Bioprocess design for D-mannitol production from low-cost substrates

Part II. Upstream and Bioreaction

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Introduction and Objectives

Mannitol is a natural occurring sugar alcohol widely used in food, pharmaceutical, medical and chemical industries. Mannitol biosynthesis through bacterial fermentation has become an interesting alternative to existing chemical production. In the fermentation, the optimization of the bioreaction and its upstream is essential to achieve high mannitol productivity, especially when a low-cost substrate such as molasses is used as carbon source. Therefore, a suitable bacterium is chosen (*Leuconostoc mesenteroides* ATCC-9135) in order to minimize the by-products generation. With this background we propose a method for the sustainable and efficient biosynthesis of this polyol.

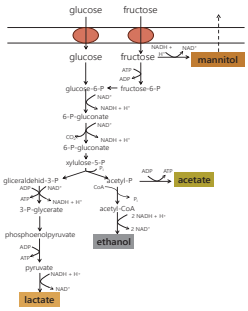
The main objective is to design a bioreaction and upstream processing for the sustainable D-mannitol biosynthesis, using industrial by-products as carbon and nitrogen sources. The efficient process design includes the election of the appropriate biocatalyst and operating mode that allows enough production to supply the market.

Leuconostoc mesenteroides ATCC-9135

Lactic acid bacteria (LAB) are known to efficiently produce mannitol from fructose, especially heterofermentative LAB. Manntiol is also produced by fungi, yeast as well as several algae and plants.

Biocatalyst alternatives

Organism	Substrates	Fermentation type	Volumetric productivity ($g\ l^{-1}\ h^{-1}$)
LAB			
Leuconostoc mesenteroides	**Fructose + Glucose**	**Batch (MCRB)**	**20.60**
Lactobacillus fermentum	Fructose + Glucose	Batch	7.60
Lactobacillus intermedius	Fructose + Glucose	Continuous	28.40
Yeast			
Candida magnoliae	Fructose	Fed-batch	1.03
Candida zeylannoides	n-Paraffin	Batch	0.52
Torulopsis mannitofaciens	Glycerol	Batch	0.18
Torulopsis versalitis	Glucose	Batch	0.23
Fungus			
Asergillus candidus	Glucose	Batch	0.08
Penicillium scabrosum	Sucrose	Batch	0.21



Heterofermentative lactic acid bacteria metabolism

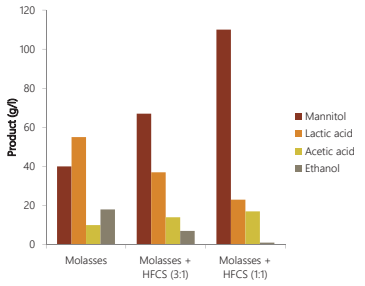
Leuconostoc mesenteroides ATCC-9135 features:

- High growth rate (0.30 h^{-1})
- Facultative anaerobe bacterium
- High mannitol volumetric productivity
- D-mannitol stereospecific
- Manntiol as an extracellular agent, easy to separate
- GRAS heterofermentative lactic acid bacterium
- Can grow on inexpensive substrates (molasses)
- and inexpensive co-substrates (corn steep liquor, soy peptone)
- Easy fermentation type
- Similar mannitol yield in a resting or slowly growing state

Carbon and nitrogen sources

Molasses is a by-product of the refining of sugarcane or sugar beets into sugar. It is composed mainly by carbohydrates. There are important crops of beet in Europe, so **beet molasses** is used as carbon source. The substrate yield of the bioreaction depends on the ratio fructose:glucose. The appropriate ratio is achieved by adding **high fructose corn syrup** (HFCS).

Molasses as heterogeneous substrate needs to be standardized in a three steps process: (1) dilution and acidification, (2) clarification and solid removal, (3) pasteurization.



Yield of different substrate ratios (Saha, 2006)

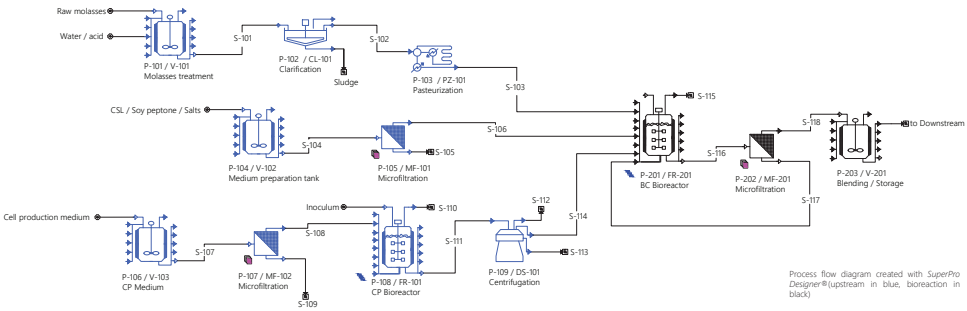
Nitrogen source is an important constituent for cell growth. **Corn steep liquor** (CSL) and **soy peptone** are used for this reason. With low nitrogen concentration a resting cell state is achieved.

Therefore, the culture media for cell production (growing state) and bioconversion (resting state) have different compositions.

Cell production and bioconversion mediums composition

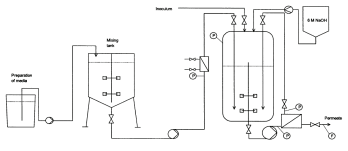
Constituent	Cell production medium ($g\ l^{-1}$)	Bioconversion medium ($g\ l^{-1}$)
Fructose	-	100
Glucose	30	50
Corn steep liquor	15	0.5
Soy peptone	-	1
K_2HPO_4	2	1
$MgSO_4$	0.2	0.2
$MnSO_4$	0.05	0.02

Process flow diagram and bioreaction details



Process flow diagram created with *SuperPro Designer*® (upstream in blue, bioreaction in black)

Membrane cell-recycle bioreactor (MCRB)


MCRB operation scheme (von Weymarn, 2003)

Mannitol is produced in a MCRB, which is a simple repeated batch operation connected to a filter for cell recovery. Biomass in resting or slowly growing state is recycled during 14 batches increasing mannitol productivity.

Operation conditions:

- pH 5.1
- Temperature = 30ºC
- Fermentation time = 7 h
- Agitation = 50 rpm
- Pressure = 1 atm
- Reactor volume = 25,000 L

Global stoichiometric reaction in BC bioreactor

2 Fructose + 1 Glucose ⇌ 2 Mannitol + 1 Lactic acid

+ 0.3 Acetic acid + 0.02 Ethanol + CO_2

BC bioreactor inputs and outputs

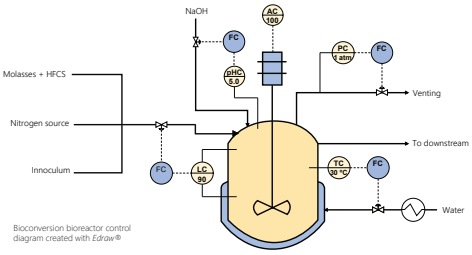
Bioconversion (BC) bioreactor inputs and outputs per batch

Inputs		Outputs	
Constituent	kg/batch	Constituent	kg/batch
Fructose	2188.82	**Mannitol**	**2079.38**
Glucose	1157.93	Sodium lactate	580.08
Biomass	352.0	Sodium acetate	377.83
Soy peptone	24.0	Ethanol	21.11
Corn steep liquor	12.0	Biomass	352.0
K_2HPO_4	24.0	Fructose	109.44
$MgSO_4$	4.8	Glucose	4.62
$MnSO_4$	0.48	Water	18261.46
Water	18349.34		

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:



Bioconversion bioreactor control diagram created with *Edraw*®

Other critical control:

- Foaming, in BC and CP bioreactors.
- Bacterial concentration.
- Molasses composition.

Conclusions

L. mesenteroides ATCC-9135 efficiently produce mannitol from a low-cost mixture of raw materials by performing the designed upstream processing and bioreaction. The bioconversion yield from fructose after a 7-hour fermentation is 95% with 14.2 g L^{-1} h^{-1} volumetric productivity. The volumetric productivity is reasonably lower than the value obtained in laboratory scale (20.60 g L^{-1} h^{-1}). Then, in order to optimize the bioreaction, a proper upstream processing design is necessary.

The annual mannitol production before downstream processing is 4.3 million kg (in 2075 batches). With this total product amount, the product recovery yield should not be lower than 92% in order to supply the expected market (see *Part III: Product recovery*).

Selected references

B. Saha, F. Racine (2011) Biotechnological production of mannitol and its applications. *Appl Microbiol Biotechnol* 89:879–891

N. von Weymarn, K. Kiviharju, S. Jääskeläinen, M. Leisola (2003) Scale-up of a new bacterial mannitol production process. *Biotechnol Prog* 19: 815-821

N. von Weymarn, K. Kiviharju, M. Leisola (2002) High-level production of D-mannitol with membrane cell-recycle bioreactor. *J Ind Microbiol Biot* 29:44-49

B. Saha (2006) A low-cost medium for mannitol production by *Lactobacillus intermedius* NRRL B-3693. *Appl Microbiol Biotechnol* 72: 676-680