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Retrosynthesis of All Available Pathways to Microbial Production of Precursors to Target Chemicals Based on Chemical Separation Characteristics

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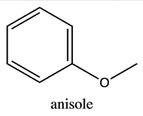
Abstract

Biological production of target chemicals is often constrained by the relative ease/difficulty of separating the chemical of interest from the bioreactor. In practice, chemicals that cannot be easily separated create inefficiencies in production that limit the viability of biological routes to production. In many cases it may be advantageous to prefer limiting the steps in biological production of material before it reaches the target chemical, in order to more easily separate material of interest from the general bioreactor substrate. In this work we use our previously reported RetSynth tool to characterize trade-offs in separation between purely biological and hybrid bio/chemical routes to synthesis of target chemicals. We define up an optimization protocol using boiling point and solubility of chemical intermediates as a proxy for separability of chemical from a liquid substrate. The RetSynth tool provides six combined biological and chemical reaction databases, with tens of thousands of biological reactions and millions of chemical reactions. This tool allows us to characterize and optimize production using differential halting, enzyme addition and hybrid routes to production. We report work characterizing alcohols, an important class of chemicals for both Spark Ignition and Mixed Compression Controlled Ignition. There are many alcohols that have the potential to be produced biologically. We demonstrate under what conditions strictly biological vs. hybrid routes to approaches to production are optimal and define a strategy for ranking paths to production more generally.

RetSynth software for discovering optimal synthetic pathways-to-production of high value chemicals

Target compound



anisole

Chassis organism

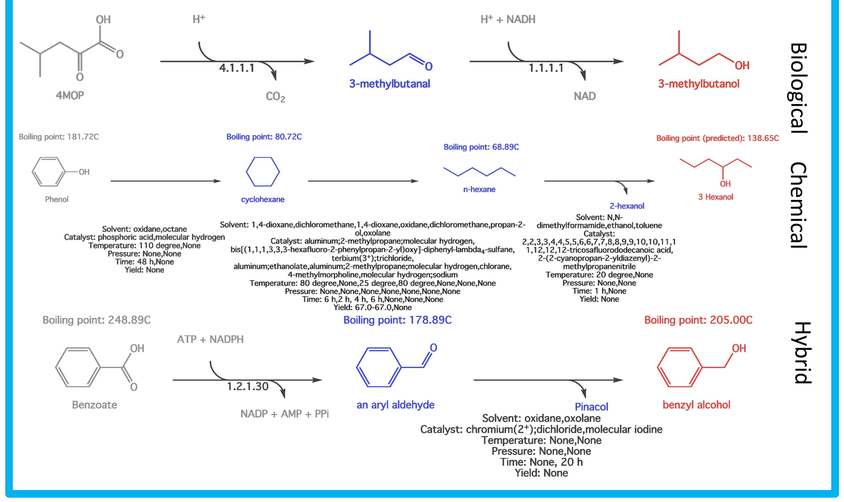
Metabolic Database

PATRIC https://www.patricbrc.org/
MetaCyc https://metacyc.org/
SPRESI https://www.spresi.com/

Software available:
https://github.com/sandialabs/RetSynth

1. Solve for synthetic pathways (optimal and sub-optimal) using novel integer program
2. Find biological, chemical and hybrid pathways to production
3. Predict maximum theoretical yields using flux balance analysis

Example output synthetic pathways for alcohols in *E. Coli*



Incorporation of separation metrics into RetSynth

- Initially, optimal pathways are defined as pathways with the minimum number of reaction steps needed to convert a native metabolite in the chassis organism into the target compound. However, post-processing steps, such as target compound separation need to be taken into consideration when designing an effective synthetic pathway.
- Separation of target compound is a difficult, costly and time consuming process. In many cases the compounds of interest and not easily extracted from the bioreactor substrate because of unfavorable characteristics such as boiling point and/or solubility. For these target compounds it may be more economically advantageous to halt biological production at an easier to separate precursor and and then proceed with chemical conversion to produce the target.
- To overcome this problem RetSynth identifies pathways optimal for separation based on compound boiling points.

Workflow for identifying optimal for separation synthetic pathways

1. For every biologically produced in a synthetic pathway, boiling points and solubility were collected from:

   PubChem NIST National Institute of Standards and Technology U.S. Department of Commerce

   Or predicted using:

   Stein and Brown group contribution method (Stein S. & Brown R., 1994)

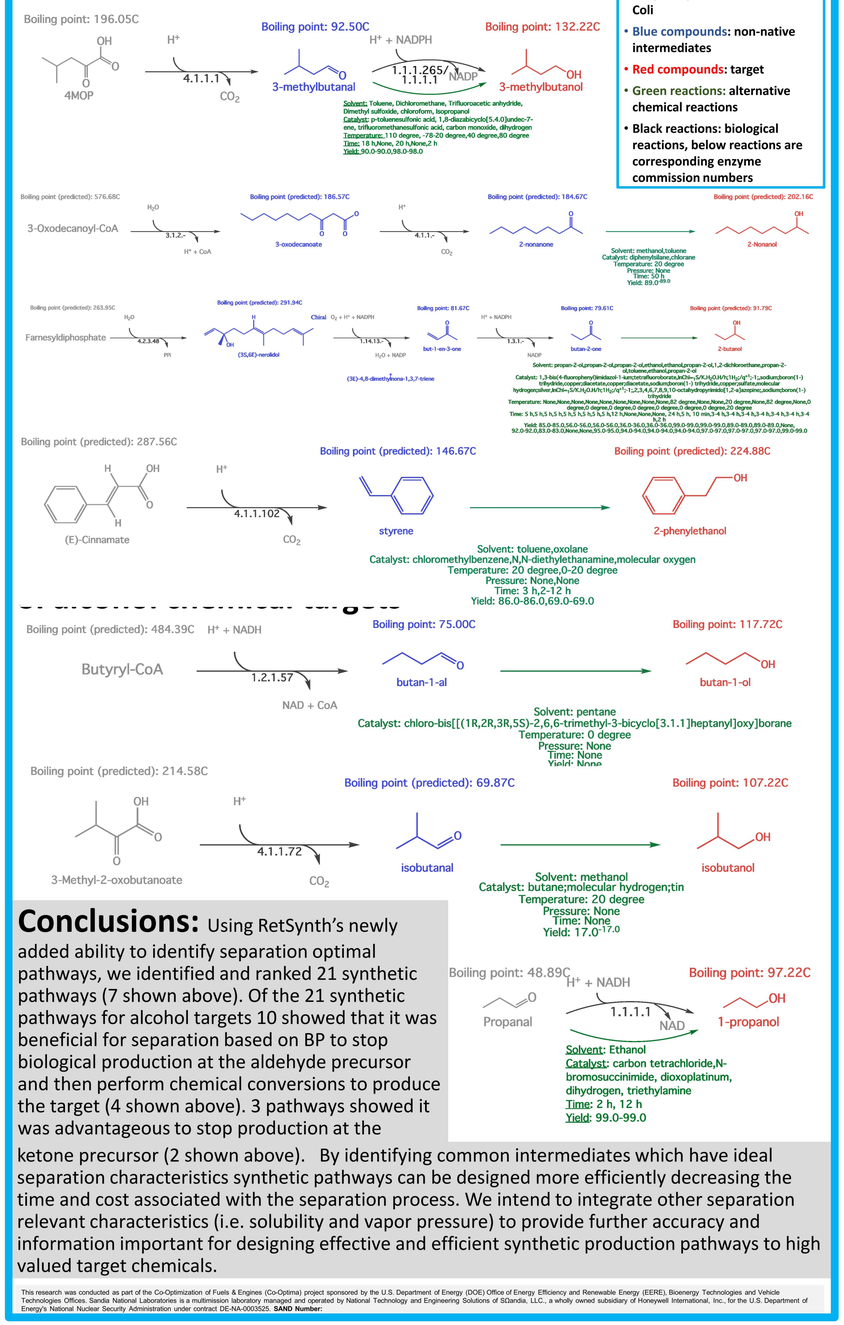
2. Identifying separation optimal hybrid pathways:
   1. Find synthetic pathway with the highest number of biological conversion steps to target synthesis
   2. Identify biologically produced intermediate with lowest boiling point
   3. From that intermediate discover chemical route to complete production of target

- This workflow was used to discover optimal synthetic pathways, ranked by separability for biofuel alcohol chemical targets for spark ignition (SI) and mixed compression controlled ignition (MCCI) engines.

Optimal separation pathways for high valued alcohol MCCI and SI targets

- Total of **40 MCCI** and **11 SI** alcohol chemical targets
- Synthetic pathways were identified in *Escherichia coli DH1* for **18 MCCI** and **8 SI** targets.
- Optimal separation synthetic pathways were identified for **15 MCCI** and **6 SI** targets

MCCI alcohol chemical target pathways



Conclusions: Using RetSynth's newly added ability to identify separation optimal pathways, we identified and ranked 21 synthetic pathways (7 shown above). Of the 21 synthetic pathways for alcohol targets 10 showed that it was beneficial for separation based on BP to stop biological production at the aldehyde precursor and then perform chemical conversions to produce the target (4 shown above). 3 pathways showed it was advantageous to stop production at the ketone precursor (2 shown above). By identifying common intermediates which have ideal separation characteristics synthetic pathways can be designed more efficiently decreasing the time and cost associated with the separation process. We intend to integrate other separation relevant characteristics (i.e. solubility and vapor pressure) to provide further accuracy and information important for designing effective and efficient synthetic production pathways to high valued target chemicals.

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