

Use of the biocatalyst Laccase in the oxidation of secondary alcohols

A00273292 Hazel Browne
Supervisor: Dr. Noreen Morris



TUS

Technological University
of the Shannon



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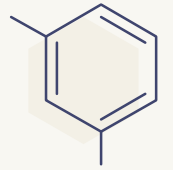
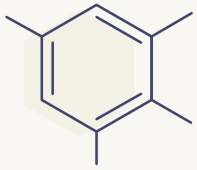
**Results and
discussion**

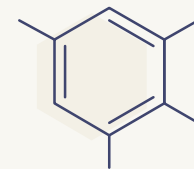
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01



Introduction

Green Chemistry,
biocatalysts, laccase and
mediators

Introduction: Aim

To look at the use of green chemistry approaches in the oxidation of secondary alcohols

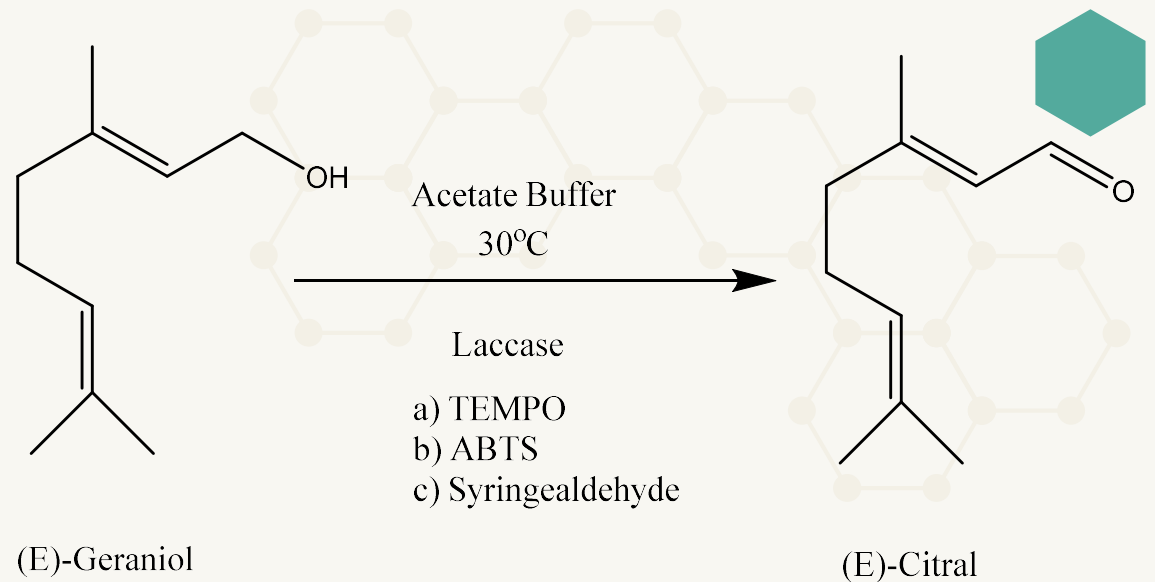


Figure 1. Oxidation of geraniol

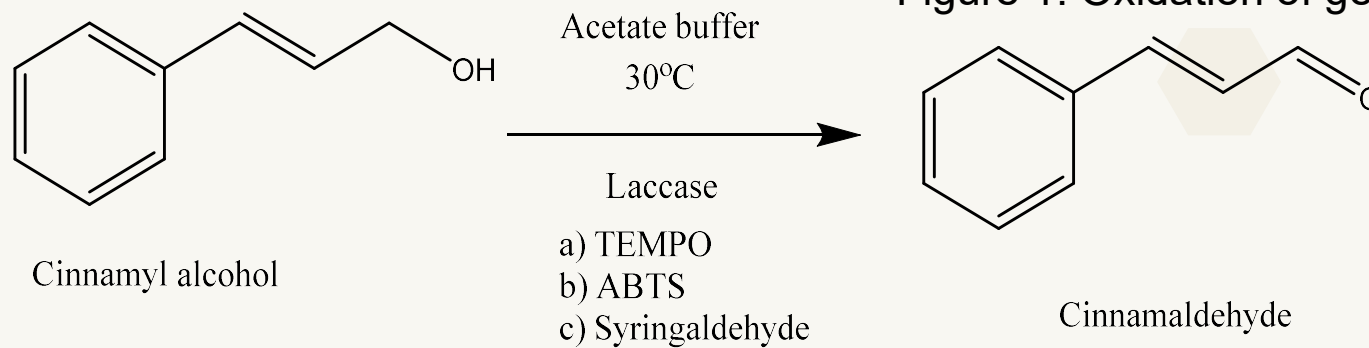


Figure 2. Oxidation reaction of Cinnamyl alcohol

Introduction: Biocatalysts



Figure 3. Use of biocatalysts

Introduction: Laccase

Laccase belongs to the family of multicopper oxidases.

- Oxidise phenolic and nonphenolic compounds.
- Over 60 strains of fungi and bacteria.
- Has a redox potential of 0.4-0.8V (Cañas and Camarero, 2010)

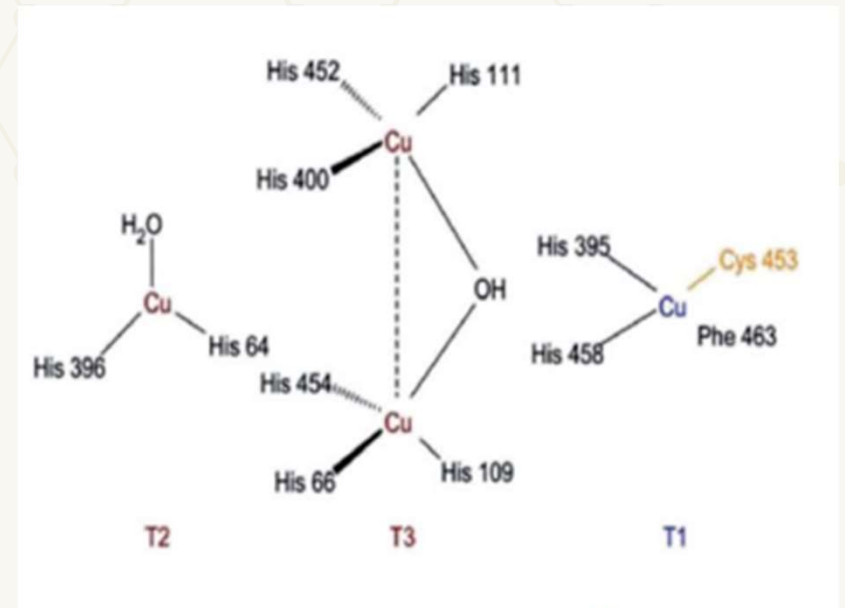
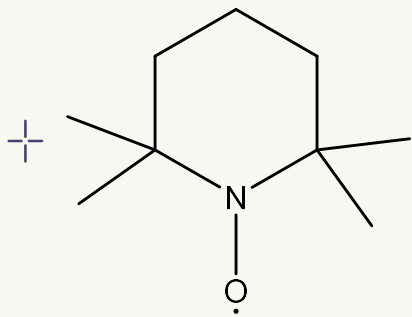


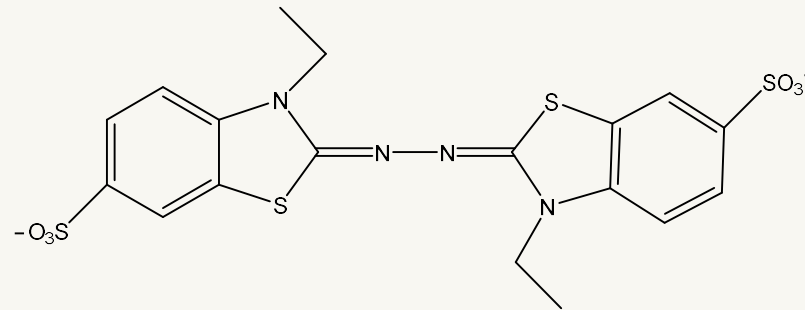
Figure 4. Laccase Structure (Gu et al. 2021)

Introduction: Mediators



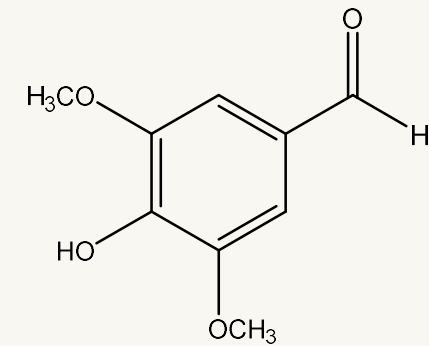
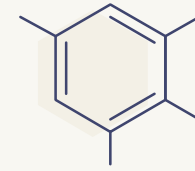
TEMPO

- Artificial
- Most commonly used



ABTS

- Artificial
- Used for the Laccase activity assay



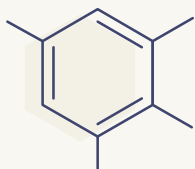
Syringaldehyde

- Natural
- By product of the paper making industry

02

Methods





Methods: HPLC



- Compounds possess chromophores
- Reverse phase used
- Mobile Phase: Water and Acetonitrile 50:50

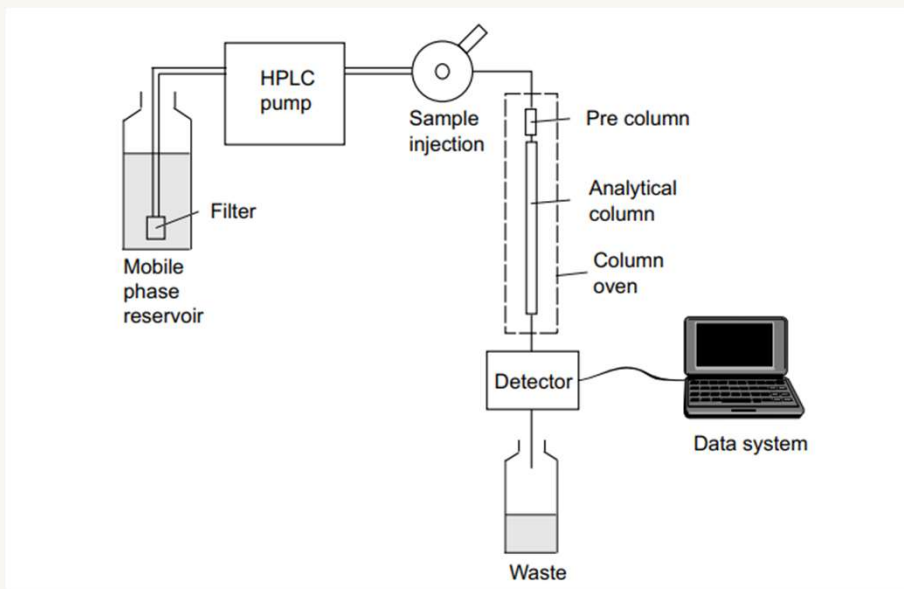
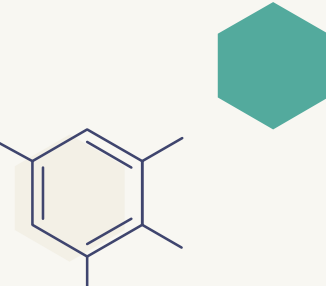


Figure 5. Block diagram HPLC (Hansen, 2012)

Instrument	Shimadzu 10A		
Column	Phenomenex C18 150 x 4.60mm 3 micron		
Size of injection	50µl	Flow rate	1ml/min
Attenuation	8	Wavelength	228nm
Mobile phase	50:50 Water: Acetonitrile (0.05%TFA)		

Instrument	Shimadzu 10A		
Column	Phenomenex C18 150 x 4.60mm 3 micron		
Size of injection	50µl	Flow rate	1ml/min
Attenuation	6	Wavelength	210nm
Mobile phase	50:50 Water: Acetonitrile (0.05%TFA)		

Methods Timeline



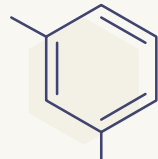
1. Laccase
Activity
assay

2.
Standard
curve

3. Oxidation
Reactions:
cinnamyl
alcohol

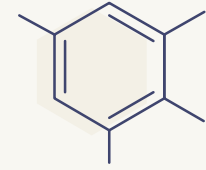


4. Oxidation
Reactions:
Geraniol



5. HPLC
analysis

1. Determined the activity of the laccase enzyme
2. Established the retention times of the substrates and products
3. Monitored over a 5 hour time period
4. Monitored over a 5 hour time period
5. Peak areas used to determine concentration of substrates/ products



03

Results and discussion

HPLC Chromatogram

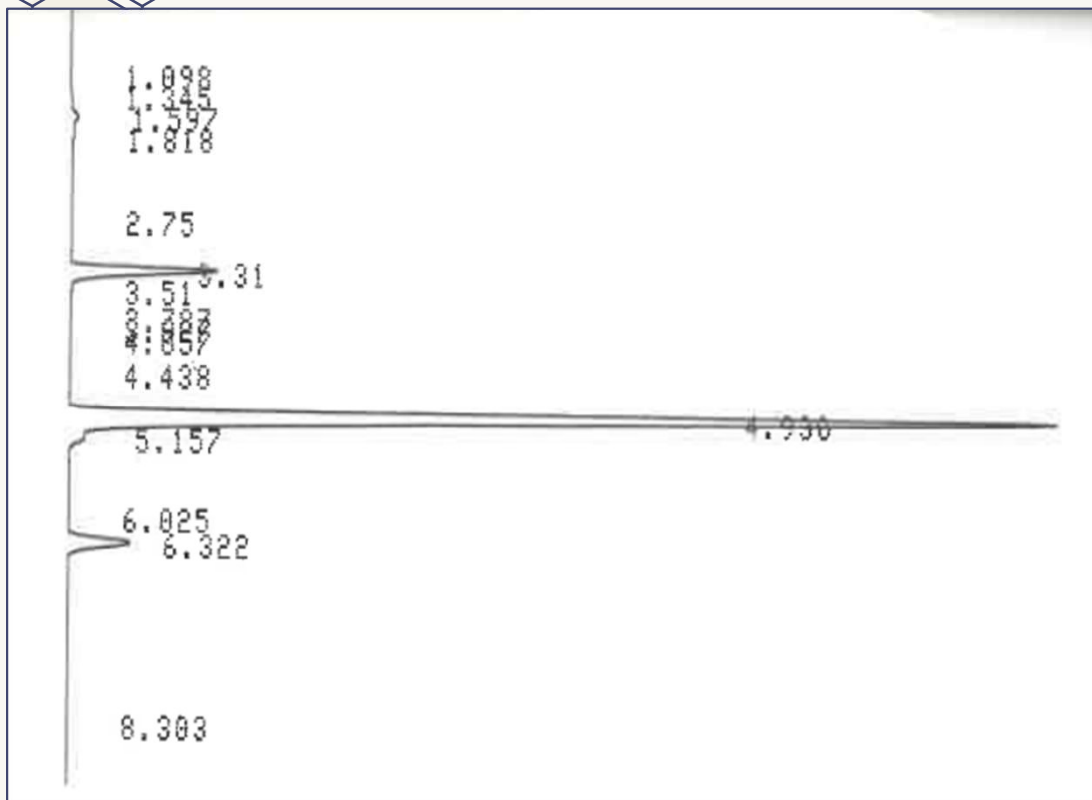
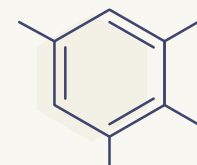


Figure 6. Cinnamyl oxidation reaction with TEMPO
Hour 3

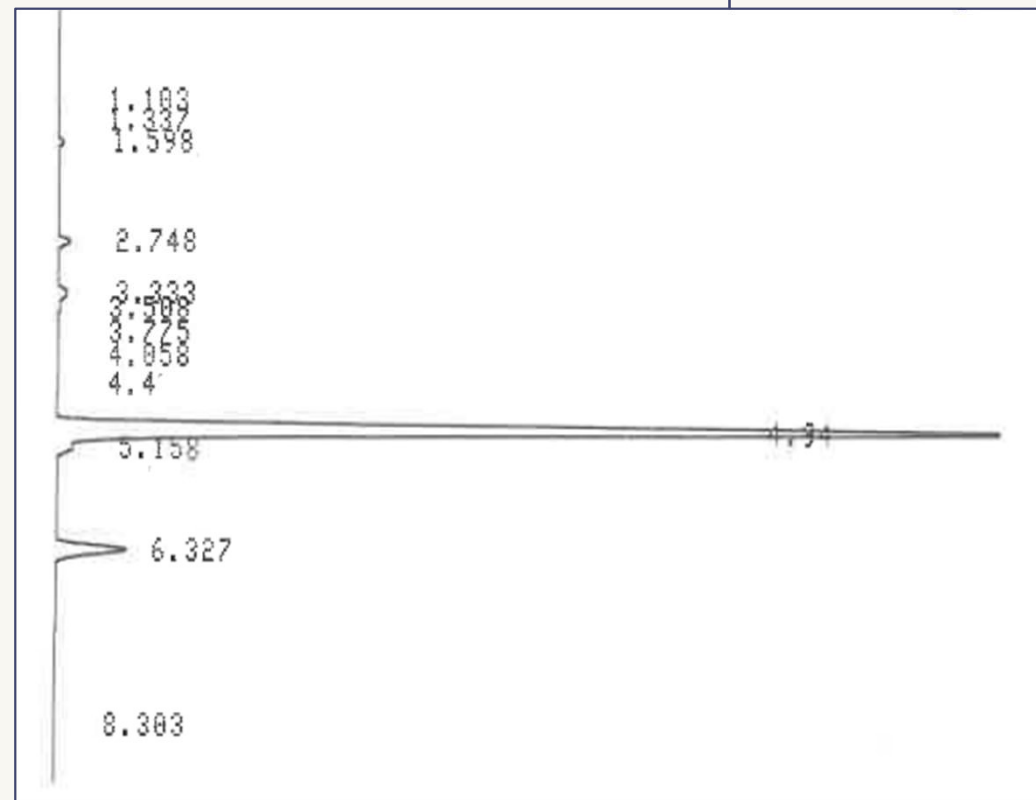


Figure 7. Cinnamyl oxidation reaction with TEMPO
Hour 5

HPLC Chromatogram

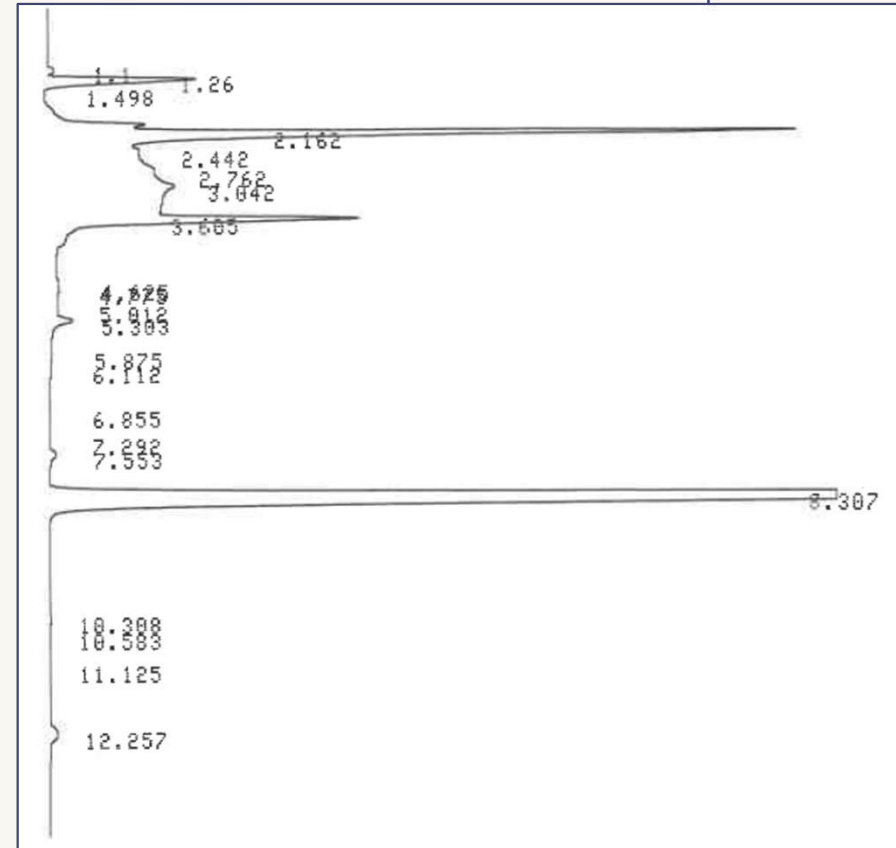
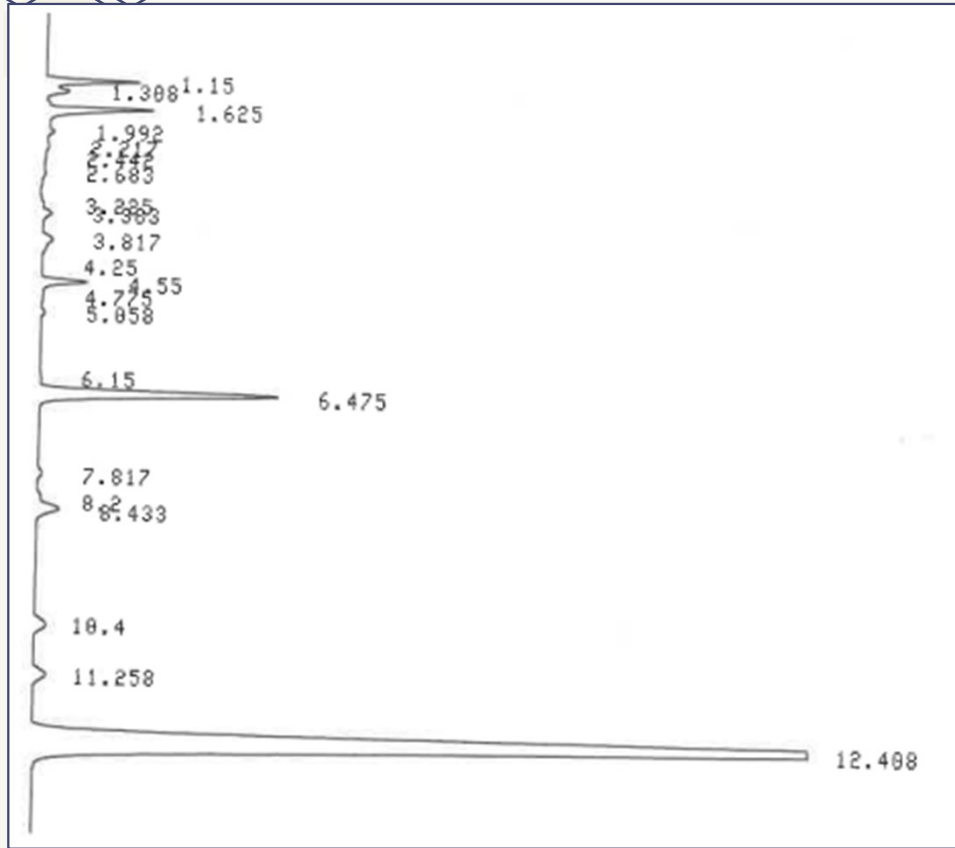
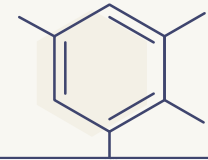


Figure 8. Geraniol oxidation reaction with TEMPO Hour 5

Figure 9. Geraniol Oxidation reaction with Syringaldehyde Hour 5

Geraniol oxidation reaction: TEMPO

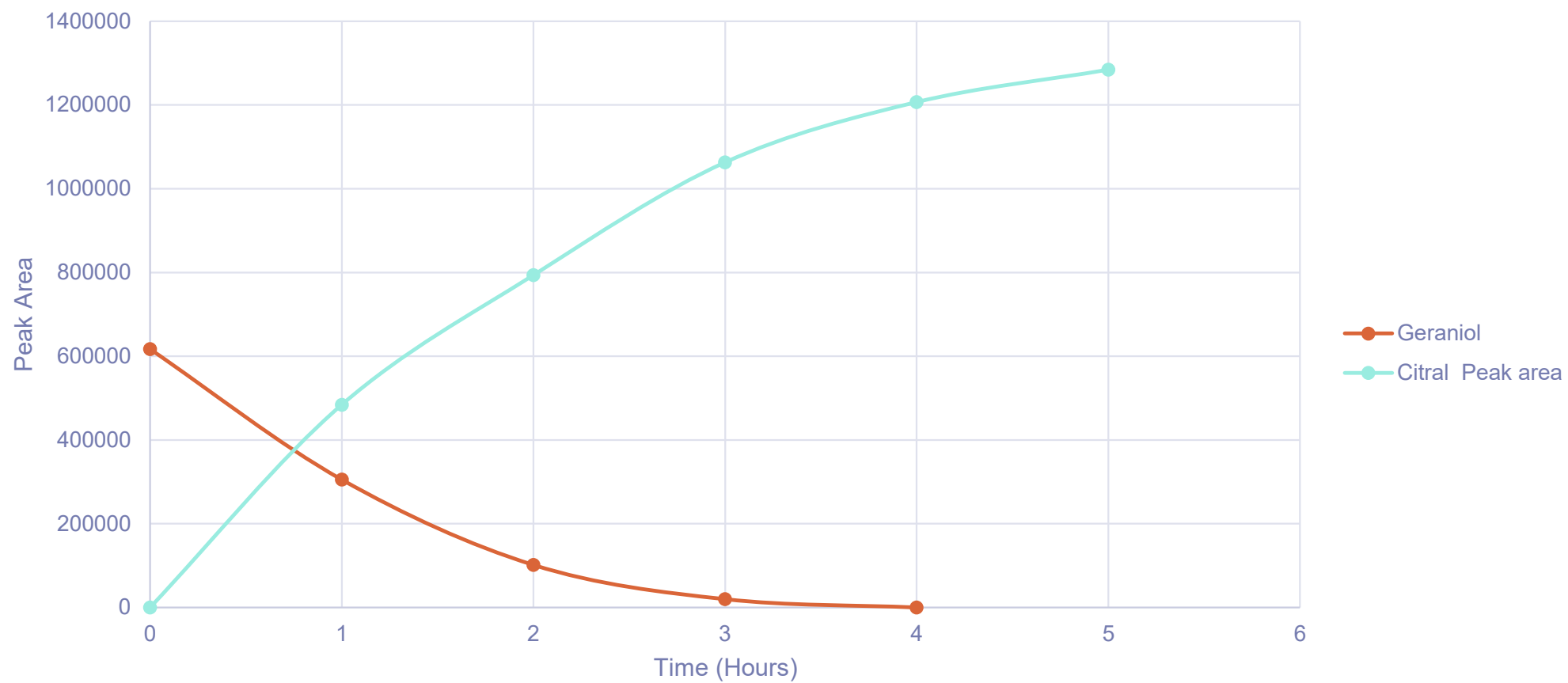


Figure 10. Geraniol oxidation reaction with TEMPO



Rate of formation of Cinnamaldehyde

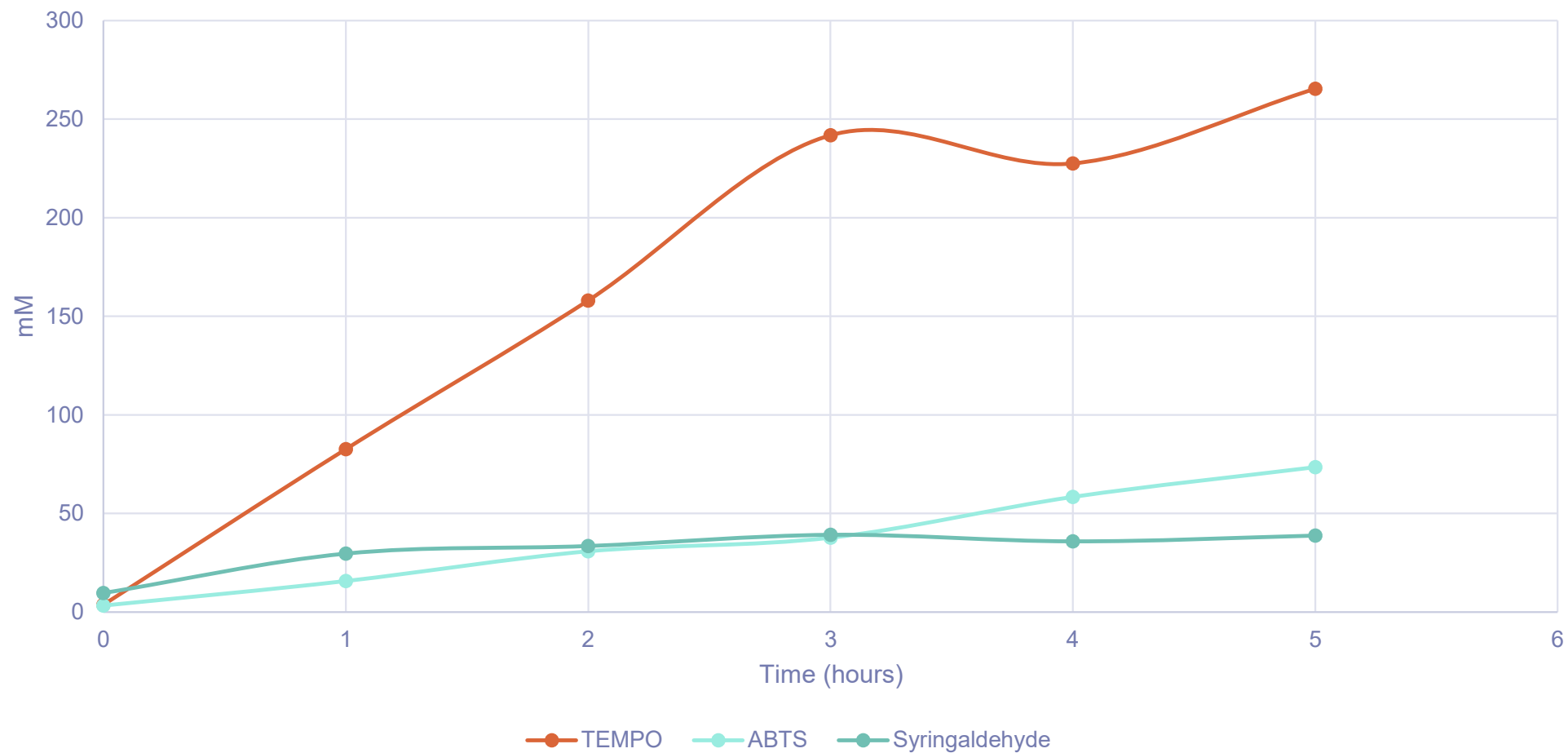
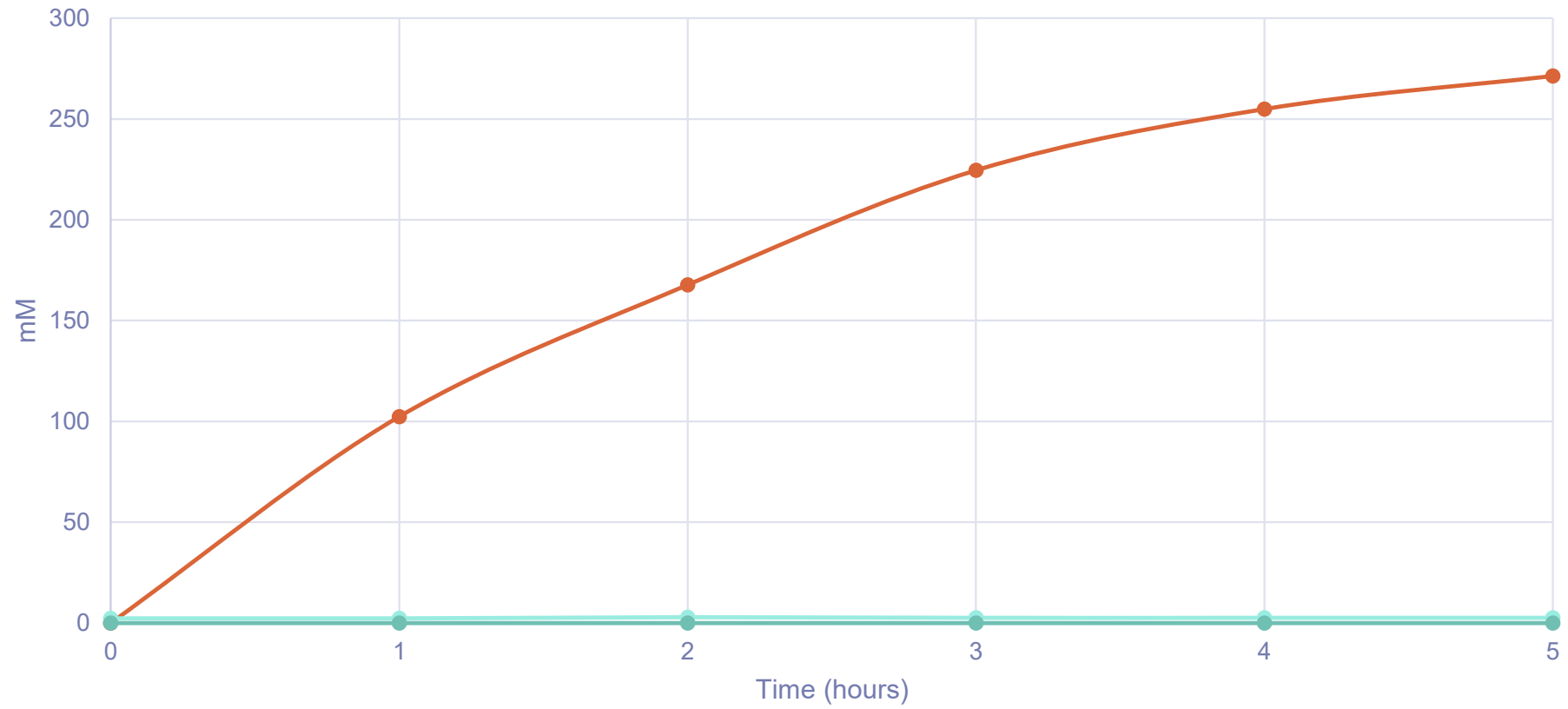


Figure 11. Rate of formation of Cinnamaldehyde

Rate of Formation of Citral

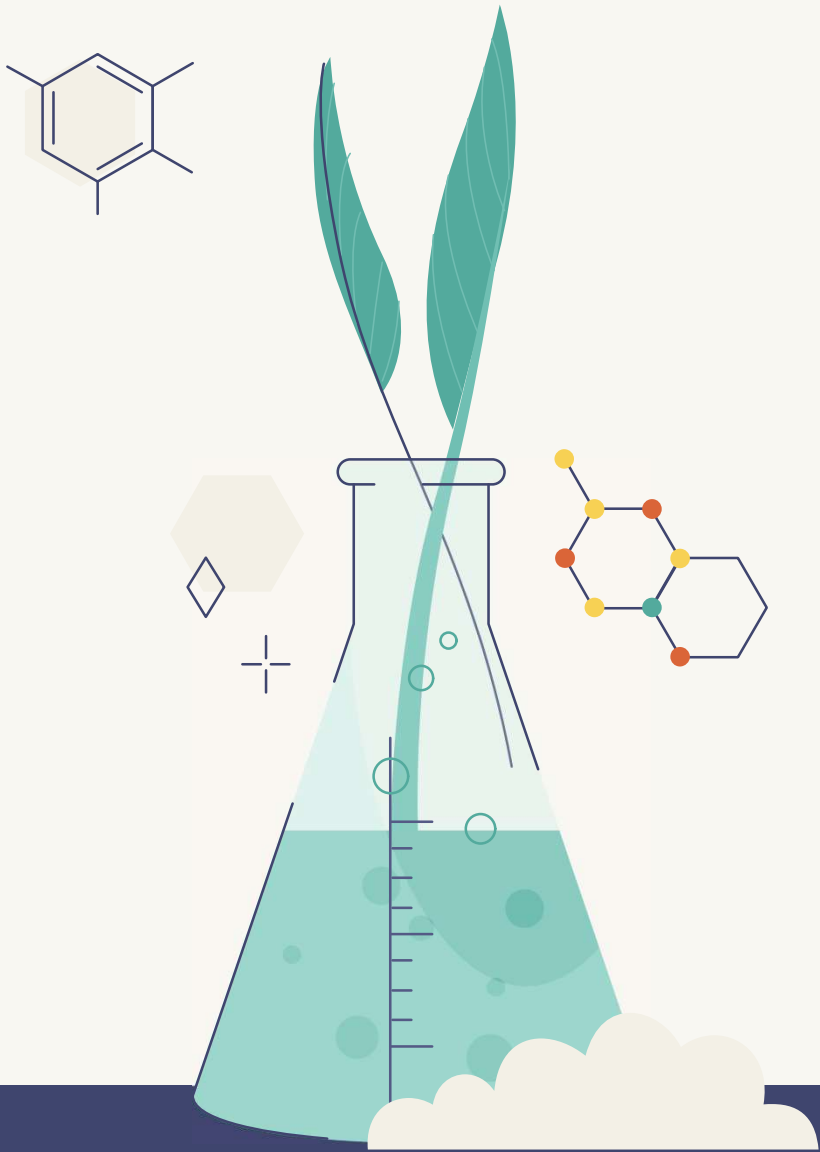


—●— TEMPO —●— Syringaldehyde —●— ABTS

Figure 12. Rate of formation of Citral

Summary of product generation

Mediators	Amount of cinnamaldehyde generated (mM)	Amount of citral generated (mM)
TEMPO	265.35	271.41
ABTS	73.48	0
Syringaldehyde	38.72	2.54



03 Discussion

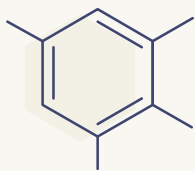
- TEMPO was the most effective mediator for both oxidation reactions
- ABTS was the second most effective mediator for the oxidation of cinnamyl Alcohol
- Syringaldehyde was the second most effective mediator for the oxidation of geraniol



04

Conclusion





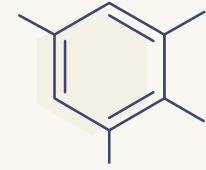
Conclusion

- TEMPO was the most effective mediator for both oxidation reaction

Areas for further research:

- Using different mediators HBT and syringaldehydes
- Wider range of substrates





05

References

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How to complete an awesome final year project

Managing time

1. Split work into manageable packages
2. Research the amount of time necessary for procedures
3. Do not overpack your timetable
4. Write up as you go along

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Thank you for listening



Any Questions?

