Continuous flow biocatalysis with in-line monitoring through Raman spectroscopy

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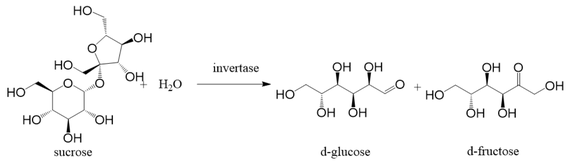
1. Biocatalysis in flow

- Compared to conventional batch processing, flow processing facilitates automation, requires less equipment, and accommodates different pressure, temperature, and reactivity conditions.
- Biocatalyzed processes in flow can benefit from increased productivity due to improved mass transport and reaction parameter control.
- More immobilized enzyme concentration leads to a higher efficiency.



Biocatalytic hydrolysis of sucrose into glucose and fructose[1]

- These bio-transformations have high activity and remarkable stereo-, regio-, and chemo-selectivity because the reaction is precisely controlled in the active site of the biocatalyst.
- Immobilization of enzymes usually employs physical adsorption, ionic interactions, or the development of covalent bonding via amino acids.

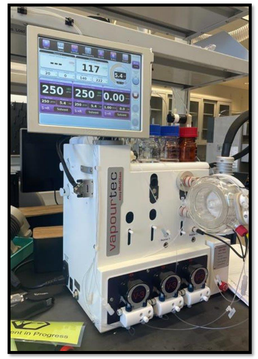
2. Raman in flow chemistry

- Raman's unique spectral fingerprint can help identify the chemical composition and molecular structure of the compounds
- These spectral images are straightforward to interpret, where the signal intensity directly correlates to the concentration.
- Water is a problematic solvent for IR monitoring because of its broad IR band.

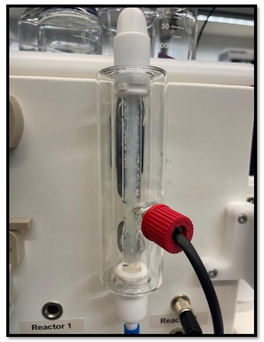
3. Hydrolysis of Sucrose in Flow

- Sucrose was hydrolyzed using a Grade VII Invertase from Bakers Yeast (*Millipore Sigma*)
- For the immobilized enzyme, a uniform mixture was prepared using sodium alginate, k-carrageenan and the enzyme.
- The mixture was dropped into a continuously stirred ice-cold calcium chloride solution using a syringe and a constant flow rate. The beads were left in the solution overnight for further hardening
- The reactions were carried out using the free enzyme solution and the beads on the bench top. For the final reaction, the beads were poured into a column reactor and the reaction was carried out by supplying a constant flow of sucrose solution on an EasyMedChem flow system from vapoutec

Calcium Alginate and K-Carrageenan beads with entrapped invertase

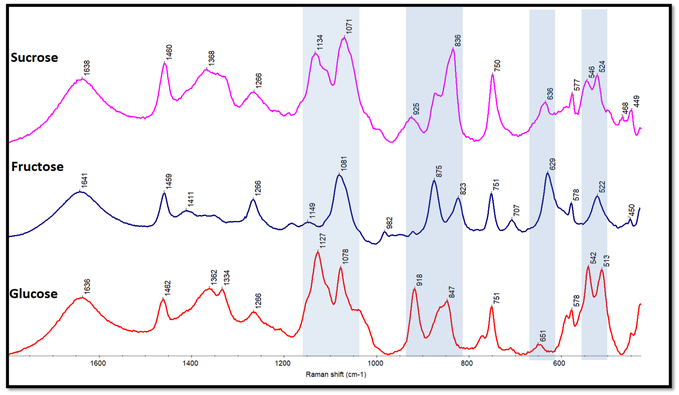


EasyMedChem™ by VapourTec™

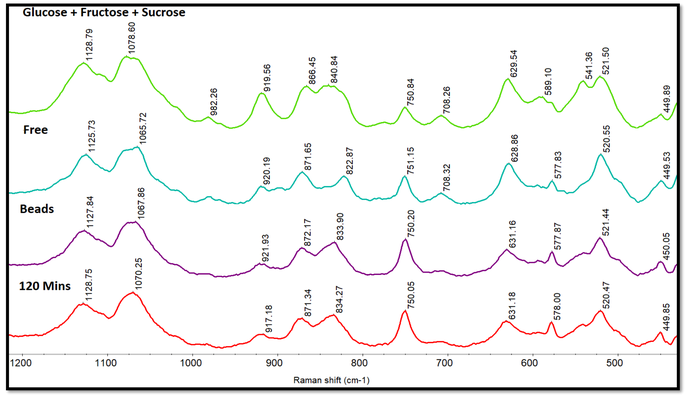


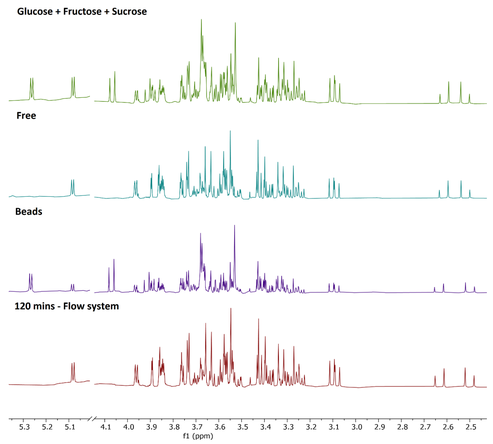
Invertase beads in a column reactor

MarqMetrix™ Raman Flow Cell



- Multiple bands were identified to differentiate between sucrose, glucose and fructose
- The biocatalysis products after 120 minutes were analyzed through Raman against commercial glucose, fructose and sucrose mixtures.





- The presence of glucose, fructose and unreacted sucrose was also confirmed through NMR on the samples.

4. Future work

- Quantification of reducing sugars by Ion Chromatography and DNSA methods – Work in progress
- Quantification of the reducing sugars using Raman only
- Immobilization of Glucose Oxidase on silica-based supports and production of hydrogen peroxide and gluconic acid in flow – Work in progress

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References

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