ITB, Stuttgart	EPP-SOP-ITB06
	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 1:short chain alcohols

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experimenter 1	Max-Philipp Fischer		1st April 2019

Instruction

Downstream processing and analysis of samples of degradation experiments 1:short chain alcohols

1 *Introduction / Purpose*

EPP-SOP-ITB04 aims at the identification of most suitable *P. putida* strains towards the stability of the short chain alcohols n-butanol, isobutanol and crotyl alcohol. Therefore, strains KT2440, GN346, EP1, EP2, EM42 and EM383 have been chosen to be analyzed. This protocol is describing the downstream processing and the analysis of the samples generated in EPP-SOP-ITB04. In that protocol, the provision of whole cell samples to be analyzed for the degradation of these alcohols 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)

Silicon mats for sealing 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

MTBE *(tert*-Butyl methyl ether) (Roth 99.5%, Cat. No. 6746.4)

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 short-chain alcohols n-butanol, isobutanol and crotyl alcohol is described in EPP-SOP-ITB04.
500 µL of the whole cell samples were provided and harvested by centrifugation at 4 °C and 4000 rpm for 20 min. All actions have to take place in the cold room due to the extreme volatility of the analytes. After harvesting, 400 µL of the supernatant were transferred into fresh 96 DWPs, taking great care not to disrupt the cell pellet. Extraction was performed by adding 400 µL cold MTBE and incubation of the carefully sealed plates at 1000 rpm for 10 min. Afterwards, the plates were centrifuged at 4000 rpm and 4 °C for 20 min. 200 µL of the organic phase were transferred into GC vials with inlets and carefully sealed. Quantification was performed via GC/MS.

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 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 70 °C for 3 min. 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]	
n-butanol	70	3.00	50	SIM	56	0.3	2.25-2.70	2.54
isobutanol	70	3.00	50	SIM	43	0.3	2.25-2.70	2.37
crotyl alcohol	70	3.00	50	SIM	57	0.3	2.25-2.70	2.57

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:*

It is highly recommended to perform the sample preparation and extraction in the cold room due to the extreme volatility of the analytes.

5 *Biosafety:*

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

6 *Acknowledgements*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635536.