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EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Analysis of 1-Decene biotransformation samples by Gas Chromatography

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Instruction

Analysis of 1-Decene biotransformation samples by Gas Chromatography

1 Introduction / Purpose

The purpose of this protocol is to analyze 1-Decene biotransformation samples by Gas Chromatography.

2 Equipment and chemicals

2.1 Equipment

Shimadzu GC2010 equipped with AOC 20s auto sampler and AOC 20i auto injector

Agilent 7890A GC/MS system (Santa Clara, US) equipped with a flame ionization detector as well as mass selective detector (Agilent technologies 5975C inert MSD, Santa Clara, US)

HP-1 dimethylpolysiloxane column (30 m, 0.25 mm, 0.25 µm; Agilent, Santa Clara, USA)

DB-5 column ((5 %-phenyl)-methylpolysiloxane, 30 m, 0.25 mm, 0.25 µm; Agilent, Santa Clara, USA)

CP-Chirasil-Dex CB column (25 m, 0.25 mm, 0.25 µm; Agilent, Santa Clara, USA)

2.2 Chemicals

Glucose (100mM)
FAD (0.3mM)
NADH
DTT
1-decene (0.5mM)
Hexanoic acid (0.5mM)
DMSO
NaOH (1M)
MTBE
1-octanol
Acetic anhydride
4-dimethylaminopyridine (DMAP)
Na_2SO_4

2.3 Bacterial strains

E. coli BL21 (DE3)

2.4 Other materials

Plasmid pET28a(+)

3 Media and buffers

3.1 Buffer for detailed analysis of MODDE-optimized conditions

100 mM glucose
0.3 mM FAD
3 mM NADH
2 mM DTT

3.2 Citrate Buffer

50mM citrate buffer (pH 6)

4 Procedures

4. 1 Conversion of 1-decene

1-Decene biotransformations were performed in 50 mM citrate buffer pH 6 at the 500 µL-scale in 2 ml glass vials as reaction tubes. E. coli BL21 (DE3) pET28a(+) served as negative control. Expression cultures were thawed on ice, resuspended in citrate buffer and disrupted by sonication for lysate assays. 50 mg mL^{-1} cell suspension and 3.8 mg mL^{-1} protein served for whole cell and lysate biotransformations, respectively. 100 mM glucose, 0.3 mM FAD, 3 mM NADH and 2 mM DTT were applied for the detailed analysis of MODDE-optimized conditions. 100 mM glucose and 0.3 mM FAD were applied for the direct comparison of whole cells and lysate under optimized and non-optimized conditions.

Reactions were started by the addition of substrate and dummy substrate (0.5 mM 1-decene and 0.5 mM hexanoic acid, respectively) with 1 % final DMSO concentration and incubated at 25 °C and 800 rpm. Biotransformation samples (triplicates) were quenched by addition of 20 µl 1 M NaOH and extracted twice with 500 µl MTBE and 500 µM 1-octanol as internal standard. After each extraction step 300 µL of organic phase was collected and directly analyzed by GC/GC-MS.

For chiral GC analysis, 250 µl acetic anhydride and a catalytic amount of 2 mg 4-dimethylaminopyridine (DMAP) were added to the organic phase (600 µl). After one hour ofincubation at 25 °C and 800 rpm 300 µl water was added. The organic phase was transferred to a newreaction tube, dried over Na 2 SO 4 and analyzed by GC-FID (CP-Chirasil-Dex CB).

4.2 Gas Chromatography analysis

1-Decene biotransformation samples were analyzed by GC without derivatization using a HP-1 dimethylpolysiloxane column (30 m, 0.25 mm, 0.25 µm; Agilent technologies, Santa Clara, USA) on a Shimadzu GC2010 (Shimadzu, Kyōto, Japan) equipped with an AOC 20s autosampler and AOC 20i autoinjector (injection volume: 1 µL, split ratio: 1:5, injection temperature: 250 °C).

Hydrogen served as carrier gas with a linear velocity of 30 cm s^{-1} and the detector temperature was 330 °C. The initial oven temperature was set to 80 °C, raised to 160 °C at a speed of 10 K mi $^{-1}$, subsequently increased to 320 °C with a rate of 50 K min^{-1} and held for 4 min. Quantification was done with 1-octanol as internal standard.

GC-MS analysis of 1-decene biotransformation samples was performed using a DB-5 column ((5 %-phenyl)-methylpolysiloxane, 30 m, 0.25 mm, 0.25 µm; Agilent technologies, Santa Clara, USA) on an Agilent 7890A GC/MS system (Santa Clara, US) equipped with a flame ionization detector as well as mass selective detector (Agilent 5975C inert MSD, Santa Clara, US).

Helium served as carrier gas (constant pressure of 16.521 psi) and the detection temperature was 320 °C. 1 µl sample was injected with a split ratio of 1:10 at an injection temperature of 250 °C. The initial oven temperature was set to 80 °C, raised to 140 °C at a speed of 15 K min^{-1} , subsequently increased to 310 °C with a rate of 40 K min^{-1} and held for 5 min.

Stereoselectivity of 1-decene hydration was analyzed by chiral GC using a CP-Chirasil-Dex CB column (25 m, 0.25 mm, 0.25 µm; Agilent technologies, Santa Clara, USA) on a Shimadzu GC2010 (Shimadzu, Kyōto, Japan) equipped with an AOC 20s autosampler and AOC 20i autoinjector (injection volume: 1 µL, split ratio: 1:20, injection temperature: 220 °C).

Hydrogen served as carrier gas with a linear velocity of 40 cm s^{-1} and the detector temperature was 230 °C. The initial oven temperature was set to 100 °C and held for 1 min, raised to 120 °C at a speed of 10 K min^{-1} and held for 2.5 min, subsequently increased to 150 °C with a rate of 10 K min^{-1} and finally raised to 200 °C (50 K min^{-1}) and held for 3 minutes.

5 remarks/troubleshooting

6 Biosafety

No biosafety issues were associated with this protocol when applied to E. coli. The protocol was developed and performed at an S1 laboratory.

7 Acknowledgements

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