

# Technical Brief

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## Novel microbial method for synthesis of C15 and C17 linear chain alkanes

### Technology Summary

Constraint-based metabolic modeling approach to bioengineer E.coli for the synthesis of highly pure C15 and C17 linear alkanes

### Background

Alkanes have widespread use in the transportation sector, being the major constituent of gasoline, diesel, and jet engine fuel. Terminal alkenes (olefins) are used for the production of lubricants, detergents, and polyethylene. However, there are many challenges associated with their sustainable and environment friendly production. Microbial production of these compounds is a promising alternative, but there are various limitations such as low yield and scalability. Thus, there is an urgent need to address these limitations and make microbial-based fuel cost-competitive to petrochemicals.

### Technology Description

A metabolic model of hydrocarbon production in E. coli was used to identify gene targets for enhanced hydrocarbon production. Based on the in silico predictions, 3 gene additions and 6 deletions were introduced into an E. coli DH5 $\alpha$  strain. For hydrocarbon production, the culture was grown in M9 modified medium supplemented with 2% glucose as the sole carbon source. Extracellular metabolites analysis was carried out using standard analytical techniques such as HPLC and GC-FID/GC-MS. Fed-batch cultivation was then used to improve the final titer of production by growing the best alkane producing strain to a high density in a 5L bioreactor with the help of substrate feeding under controlled condition. The total accumulation of alka(e)ne and hydrocarbon (sum of long chain alkane, alcohol and aldehyde) in the culture had reached to 2.54g/L and 5.68g/L respectively, by 84h of cultivation, where the major contributions were from heptadecene (C17:1, 55%) and pentadecane (C15, 40%).

### References

Model-assisted metabolic engineering of Escherichia coli for long chain alkane and alcohol production. Zia Fatma, Hassan Hartman, Mark G Poolman, David A Fell, Shireesh Srivastava, Tabinda Shakeel, Syed Shams Yazdani, Meab Eng 46(2018), 1-12

Microbial engineering to produce fatty alcohols and alkanes. Ashima Sharma and Syed Shams Yazdani, Journal of Industrial Microbiology and Biotechnology, 2021, 0, 1-18 DOI: 10.1093/jimb/kuab011

### Value Proposition

- Highly pure C15 and C17 alkanes without branched and cyclic alkanes, aromatics, and other contaminants - uniform and consistent product quality
- highest reported titers of long chain alkanes in E. coli: 2.54 g/L after 84 h of cultivation, with major contributions C17:1 (55%) and C15 (40%).
- Green process of production from a renewable source
- Use of glucose as the carbon source
- Ease of extraction due to extracellular synthesis of the linear chain alkane

### Market Potential

The market size for oleochemicals is expected to grow at a compound annual growth rate (CAGR) of 5.8% from 2020 to 2027.

### Applications

Long chain linear alkanes are used in Cosmetic and pharma applications. Industrial applications as solvent and lubricants, food coatings, and as a key ingredient of jet fuel

### Technology Status

- Demonstrated at lab scale using a 5 L fermenter
- Patent protected
- Seeking interested industry partners

