**Bioproduct Production**

**Expectations**

**Learning Objectives**

Each student should be able to:

* Explain the assumptions and limitations of strain design using constraint-based metabolic reconstructions.
* Explain the process of bioproduct identification.
* Explain the process of selecting a host strain.
* Explain the process of defining a bioproduct pathway.
* Explain the strain design process.
* Explain the purpose of understanding the bioproduct maximum production.
* Explain the purpose of carbon source selection.
* Explain the purpose of identifying nutrient & amino acid limitations.
* Explain the purpose of identifying undesired by-products.
* Explain the purpose of growth coupling.
* Explain the purpose of cofactor balancing.
* Explain the purpose of sampling analysis.

**Prerequisites**

* Course Introduction
* Matlab Tutorial
* Flux Balance Analysis Overview
* *E.coli* Core Model
* Cobra Toolbox
* Robustness Analysis & Phenotype Phase Plane Analysis
* Flux Variability Analysis & Parsimonious Analysis
* Gene/Reaction Knockouts
* Randomized Sampling
* Dynamic FBA
* Transcriptional Regulatory Networks

**Resources**

**Required Readings**

1. [Feist, A. M., D. C. Zielinski, et al. (2010). "Model-driven evaluation of the production potential for growth-coupled products of Escherichia coli." Metabolic engineering 12(3): 173-186.](http://www.ncbi.nlm.nih.gov/pubmed/19840862)
2. Systems Biology: Constraint-based Reconstruction and Analysis, Bernhard O. Palsson, Cambridge University Press, 2015, Chapter 25, 26, 27.

**Recommended Readings**

1. [Monk, J. M., P. Charusanti, et al. (2013). "Genome-scale metabolic reconstructions of multiple Escherichia coli strains highlight strain-specific adaptations to nutritional environments." Proc Natl Acad Sci USA  110(50): 20338-20343.](http://www.ncbi.nlm.nih.gov/pubmed/24277855)
2. [Lee, J. W., D. Na, et al. (2012). "Systems metabolic engineering of microorganisms for natural and non-natural chemicals." Nature chemical biology 8(6): 536-546.](http://www.ncbi.nlm.nih.gov/pubmed/22596205)

**Classroom Activities**

**Presentations**

* Lecture Presentation *(“Bioproduct Production-2021.pdf”)*
* Lecture Supporting Matlab Files *(“Bioproduction Matlab Files 2021.zip”)*

**Laboratory**

* Lab #9 *(“Lab-9.docx”)*

**Reinforcement Activities**

Examples

* Related websites
  + [Chemical & Biological Systems Optimization Lab; Dr. Costas D. Maranas at Penn State](http://maranas.che.psu.edu/index.htm) (OptCom, OptForce, OptKnock, OptCom, k-OptForce)
  + [Dr. Jennifer Reed Lab at the University of Wisconsin](http://reedlab.che.wisc.edu/index.html) (SimOptStrain, BiMOMA, OptOrf)
  + [Laboratory of Metabolic Systems Engineering, Dr. Radhakrishnan Mahadevan](http://www.labs.chem-eng.utoronto.ca/mahadevan/) (Emilio)
  + Pathway Selection
    - [FMM Server](http://fmm.mbc.nctu.edu.tw/index.php)

**Assessment**

* Reflective Questions
  1. What are the main Cobra tools for determining the knockouts that can improve bioproduct production?
  2. What is the Cobra function for adding a reaction?
  3. Can a host cell be growth-coupled to all new pathways created by adding reactions?
  4. Why can a cell produce a bioproduct when a gene is added to a host cell even though the host is not growth-coupled to the bioproduct?
  5. What governs the maximum production of a bioproduct in a given host cell?
  6. What role does the media play in the maximum production of a bioproduct?
  7. What is cofactor balancing?
  8. In addition to the designated carbon source in a growth media what other components of the media can act as carbon sources?
  9. What components in a growth media can impact the maximum bioproduct production?
  10. How is the M9 minimal media modeled?
  11. In the standard E.coli models (textbook, iAF1260, and iJO1366) what are the typical default lower bounds for most amino acids and minerals? What are the default upper bounds?
  12. What relationship do the amino acids have with the biomass function?
  13. How can amino acid and mineral uptake rates impact growth rate and bioproduct production?
  14. How can reduced costs be used to understand the impact of an amino acid or mineral on bioproduct production?
  15. Are regulatory constraints included in the standard E.coli constraint-based models?
  16. What impacts can be observed by adding a plasmid to a host cell?
  17. How can plasmids be modeled?
  18. After a gene has been knockout or a new gene has been added to a host cell does the maximum theoretical performance match the laboratory results?
  19. What are the components of the host strain design process?
  20. What are the assumptions and limitations of bioproduction modeling?
  21. What are the differences between natural-inherent chemicals, natural-noninherent chemicals, non-natural noninherent chemicals, and nonnatural-created chemicals?
  22. Why is *E.coli* a good host organism?
  23. What are uncoupled bioproducts?

**References**

**Bioproduct Optimization**

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2. [Zomorrodi, A. R. and C. D. Maranas (2012). "OptCom: A Multi-Level Optimization Framework for the Metabolic Modeling and Analysis of Microbial Communities." PLoS computational biology 8(2): e1002363. - **OptCom**](http://www.ncbi.nlm.nih.gov/pubmed/22319433)
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4. [Xu, P., S. Ranganathan, et al. (2011). "Genome-scale metabolic network modeling results in minimal interventions that cooperatively force carbon flux towards malonyl-CoA." Metabolic engineering 13(5): 578-587.](http://www.ncbi.nlm.nih.gov/pubmed/21763447)
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**Strain Design**

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