# Bioprocess dessign for D-Mannitol production from low cost substrate

## Part I. Analysis of production alternatives and project overview

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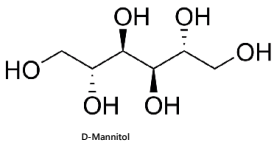
## Introduction

D-Mannitol is a polyol occurring naturally in algae, mushrooms and some bacteria. It has a sweet and refreshing taste and even it is just half as sweet as sucrose, it is still popular among alimentary sweeteners. Mannitol a is not well methabolitzed, nor interacts with insulin, so it is suitable for diabetics and adequate as a low caloric food ingredient. To provide for the alimentary market, a purity of 97.8% is achieved in the designed plant.
Mannitol has also direct clinical application thanks to its osmoregulatory capabilities. It is widely used in episodes of cerebral edema or raised intracranial pressure. Mannitol is conferes renal protection during cardiac and vascular surgery and is also used in the management of rhabdomyolysis (skeletal muscle breakdown). For pharmaceutical applications an additional purification step is added to the food grade product in order to raise its purity to 99.7%.
The aim of this project is to design a production plant for D-Mannitol, using a bio-based method and by employing cheap industrial residues as a carbon source. The purity obtained must match food industry and pharmaceuthical standards.

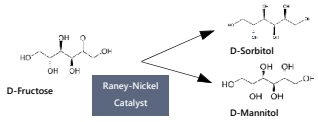


D-Mannitol

## Production alternatives

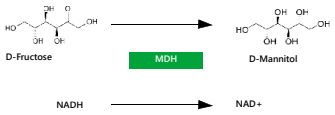
### Chemical path

The chemical path for the synthesis of D-mannitol involves hydrogenation of fructose using high temperatures and pressure in presence of a Raney nickel catalyst and by using pure hydrogen gas. Traditional substrates include 50-50 glucose-fructose syrup, which yields a racemic mixture of mannitol and its stereoisomer sorbitol, as α-fructose is reduced to sorbitol while β-fructose is transformed into mannitol. This path's mannitol yield is approximately 17%w/w. Alternative substrates for the reaction such as high fructose syrup or pure fructose achieve better yields, up to a maximum of 50%w/w.



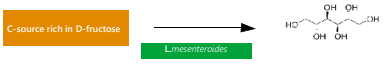

### Biocatalyst path

Mannitol can be enzymatically produced with immobilized enzymes. Mannitol dehydrogenase (MDH) is a ubiquitous enzyme found and easily isolated from many species. NADH-dependent and NADPH-dependent enzymes have been characterized. The need for cofactor supply renders this path economically unfeasible unless a cofactor regeneration is used. A formate dehydrogenase (FDH) system can be applied used. It oxidizes formate to $CO_2$ regenerating one molecule of NADH.

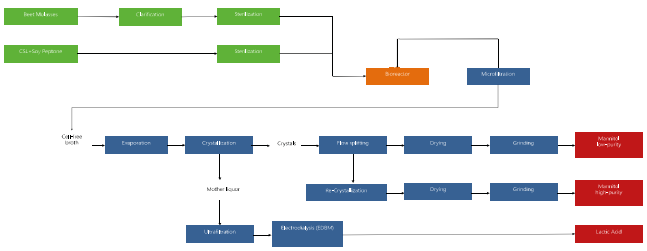



### Whole cell bioconversion

Using whole cells as biocatalyst is a simple method for overcoming the cofactor regeneration needed in the enzymatic hydrogenation of fructose. Glucose added in the reaction media serves this purpose of regenerating $NAD^+$ into NADH. Whole cells can also adapt to a wider variety of substrates or even resist fluctuations in its composition thanks to the self-regulatory capabilities of live cells. Whole cell bioconversion is the method which has been found most suitable for mannitol production due to its simplicity and robustness and therefore used in this project. The bocatalys is produced in situ to reduce dependency on external industries.




## Block Flow diagram




## Whole cell biocatalysis: plant dessign overview

### Biocatalyst production

*Leuconostoc mesenteroides* is the bacteria used as a whole cell biocatalyst. In this project ATTC-9135 stain has been naturally selected for its increased bioconversion of fructose into mannitol. L. mesenteroides is widely used in alimentary fermentations thus being generally recognized as safe (GRAS) by US FDA. In the upstream section of the plant, a fresh starter is periodically produced in a separate reactor (1.5m3) and transferred into the bioconversion reactor.

### Bioconversion reactor

Three 24m3 membrane cell recycle bioreactor (MCRB) are used in a staggered mode. Operating a MCRB consists in installing a microfiltration system so cells remain confined in the reactor during multiple batches, while product can be transferred out to the downstream. Cells are kept alive and maintained in a nitrogen-limited media so that a resting cell state is achieved. Biomass can be reused for up to 14 batches with little decay of the biocatalyst activity.

### Operation mode

The bioreactor runs 17.6h batches to allow for a 95% bioconversion of the feed fructose. The reactor achieves a concentration of 98g/l of mannitol, making 2080kg of mannitol per batch. Additional by-products include lactic acid 27g/l and acetic acid 17g/l.

### Mannitol recovery

Due to its differential solubility with respect to other salts abundant in the media, crystallization of the super saturated solution is a cost-effective method for obtaining pure mannitol. For the production of pharmaceutical grade mannitol, a further recrystallization step is used, as shown in the block flow diagram. At the end both pharmaceutical and food grade mannitol are fluid bed dried, grinded and packed.

### Byproduct revaluation

Lactic acid is retrieved by using bipolar membranes electrodyalisis. This permits the recovery of 460kg of pure lactic acid every batch, greatly reducing waste treatment costs and providing with additional revenues.

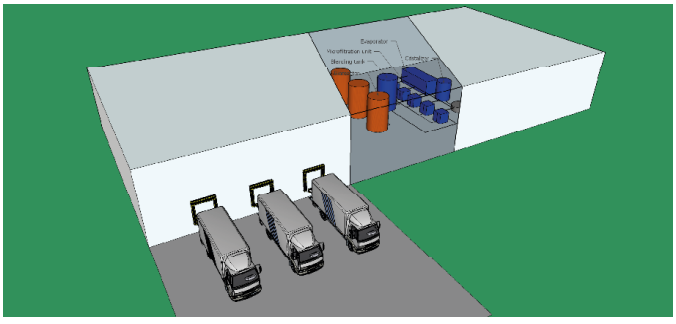
## Scope

The mundial mannitol market is of 50 000 tn $year^{-1}$, rising 2-3% anually.
With a production set as high as 4000 tn per year, our project plans to get a leading position in the European sector, covering a 8% of both the pharmaceutical demand and the alimentary market. Bio based production methods descrived in this project allowto sell food grade mannitol at 3.5 USD $kg^{-1}$.
Pharmaceutical grade mannitol is sold at 42 USD $kg^{-1}$. Both products are of higher purity and can be sold for a lower price than the present counterparts.

## Localization

This plant needs a constant and robust supply chain able to provide for the plant's constant needs on beet molasses, high fructose syrup and corn step liquor. Tarragona harbor zone is chosen as it acomodates multitude of chemical plants, has sea transportation avialable and is geographicaly close to France, the second world productor in beet molases.
Localizing the plant in Tarragona allows for low transport fees while benefiting local economy.

## Layout




This production plant layout is based on product flow and sequence of operations. Equipment stacking has been keept to a minimum to ensure ease of operation and mateinance. A 3D model of the factory layout has been constructed for the project.

## Conclusion

Bio production of D-mannitol using *Leuconostoc mesenteroides* whole cells benefits from several advantages:

- Mild operating conditions, reducing energy consumption and safety concerns
- Substrate adaptability. Live cells can metabolize various types of substrate thanks to the wide range of enzymes coded in the genome.
- Higher mannitol yield per g of substrate than when using chemical hydrogenation thanks to the enzyme enantioselectivity.
- The biocatalyst microorganism can be produced in situ. This grants independence from external enzyme providers.

The biotechnological approach in the manufacture of mannitol is feasible and provides many benefits over the traditional chemically based strategy, not only in terms of ecological and social responsibility, but also in terms of economic viability. As shown in part IV of this project, further improvements in the system can be made, further increasing its strengths.

## References

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