ENGINEERING *ESCHERICHIA COLI* FOR BIOFUEL PRODUCTION

Reactor Simulation and Operation

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Problem Statement

Engineered *E. coli* strain CPC-PrOH3 was cultivated anaerobically in a bioreactor with 30.7 g/L of glycerol for approximately 40 hours. Refer to Appendix B of the case study for a full data set [1]. The culture was inoculated in a 1-L stirred-tank bioreactor containing the medium described in Table 1 and operated anaerobically at 30°C with stirring at 430 rpm with a single Rushton turbine. Anaerobic conditions were maintained by constant bubbling of nitrogen. The pH of the production culture was maintained at 7.0 ± 0.1 with 30 % (v/v) NH_4OH and 15 % (v/v) HNO_3.

1. Suggest a mode of operation to continue to the fermentation, to extend the culture, and maximize the production of solvents.

    a. Should you decide to provide additional nutrients to extend the culture, explain the choice of nutrients in the feed.

2. Develop material balances around the various components for the fermentation that will allow you to simulate the extension of your fermentation.

3. Prepare a short MATLAB script that will show the fermentation extension.

4. What are the pitfalls of your approach that would make your anticipated outcome deviate from reality?

Table 1 – One litre stirred-tank bioreactor components and concentrations

Component	*Concentration*
carbon source (i.e. glycerol)	30 g/L
K_2HPO_4	0.23 g/L
NH_4Cl	0.51 g/L
$MgCl_2$	49.8 mg/L
K_2SO4	48.1 mg/L
FeSO4	1.52 mg/L
$CaCl_2$	0.055 mg/L
NaCl	2.93 g/L
tricine	0.72 g/L
yeast extract	10 g/L
$NaHCO_3$	10 mM
cyanocobalamin (vitamin B_{12})	0.2 µM
Trace Elements	
H_3BO_3	2.86 mg/L
$MnCl_2•4H_2O$	1.81 mg/L
$ZnSO_4•7H_2O$	0.222 mg/L
$Na_2MoO_4•2H2O$	0.39 mg/L
$CuSO_4•5H_2O$	79 µg/L
$Co(NO_3)_2•6H_2O$	49.4 µg/L

References

[1] Kajan Srirangan, Lamees Akawi, Lyndia Stacey, Cheryl Newton, Module 01. "Engineering *Escherichia coli* for Biofuel Production". Waterloo Cases in Design Engineering (WCDE), University of Waterloo.