

Utilizing a Modified Wastewater-Based Medium as a Feedstock for Engineered *Saccharomyces cerevisiae* to Biologically Produce Fatty Alcohols and Carboxylic Acids as Alternatives to Petrochemicals

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Petroleum-based chemistry is responsible for the raw materials needed to produce everyday items, ranging from personal care and pharmaceutical items to industrial solvents. A more environmentally-friendly, economically competitive approach is needed. The metabolic engineering of microbial hosts, specifically heterotrophic yeast *Saccharomyces cerevisiae*, using a cost-effective wastewater-based growth medium could potentially provide a solution. Manipulation of the fatty acid biosynthesis pathway is key to inducing the production of fatty alcohols that can act as direct replacements to standard petrochemicals. Three genes responsible for the production of the enzymes acetyl coa carboxylase (ACC1), fatty acid synthase (FAS), and fatty acid reductase (mFAR1), were transfected into *S. cerevisiae* cells. Transfected cells were hypothesized to have an increase in fatty acid and fatty alcohol content and an ability to grow in a modified wastewater-based medium while degrading dissolved organics. To appropriately affirm the transfection of the genes, a spectrophotometric assay predicated on the oxidation of NADPH to NADP⁺ was performed to determine enzymatic activity along with a high performance liquid chromatography (HPLC) analysis to identify select fatty alcohols and fatty acids in the yeast. Gas chromatography-mass spectrometry (GC-MS) portrayed a reduction in harmful organics that were metabolized by *S. cerevisiae* in wastewater media that was deemed competitive with current petroleum production processes through cost analysis. The study adds to research on renewable energy alternatives, but more importantly, demonstrates a unique method in which *S. cerevisiae* can produce valuable specialty and commodity chemicals while removing pollutants from wastewater.

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