ITB, Stuttgart	EPP-SOP-ITB04
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EPP - Standard Operating Procedure

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

Title: Optimization of reaction conditions using MODDE for statistical experiment design.

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Instruction

Optimization of reaction conditions using MODDE for statistical experiment design.

1 Introduction / Purpose

The purpose of this protocol is the design of experiments for optimization of reaction conditions using MODDE for statistical experiment design.

2 Equipment and chemicals

2.1 Equipment

MODDE™ software for statistical experiment design (Version 11; MKS Umetrics AB, Sweden)

2.2 Chemicals

Glucose (0-100 mM)

NaCl (0-100 mM)

FAD (0-0.5 mM)

DTT (0-5 mM)

NADH (0-5 mM)

Glutathione (0-5 mM)

BSA (0-0.5 mg/ml)

0.5 mM (Z)-undec-9-enoic acid, 1 % finale DMSO concentration

1M HCl

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

Reaction conditions were optimized using MODDE for statistical experiment design (Version 11; MKS Umetrics AB, Sweden).

All biotransformations were performed using whole cells (50 mg mL^{-1}) at the 500 µL-scale in 1.5 mL reaction tubes. Expression cultures were thawed on ice and resuspended in 50 mM citrate buffer pH 6.

Compositions were designed by MODDE according to the selected interaction model (FracFac Res V+, Full Fac (2 levels)). Up to five conditions were analyzed simultaneously in four rounds of optimization.

The following components and concentrations were tested:

Glucose (0-100 mM), NaCl (0-100 mM), FAD (0-0.5 mM), DTT (0-5 mM), NADH (0-5 mM), glutathione (0-5 mM) and BSA (0-0.5 mg/ml).

1. In the first round, glucose, NADH, FAD, DTT and glutathione concentrations were analyzed.

2. Due to the obtained results, glutathione was excluded from further analysis and the glucose concentration was set to 100 mM in the second round of optimization varying once again the concentrations of NADH, FAD and DTT.

3. In the third round, glucose, NADH and FAD concentrations were fixed, while the amount of DTT was adjusted.

4. The four parameters were fixed in the last round of optimization analyzing different NaCl and BSA concentrations at 25, 30 and 37 °C.

Biotransformations were started with the addition of substrate (0.5 mM (Z)-undec-9-enoic acid, 1 % finale DMSO concentration).

After 28 hours of incubation at 800 rpm reactions were quenched by the addition of 20 µl 1M HCl and extracted as described above. Substrate conversion and product formation was analyzed by GC-FID. Optimized conditions for (Z)-undec-9-enoic acid conversion were evaluated by MODDE applying tools for analysis and prediction.

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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