BIOREFINERY: A solution for a sustainable future

Part III. Genetically modified *E.coli* strain to produce 1,3-propanediol

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INTRODUCTION AND OBJECTIVES

Objective:

The aim of this project is to improve the production of 1,3-propanediol (PDO) in batch process with *Klebsiella pneumonia* using genetic engineering techniques in a microorganism and to increase the economic benefits.

Strain:

Escherichia coli is a good option because:

- It is no-pathogenic.
- Its metabolism has been studied in detail.
- It has a rapid growth rate.

The natural producers of 1,3-propanediol from glycerol are in general *Klebsiella*, *Clostridia*, *Citrobacter*, *Enterobacter* and *Lactobacillus*.

There are two different PDO synthesis ways:

1 **Glycerol**

↓ Vitamin B_{12}-dependent glycerol dehydratase **(gen *dhaB*)**

3-hydroxypropionaldehyde (3-HPA)

↓ NADH-dependent 1,3-propanediol oxidoreductase **(gen *dhaT*)**

1,3-Propanediol

PROBLEMS

- Large amounts of vitamin B_{12}, a high cost molecule, is required.
- Glycerol dehydratase is inactivated by glycerol.
- High levels of 3-HPA inhibits glycerol dehydratase.

2 **Glycerol**

↓ Vitamin B_{12}-independent glycerol dehydratase **(gen *dhaB1*)**

3-hydroxypropionaldehyde (3-HPA)

↓ NADP-dependent 1,3-propanediol oxidoreductase **(gen *yqhD*)**

1,3-Propanediol

PROBLEMS

- An activating factor for vitamin B_{12}-independent glycerol dehydratase is required (gen *dhaB2*).

PLASMID

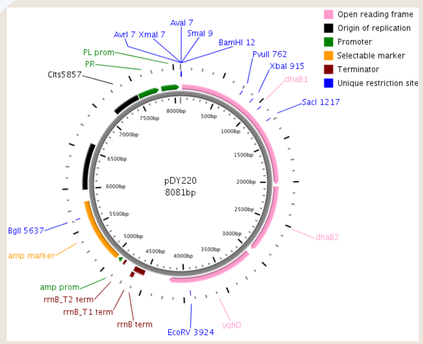


Figure 1. Recombinant pDY220 plasmid with interested genes inserted. Created using PlasMapper.

The vector pBV220 contains:

- A **MCS** (Multiple Cloning Site).
- The promoter $\mathbf{P_LP_R}$ obtained from λ phage.
- **rrnB**, a strong transcriptional stop sequence from *E. coli* ribosomal protein gen rrnB.
- The **ampicillin resistant gene** derived from *Salmonella paratyphi*.
- The ***CIts* gene** that encodes a temperature-sensitive inhibitor from λ phage.

The PDO operon constructed and introduced in the MCS contained the following genes:

- ***dhaB1*** and ***dhaB2*** from *Clostridium butyricum*.
- ***yqhD*** from *E. Coli.*

The transformation is done by electroporation and the transformed cells are selected with ampicillin.

STRAIN

1 Glycerol + 0,2 NH_4OH →

0,86 PDO + 0,045 Acetic acid + 0,02 Biomass + 0.02 Formic acid + 0,02 Lactic acid

Substrate	Productivity [g/(l·h)]	Conversion rate [% g PDO/g Glycerol]
Pure glycerol	2,61	90,2
Crude glycerol	1,56	50,5

Some crude glycerol impurities impair E.Coli metabolism and consequently the productivity and conversion rate decrease.

ECONOMIC ANALYSIS

Concept	Batch process with *Klebsiella*	Batch process with recombinant *E. coli*	Difference
Total investment ($)	91.531.000	133.513.000	+ 45,90 %
Annual Operating Cost ($)	27.789.000	49.137.000	+ 76,82 %
Revenues ($/yr)	233.911.000	382.190.000	**+ 63,39 %**
Unit production cost ($/kg)	7,37	7,71	+ 4,6 %
Gross margin	88,1 %	87,4 %	- 0,79 %
Return on investment	142 %	162 %	**+ 14,08 %**
Payback time	0,69 yr	0,62 yr	**- 10,14 %**
NPV (net price value at 7% interest) ($)	847.787.000	1.407.044.000	**+ 65,96 %**

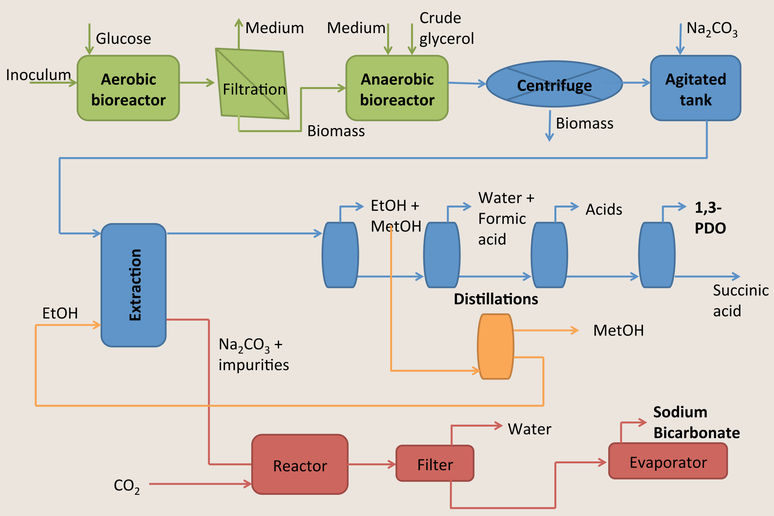
Total investment and annual operating cost increase significantly because:

- The new process has 2 stages → more bioreactors.
- In the first stage glucose is used → it represents 55% of the annual material cost.
- Both process use the same quantity of crude glycerol → Increase the production and the consumption of raw materials → The waste increases →The cost of waste treatment increases fourfold.
- Biomass can not be recirculated.

NPV increase considerably because the yield and the production increase too.

The process with recombinant cells has more economics benefits than the batch with *Klebsiella*

PROCESS



The fermentation to produce 1,3-PDO by this recombinant *E. coli* is divided into two stages:

- **Aerobic fermentation:** is used to obtain a high-density cell culture. The cells are cultivated at 30ºC during 10h and glucose is maintained to 25g/L in a fed-batch.
- **Anaerobic fermentation:** is used to promote the production of 1,3-PDO from glycerol at 42ºC during 30h. Residual glucose medium from the first stage is completely replaced by new medium and crude glycerol. Crude glycerol is added by using a fed-batch in order to maintain a glycerol concentration of 35 g/L. E.coli is able to excrete 1,3-PDO to the medium and cell disruption is not necessary.

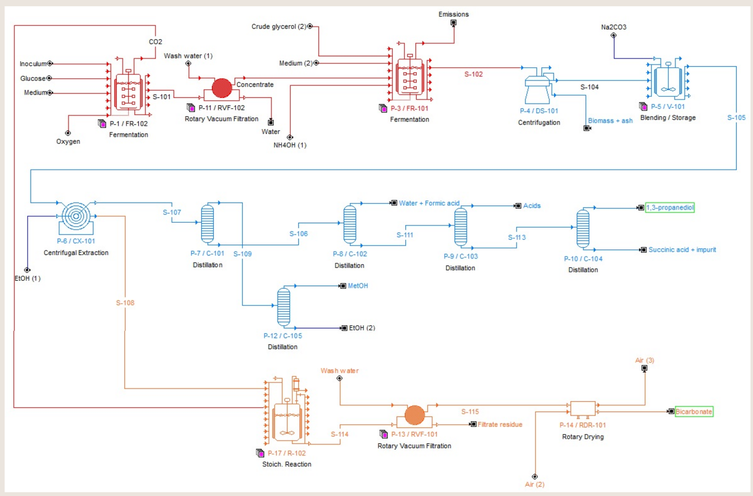


Figure 1. Flux diagram of process with recombinant E.coli designed with SuperPro. All flows and equipment have been characterised in detail.

SAFETY CONSIDERATIONS

Our industrial plant requires to comply good manufacturing practice (GMP) to be sure that the products are of high quality and do not have any risk to the consumer.

In this project recombinant cells are used. Thus it is important to avoid any leak and have safety measures to act in case of accident.

CONCLUSIONS

Once the plant has been analysed and compared with the batch process with *Klebsiella pneumonia*, it can be concluded that using this recombinant *E. Coli* the conversion of crude glycerol to PDO increase more that a 60%. Despite the cost of the plant and the annual operation cost increase, the benefits are more favourable than the benefits using *Klebsiella.*

SELECTED REFERENCES

- Xueming T., Yongsong T., Hong Z., Kai Z., Wei S. “Microbial conversion of glycerol to 1,3-propanediol by an engineered strain of Escherichia coli” Applied and emvironmental microbiology Mar p. 1628-1634 (2009)
- M. Zheng, R. Zhiming, X. Liyu, L. Xiangru, F. Huiying, Z. Bin, Z. Jian. “Production of 1,3-propanediol from glycerol by engineered Escherichia coli using a novel coexpression vector”. African Journal of Biotechnology Vol. 8 (20) pp. 5500-5505 (2009)