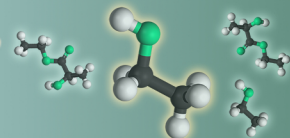


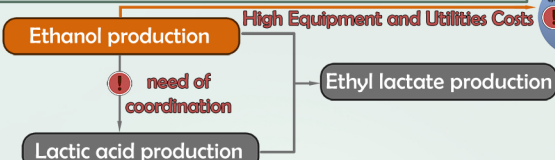
# BIOPROCESS DESIGN FOR GREEN ETHYL LACTATE II: ETHANOL FERMENTATION



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## OBJECTIVES

INITIAL PLANT



High Equipment and Utilities Costs

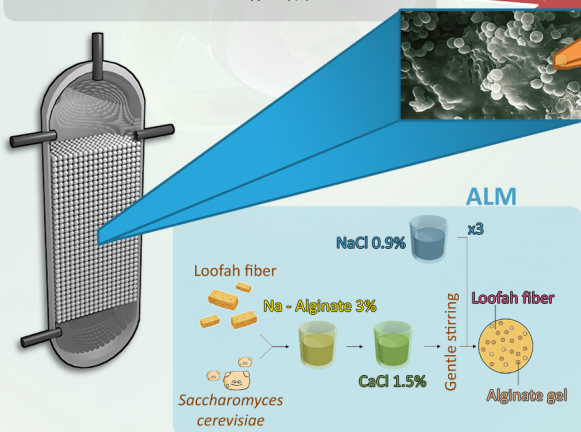
FINAL OBJECTIVES

- Decoupling the two sections and convert the Ethanol from discontinuous to continuous fermentation.
- Improve the strain yield and productivity:
  - Cells immobilization
  - Strain genetic improvement

## IMPROVEMENTS

### BIOREACTOR

- Continuous PFR.
- PBR (packed bed reactor) with immobilized cells in loofah-reinforced alginate matrix (ALM). Cells immobilization leads to increase productivity and to improve purification.
- ALM dimensions will be 20x20x3 mm<sup>3</sup>. These occupy 32% (v/v) of the PBR bed volume.



$Y_{P/S} = 0.373$

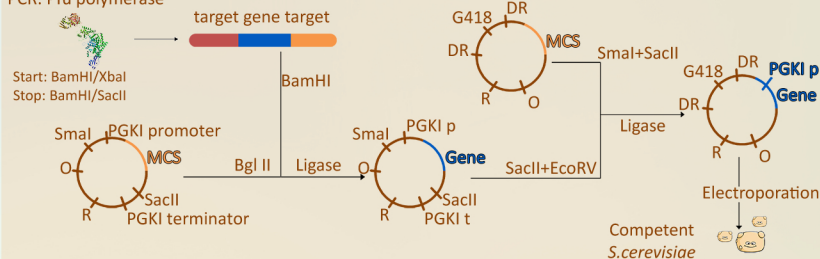
Yield increase: 10%

### GDH1 DELETION

A plasmid is obtained, in which one fragment of *gdh1* gene with 1.0 Kb has been replaced with another fragment of *ura3* ORF (genetic marker) with 1.1 Kb. This plasmid is transformed into *Ura* or uridine auxotrophic *S. cerevisiae* to do positive selection with the marker.

### GLN1 & GLT1 OVEREXPRESSION

PCR: Pfu polymerase



Total  $Y_{P/S} = 0.41$

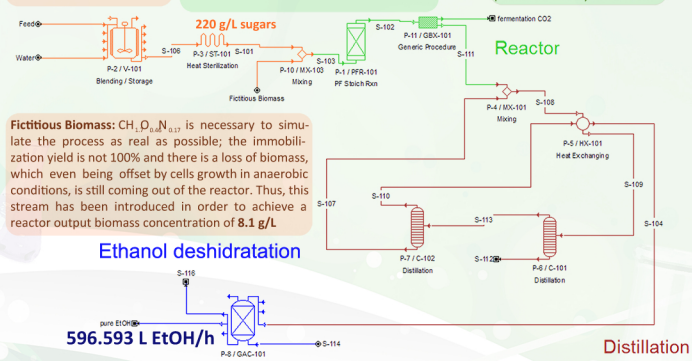
## DESIGN

### Pretreatment

Feed: Mixture composed by Molasses (defined as glucose) and Others

Reactor: Molasses ( $C_6H_{12}O_6$ )  $\rightarrow 2 C_2H_5OH + 2 CO_2$   
 •  $V_s = 4.8$  cm/h  
 • Ethanol concentration achieved: 90.266 g/L  
 •  $\theta = 9.10$  h  
 •  $V = 46.62$  m<sup>3</sup>  
 • Continuously anaerobic conditions at 32°C

A Black box has been added necessarily to separate  $CO_2$ , which actually comes out of reactor, because SuperPro Designer did not allow this output to be directly entered in it.



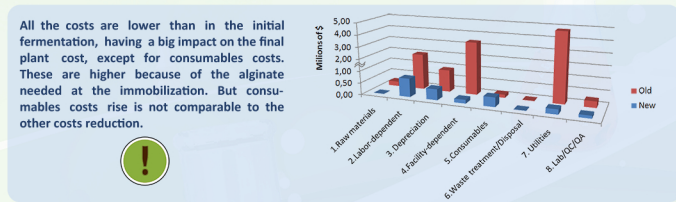
Distillation

This ethanol is needed to load the esterification section to produce ethyl lactate, it has been established that the purity is 100% in order to simplify the simulation process.

## RESULTS



The importance of Equipment and Utilities costs has been reduced, but the importance of labor-dependent costs has been outweighed.



	PREVIOUS DESIGN	MODIFIED DESIGN
Bioreactor used	3 x 268 m <sup>3</sup> STRC	1 x 46.62 m <sup>3</sup> PFR
Inoculum's Tank	2 x 76 m <sup>3</sup> STRC	Not needed
Use of consumables	No consumables	Alginate
Total annual costs	11 million dollars	2 million dollars

## CONCLUSIONS

Equipment costs have been dramatically reduced, as a consequence utilities and facility-dependent costs have been reduced too.

The improvements have solved all the problems previously identified, and contribute to improve the overall process.

A non-viable process will be transformed in a profitable one due to this new section and improvements done in other sections (lactic acid fermentation and esterification).



## REFERENCES

- [1] - Bangrak.P, Limtong. S, Phisalaphong.M, "Continuous ethanol production in yeast cells entrapped in loofa-reinforced alginated carriers," 2011.
- [2] - Torben.L, Morten.C, Kielland-Brandt et al., "Optimization in *Saccharomyces cerevisiae* by Metabolic Engineering of the Ammonium Assimilation," *Sciencedirect*, 2000.