IBVT Universität Stuttgart	EPP-SOP-IBVT03
	Version 1.0

EPP - Standard Operating Procedure

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

Title: Sampling for nucleotide analysis from chemostat cultivation with *P. putida*

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	name	signature	date
experimenter 1	Andreas Ankenbauer		11th March 2019

Instruction

1 Introduction / Purpose

This SOP is a standardized protocol for sampling for nucleotide analysis (e.g. NADP, NAD, AMP, ADP ATP) from chemostat cultivations with *Pseudomonas putida* in a lab scale bioreactor. Afterwards samples can be analyzed for quantitative amounts of intracellular nucleotides using HPLC and calculating energy charge. This protocol is adopted from (Cserjan-Puschmann et al. 1999)

2 Equipment and chemicals

2.1 Equipment and Media

- ⇨ 15 mL reaction tubes
- ⇨ PCA solution = Perchloric acid (35 % v/v) containing 80 µM EDTA, keep at -20 °C; ! ATTENTION! PCA is quite reactive when mixing with aqueous solution. Make sure to keep it cold and mix it carefully with appropriate safety precautions!
- ⇨ 1 M K_2HPO_4
- ⇨ 5 M KOH

2.2 Bacterial strains

Pseudomonas putida KT2440

3 Media and buffers

3.1 HPLC buffers and method

Buffer A: 0.1 M KH_2PO_4, 0.1 M K_2HPO_4, 4 mM TBAS; pH6
adjust pH 6 with phosphoric acid (H_3PO_4) / potassium hydroxide (KOH)

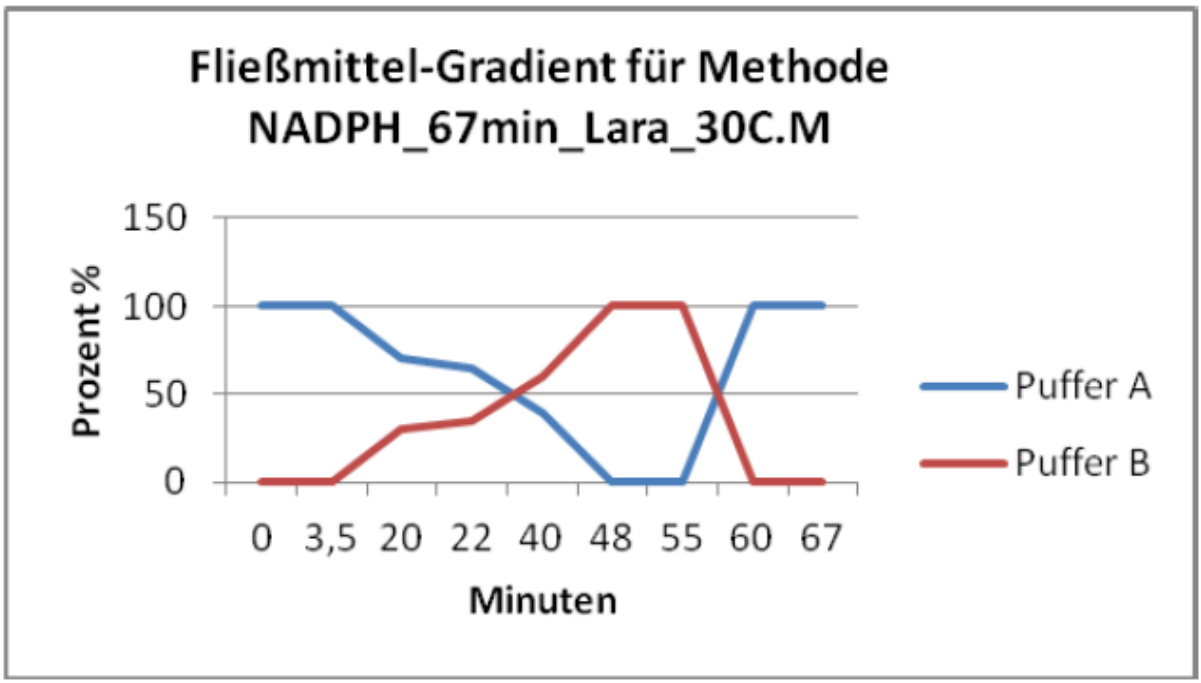
Buffer B: 0.1M KH_2PO_4, 0.1 M K_2HPO_4, 4 mM TBAS; pH7, 2 + 30% methanol
Adjust pH 7.2 with phosphoric acid (H_3PO_4) / potassium hydroxide (KOH) **prior to addition of methanol!**

Eluent consumption (flow: 1.0 mL / min
In the course of a measurement (duration of the method 67 minutes and start to the new measurement about 70 minutes), about 70 mL eluent consumed (ratio: 58% buffer A and 42% Buffer B).

Number of measurements	Amount of buffer A (ml)	Amount of buffer B (ml)
1	40.6	29.4
10	406	294
50	2030	1470
100	4060	2940

Method for sample measurement:
Gradient: starting with 100% A, Flow 1 ml /min, duration: 67 min , 50 µL injection volume
Column: Supelcosil LC-18-T (4,6*150mm, 3µm), 30 °C

Eluent gradient:



4 Procedures

4. 1 Preparation

Label each 15 mL reaction tube and add 0.5 mL of PCA solution. Weigh tube containing PCA solution and write down weight. Keep tubes at -20° C until usage!

4.2 Sampling from bioreactor cultivation

This sampling protocol was adjusted for a cultivation culture with *P. putida* at OD 20- 24 in chemostat:

1. Keep the pre-weighed reaction tubes containing 0.5 mL PCA solution at -20 °C
2. Add 2 mL of culture broth and mix immediately. Incubate while gently shaking for 15 min at 4 °C
3. Weigh tube and write down weight
4. Add 0.5 mL of 1 M K_2HPO_4 and mix it
5. Add 0.6 mL of 5 M KOH and mix it -> pH around 7
6. Centrifuge at 4 °C for 5 min at 7197 g
7. Transfer supernatant in labelled 2 mL reaction vial and deep freeze in liquid nitrogen. Keep stored at -70 °C until measurement on HPLC

4.3 Measuring nucleotide concentration on HPLC

Thaw samples on ice. Adjust pH at 7 ± 0,05 using HCl/KOH. Prepare standards for calibration in concentrations from 1 – 100 µM. Measure samples and standards on HPLC.

To calculate the actual concentration in your cells, you must consider the dilution from cell suspension in PCA solution. Therefore use the weight before and after adding cell suspension into reaction tubes.

5 remarks/troubleshooting:

6 Biosafety

Cells must be handled according to S1 lab safety.

P*ublication bibliography*

Cserjan-Puschmann, M.; Kramer, W.; Duerrschmid, E.; Striedner, G.; Bayer, K. (1999): Metabolic approaches for the optimisation of recombinant fermentation processes. In *Applied microbiology and biotechnology* 53 (1), pp. 43–50.

7 Acknowledgements

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