IOWA STATE UNIVERSITY

Department of Chemical and Biological Engineering

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Effect of Oxo-Degradation Products on Yeast Growth

Introduction

The purpose of this research is to determine the effects of degraded plastic products on yeast and bacteria growth. The work done fits into a larger project funded by DARPA with the goal of turning plastics into edible nutrients.

Petrochemical products, such as plastics, are notorious for accumulating in landfills and being resistant to degradation. Dr. Robert Brown and his team at the Bioeconomy Institute are pioneering a new process that uses a high temperature reactor to break down plastics in the presence of oxygen to make "oxo-degradation products." The goal is to use these products as a substrate for yeast and/or bacteria. While most of the products are viable substrates, there are a few that can be toxic.

The goal of this project is to quantify how the effects of oxo-degradation products affect the growth of different candidate yeast and bacteria strains. One result will be a list of organisms that grow well on the oxo-degraded substrate and can be used as a platform for optimization through genetic engineering. The other results will be a list of compounds to target for oxo-degradation products, as well as a list of compounds to avoid.

Methods

The methods used for this project were a combination of conventional and custom biological lab practices. In addition, a detailed literature review was performed when the lab was closed due to the COVID-19 pandemic.

The oxo-degraded products were not directly available due to a redesign of the reactor, but there was composition information determined by GC-MS. This information was used to build a list of "model compounds" to be tested individually. The model compounds can be seen in the figure.

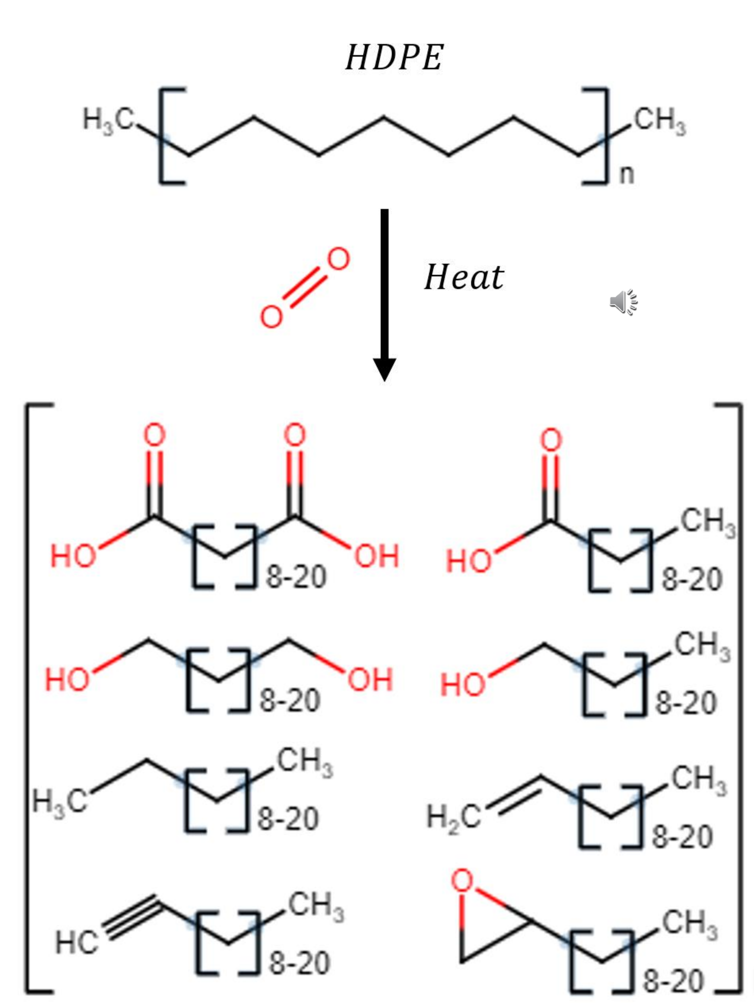
Due to the nature of polymeric plastics, many of the model compounds were highly insoluble, which led to development of nonconventional methods to measure growth. Many high-throughput growth quantifications use light to measure the density of a solution. Since the model compounds were insoluble, they would significantly interfere with the measurement. This can be seen in the graphs. This also made them difficult to accurately dose.

The first experimental iteration involved performing a serial dilution on a sterilized, melted emulsion of a model compound. Then measuring the optical density using a microplate reader. The most soluble compounds were used to test this method paired with our top yeast candidate. Unfortunately, this was the last iteration before the lab closed due to the COVID-19 pandemic.

Results

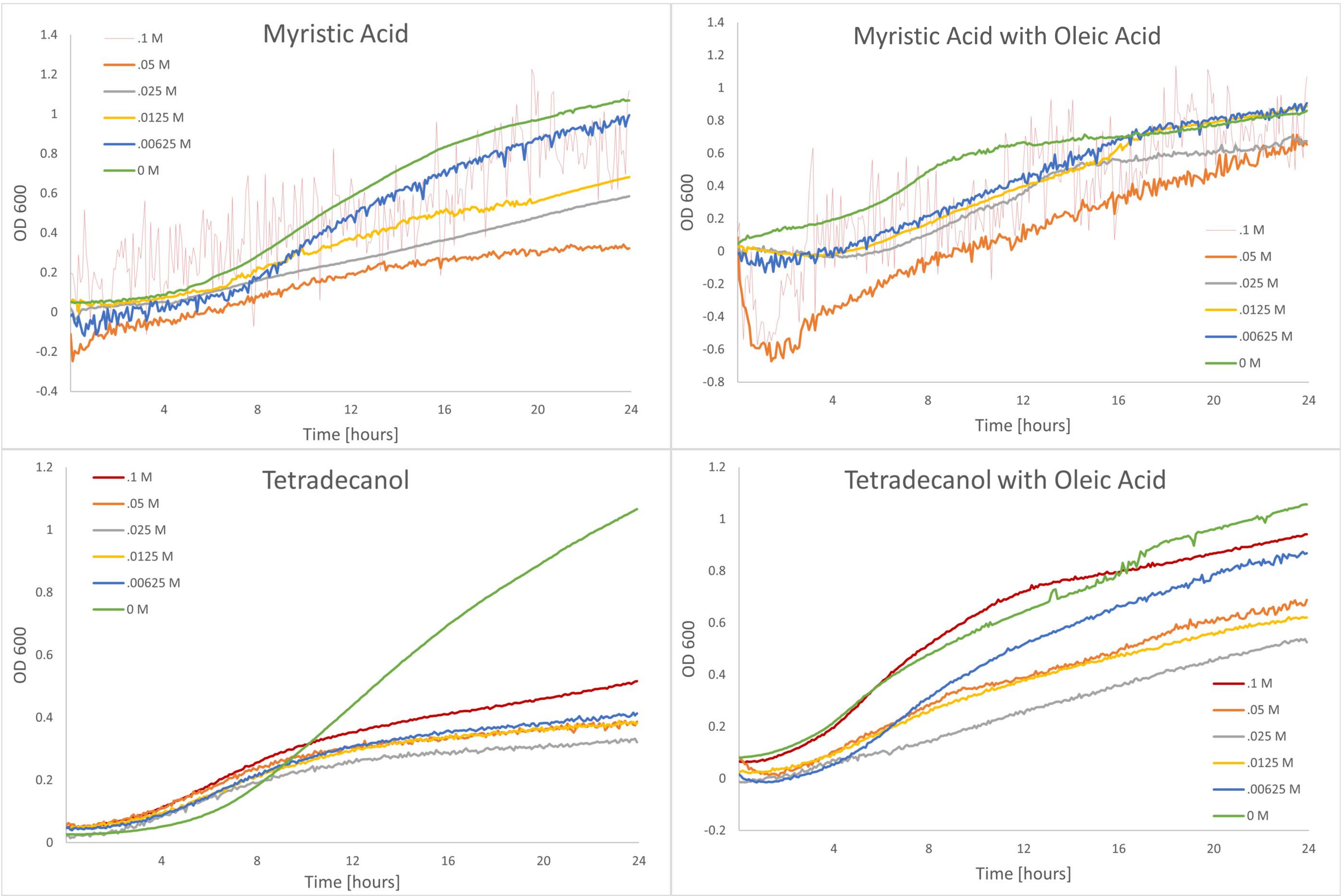
The organism in the shown results is *Yarrowia lipolytica*, a yeast known for its utilization of fatty substrates. The two model compounds used were myristic acid (14 carbon fatty acid) and tetradecanol (14 carbon alcohol). A condition was also run with a small aliquot (1 µL) of oleic acid, which as been reported to stimulate fatty substrate utilization in *Y. lipolytica.* The results show a direct increase in interference with model compound concentration. The model compounds appear to be inhibitory by themselves, but when supplemented with oleic acid, the growth increases substantially. This suggests that the oleic acid did enable metabolic pathways that are otherwise suppressed.

In addition to this data, a literature review was performed to collect published information related to the topic. The thermal oxo-degradation of plastic is known to release highly toxic compounds, but many of them are noncondensable or polar enough to be scrubbed out by water filtration. There are also several genes in certain yeast that significantly improve fatty substrate utilization. This shows potential for heterologous expression and control via genetic engineering.



This figure shows a summary of the model compounds that were selected. The results were concluded based on knowledge of pyrolysis and mass spectroscopy.

The graphs to the right were generated from data collected by a microplate reader over 24 hours.



Conclusion and Future

From these results, it was determined that the method being used was inadequate for characterizing the rest of the molecules. The entire experiment was completed by manually checking the growth at certain time intervals. It was much more labor intensive and was completed by a grad student after the pandemic began. This project aided in determining potential organism/compound combinations that could be exploited through engineering.

Acknowledgements