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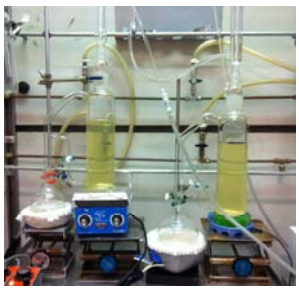

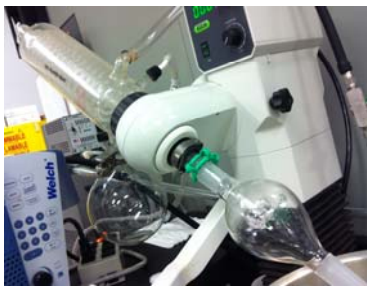

Extraction and Characterization of Organics in Fungal Broth

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July 31st, 2013

Background

For this project, we are collaborating with teams in the Biomass Science & Conversion Technologies, Systems Biology, and Combustion Chemistry Department. Their research involves using a type of endophyte fungus, specifically *hypoxylon* and other closely related species. This fungus has the unique ability to produce terpenes and volatile organic compounds (VOCs) such as 1,8-cineol. These compounds have the possibility to perform similar to, if not more efficient than diesel fuel when burned in an engine. The biological team has already sequenced the genes that code for the production of terpenes, and have characterized the organic compounds found in the head space. They have requested our help in extracting the VOCs from mass quantities of fungal broth, and to characterize the remaining liquid organics.

Figure 1

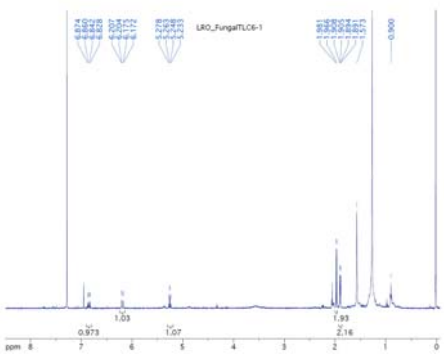

Figure 1 shows an NMR spectrum from one of the chemicals separated using TLC. Based on the GC-MS data and preliminary NMR spectra, we believe this compound to be 2-(2-methyl-2-propenyl)-2-cyclohexen-1-one.

Results

The fermentation product, 1,8-cineol, is an interesting target as a potential diesel feedstock. The biological team's intent was to collect this product, however it is not the main component. Identification of the isolated component mixtures with GC-MS showed that the results vary from batch to batch, but in all GC-MS spectra collected, the largest peak observed is assumed to be 2-(2-methyl-2-propenyl)-2-cyclohexen-1-one. In some GC-MS samples from the most recent fermentation batch, no 1,8-cineol was present.

Presently the approximate quantity of material isolated is roughly 0.5g, or 0.25 mL of material for every 2 L of broth that is processed. The plan is to isolate close to 10 mL of extract, which will require us to process 40L of broth. The collected materials will then be given to the Combustion Research Lab to test its performance as a potential diesel alternative.

Method

Initial attempts to isolate the organic content of the fungal fermentation broth utilized a combination of steam distillation and liquid-liquid extraction. The concept was to first concentrate the organic components in the aqueous layer in an effort to separate the materials by phase separation. Since the concentration of organic components was low, a liquid-liquid extraction of the aqueous layer with diethyl ether was performed. In later separations the stream distillation process was deemed unnecessary. The organics were extracted with the solvent diethyl ether using a continuous liquid-liquid extraction vessel. The solvent containing the organics was collected, dried over magnesium sulfate , and concentrated using a rotavap to collect the less volatile organic components.

The individual constituents of the collected samples were identified by gas chromatography mass spectroscopy (GC-MS). Between 0.25-0.50 µL of the collected materials was added to 1 mL of methanol. Figure 2 shows a representative GC-MS chromatogram of one of the first amounts of extracted organics. Integration performed on the chromatogram was used to determine the relative percentages of the different components. The results vary between fermentation batches; common percentages included 1.88% 1,8-cineol, 8.33% β-pinene, 22.48% 2-(2-methyl-2-propenyl)-2-cyclohexen-1-one, and 8.49% BHT (a stabilizer present in the solvent that is concentrated through processing).

Semi-preparative TLC experiments were also run with 3% ethyl acetate in hexanes in order to isolate the organic compounds. The separate spots on the plate were identified under 254 nm light and silica gel with the adsorbed components was then scraped off with a razor blade. The silica gel was washed with deuterated chloroform ($CDCl_3$) to remove the organics from the gel. This $CDCl_3$ solution was then run through proton NMR to produce the spectra shown in Figure 1.

Figure 2

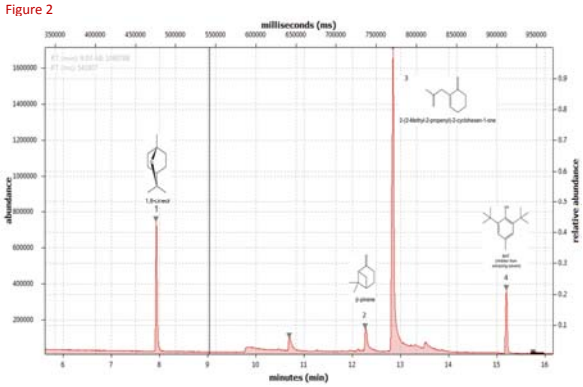

Figure 2 depicts a GC-MS spectrum of a sample of the collected organics from the fungal broth. Using the compounds that the biological team has identified as a reference, the molecules shown where able to be characterized based on the mass-spec data.

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