Supporting Information

Cascading Old Yellow Enzyme, Alcohol Dehydrogenase and Glucose Dehydrogenase for Selective Reduction of (*E/Z*)-Citral to (*S*)-Citronellol

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Supplementary tables

Table S1. The primers for the construction of the plasmid pACYCDuet-1-Y*sADH*

Primer	Sequence
pET28b-*YsADH*-F	5′-TAATAAGGAGATATACCATGGGCATGTCTATTATAAAAAGCTATGCCGC-3′
pET28b-*YsADH*-R	5′-TAAGCATTATGCGGCCGCAAGCTTAAAGTCGGCTTGCAGTACCACG-3′
pACYCDuet-1-F	5′-CGGCATAGCTTTTTATAATAGACATGCCCATGGTATATCTCCTTATTAAAGTTAAACAAAATTATTTC-3′
pACYCDuet-1-R	5′-TGGTACTGCAAGCCGACTTTAAGCTTGCGGCCGCATAATGCTTAAG-3′

Table S2. The primers for the construction of the plasmid pACYCDuet-1-Y*sADH-NemR-PS*

Primer	Sequence
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pACYCDuet-1-*YsADH*-F	5′-CTTTTTCTGGCTCATCATATGTATATCTCCTTCTTATACTTAACTAATATACTAAGATGGG-3′
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>OYE3

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Figure S1. The codon-optimized nucleotide sequences encoding old yellow enzymes, alcohol dehydrogenase and glucose dehydrogenase.
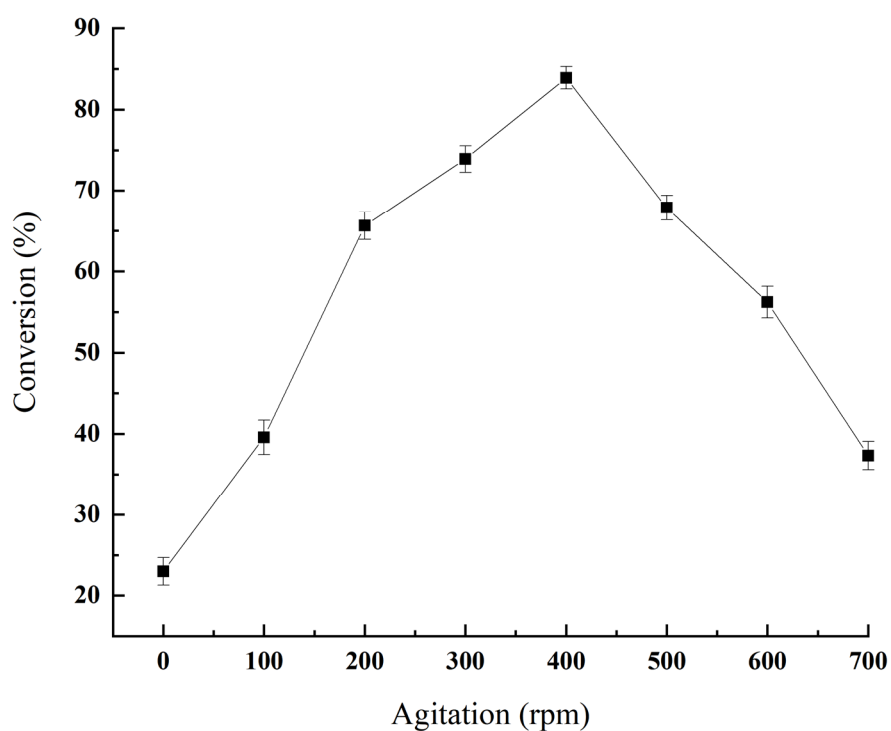
Figure S2. The effect of agitation speed on enzymatic double reduction of (*E/Z*)-citral to (*S*)-citronellol. The reaction mixture (10 ml) contained 200 mM (*E/Z*)-citral, 600 mM glucose, 1 g wet cells, 0.2 mM NADP$^+$, 20%(v/v) isopropanol and 50 mM PIPES buffer (pH 6.5). The reactions were conducted at 30 °C and 0-700 rpm for 12 h. Standard deviations are indicated in the diagram (n=3).
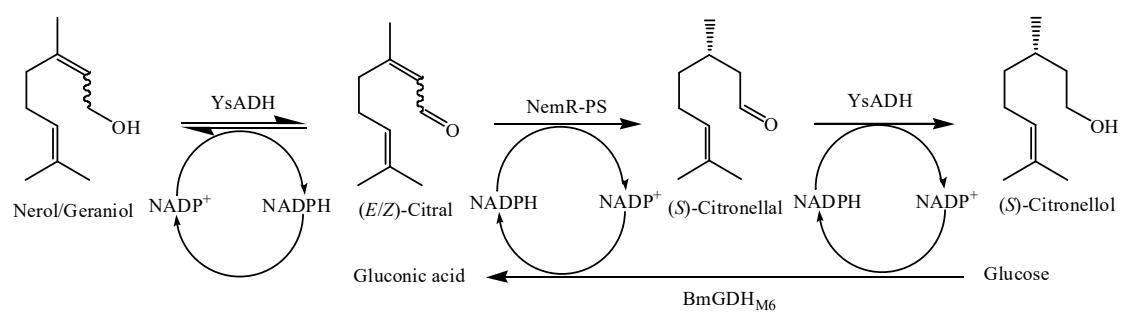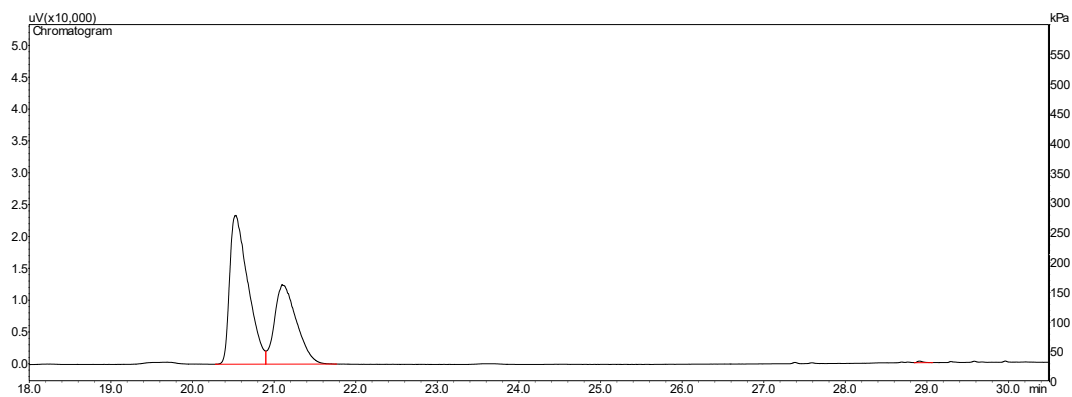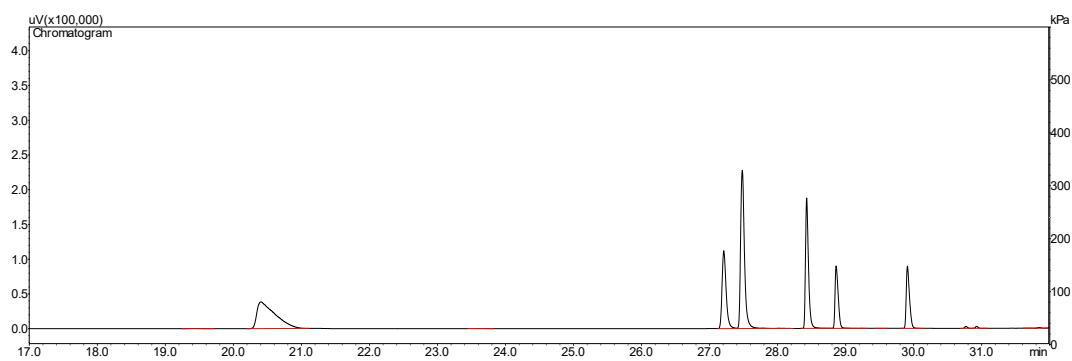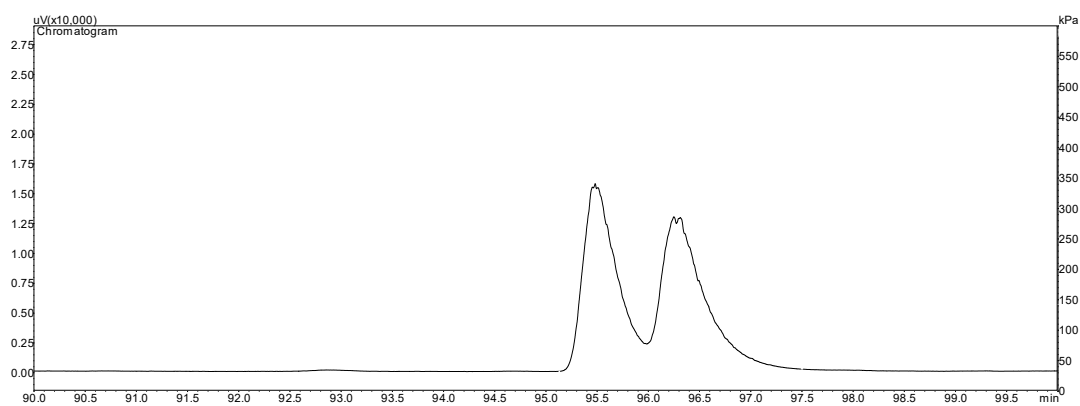
Figure S3. Schematic formation and consumption of the intermediate (*S*)-citronellal and the by-products nerol and geraniol.

(a)

(b)

(c)
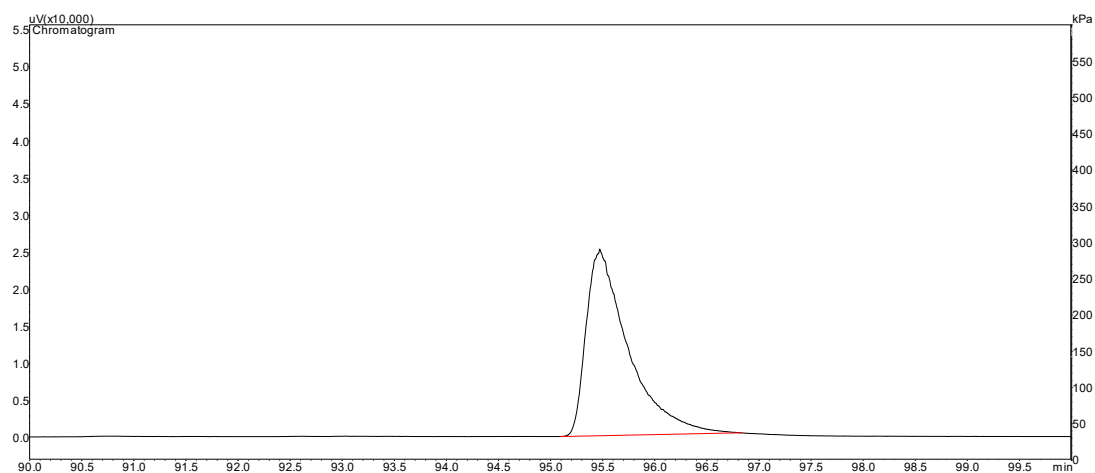
(d)

Figure S4. GC chromatogram for various standards used in the study and the product extracted from the reaction mixture. (a), GC chromatogram for (*S*)-citronellal (20.128 min) and (*R*)-citronellal (21.098 min). (b), GC chromatogram for (*S*)-citronellal (20.128 min), nerol (27.208 min), citronellol (27.480 min), geraniol (28.419 min), (*E*)-citral (28.842), (*Z*)-citral (29.890 min). (c) GC chromatogram for (*S*)-citronellol (95.481 min) and (*R*)-citronellol (96.249 min). (d) GC chromatogram for the product (95.481 min) extracted from the reaction mixture.
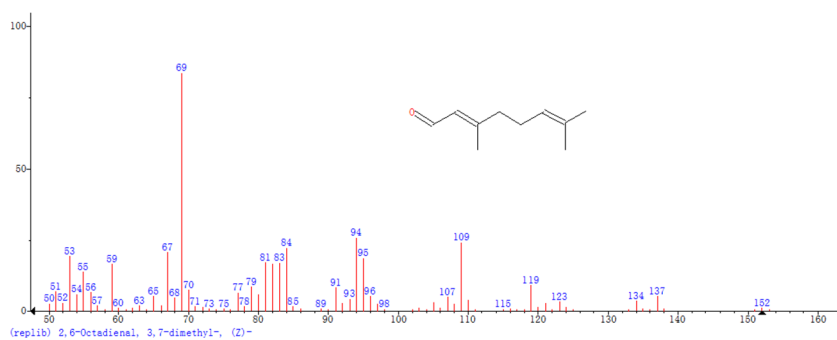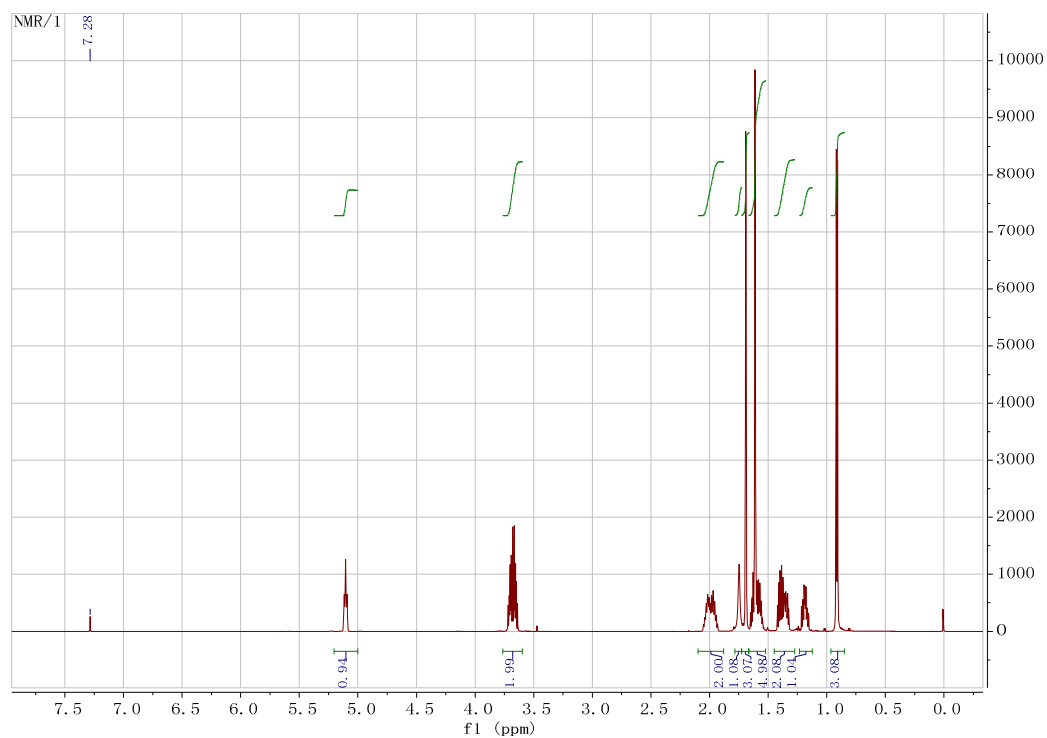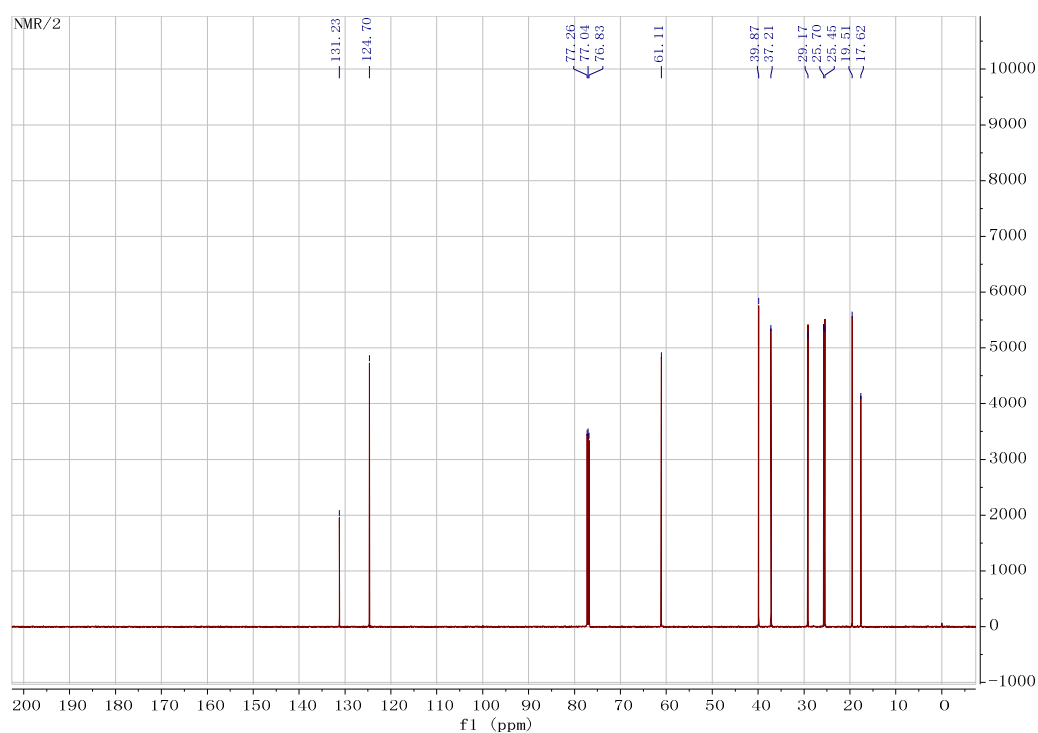
(a)
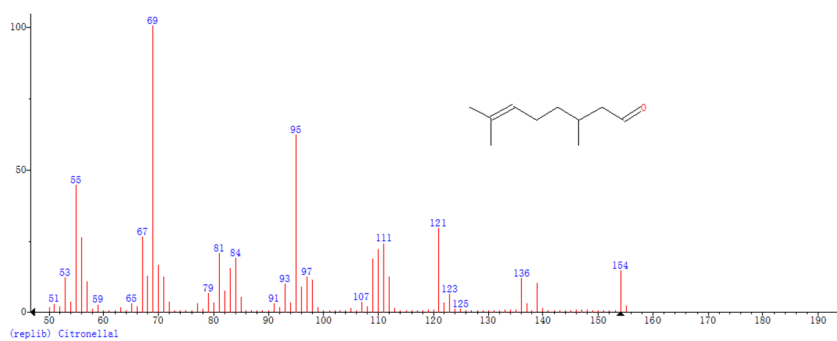
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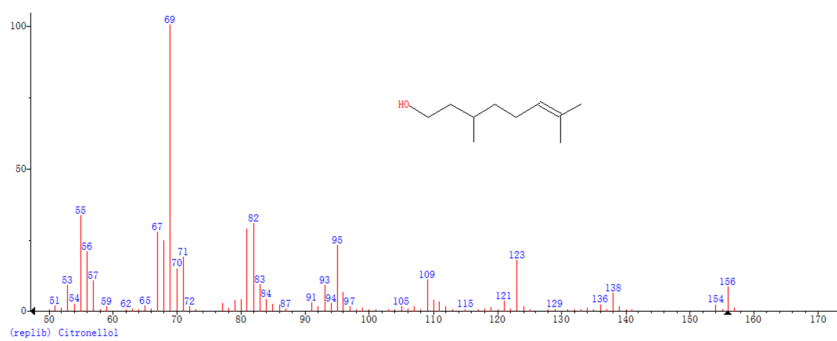
(c)

Figure S5. GC-MS analyses of substrate, intermediate and product. (a), GC-MS chromatogram for citral (MW 152). (b), GC-MS chromatogram for citronellal (MW 154). (c), GC-MS chromatogram for citronellol (MW 156).

(a)

(b)

Figure S6. The ¹H NMR (a) and ¹³C NMR (b) analyses of the product (*S*)-citronellol. (a), ¹H NMR (600 MHz, Chloroform-d) δ 5.08 (dddd, J = 8.6, 5.8, 2.7, 1.3 Hz, 1H), 3.71 – 3.60 (m, 2H), 1.99 – 1.90 (m, 2H), 1.66 (d, J = 1.6 Hz, 3H), 1.62 – 1.58 (m, 3H), 1.58 – 1.52 (m, 2H), 1.40 – 1.29 (m, 2H), 1.16 (dddd, J = 13.4, 9.5, 7.7, 5.8 Hz, 1H), 0.89 (d, J = 6.6 Hz, 3H). (b), ¹³C NMR (151 MHz, CDCl₃) δ 131.23, 124.70, 61.11, 39.87, 37.21, 29.17, 25.70, 25.45, 19.51, 17.62.

Figure S7. The setup of the reactor with constant pH auto-titration system.