Succesful use and handling of FeedPlate® SMFP08002

96-square well, Organism: *E.coli*, Media: Wilms-MOPS

Kuhner EquipNote

by Kuhner shaker

HOW DOES THE FEEDPLATE® WORK?

The FeedPlate® is a polymer-based slow release system, which enables fed-batch conditions in small scale bioreactors [Anderlei et al., 2015]. The FeedPlate® is applied in biological cultivations in microtiter plates, high-throughput screening, as well as in the process development of shaken bioreactor systems. To start the substrate release, no additional technical equipment is required. After filling the FeedPlate® with cultivation broth/media/ liquid, glucose will be released from the polymer. Due to a diffusion driven substrate release, the amount of release substrate depends on applied type of media, pH and osmolality. This application note describes the operation of the FeedPlate® in cultivations of *E;Coli* with the synthtic Wilms-MOPS medium. [For recipe see: Appendix]

Note: The FeedPlate® is delivered sterile and can be used like a conventional microtiter plate. Please do not autoclave the FeedPlate® or reuse it after cultivation. The glucose release in Wilms-MOPS media (pH 7.5) is 7.8 mg/well within 48 h. Other media will show other release kinetics. Therefore, please check your individual substrate release first.

APPLYING THE FEEDPLATE®

Fill the FeedPlate® with liquid. A liquid volume of 250 – 700 µL per well (96 deep well plate) is recommended. Thus, oxygen transfer rates (OTR) of 2-10 mmol/(L*h) can be achieved during the fed-batch phase.

For a fed-batch cultivation, the initial glucose concentration in the medium should be reduced. In case of the Wilms-MOPS medium, the glucose stock solution is replaced by DI-water. [see: Appendix]

Note: During fed-batch cultivation, a further secondary substrate limitation (e.g. nitrogen) might occur. For Wilms-MOPS media, ammonia will be limited if 26.7 g/L glucose are metabolized. Please check your cultivation medium for a sufficient ammonia supply.

EN4A - All data are kindly provided by Timm Keil, AVT-BioVT RWTH Aachen University

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Batch preculture in standard microtiter plate

First Preculture

Filling volume:	500 µL Wilms-MOPS or complex media with 20 g/L glucose
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	between 8 – 24 hours
End biomass conc.:	~ 5 – 10 g/L

Second Preculture

Filling volume:	500 µL Wilms-MOPS media with 20 g/L glucose
Inoculation conc.:	~ 0.1 g/L
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	8 hours

OR

Fed-Batch preculture in FeedPlate®

Preculture (2x)

Filling volume:	500 µL Wilms-MOPS media with 1-2 g/L glucose
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	24 – 30 hours

Evaporation of water should be payed attention in small scale bioreactors such as microtiter plates. High evaporation rates might negatively influence the cultivations results (e.g. increased final product titer). Therefore, cultivation experiments should be run under humidity-controlled conditions (> 80 %). Moreover, the applied sealing foil is essential to reduce evaporation from the wells (see Sieben et al. 2016). With AeraSeal film covers (Sigma-Aldrich), the following evaporation rates per well in a 96-well microtiter plate are known:

37 °C: 3.4 – 3.9 µL/h

30 °C: 2.4 – 2.8 µL/h

Finally, the polymer matrix in the FeedPlate® is known to absorb water. For the 96-well FeedPlate®, a total amount of 60 µL liquid per well will be lost during cultivation.

PRECULTURE

Two successive cultivations of precultures are recommended to be performed successively. The best inoculation time for *E.coli* is at highest metabolic activity of the microorganisms. This is reached at exponential or fed-batch growth of the microorganisms. For the first preculture, complex media or Wilms-MOPS can be applied. The second preculture should be run with the same medium which is later applied in the main culture (here Wilms-MOPS medium). If no on-line signal for growth monitoring is available, you should use the following procedures to run precultures (refer to the left side of this page).

Note: If your initial cultures are picked from agar plates and transferred into the FeedPlate®, please apply an initial glucose amount of 1–2 g/L glucose in the medium to ensure initial growing of the microorganisms. Wait at least for 24 h before inoculating a second preculture or main culture.

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Fed-Batch preculture in FeedPlate®

Mainculture

Filling volume:	250 -700 µL Wilms-MOPS media with 0 g/L glucose
Shaking conditions:	350 rpm, 50 mm shaking diameter, 37 °C, 80 % humidity
Duration:	without nitrogen limitation 41 – 115 hours possible

MAIN CULTURE IN FeedPlate®

Starting ODs in main culture should be between 0.1 - 2 (~ 0.05 - 1 g/L biomass), no additional glucose should be present in the cultivation medium. In Figure 1 the influence of the initial biomass concentration on the dissolved glucose concentration and the initial batch phase during cultivation with FeedPlate® is shown. The oxygen transfer rate is a good indicator to distinguish between batch and fed-batch phase [Jeude et al. 2006]. At high initial biomass concentrations (1 g/L) the batch phase occurs over a time period of approximately 2 h. During that period, glucose concentrations stay below 0.5 g/L. In contrast at initial biomass concentrations of 0.05 g/L the batch phase occurs over 10 h and glucose concentrations of 7 g/L are reached.

During batch phase oxygen limitation is possible. If too much glucose is accumulated during the initial growth of the microorganisms, the cultures might get oxygen limited and produce undesired by-products. By using the FeedPlate® oxygen limitation can be avoided. Due to the reduced substrate release at fed-batch conditions, no oxygen limitation will occur if the following shaking conditions are adjusted (refer to the left side of this page).

To avoid oxygen limitations at all, we recommend an inoculum biomass concentration of more than 0.25 g/L and sticking to the procedure of preculturing mentioned above.

Note: Glucose is accumulating during the initial cultivation time in the FeedPlate®. At extended microbial lag-phases (e.g. due to delayed harvesting of the preculture), the glucose concentration in the medium might become critical and by-product might be formed. For example, *E.coli* is able to produce acetate which in turn might inhibit the microbial growth.

To avoid critical glucose concentrations:

- Apply high inoculum concentration (best between 0.1 – 1 g/L) and run precultures in the above-mentioned way
- Use FeedPlate® with lower glucose release
- Increase culture volume (up until 700 µL)

References

- Jeude M, Dittrich B, Niederschulte H, Anderlei T, Knocke C, Klee D, Büchs J. **Fed-batch mode in shake flasks by slow-release technique**. BiotechnolBioeng, 2006, 95(3):433-45
- Sieben M, Giese H, Grosch J, Kauffmann K, Büchs J **Permeability of currently available microtiter plate sealing tapes fail to fulfil the requirements for aerobic microbial cultivation**. Biotechnol.J., 2016, 11:1-14
- Anderlei T, Laidlaw D, Bruellhoff K, Selzer S **Slow Release Technology for Small-Scale Bioreactor Operations**. Gen Bioprocessing, 2015, Mar 15

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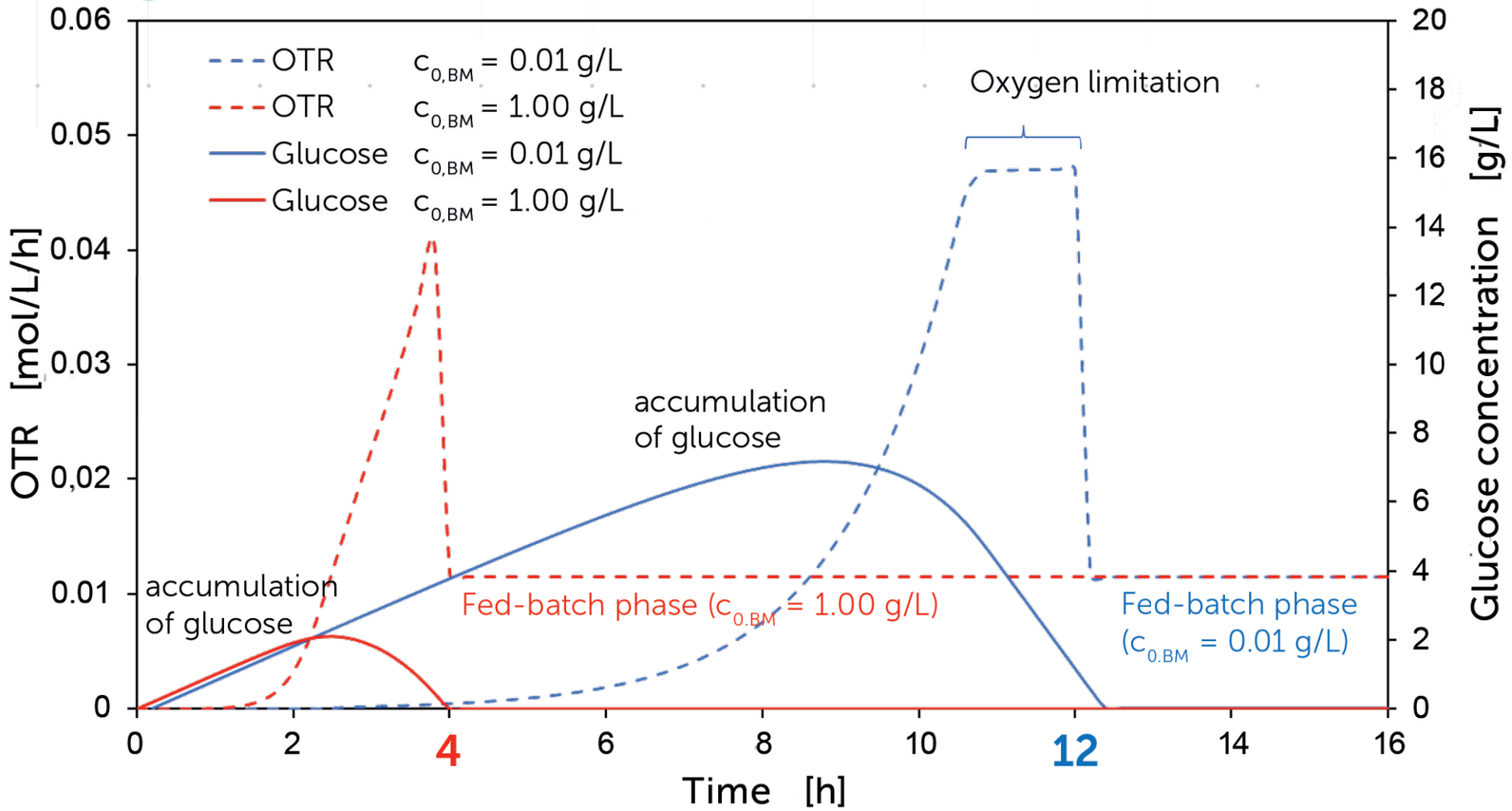
APPENDIX: Recipe Wilms-MOPS media

Main solution					
Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for **947 mL [g]**	Volume per 1L medium
$(NH_4)_2SO_4$	132.02	52.87	6.98	6.98	**947 mL**
K_2HPO_4	174.18	17.22	3.0	3.00	
Na_2SO_4	142.04	14.08	2.0	2.00	
MOPS	209.27	200.00	41.85	41.85	
➔ Components in ca. 900 mL DI-water, adjust pH with NaOH to 7.5, fill up to 947 mL. autoclave; storage at RT					
1000x Trace elements					
Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for **50 mL [g]**	
$ZnSO_4 \times 7H_2O$	287.45	0.0019	0.00054	0.027	**1 mL**
$CuSO_4 \times 5H_2O$	249.60	0.0019	0.00048	0.024	
$MnSO_4 \times H_2O$	168.99	0.0018	0.00030	0.015	
$CoCl_2 \times 6H_2O$	237.38	0.0023	0.00054	0.027	
$FeCl_3 \times 6H_2O$	270.30	0.1545	0.04176	2.088	
$CaCl_2 \times 2H_2O$	147.02	0.0135	0.00198	0.099	
$Na_2EDTA \times 2H_2O$	372.24	0.0897	0.03340	1.670	
➔ Steril filtration, storage without light at 4 °C.					
25x Glucose (500 g/L pure Glucose) **IMPORTANT: Only used for batch cultivation.**					
Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for **500 mL [g]**	
Glucose-Monohydrate	198.17	111.01	22	275	**40 mL**
➔ Autoclave (22 g/L Glucose-Monohydrate = 20 g/L pure glucose), storage at RT					
100x Magnesiumsulfate (50 g/L)					
Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for **50 mL [g]**	
$MgSO_4 \times 7H_2O$	246.36	2.03	0.5	5.0	**10 mL**
➔ Autoclave, storage at RT					
1000x Thiamin (10 g/L)					
Component	Molmass [g/mol]	Molarity in medium [mM]	Concentration in medium [g/L]	Weighed portion for **10 mL [g]**	
Thiamin-Hydrochloride	337.27	0.03	0.01	0.1	**1 mL**
➔ Steril filtration, storage at 4 °C					



Simulation of oxygen transfer rate (OTR) and Glucose concentration of fed-batch cultivation in microtiter plate with 1 g/L and 0.01 g/L inoculum biomass concentration.

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