Laccase Oxidation Studies: Method Development for following the Benzyl Alcohol oxidation reaction using the Laccase/TEMPO system

Hong Ann Gan[1], Dr. Sean Reidy[1], Dr. Noreen Morris[1], Dr. Luc Selve[2], Prof. Tom Moody[3]

1. Dept of Pharmaceutical Science and Biotechnology, Technological University of Shannon, Athlone
2. Arran Chemical Company, Athlone (a member of the Almac Group)
3. The Almac Group, Craigavon

1. Background: Biocatalysis and Laccase

Biocatalysis is the use of nature's biological catalysts – "enzymes" to perform synthetic chemical reactions, which are generally "greener" than traditional chemical methods. Laccases are a group of oxidoreductase enzymes considered "green catalysts" as they perform oxidation by consuming oxygen and only producing water as a byproduct.[1]

Although Laccases are environmentally friendly catalysts, they are limited in the range of substrate they can react with (i.e. phenolic substrates). This range can be expanded to non-phenolic substrates with a mediator such as TEMPO. The whole system is termed a Laccase-Mediator system (LMS).[2] (Figure 1)

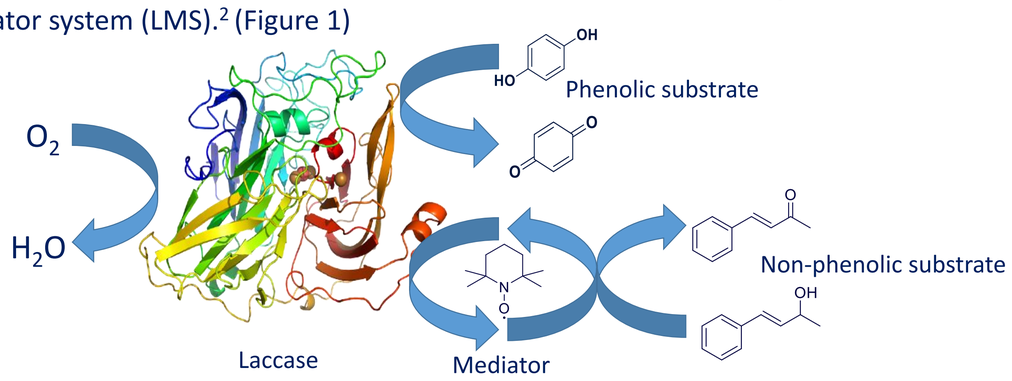

Figure 1: General reaction scheme for laccase oxidation (top) and LMS (bottom)

2. Benzyl Alcohol Oxidation

The oxidation of benzyl alcohol to benzaldehyde and benzoic acid has been reported using various methods, but many require the use of toxic organic reagents, heavy metals or they generate large amount of waste due to use of organic based solvents.

For our current project, we have oxidized benzyl alcohol using the Laccase/TEMPO system. The advantage of this system is its mild conditions (i.e. low temperature and pressure) and its non-toxic nature (i.e. aqueous buffer, no toxic metals or reagent used). (Figure 2)

The aim of this project is to develop experimental and analytical methods to monitor all components of the reaction over a period of time intervals. The ultimate goal of this project is to optimize reaction conditions to result in maximum conversion of benzyl alcohol into benzaldehyde, while minimizing benzoic acid formed by over-oxidation of benzaldehyde.

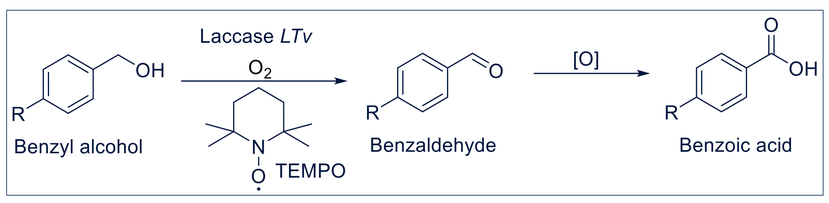

Figure 2: Reaction scheme of benzyl alcohol oxidation via laccase/TEMPO LMS system

3. Results and Discussion: GC and HPLC Method Development

Gas chromatography (GC) was initially employed to monitor benzyl alcohol oxidation. A temperature program was developed and optimized to enable separation of benzyl alcohol, benzaldehyde and TEMPO concentrations at a relatively short run time.

An internal standard was incorporated into the standard curve to enable accurate and replicable representation of peak area with the concentration of each component (Figure 3 and 4).

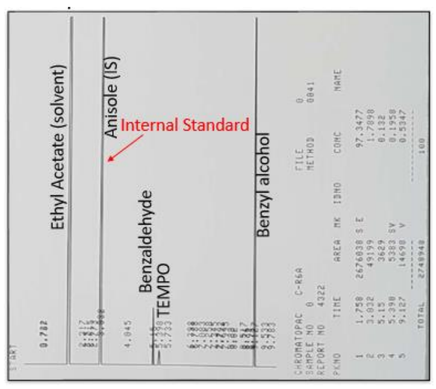

Figure 3: An example of GC chromatogram of benzyl alcohol oxidation utilizing internal standard with a 10 min run time

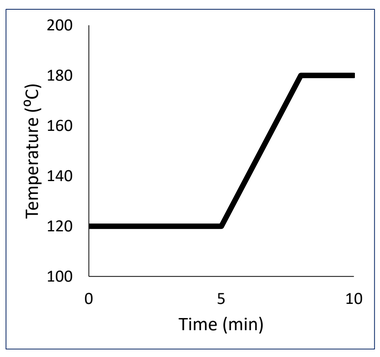

Figure 4: GC temperature program optimized to obtain chromatogram in Figure 3

Figure 5: An example of GC chromatogram solely for benzoic acid with a separate temperature program (isothermal 230°C 5 min)

Using this method, it was noticed that the total concentration of the reaction was decreasing over time. The most likely scenario was the over-oxidation of the product benzaldehyde to benzoic acid, which was not being detected properly.

Efforts in developing a temperature program to include benzoic acid proved to be difficult due to its long retention factor and poor response to the FID detector. A separate temperature program was developed solely for benzoic acid but the response was still unsatisfactory (Figure 5).

Eventually, the method for monitoring benzyl alcohol was switched over to high performance liquid chromatography (HPLC). Paired with a UV detector, benzoic acid had greater response in HPLC compared to FID detector in GC.

Wavelength scans with a PDA detector was used to select the optimum wavelength for following this reaction. Finally, 210nm was chosen as it provided a good relative response factor (RRF) between all three compounds. (Figure 6 and 7)

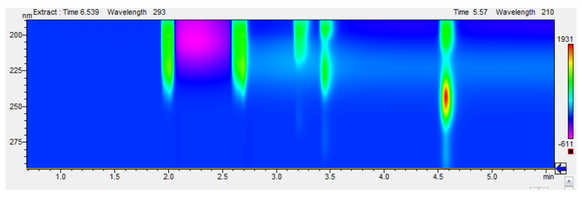

Figure 6: Contour view of PDA detector visualizing benzyl alcohol, benzaldehyde and benzoic acid.

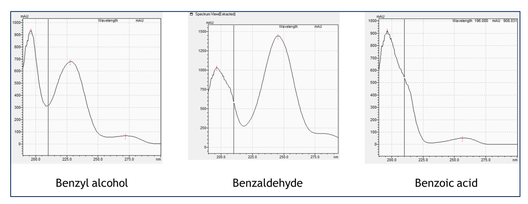

Figure 7: Wave scans of each component obtained using PDA detector.

With all the components including benzoic acid being visible using HPLC-UV@210nm, an optimum gradient condition was developed and optimized to enable good separation with a relatively short run time (Figure 8).

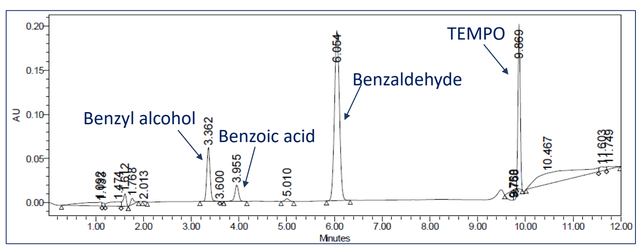

Figure 8: An example of HPLC chromatogram showing all components using gradient with a 12 min run time

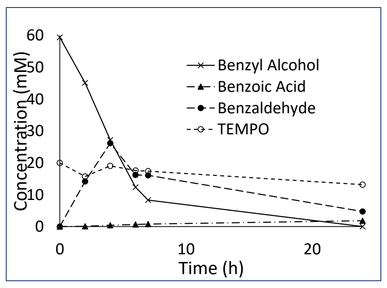

Figure 9: An example of 24h reaction timescale followed using HPLC

With good method development for following benzyl alcohol oxidation, the next step of this work will be adjusting reaction conditions to achieve maximum conversion to benzaldehyde while limiting over-oxidation to benzoic acid.

4. Future Works and Applications

- Novel downstream transformation with benzyl alcohol oxidation product via laccase/TEMPO system, leading to the development of biocatalytic or chemoenzymatic one-pot multi reaction cascade.

- Incorporation of state of the art flow chemistry and enzyme immobilization to improve yields of novel Laccase/TEMPO system reactions, greater recyclability and potential scale up to kilogram scale.[3] (Figure 6)

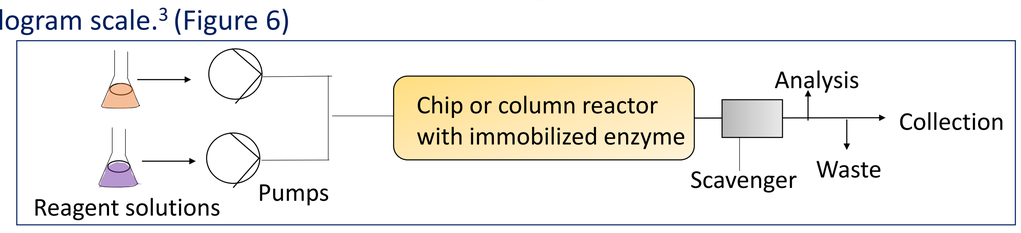

Figure 6: A typical flow approach set up with an immobilized enzyme reactor

5. Acknowledgement

I would like to thank Dr. Sean Reidy, Dr. Noreen Morris for their continuous support and guidance. I would like to thank Arran Chemical Company for their assistance and supply of materials, especially Laccase enzymes, Prof Tom Moody and Dr Luc Selve for their continuous assistance on the method development.

6. References

1. Bassanini, I.; Ferrandi, E.E.; Riva, S.; Monti, D. Biocatalysis with Laccases: An Updated Overview. *Catalysts* **2021**, *11*, 26.
2. Tromp, S. A.; Matijošytė, I.; Sheldon, R. A.; Arends, I. W. C. E.; Mul, G.; Kreutzer, M. T.; Moulijn, J. A.; de Vries, S. Mechanism of Laccase-Tempo-Catalyzed Oxidation of Benzyl Alcohol. *ChemCatChem* **2010**, *2* (7), 827–833.
3. Britton, J.; Majumdar, S.; Weiss, G. A. Continuous Flow Biocatalysis. *Chemical Society Reviews* **2018**, *47* (15), 5891–5918.