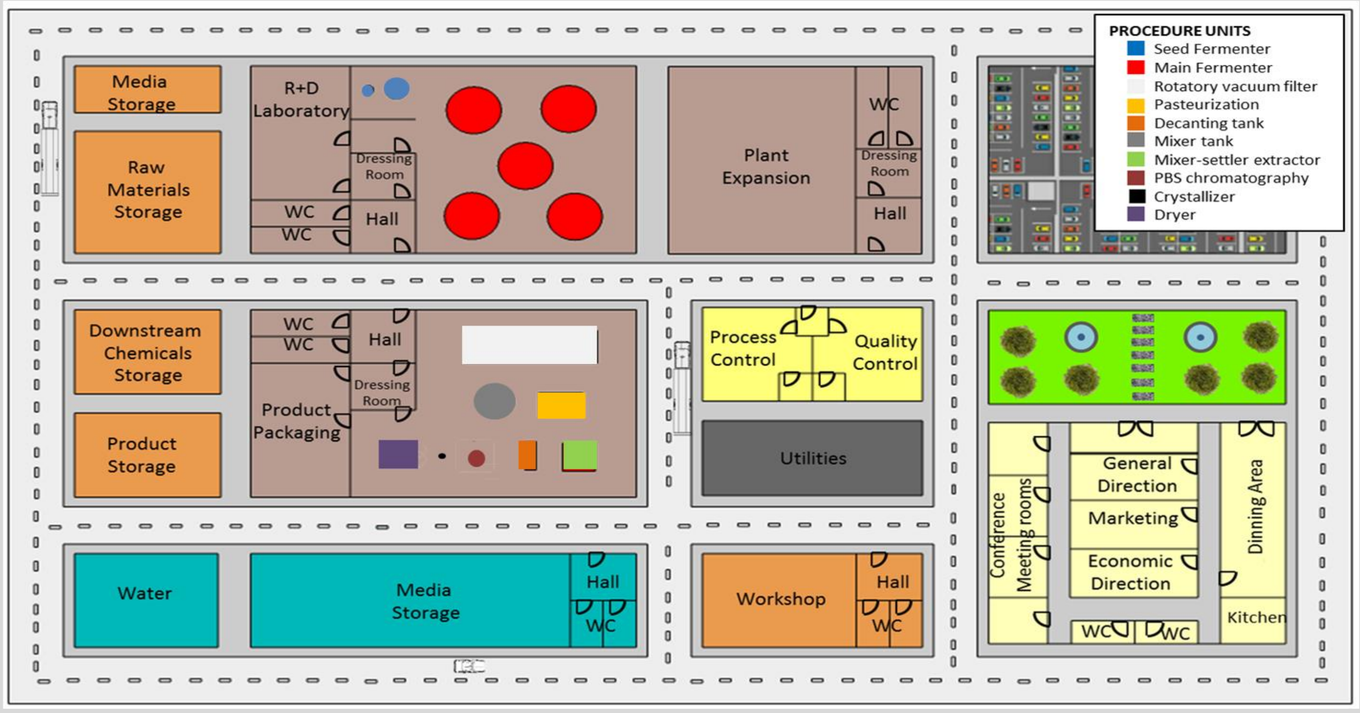

Figure 4. *Layout proposal for the vitamin B12 industrial plant. The final land usage goes up to 9030 m^2 with a 13 different buildings. All safety and efficiency considerations are taken into account in the design of the layout*

Bioprocess improvement

Three different approaches are taken into consideration:

Genetic improvement → **Genetically modified strains**

Metabolic optimization → **Component addition to the medium**

Downstream reduction → **Use of activated carbon**

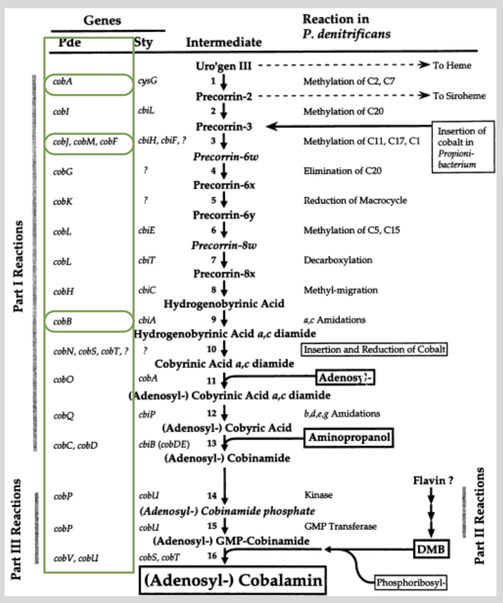

Figure 5. Genes implied in vitamin B12 biosynthesis carried by *P. denitrificans*[1]. Enhanced genes *in green.*

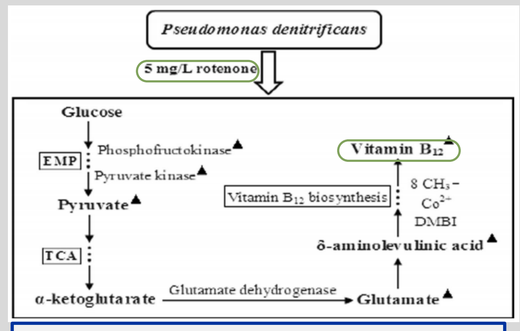

Figure 6. Metabolic optimization by the addition of rotenone in *P. denitrificans*[2]

To sum up

- Genetic improvement → Higher production but the plant security should be enhanced
- Metabolic optimization → Increased B12 production. No industrial data.
- Downstream reduction → Reduction of the separation time. No industrial data

Conclusions

Taking into account the information mentioned above, the vitamin bioprocess design is a suitable project. The economic data shows good future perspectives with proper assessment metrics. The environmental assays done to the process also demonstrate that all the hazardous substances are controlled and treated in an optimal way. On the other hand, the process can be improved following the proposals above, but further experimentation will need to be carried in order to implement them in an industrial scale

1. Cheng, X. et al., 2014. Improved vitamin B12 fermentation process by adding rotenone to regulate the metabolism of Pseudomonas denitrificans. *Applied biochemistry and biotechnology*, 173(3), pp.673–81.
2. Roth, J.R. et al., 1993. Characterization of the cobalamin (vitamin B12) biosynthetic genes of Salmonella typhimurium. *Journal of bacteriology*, 175(11), pp.3303–16.