Dehalogenation of 1,2,3-trichloropropane in microfluidic environment via Raman spectroscopy

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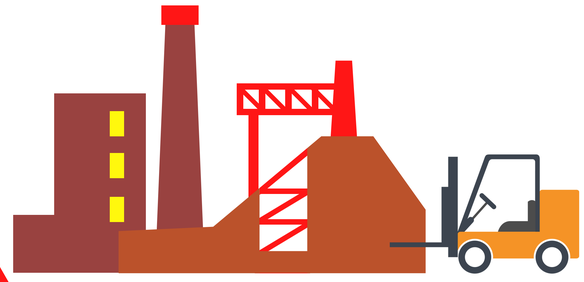
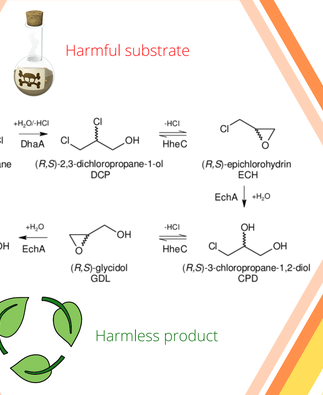
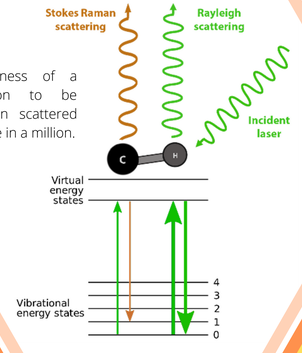
1,2,3-Trichloropropane (TCP) is a chemical widely used in industry as a solvent, extraction agent, and is produced during epichlorhydrine manufacturing. It is also considered a likely carcinogen [1, 2] that can leak into soils and groundwater supplies [3]. This experiment focuses on optimization of the dehalogenation processes perpetuated by:
haloalkane dehalogenase (DhaA),
halohydrine dehalogenase (HheC),
and **epoxid hydrolase** (EchA).
The optimal ratio of the enzymes will be established by the detection of the glycerol production by Raman microspectroscopy. High power laser is used to compensate the low sensitivity of Raman. The reaction will be held within droplets in microfluidic tubes with the detection in a glass capillary attached to the end of the tube.



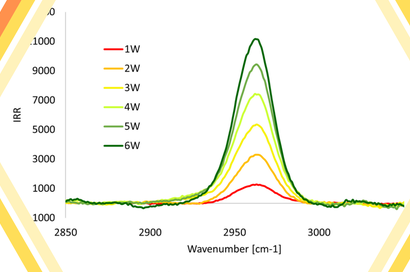


Basic principle behind Raman spectroscopy

Likelyness of a photon to be Raman scattered is one in a million.

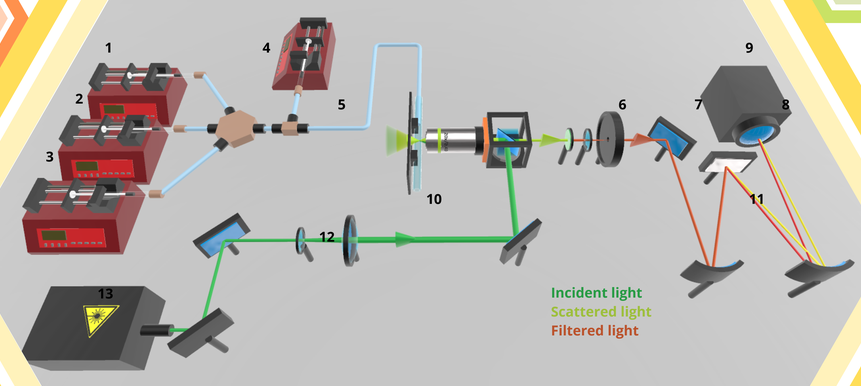


Relation between incident laser power and TCP peak relative Raman intensity



In general the more power was used, the higher signal was acquired in a linear fashion.

Model of the instrumentation for the detection of enzymatic degradation of TCP



1, **2**, **3** - enzymes DhaA, HheC, EchA; **4** - oil with substrate; **5** - microfluidics; **6** - 20x objective; **7** - beam splitter; **8** - notch filter; **9** - CCD; **10** - glass capillary; **11** - diffraction grating; **12** - beam expander; **13** - laser source (532 nm)

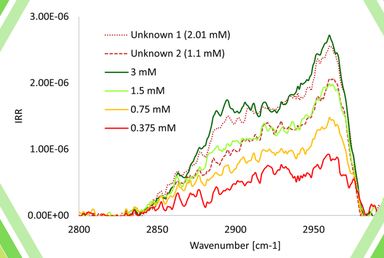
The presented model will be the last stage of the experiment. Current stage setup utilizes only three pumps and does not yet include enzymes from the reaction. Instead, concentration gradient of glycerol was measured within simulated reaction conditions to test the potential of the method.

An example of the detection process can be seen in the video (accessible through the QR code).

Spectra obtained from oil phase are discarded through software that automatically recognizes and marks them.

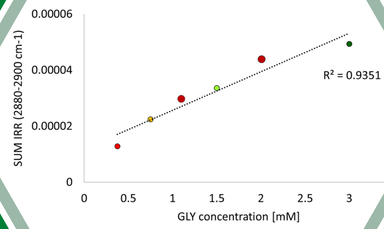
In house built Raman spectroscope was used for detection of glycerol. Incident laser (532 nm) was set to 6 W (3.5 W measured behind the objective), integration time was 0.1 s and 10 accumulations were acquired per spectrum. 75 spectra were collected. Flowrate was set to 900 µl/h and the ratio of aquaeous and oil phases was 1:1. BSA was used to mimick the presence of enzymes, TCP was in the oil phase in high concentrations (50 mM) and buffer was enriched with DCP. This should provide similar conditions as if dehalogenases enzymes were used. Then, concentration gradient of glycerol (including two random concentrations as our unknown samples) was measured and calibration curve created.

Glycerol spectra for different concentrations



Spectra after smoothing, normalization, and blank and manually-fitted baseline subtraction.

Glycerol calibration curve



Each point represents the sum of all relative Raman intensities within the 2880-2900 cm-1 range (1st glycerol peak) in the spectra.

Submilimolar detection of glycerol was achieved in microfluidic environment and within complex matrix of the reaction solution. Using **high power** laser capable of producing 6 W in continuous mode helped to reach these concentrations, which were otherwise below LOD with conventional Raman spectrometers. Although, the linearity of the response was adversely affected by those lower concentrations, where glycerol peaks were harder to find and process, making more room for an error. Data fluctuation could be dealt with by averaging multiple measurements. For higher concentrations (>0.5 mM) the method was more precise. The final experiment is expected to at incorporate the actual dehalogenases and detect **milimolar** concentrations of glycerol.

1. Yan J, Rash BA, Rainey FA, Moe WM. Isolation of novel bacteria within the Chloroflexi capable of reductive dechlorination of 1,2,3-trichloropropane.
2. Sarathy V, Salter AJ, Nurmi JT, Johnson GOB, Johnson RL, Tratnyek PG. Degradation of 1,2,3-Trichloropropane (TCP): Hydrolysis, elimination, and reduction by iron and zinc.
3. Bosma T, Damborský J, Stucki G, Janssen DB. Biodegradation of 1,2,3-trichloropropane through directed evolution and heterologous expression of a haloalkane dehalogenase gene.