ENGINEERING *ESCHERICHIA COLI* FOR BIOFUEL PRODUCTION

Case Study

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The depletion of fossil fuels and environmental concerns associated with their consumption necessitate the need for alternative renewable energy carriers [1]. As a result, much research has been directed towards implementing ethanol as a sustainable biofuel. However, ethanol cannot be transported in existing gas pipelines [2]; 1-propanol (Figure 1) holds far greater promise as an alternative fuel due to its higher energy content and better physicochemical properties [3]. Currently, the most economical model for 1-propanol production is through a non-renewable petrochemical process. Beginning in 2012, a bio-manufacturing research group in the Department of Chemical Engineering at the University of Waterloo (Waterloo) analyzed the production of 1-propanol from engineered *Escherichia coli* as an alternative biofuel. Over the last two decades, significant progress has been made in an attempt to overcome inherent biotechnological limitations associated with the use of microbial conversion platforms. The development of these particular strategies, such as novel systems biology, synthetic biology, and metabolic engineering, has made this achievable. Research at Waterloo has manipulated a novel metabolic pathway for the synthesis of 1-propanol and 1-butanol in the genetically tractable bacterium *E.coli*.

Kajan Srirangan, a PhD graduate student, and Lamees Akawi, a MASc student, in the Department of Chemical Engineering, investigated the production of 1-propanol in engineered *E. coli*.

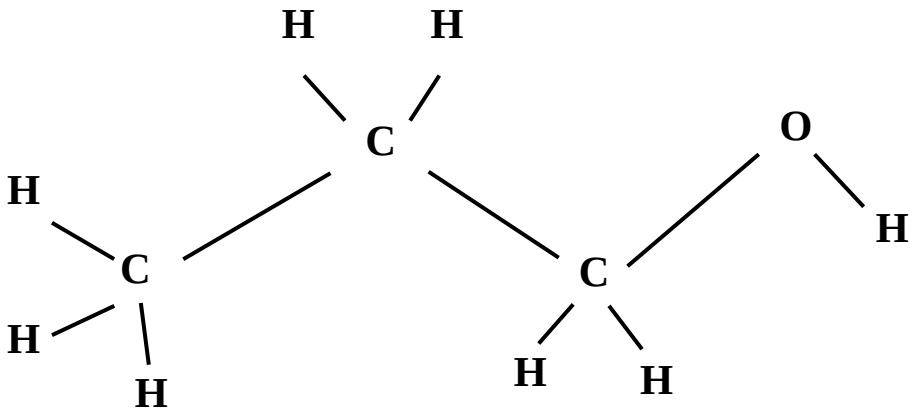

Figure 1 – Structural formula of 1-propanol

Waterloo (Bio) Chemical Engineering Group

With a diversity of interests including water treatment, food processing, commodity and specialty biochemicals, biofuels and human therapeutics, the Waterloo (Bio) Chemical Engineering Group embodies modern day bioprocessing. The group combines elements of traditional chemical processes with emerging fields such as synthetic biology. Professors Chou and Aucoin are regular instructors of Bioprocess Engineering and other bioengineering courses delivered at Waterloo. Professor Chou has held a Canada Research Chair in Biomanufacturing and has a significant interest in engineering bacteria, including *E. coli*, for the production of fuels, solvents and biopolymers. Professor Aucoin has a significant interest in the production of antibodies and propagation of viruses, as well as engineering viruses, primarily baculoviruses, for the infection of cells and subsequent production of complex biologics. He is also interested in large dataset analyses resulting from metabolomic analytical techniques such as NMR/LCMS.

Natural System vs. Engineered

Motivated by the potential production of specialty chemicals and using the tools of genetic engineering, metabolic engineering makes it possible to promote an organism to make a new pathway or amplify an existing pathway [4]. Organisms used in biomanufacturing can be either a native producer (natural) of a target product or, using genetic engineering tools, a microorganism can be manipulated to produce a novel product (engineered).

Metabolic Engineering of *Escherichia coli* for 1-propanol production

Recently, 1-propanol has been identified as a promising alternative biofuel [5]. A summary of 1-propanol properties and comparisons to other biofuels is provided in Appendix A. Compared to ethanol, an established and successful biofuel, 1-propanol is a longer-chain alcohol that tends to have higher energy content and would be compatible with existing biofuel infrastructures and pipelines [6]. No microorganism has been identified as a natural 1-propanol producer, but it can be produced using engineered strains of *E. coli (*Figure 2*). E. coli* can grow under aerobic or anaerobic cultivation conditions.

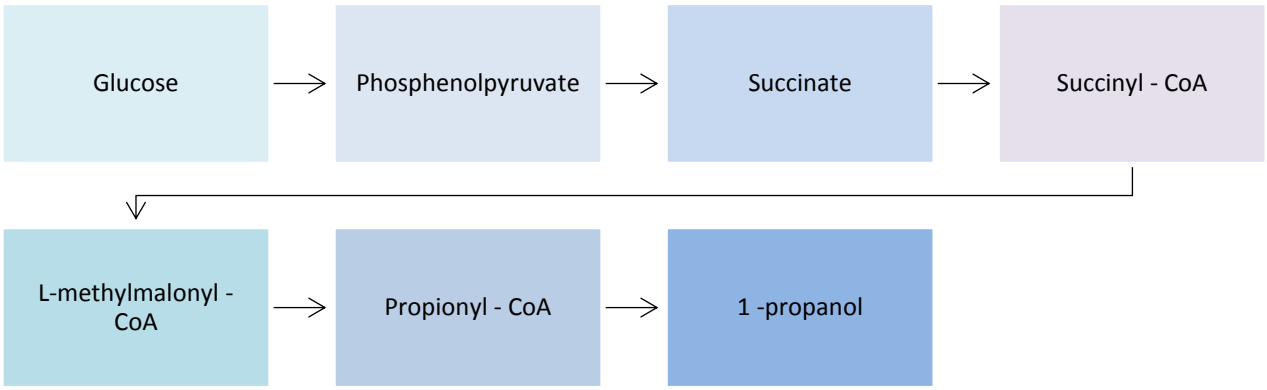

Figure 2 - Simplified 1-propanol fermentative pathway in *E. coli*

Sleeping beauty mutase Pathway

It was demonstrated that a silent, yet functional operon (termed the Sleeping beauty mutase, or Sbm operon) in *E. coli* can be genetically activated to dissimilate endogenously produced succinate into the intracellular precursor propionyl-CoA [7]. The synthesized propionyl-CoA can then be reduced via a variety of endogenous and heterologous alcohol dehydrogenases to enable production of the biological fuel 1-propanol (Figure 2). For greater detail of the metabolic pathways, see Appendix B.

Current production levels at Waterloo of 1-propanol in engineered *E. coli* are approximately 2.5 g/L under anaerobic conditions. Although this has a potential for commercialization [8][9], there are still improvements that need to be made and assessed, such as eliminating bottlenecks in the pathway, removing unwanted byproducts (Table 1), and determining the range of cultivation conditions supporting production of 1-propanol in *E. coli* [2].

Table 1 – Culture performance of a 42.5 h batch cultivation in a bioreactor for CPC-PrOH3 using glycerol as the major carbon source

	Glycerol	Dry Cell Weight (Biomass)	Succinate	Acetate	Propionate	Ethanol	1-Propanol
Initial Concentration (g/L)	30.73	2.638	0.318	0.259	0.370	0.363	0
Final Concentration (g/L)	0	5.474	0.906	4.297	1.152	9.897	2.438

Problem Statement

Kajan and Lamees needed to determine whether it was feasible to efficiently produce 1-propanol in order to decide whether or not they should start their own company.

References

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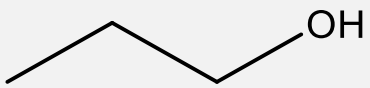
[9] Srirangan, K., Moo-Young, M., Chou, C.P. (2015) Biochemical, genetic, and metabolic engineering strategies to enhance coproduction of 1-propanol and ethanol in engineered *Escherichia coli*. U.S. Patent Application No. 62/150,257, filed April 20, 2015.

Appendix A – Comparison of the properties of 1-propanol to other advanced fuels

Properties	1-propanol	1-propanol structure
Melting point (°C)	-126.0	
Boiling point (°C)	97.5	
Ignition temperature (°C)	371.0	
Flash point (°C)	22.0	
Density at 20°C (g/ml)	.80	
Critical pressure (MPa)	51.7	
Critical temperature (°C)	263.5	



	Fuels				
	1-pentanol	**1-butanol**	**1-propanol**	**Gasoline**	**Ethanol**
Energy density (MJ/kg)	37.7	36.1	33.6	42.7	29.7
Air-fuel ratio	12.5	11.2	21.4	14.6	9
Vapor Pressure (psi))	0.04	0.08	0.29	0.1-30	1.1
Average Octane (AKI rating/RON)	84/113	97/103	108/118	85-96/90-105	99.5/108.6

Appendix B – Major metabolic pathways

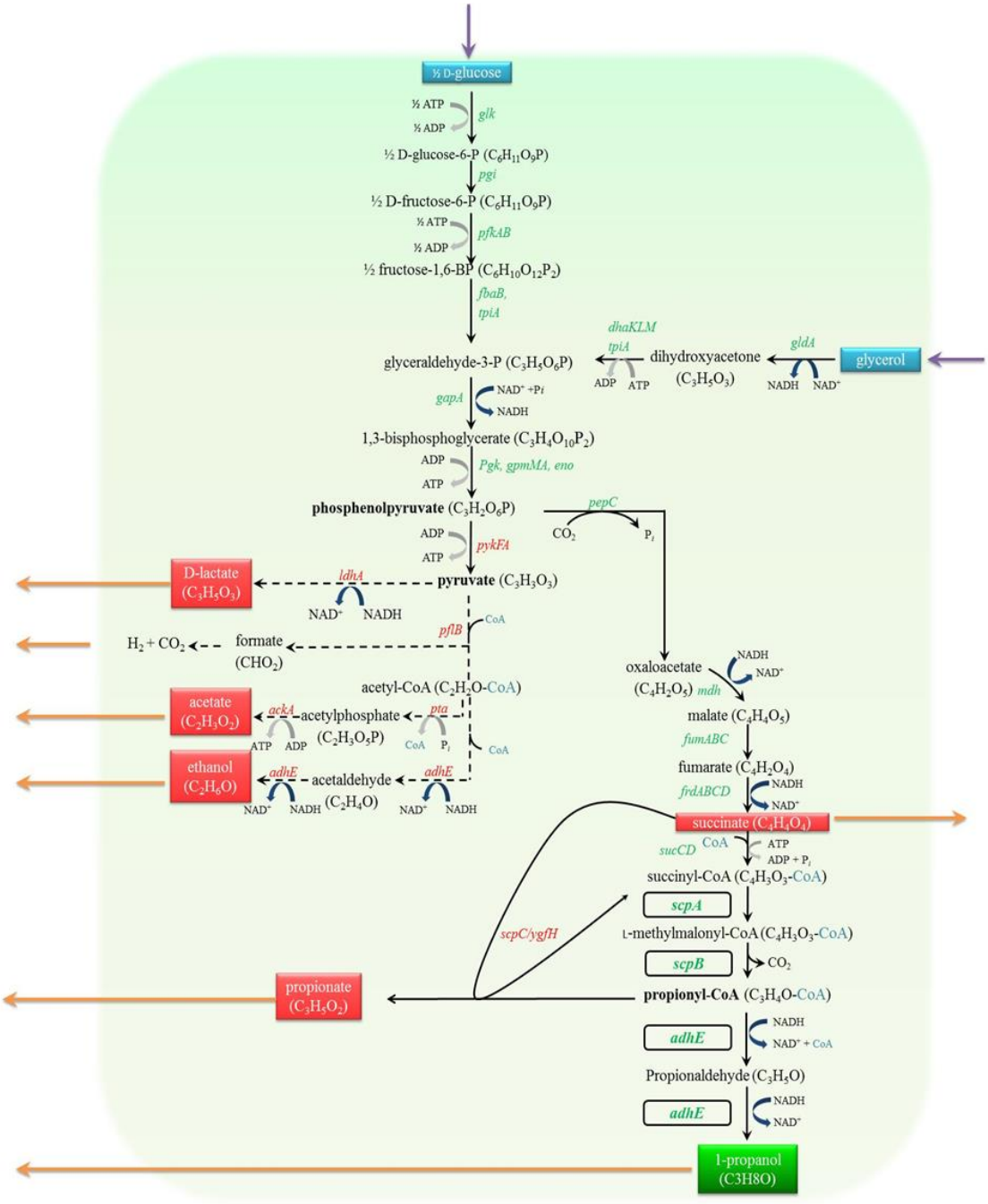


Figure 3 - Major metabolic pathways for anaerobic fermentation and the activated Sbm pathway for extended dissimilation of succinate to form 1-propanol. Red colored gene names above or beside dashed lines represents diverting pathways; metabolites in red boxes are unwanted.

Appendix C – Glycerol consumption and metabolite profile of CPC-PROH3 during anaerobic batch cultivation (Raw data)

Time Point		Glycerol	Biomass	Succinate	Lactate	Acetate	Propionate	Ethanol	1-Propanol
I 0 h	Concentration (g/L)	30.73	0	0	0	0	0	0	0
II 4 h	Concentration (g/L)	30.25	0.52	0.16	0	0.98	0.19	1.3	0.08
III 16 h	Concentration (g/L)	21.43	1.37	0.16	0	2.23	0.39	5.68	0.73
IV 30 h	Concentration (g/L)	8.21	1.78	0.14	0	2.94	0.01	7.33	1.96
V 42. 5 h	Concentration (g/L)	0.07	2.80	0.56	0	4.38	0.72	9.51	2.43