ITB, Stuttgart	EPP-SOP-ITB07
	Version 1.0

EPP - Standard Operating Procedure

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

Title: Downstream processing and analysis of samples of degradation experiments 2:terpenoids

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experimenter 1	Max-Philipp Fischer		1st April 2019

Instruction

Downstream processing and analysis of samples of degradation experiments 2:terpenoids

1 *Introduction / Purpose*

EPP-SOP-ITB05 aims the identification of most suitable *P. putida* strains towards the stability of the terpenoids nerol, R/S-citronellol, geraniol and linalool in strains EP1 and EM383 harboring CRISPRi constructs for the downregulation of all 19 ADHs. This protocol is describing the downstream processing and the analysis of the samples generated in EPP-SOP-ITB05. In that protocol, the provision of whole cell samples to be analyzed for the degradation of these terpenoids is described. This SOP, however, is regarding the processing of those samples and their analysis.

2 *Equipment and chemicals*

Equipment

2 mL Deep Well Plates (DWPs)

Eppendorf Centrifuge 5810R (for DWPs)

2 mL autosampler vials (11 mm)

Shimadzu GC/MS-QP2010 equiped with PAL AOC-5000 Auto Injector
column: HP-5msi (30 m, 0.25 mm, 0.25 µm; Agilent technologies)

Chemicals

Cyclohexane (99.5 %, Roth)

3 *Procedures*

Preparation of cells and extraction

The preparation of cells and the procedure of taking samples in order to determine the degradation of the terpenoids nerol, R/S-citronellol, geraniol and linalool is described in EPP-SOP-ITB05. 1 mL of the whole cell samples were provided and harvested by centrifugation at 4 °C and 4000 rpm for 20 min. After harvesting, 800 µL of the supernatant were transferred into GC vials, taking great care not to disrupt the cell pellet, and immediately overlayed with 800 µL cyclohexane. Extraction was performed by incubation of the carefully sealed vials at 180 rpm for 1 h. Afterwards, the vials were transferred to GC/MS and analyzed according to EPP-SOP-ITB01.

Quantification via GC/MS

Quantification was performed by GC/MS using Shimadzu GC/MS-QP2010 equiped with PAL AOC-5000 Auto Injector with HP-5msi column (30 m, 0.25 mm, 0.25 µm; Agilent technologies). 1 µL was

injected directly out of the organic phase at 250 °C injection temperature at a split ratio of 1:50. Helium served as carrier gas with a constant pressure of 26.7 kPa and the detection temperature was 280 °C. Measurements were performed isothermally at 160 °C for 3.25-4.25 min according to the analyte. Table 1 gives the detection methods in detail.

Table 1: GC/MS programs. ET, event time.

Compound	GC			MS				Ret. [min]
	T [°C]	t [min]	Split	Modus	m/z	ET [s]	t [min]	
nerol	160	4.00	50	Scan	15-160	0.3	3.50-4.00	3.83
R-citronellol	160	4.00	50	Scan	15-160	0.3	3.50-4.00	3.77
S-citronellol	160	4.00	50	Scan	15-160	0.3	3.50-4.00	3.77
geraniol	160	4.25	50	Scan	15-160	0.3	3.75-4.25	3.99
linalool	160	3.25	50	Scan	15-160	0.3	2.75-3.25	3.04

Semi-quantification was performed by normalizing the obtained areas relative to the areas of the buffer controls of each sample and timepoint.

4 *remarks/troubleshooting:*

Terpenoids tend to be integrated into the cell membranes as well as plastics. Therefore, one has to plan the experiments accordingly and use the right controls in order not to obtain unexplainable results.

5 *Biosafety:*

No biosafety issues were associated with this protocol. The protocol was developed and performed at an S1 laboratory. The pipetting of cyclohexane was performed in the fume hood due to the harmfulness of vapors (H225-304-315-336-410). For protection against the extraction agent cyclohexane, nitrile gloves are to be worn at all time when handling the samples.

6 *Acknowledgements*

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