Dehalogenation in microfluidic environment through Raman spectroscopy

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1) Introduction - a)

1,2,3-Trichloropropane (TCP) is a chemical that is widely used as an intermediate in syntheses, as a solvent and also as an extraction agent. Furthermore it is produced as a byproduct during epichlorhydrine manufacturing. TCP is considered a likely carcinogen[1 2] and is able to permeate soils, leaking then into groundwater supplies[3]. The promising strategy for remediation of TCP-contaminated environment is biocatalytic **degradation by dehalogenases enzymes**[4], as they convert wide range of haloalkanes to their respective alcohols[5]. **Microfluidic devices** offer an option for related analyses and can provide fast detection and identification, low chemicals consumption and hold a potential for environmental use[6 7]. TCP's total enzymatic degradation consist of five-step reaction perpetuated by three enzymes: **haloalkane dehalogenase** (DhaA), **halohydrine dehalogenase** (HheC) and **epoxid hydrolase** (EchA) and ends with production of harmless **glycerol** (GLY, GLC) (*Fig. 1*).

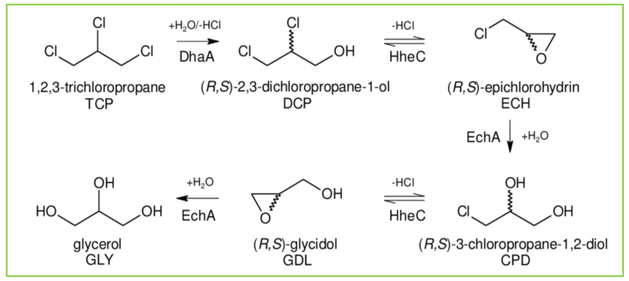


Fig. 1 – *Scheme of TCP total dehalogenation process to glycerol*

Raman spectroscopy is a method convenient for analysis of TCP degradation. It is fast, broad, contactless, noninvasive and fitting for e.g. on-line experiments of biotechnological processes[8] and is suitable for nutrient dynamics and **real-time metabolism measurements**[9].

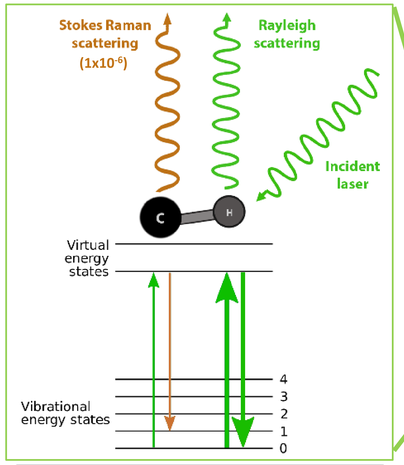


Fig. 2 – *Scheme of Raman principle*

2) Methods

Raman spectrometers for measurements:
1) Homemade system developed at ISI CAS with Verdi V6 laser from Coherent Inc. was used for most measurements (named **Verdi**)
2) InVia Renishaw Raman spectroscope (well established, defined and well-behaved instrument) was used for reference measurements (named **Reni**)

Setup: 10 s integration time, 532 nm laser, 20x objective, maximum laser intensity (unless specified otherwise) – 6 W for Verdi (3.5 W beyond objective), 35 mW for Reni (30 mW beyond objective).
Microfluidic environment: glass capillary with 0.5 mm inner square section.

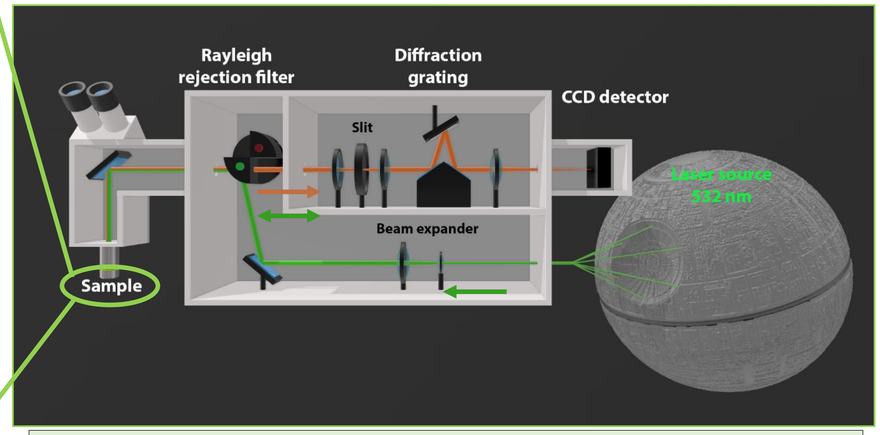


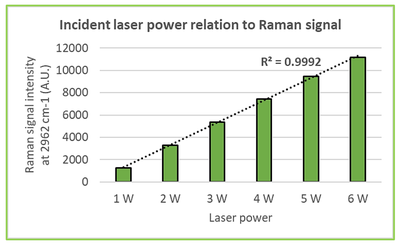
Fig. 3 – *Scheme of Raman spectroscope – inspired by Reni design, but relatable for other spectroscopes as well*

3) Goals

Developing a platform (using Raman spectroscopy and microfluidics) in which we would be able to observe the whole process of TCP dehalogenation, meaning that we would obtain levels of all present metabolites at any given moment of the reaction. That would help us to determine the best optimization for the process (e.g. enzymes concentrations) and once established for this substrate, we would like to expand the usage for other enzymatic reactions as well.

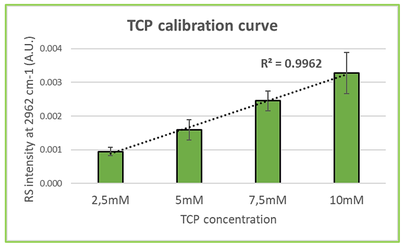
4) Results

TCP is **not** very **Raman-active** molecule, thus the decision to use **Verdi** system with 6 W incident laser for better detection of lower concentrations. But as effect of laser this powerful was uncertain, we tested one sample of 10 mM TCP with increasing laser power. The resulting dependency was nearly perfectly linear with coefficient of determination $R^2 = 0.999$ (*Graph 1*).



Graph 1 – *Effect of high power laser on Raman signal*

To further examine **detection limits**, we measured a concentration gradient of TCP (other compounds are yet to be tested) to get a calibration curve (*Graph 2*). That resulted in $R^2 = 0.996$, which means that our system is very responsive to concentration changes and thus is suitable for **quantitative analyses** as well.



Graph 1 – *Effect of TCP concentration on Raman signal*

As mentioned in Methods section, we analysed **neat compounds** with **Reni** to get reference spectra (*Chart 1 - left*) and then proceeded to **10 mM solutions** with **Verdi** (*Chart 1 - right*). After data processing the outcoming spectra resembled their respective references very closely, so even for lower concentrations we were still able to distinguish metabolites solely according to their varying C-H bond vibrations.

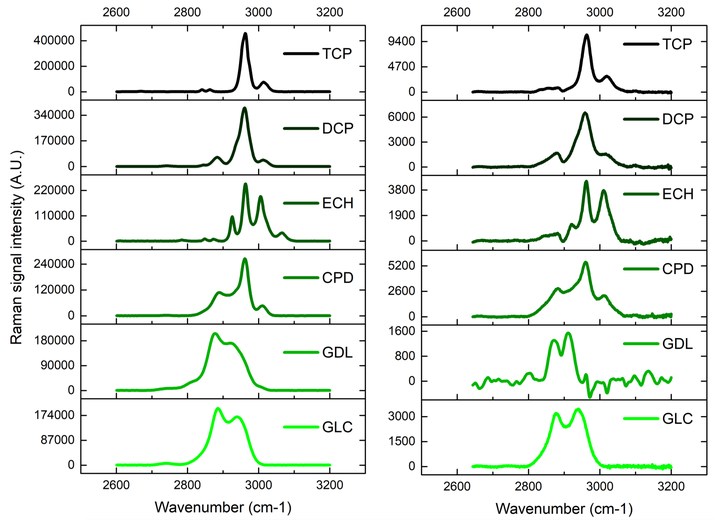


Chart 1 – *Detection of neat (left - Reni) and 10 mM (right - Verdi) metabolites*

5) Conclusion & future plans

So far, we examined the effect of high-power laser, detection of individual intermediates through the cascade of the enzymatic dehalogenation of TCP and reliability for the compound when present only in their lower concentrations. We achieved rather nice calibration curve for TCP and collected data from various other measurements (gradients of other metabolites, their cross-gradients, etc.), which were not shown here, but suggest that we should be able to identify multiple compounds simultaneously. Plan is to progressively and deeply examine each step of the reaction individually at first, identify and define the changes within them, and later combine all these fractions into the whole reaction analysis.

6) References

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