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Supporting information

Integrating Fermentation Engineering and Organopalladium Chemocatalysis for the Production of Squalene from Biomass-derived Carbohydrate as the Starting Material

Cuicui Wu, Kaifei Tian, Xuan Guo * , Yunming Fang *

National Energy R&D Research Center for Biorefinery, Department of Chemical Engineering, Beijing University of Chemical Technology, Beijing 100029, China; wu941210@buct.edu.cn (C.W.); tiankfei@126.com (K.T.)

* Correspondence: Corresponding author: Dr. Xuan Guo, guoxuan19930426@163.com; Prof. Yunming Fang, fangym@mail.buct.edu.cn

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Materials and Methods

Solution composition

Vitamin solution included 50 mg/L of biotin, 80 mg/L of NaOH, 1 g/L of D-pantothenic acid, 1 g/L of thiamin HCl, 1 g/L of pyridoxin HCl, 1 g/L of nicotinic acid and 1 g/L of 4-aminobenzoic. Trace metal solution included 5.75 g/L of $ZnSO_4 \cdot 7H_2O$, 0.32 g/L of $MnCl_2 \cdot 4H_2O$, anhydrous 0.32 g/L of $CuSO_4$, 0.47 g/L of $CoCl_2 \cdot 6H_2O$, 0.48 g/L of $Na_2MoO_4 \cdot 2H_2O$, 2.80 g/L of $FeSO_4 \cdot 7H_2O$, 2.90 g/L of $CaCl_2 \cdot 2H_2O$ and 0.5 M 80 mL/L of EDTA (pH = 8).

Preparation of preculture and shake flask cultivations

1. 20 µL of frozen cells were transferred to 4 mL Yeast Peptone Dextrose medium in a 50 mL glass tube, cultivated in the shaker at 200 rpm and 30 °C overnight.
2. The resulting (1) bacterial solution was streaked on an agar plate and placed in a 28 °C incubator for 2-3 days, in order to further activate the strains.
3. Excellent single colonies were screened, further introduced to 5 mL Yeast Peptone Dextrose medium in a 50 mL glass tube. After cultivation overnight at 200 rpm and 30 °C, a primary seed solution was obtained.
4. 1 mL of primary seed solution was inoculated into a 250 mL shake flask containing 50 mL seed medium and cultured in a shaker for 8-12 h to get a secondary seed solution with OD_{600} of 1-2 used for 2.5 L bioreactor cultivations.

Table S1. Biobased β-farnesene of the ^{1}H and ^{13}C NMR-chemical shifts.

Atom marked	δ ^{1}H-NMR (ppm)	δ ^{13}C-NMR (ppm)
1	5.02-5.30	115.71
2	6.37-6.44	146.13
3	-	139.08
4	5.02-5.30	112.94
5	1.99-2.29	31.49
6	1.99-2.29	26.78
7	5.02-5.30	124.47
8	-	135.29
9	1.63-1.74	16.0
10	1.99-2.29	39.79
11	1.99-2.29	26.69
12	5.02-5.30	124.13
13	-	131.13
14	1.63-1.74	17.65
15	1.63-1.74	25.69

Table S2. Concentration gradient of standard curve.

Compounds	Concentration gradient					
β-farnesene	0.5 g/L	1 g/L	2 g/L	3 g/L	4 g/L	5 g/L
tetradecane	900 mg/L	900 mg/L	900 mg/L	900 mg/L	900 mg/L	900 mg/L

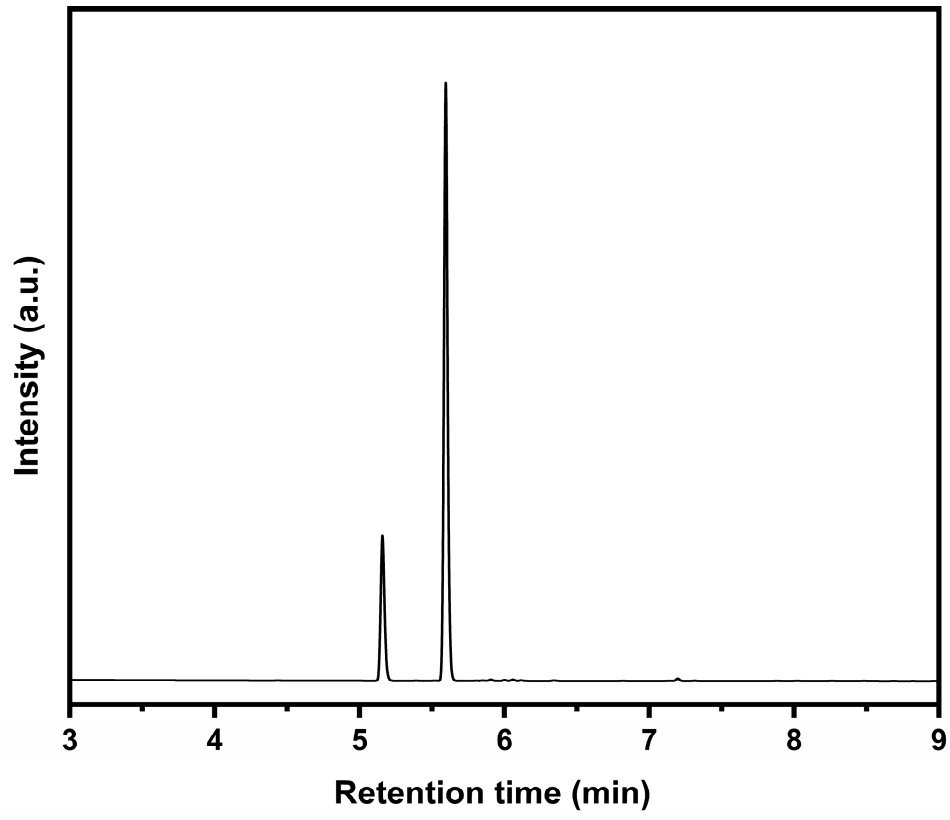

Figure S1. GC chromatogram of β-farnesene using internal standard method (Retention time 5.102 min: tetradecane; Retention time 5.545 min: β-farnesene).

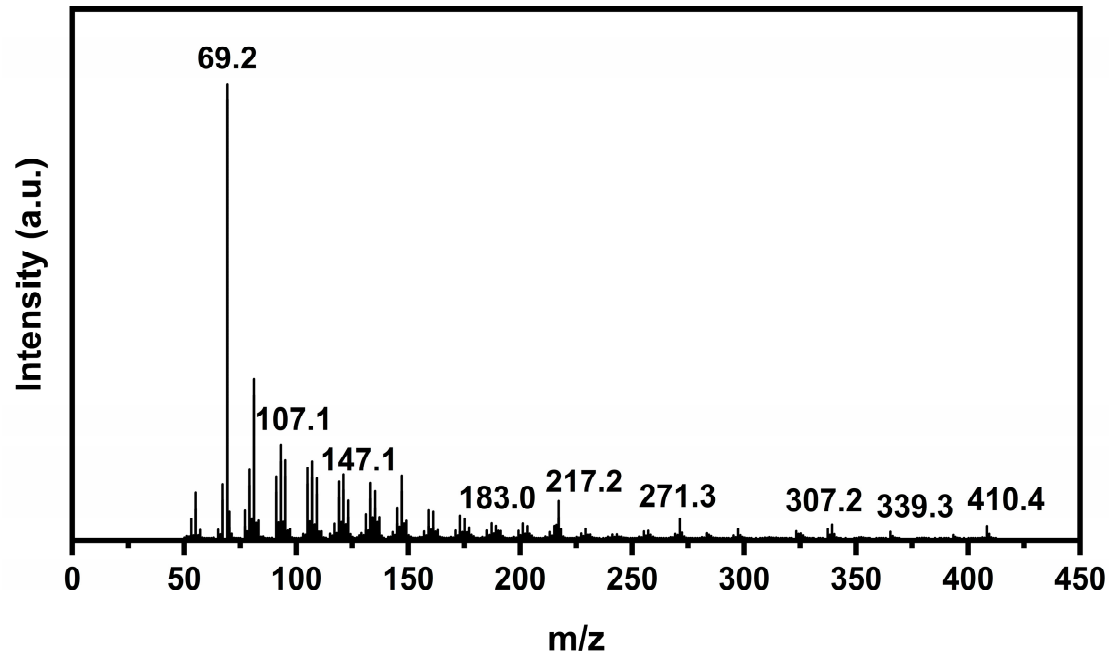

Figure S2. Mass spectrum of the purified squalene product.

Table S3. Purified squalene of the ^{1}H and ^{13}C NMR-chemical shifts.

Atom marked	δ ^{1}H-NMR (ppm)	δ ^{13}C-NMR (ppm)
1	1.29-1.35	22.67
2	-	135.14
3	5.10-5.20	124.41
4	1.99-2.84	26.75
5	1.99-2.84	40.01
6	-	129.44
7	5.10-5.20	124.43
8	1.99-2.84	26.49
9	1.99-2.84	39.93
10	-	132.32
11	5.10-5.20	124.35
12	1.99-2.84	26.77
13	1.99-2.84	26.85
14	5.10-5.20	124.41
15	-	132.82
16	1.99-2.84	39.75
17	1.99-2.84	26.42
18	5.10-5.20	124.26
19	-	131.17
20	1.99-2.84	39.95
21	1.99-2.84	26.57
22	5.10-5.20	124.08
23	-	135.19
24	1.29-1.35	22.75
25	1.68-1.71	16.02
26	1.63-1.66	17.67
27	1.63-1.66	17.58
28	1.63-1.66	17.55
29	1.63-1.66	17.70
30	1.68-1.71	16.08

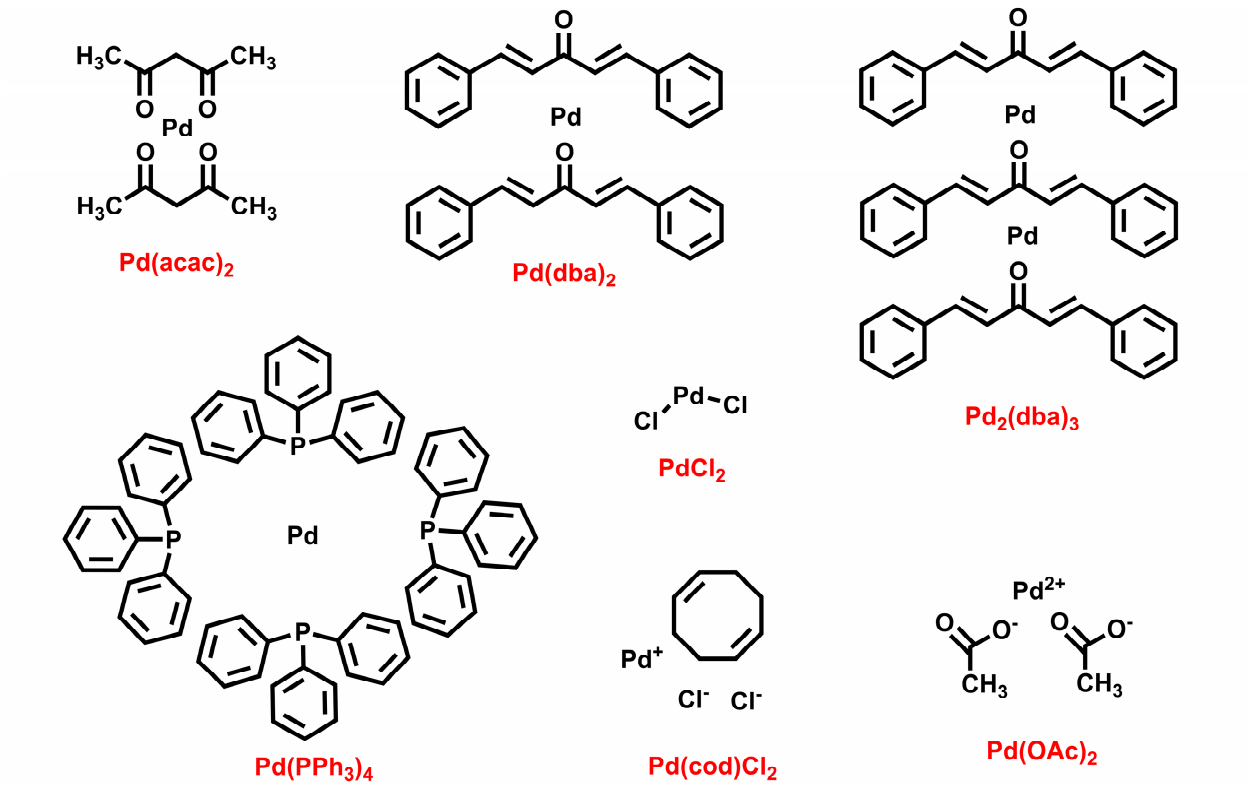

Figure S3. Molecular structure of homogeneous palladium catalysts.

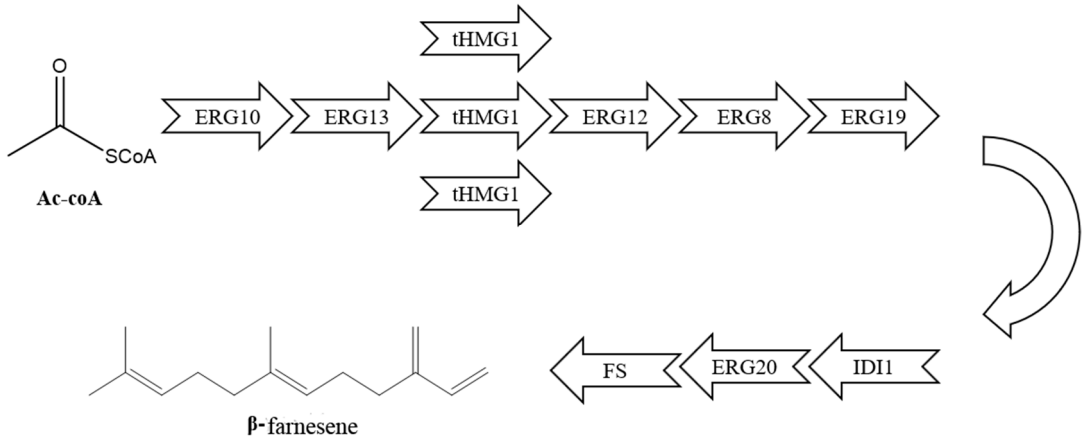

Figure S4. Strain transformation route.