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KGOs: the future of sustainable chemistry

Assessment of the applicability of KGOs in biocatalysis

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Introduction

In organic synthesis, biocatalysis is necessary for green chemistry, but there is a lack of commercially available enzymes with broad selectivity. Fe(II)/α-ketoglutarate-dependent oxygenases (KGOs) show potential for site-selective functionalisation of C-H bonds.

A total of 19 KGOs and a control group consisting of an empty vector were obtained from Aminoverse B.V.

The company aims to identify promising enzyme families and to develop screening kits that could be commercially available.

The main objective of this research is to determine the activity of KGOs for application in biocatalysis by screening 20 amino acids, 4 terpenes, 3 benzenes and 1 lactone on High Performance Liquid Chromatography (HPLC).

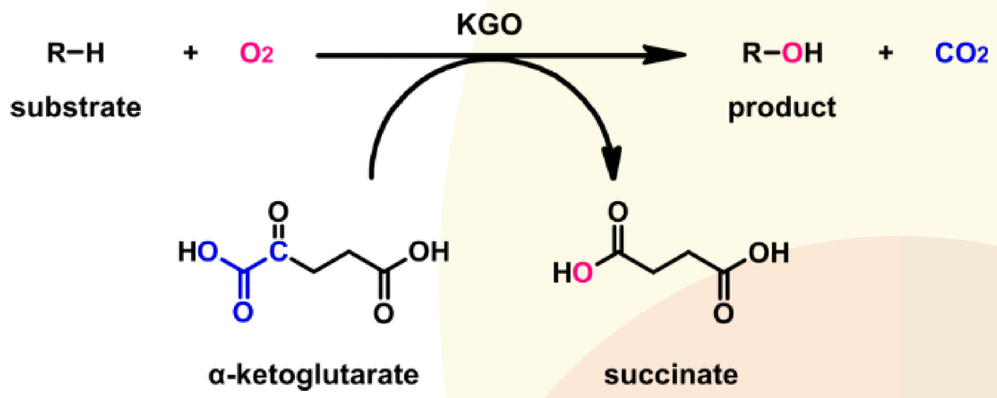

Figure 1: Reaction catalysed by KGOs. *One atom of O_2 is incorporated into the substrate (R-H), yielding the hydroxylated product (R-OH), whereas the second atom of O_2 is incorporated into succinate.*

Materials and methods

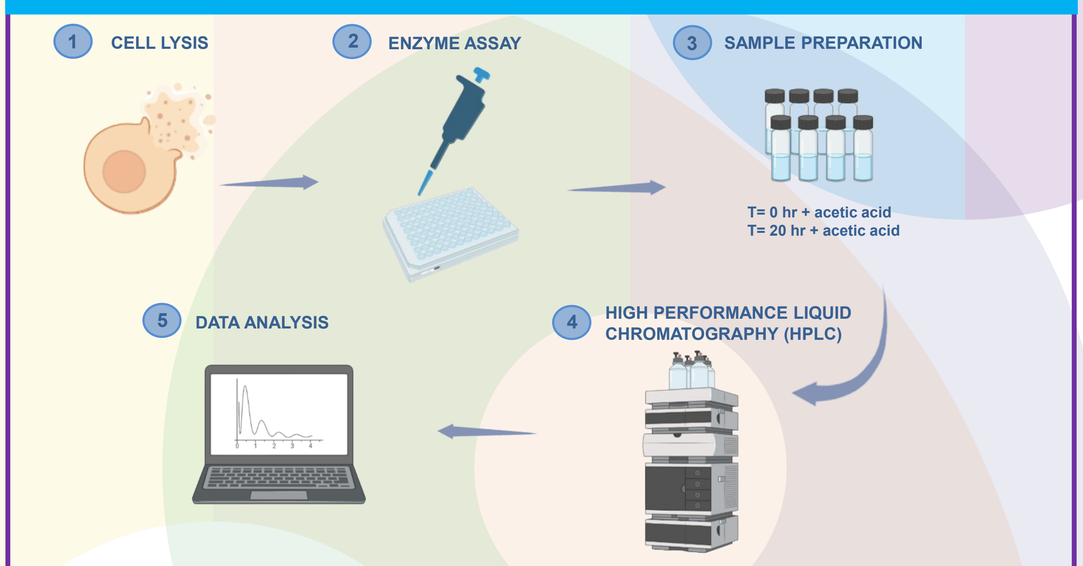

Figure 2: *1) Cell lysis is initiated to obtain entry to target proteins, which 2) are subsequently combined with a substrate and other substances to form products gradually. 3) Samples of the enzyme assay are mixed with acetic acid and taken at 0 hr and 20 hr. 4) HPLC is used to analyse the samples after incubation. 5) The results are taken and analysed using ANOVA on excel.*

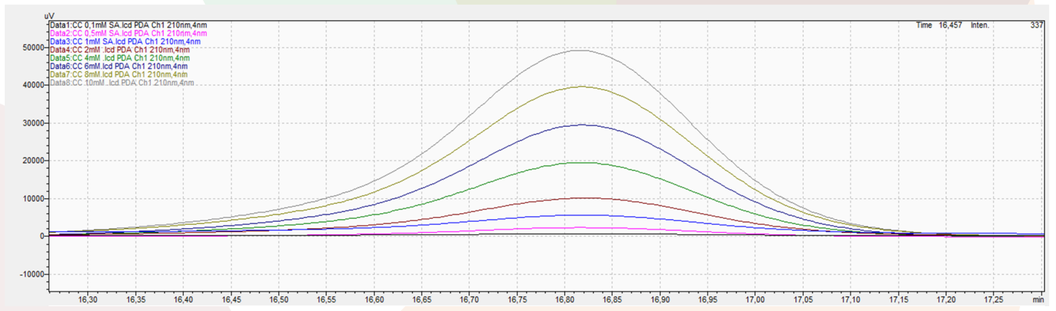

Figure 3: *Stacked chromatogram of the succinate calibration curve to determine an interval.*

Results

Table 1. Results obtained through HPLC analysis. *Green: Active; Grey: Not active.*

	SUBSTRATE	KGO1	KGO4	KGO7	KGO8	KGO11	KGO18	KGO25	KGO27	KGO28	KGO32	KGO 34	KGO37	KGO40	KGO43	KGO44	KGO45	KGO47	KGO49	KGO50	E.V.
Amino acids	Arginine																				
	Histidine																				
	Lysine																				
	Aspartic acid																				
	Glutamic acid																				
	Alanine																				
	Valine																				
	Isoleucine																				
	Leucine																				
	Methionine																				
	Phenylalanine																				
	Tyrosine																				
	Tryptophan																				
	Serine																				
	Threonine																				
	Asparagine																				
	Glutamine																				
	Glycine																				
	Cysteine																				
	Proline																				
Benzenes	Ethylbenzene																				
	Isopropyl benzene																				
	2-pheonyxpropionic acid																				
Terpenes	Linalool																				
	Limonene																				
	β-ionone																				
Lactones	ε-caprolactone																				

Conclusion & Recommendations

Conclusion: active KGOs

- Amino acids: KGO 1, 4, 7, 8, 11, 18, 25, 27, 28, 32, 34, 40, 43, 45, 47 and 50
- Benzenes: 1, 18, 25, 32, 40, 45, 47 and 50
- Terpenes: KGO 1, 7, 11, 18, 25, 27, 45, 47 and 49
- Lactones: 1, 7, 11, 18, 25, 32, 45 and 47
- KGO 25 was active on 74.1% of the substrates and KGO 45 on 55.6% of the substrates

Recommendations:

- Calibrate HPLC
- Spiking
- LC-MS/MS
- Fresh reagents for the enzyme assay

References

[1] Aminoverse B.V., *KGO-KIT - Establishing the information base for a commercial biocatalysis kit containing α-ketoglutarate-dependent oxygenases,* Heerlen, 2022.

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