Cultivation and Bioprocessing Techniques & Design of Experiments (DoE)

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Cultivation & Bioprocessing Techniques

1. Closed batch (serum bottles, usually anaerobic)

2. Batch (e.g. Erlenmeyer flask, uncontrolled conditions)

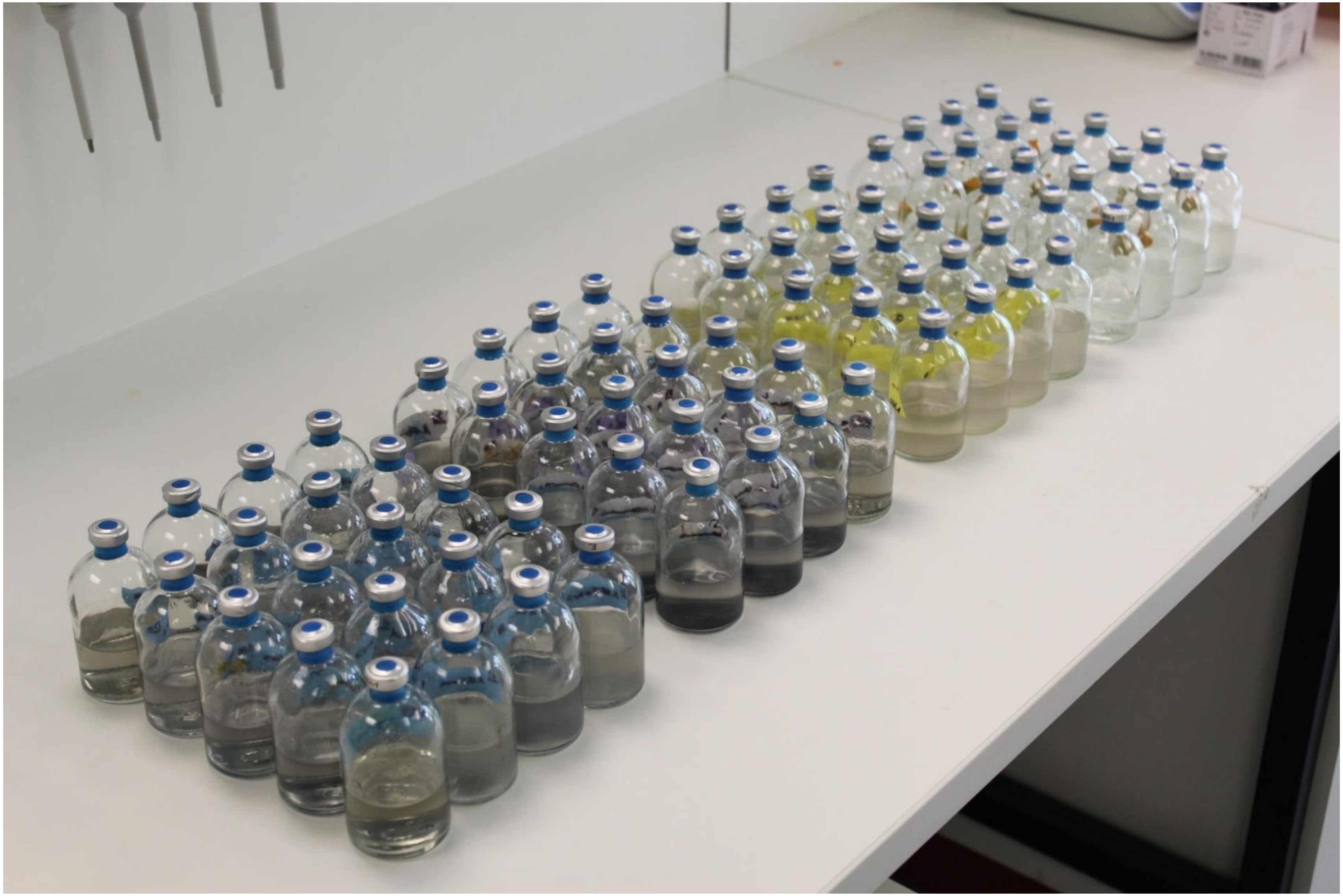
3. Batch (bioreactor, (un)controlled conditions)

4. Fed-batch (bioreactor, controlled conditions)

5. Continuous culture (bioreactor, controlled conditions)

Rittmann *et al*. 2012, Rittmann *et al*. 2015

Closed batch



Closed batch cultivation of *Methanothermobacter marburgensis*, *Methanothermococcus okinawensis*, *Methanocaldococcus villosus* and *Methanosarcina soligelidi* in 120 mL serum bottles.

Closed batch

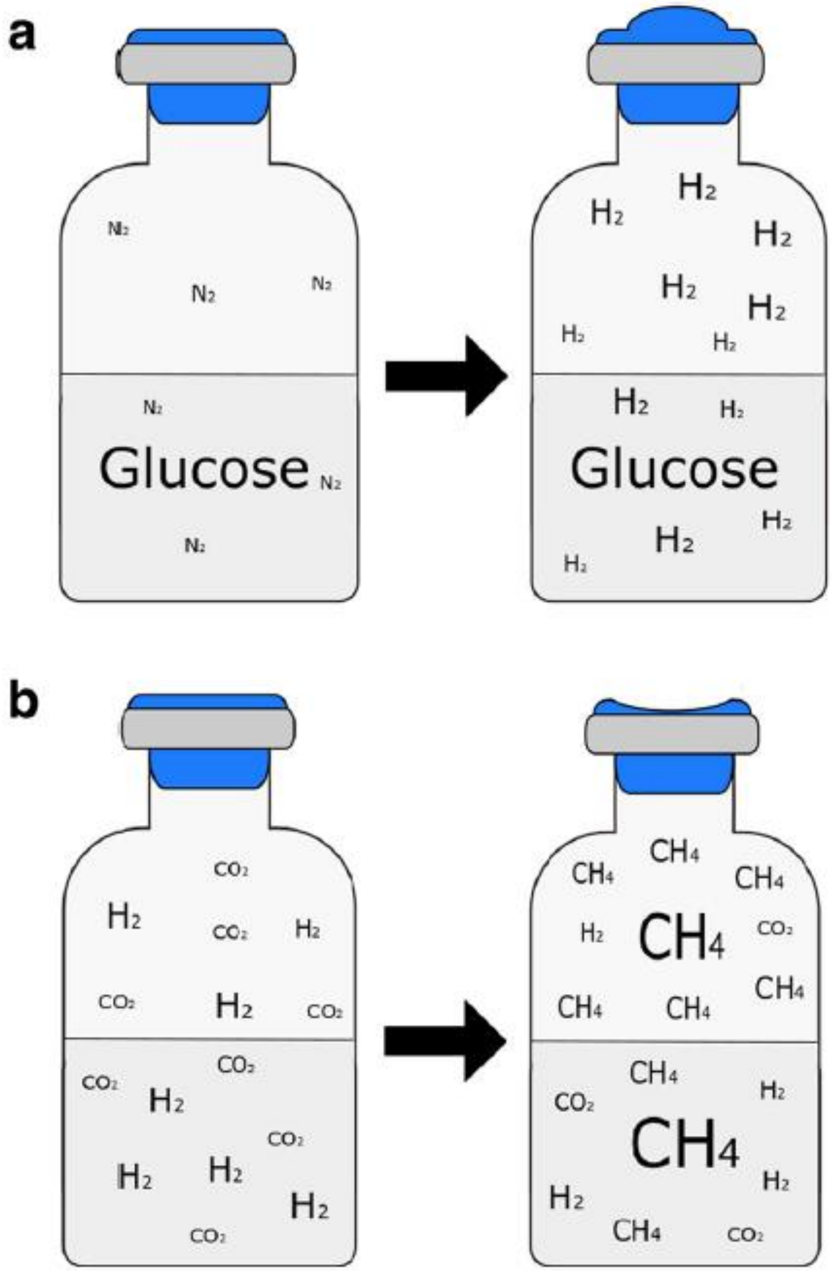
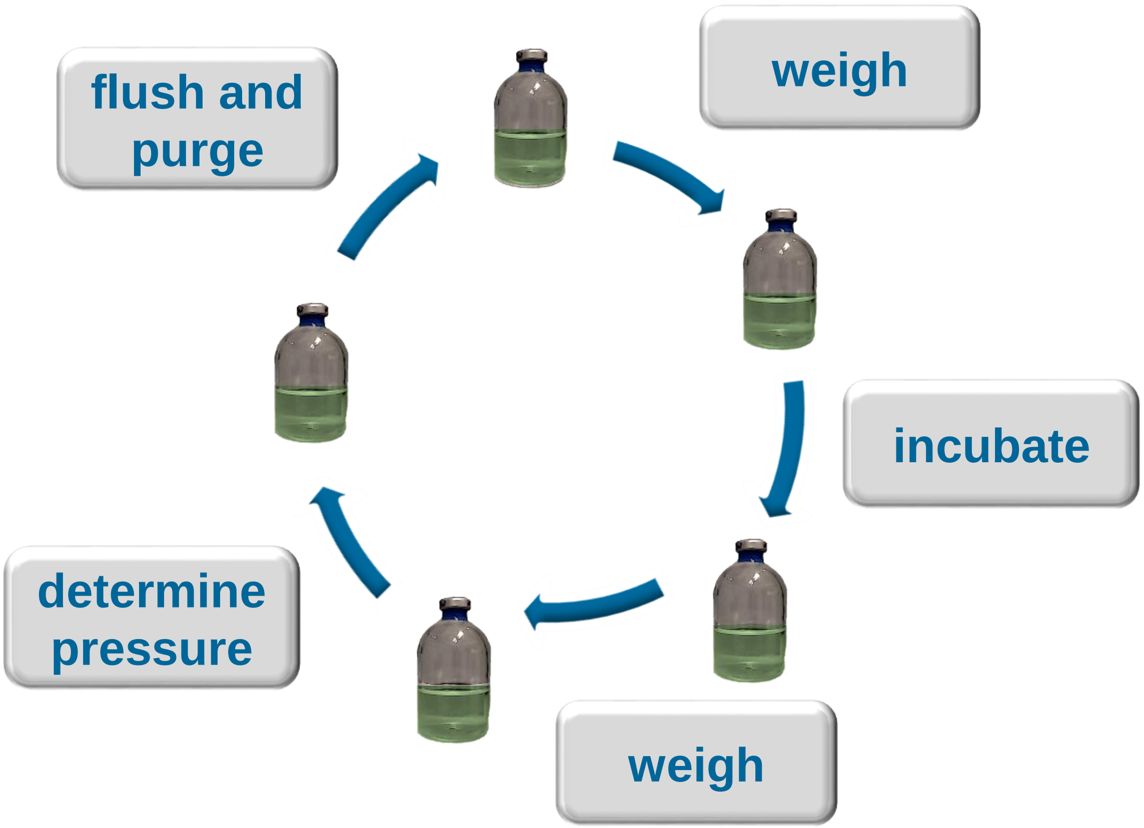
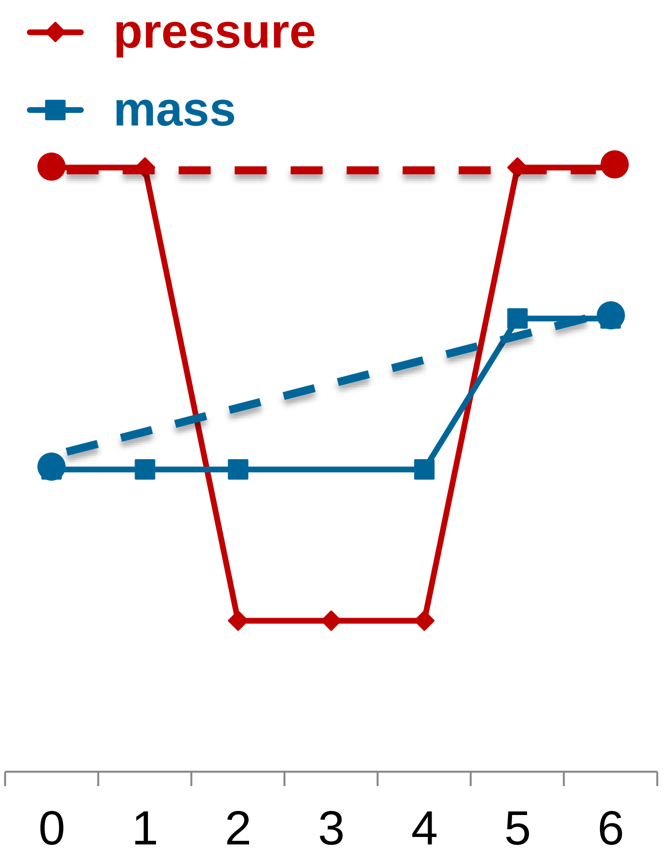


Fig. 4 Anaerobic closed batch cultivation set-up (serum bottle) supplemented with (**a**) a liquid substrate, glucose, and (**b**) a gaseous substrate, H_2/CO_2. **a** Cultivation of a H_2-producing microorganism: H_2 production from glucose leads to a pressure increase in the serum bottle. **b** Cultivation of a methanogenic archaeon: closed batch cultivation with discontinuous H_2/CO_2 gassing. The conversion of H_2/CO_2 to CH_4 leads to a pressure drop in the cultivation device due to the following stochiometric formula ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$)

Mauerhofer *et al.* 2018

Closed batch

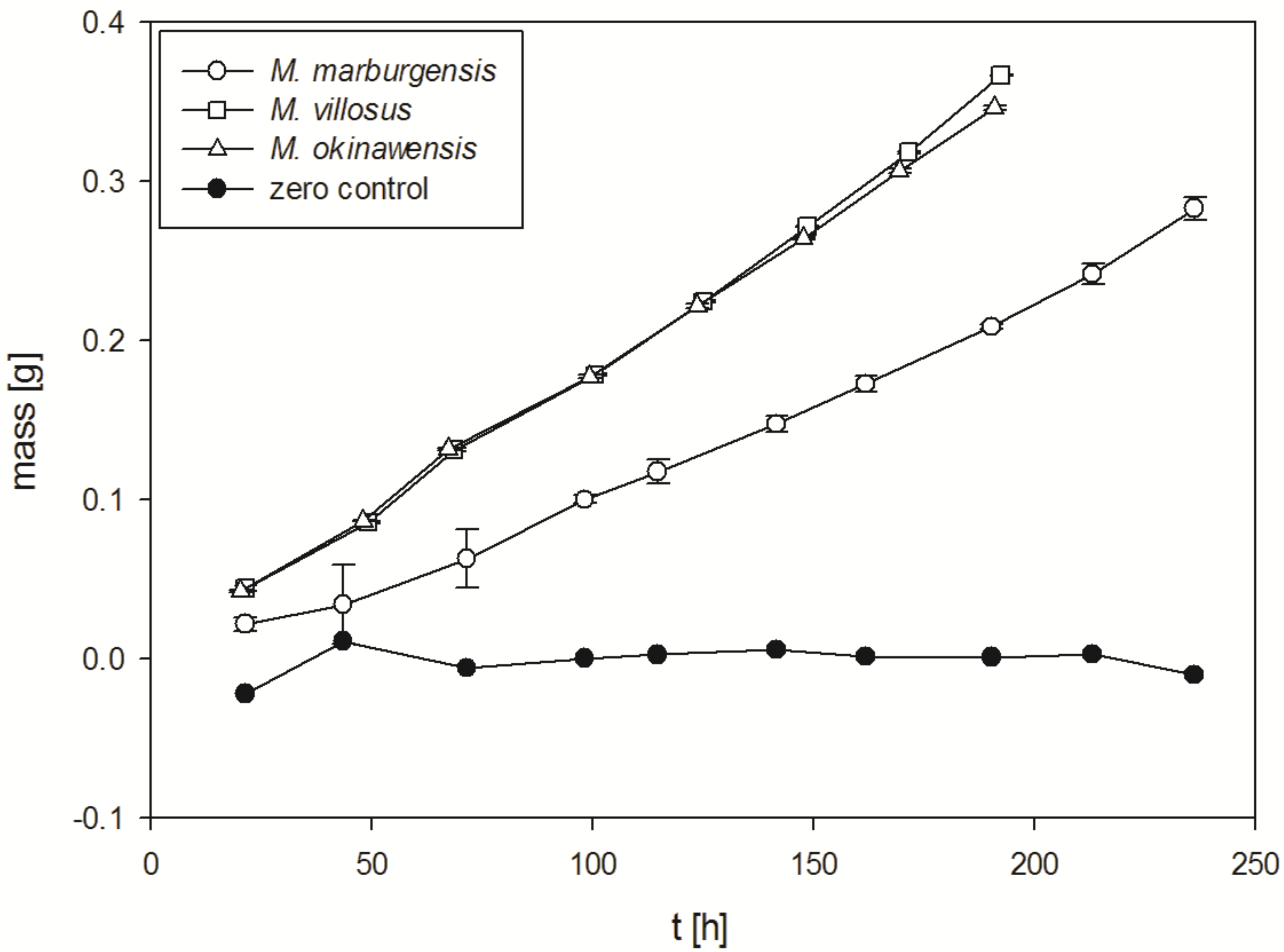




$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

Taubner & Rittmann, 2016

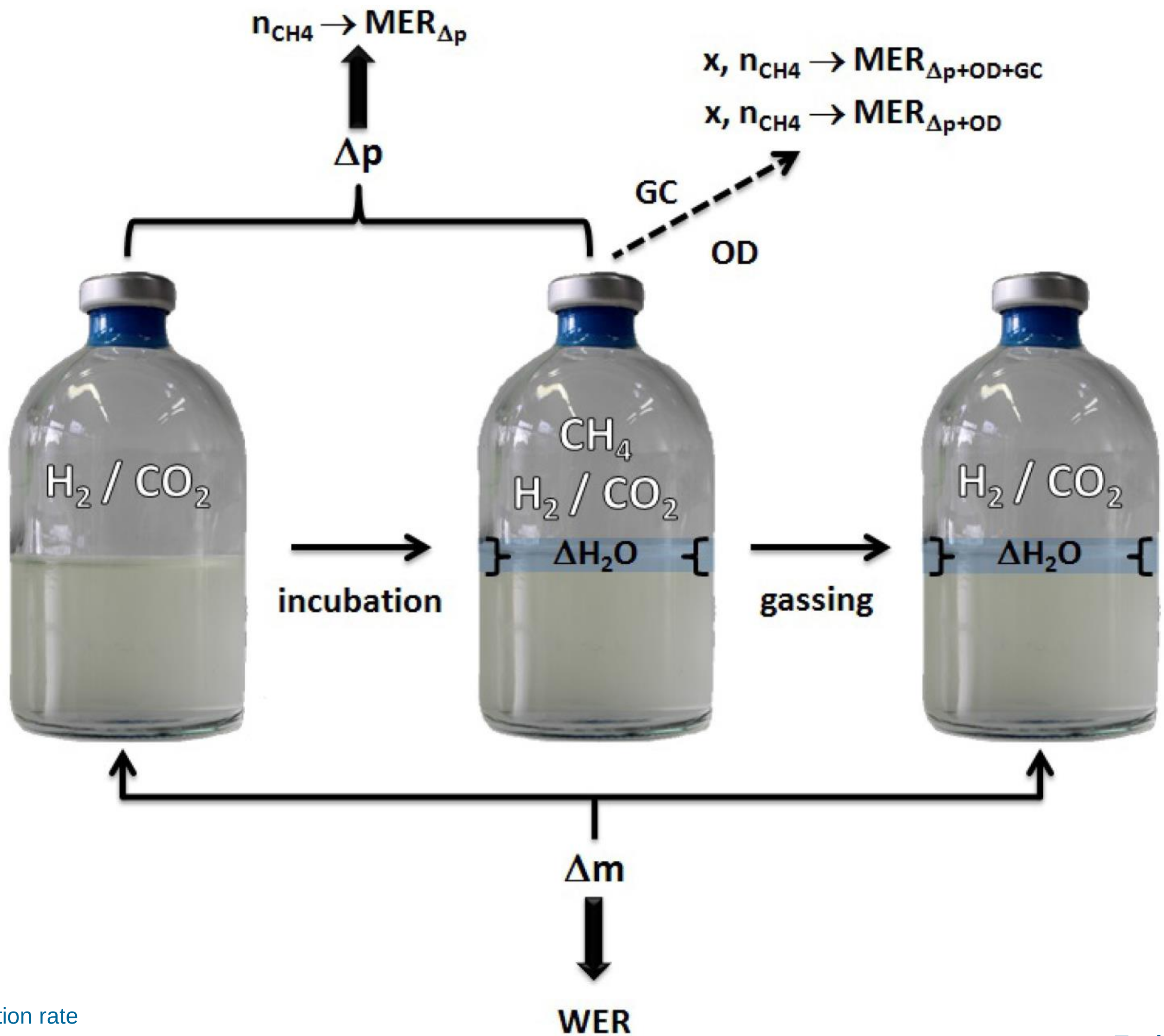
Closed batch



Cummulative H_2O production of *M. marburgensis, M. villosus* and *M. okinawensis* in 120 mL serum bottles.
Growth conditions: T = 65, 80 and 60°C, respectively, V = 50 mL, n = 9, 3 and 3, respectively.

Taubner & Rittmann, 2016

Closed batch



MER = methane evolution rate
OD = optical density

Taubner & Rittmann, 2016

General mass balance

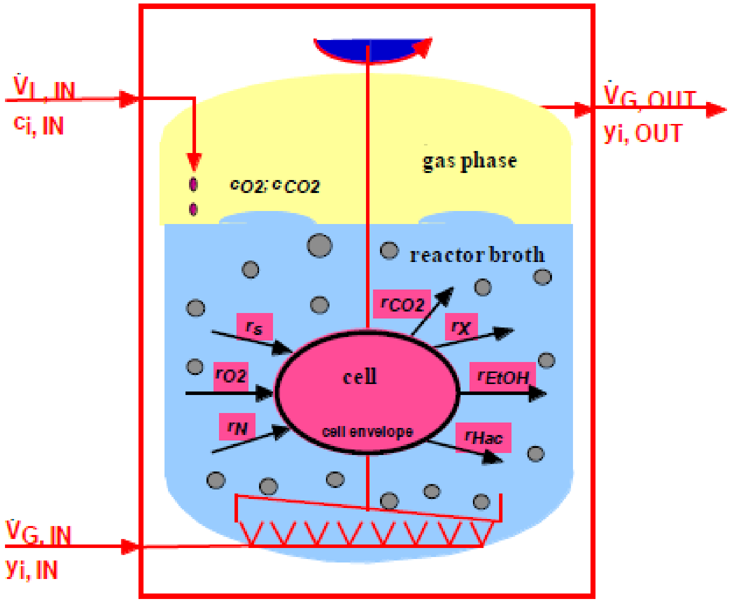
$$\dot{V}_{In} c_{i,In} - \dot{V}_{Out} c_{i,out} + V_R r_i = V_R \frac{\partial c_i}{\partial t} + c_i \frac{\partial V_R}{\partial t}$$

c_i = concentration of component i

V_R = reactor volume

$\dot{V}$ = volumetric flowrate

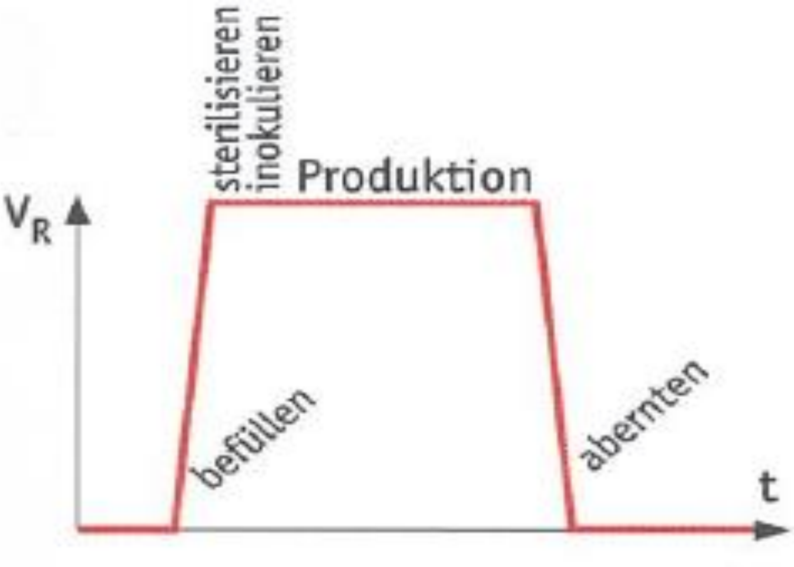
r_i = reaction rate of component i = production – uptake

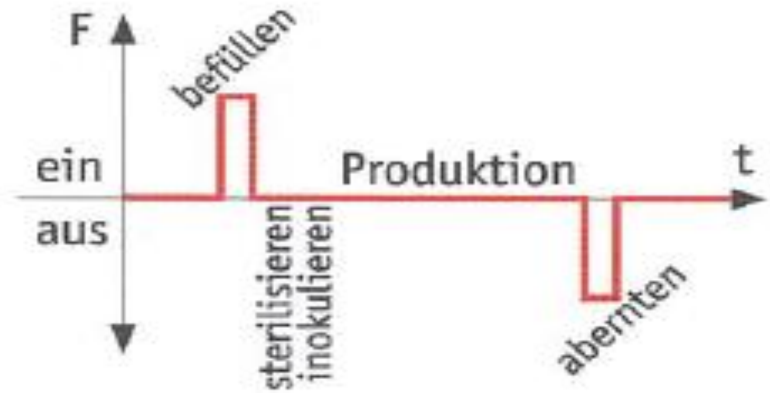


Batch & Fed-batch

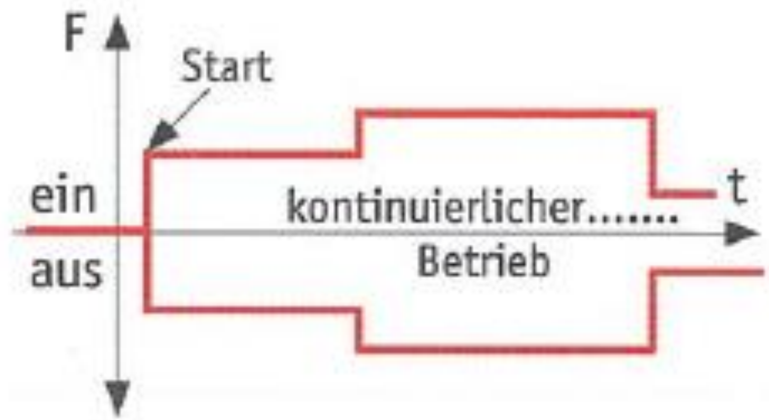
Spektrum
Akademischer
Verlag, 2006

Satzverfahren (batch)





Kontinuierliche Kultur (continuous culture)



Batch & Fed-batch

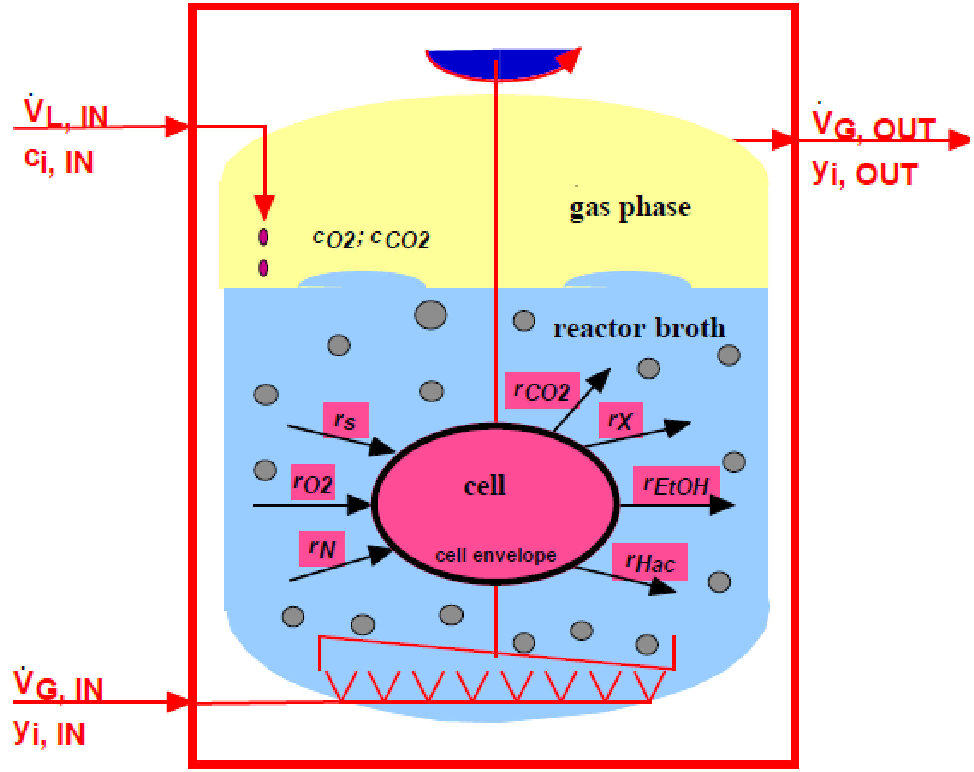
Mass balance for batch and fed-batch

- Batch
  - i = liquid component!

$$V_R r_i = V_R \frac{\partial c_i}{\partial t}$$

- Fed-Batch
  - i = limiting added component!

$$\dot{V}_{In} c_{i,In} + V_R r_i = V_R \frac{\partial c_i}{\partial t} + c_i \frac{\partial V_R}{\partial t}$$



Exponentieller Feed

$$\dot{V}_{In} = \dot{V}_0 \exp(\mu t)$$

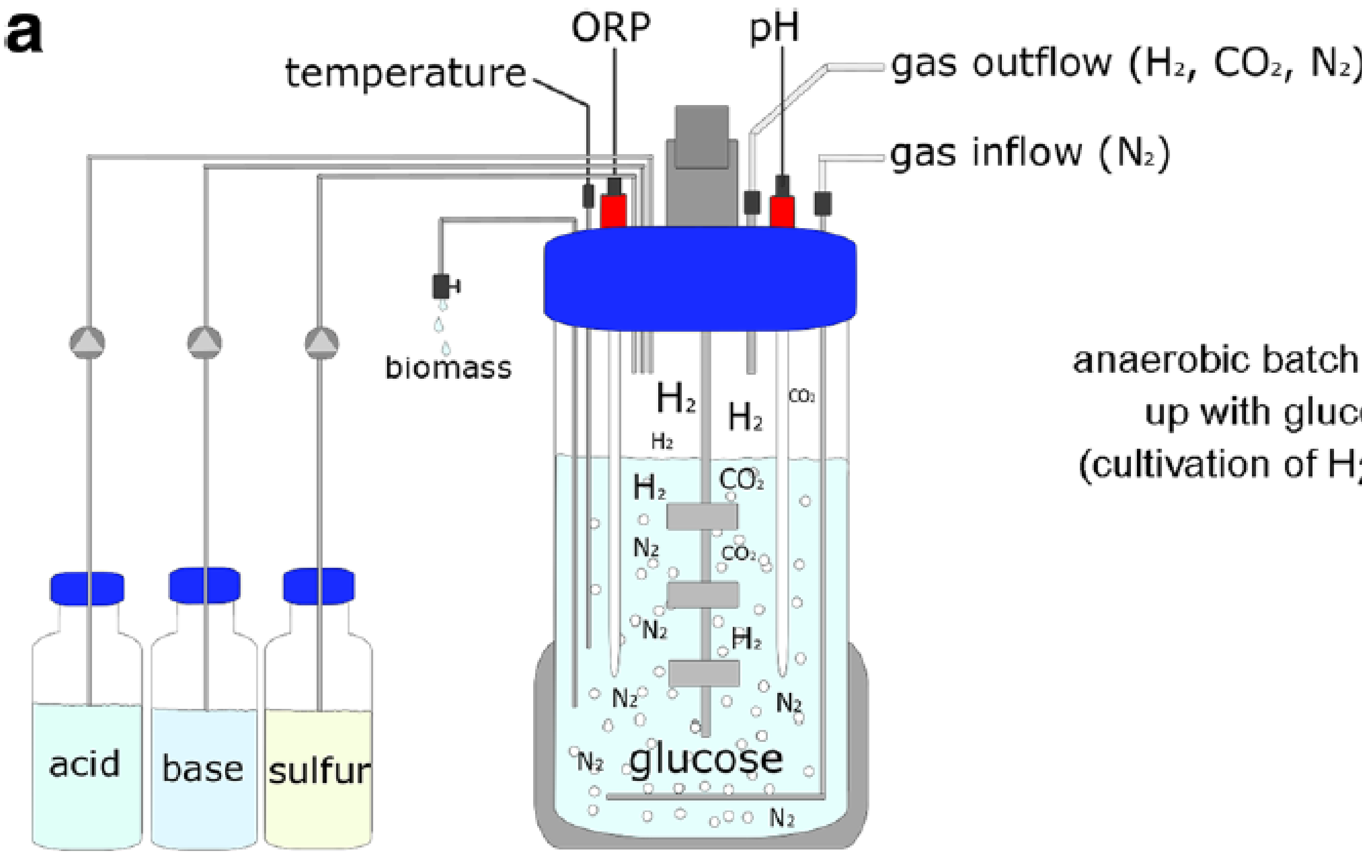
Beschleunigter Feed (Accelerostat)

$$\dot{V} = \dot{V}_0 \exp((\mu + \alpha t)t)$$

Konstanter Feed

$$V_R(t) = V_{R,0} + \dot{V}_{In} t \; und \; \dot{V}_{In} = \frac{\partial V_R}{\partial t} = const.$$

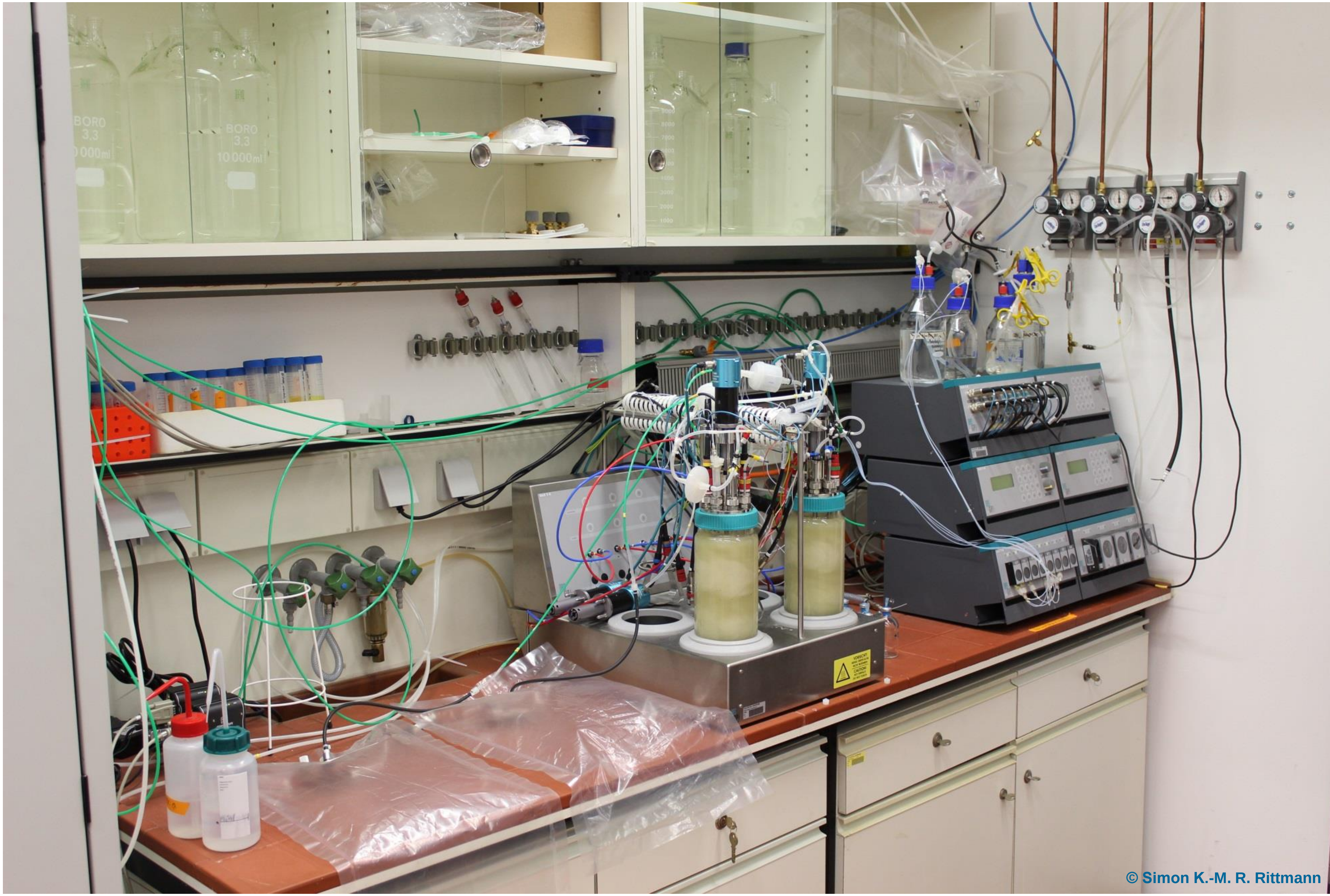
Batch



anaerobic batch fermentation bioreactor set-up with glucose as a liquid substrate (cultivation of H_2-producing microorganism)

Mauerhofer *et al*. 2018

Fed-batch



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Fed-batch

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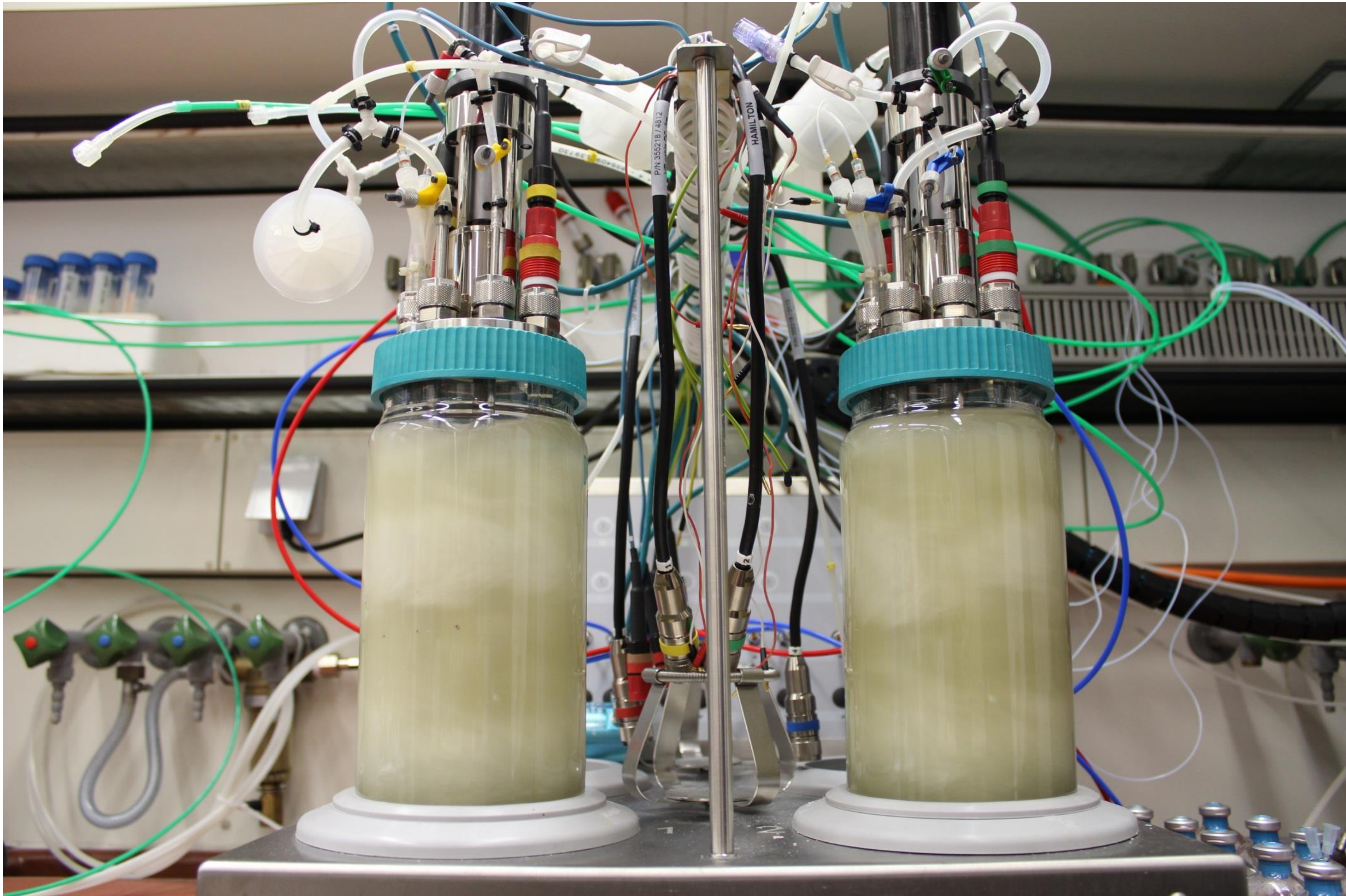
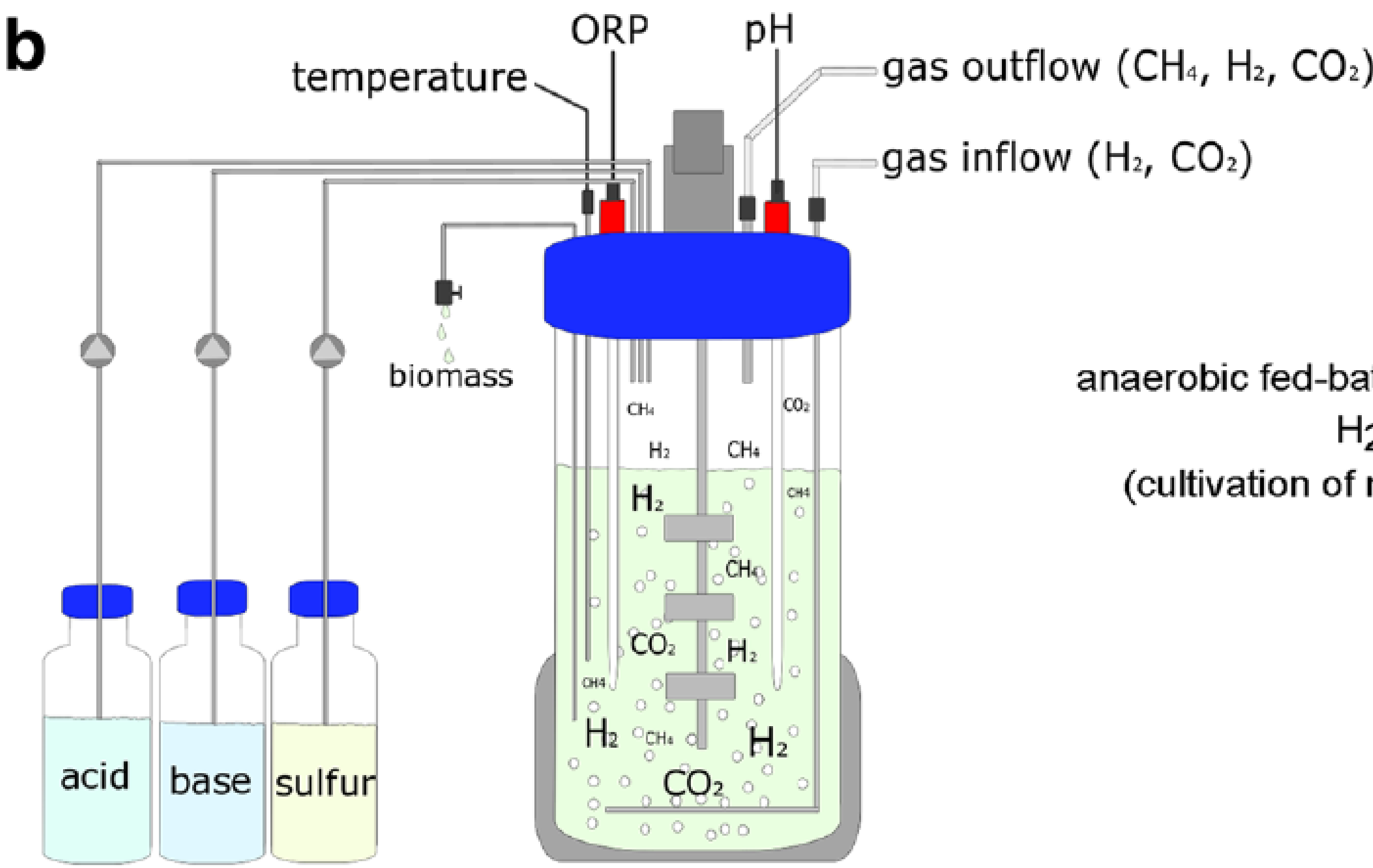


Fed-batch fermentation of *Methanothermobacter marburgensis* in the Eppendorf bioreactor system.

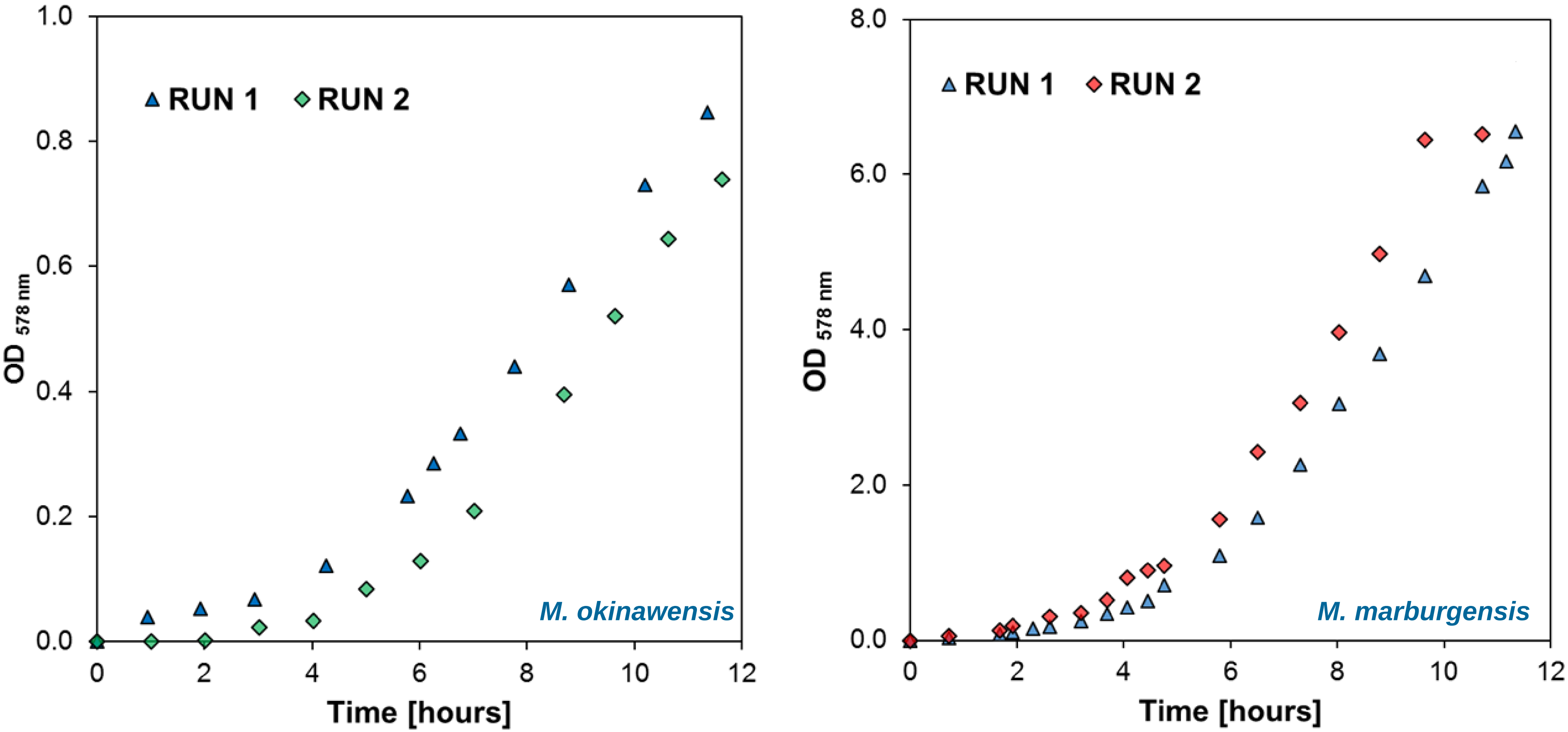
Fed-batch



anaerobic fed-batch bioreactor system with H_2/CO_2 feed (cultivation of methanogenic archaea)

Mauerhofer *et al*. 2018

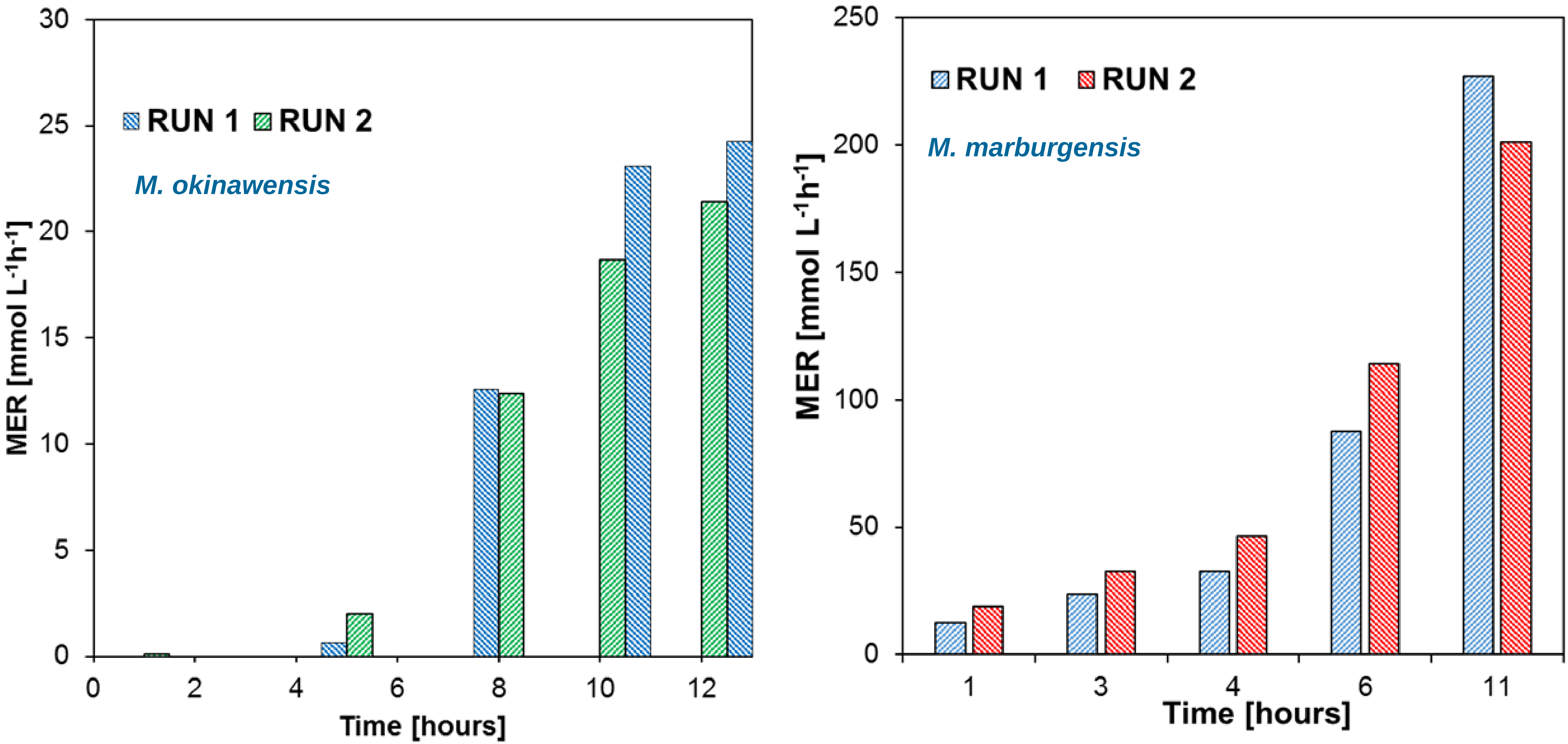
Fed-batch



Growth (OD_{578nm}) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates.

Abdel Azim *et al.*, 2017

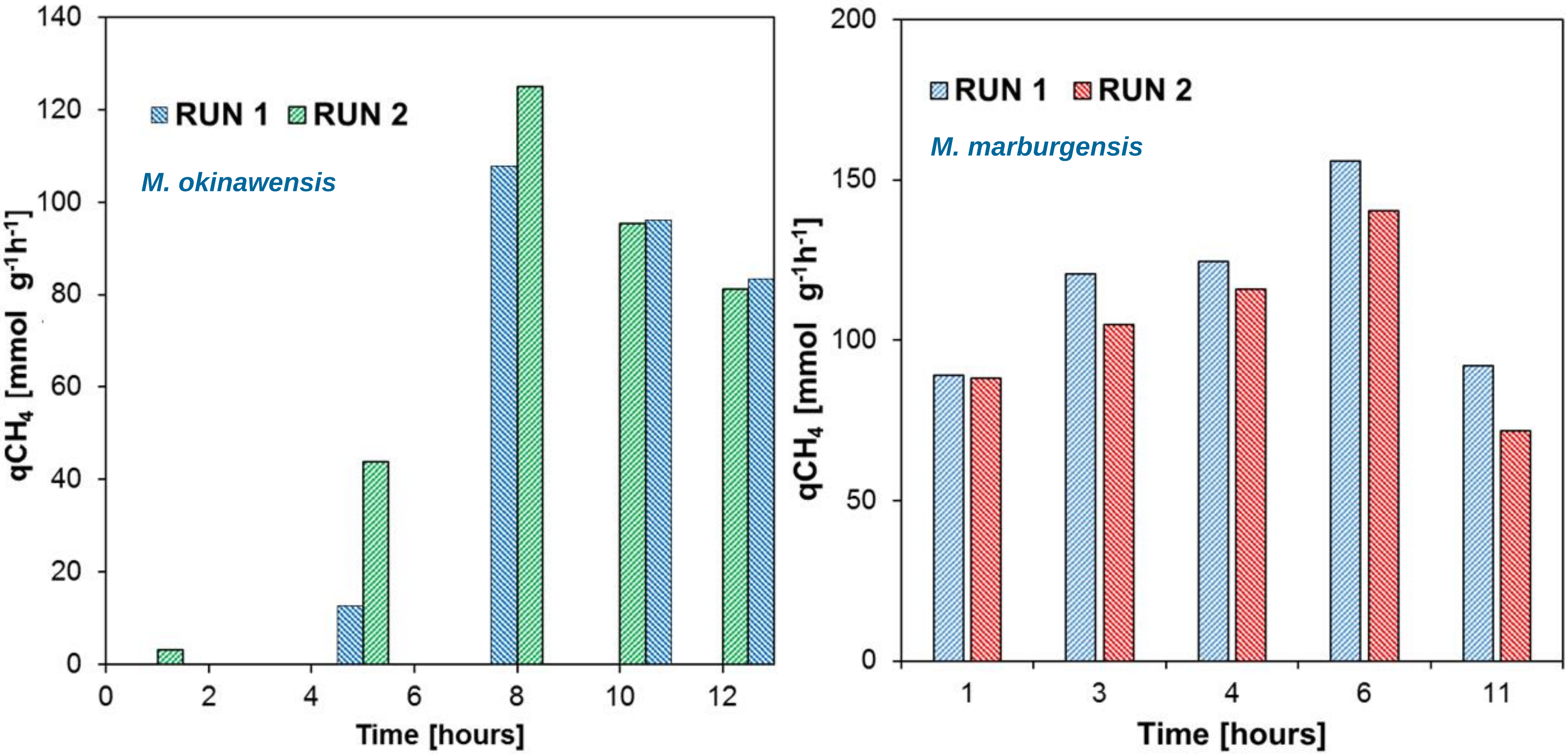
Fed-batch



Methane evolution rate (MER) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates.

Abdel Azim *et al.*, 2017

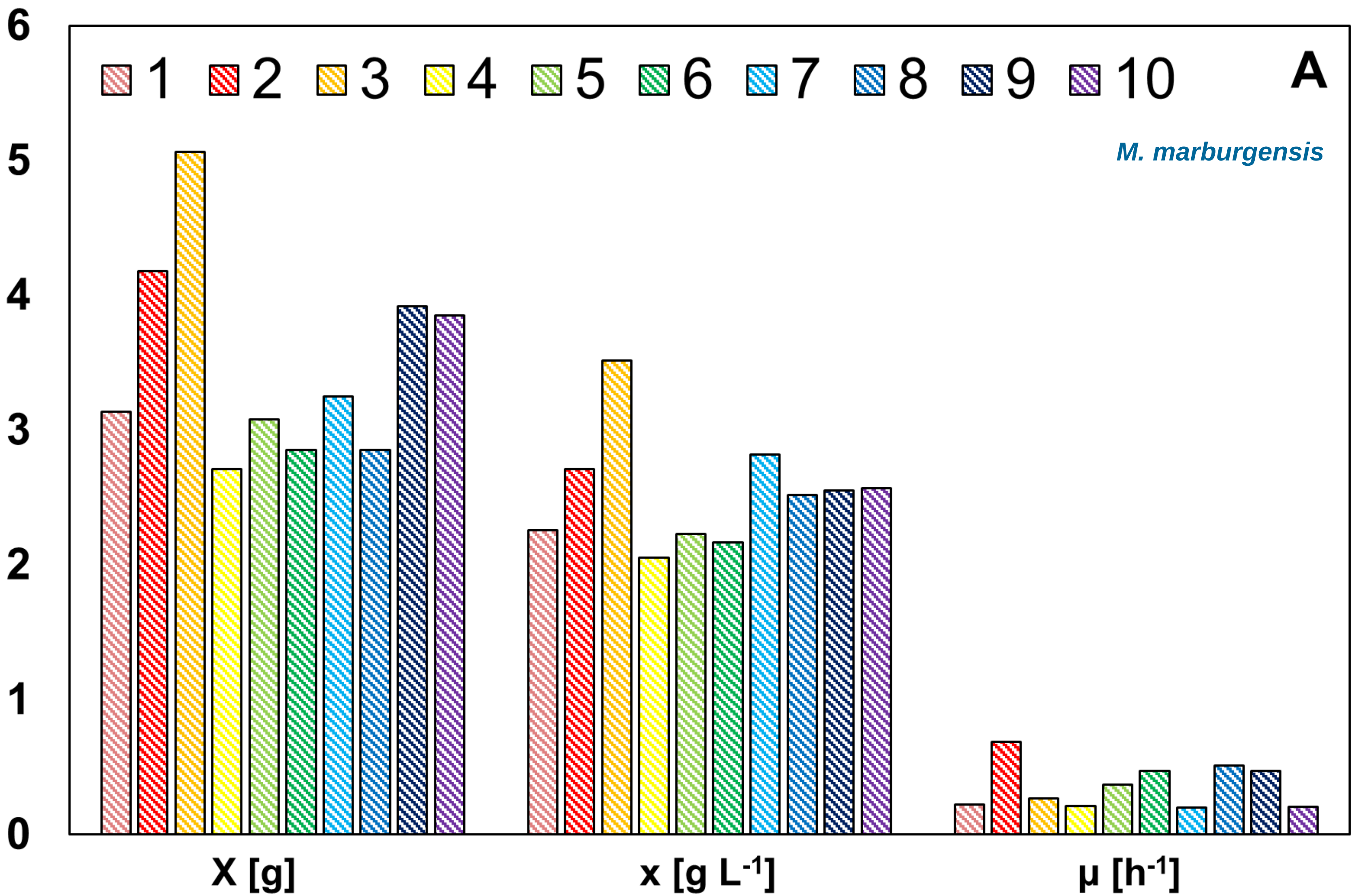
Fed-batch



Specific methane evolution rate (qCH_4) of *M. okinawensis* (left) and *M. marburgensis* (right) in fed-batch cultivation mode. Run 1 and run 2 are replicates.

Abdel Azim *et al.*, 2017

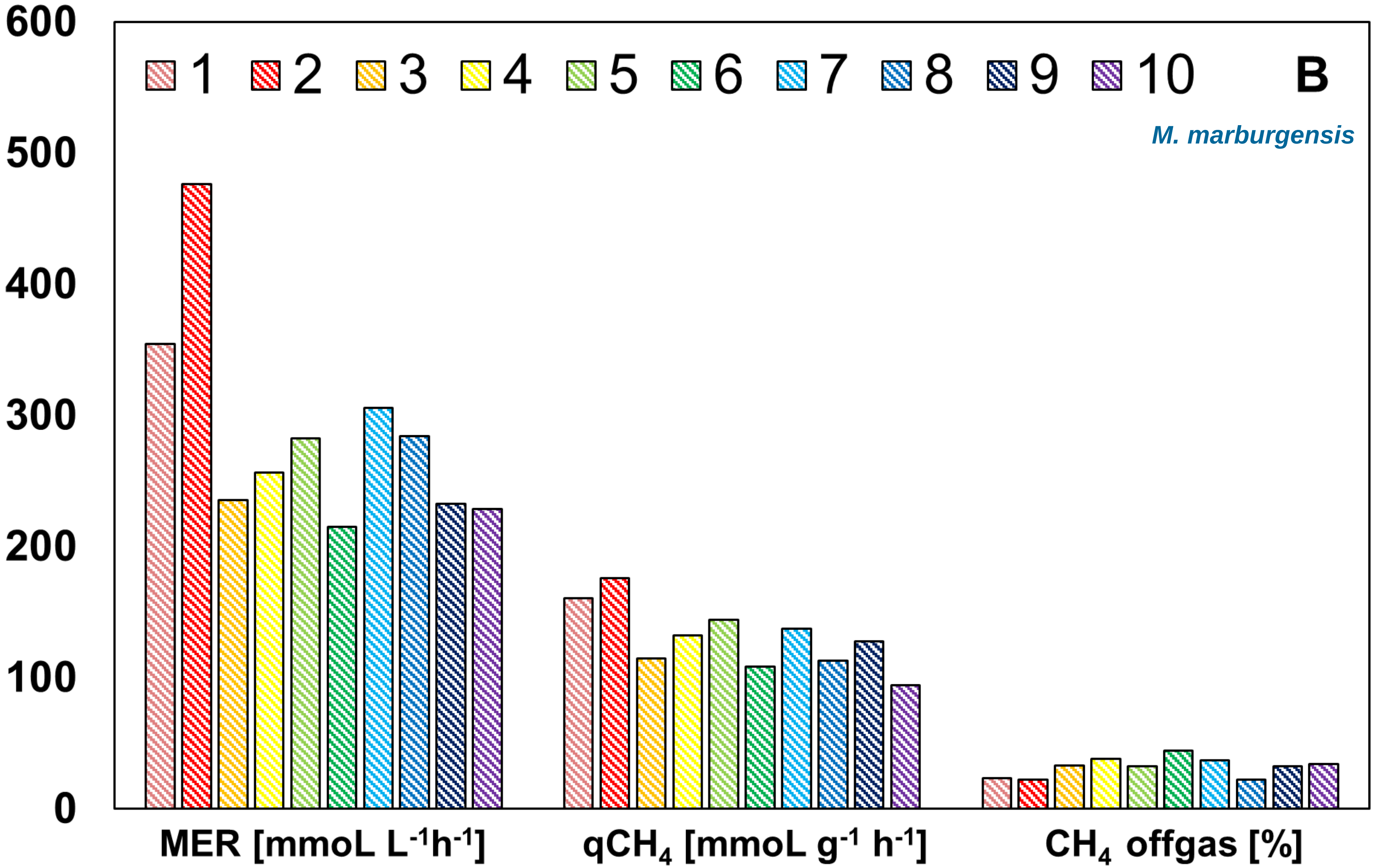
Exponential fed-batch



Results from the exponential fed-batch cultivation using *M. marburgensis*. For each run (colour legend) are presented the values of X, x, μ on the x-axis. Run 3 (orange bar) had the highest biomass (X [g]) and biomass concentration (x [g L^{-1}]).

Abdel Azim *et al*., 2017

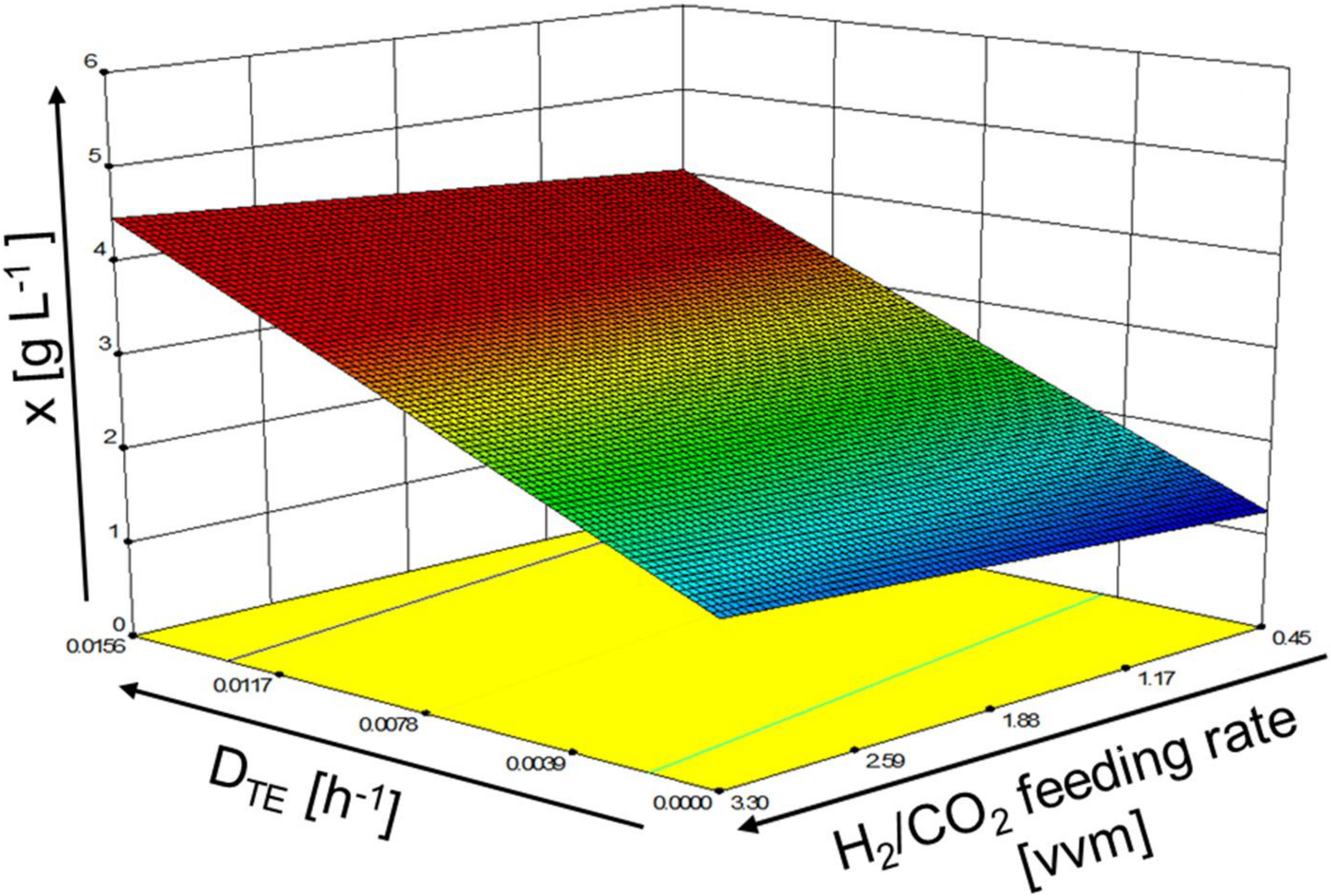
Exponential fed-batch



Results from the exponential fed-batch cultivation using *M. marburgensis*. For each run the values MER, qCH_4, CH_4 offgas are presented on the x-axis. Run 2 (red bar) showed the highest MER and qCH_4. During run 6 the highest CH_4 off-gas concentration was obtained.

Abdel Azim *et al.*, 2017

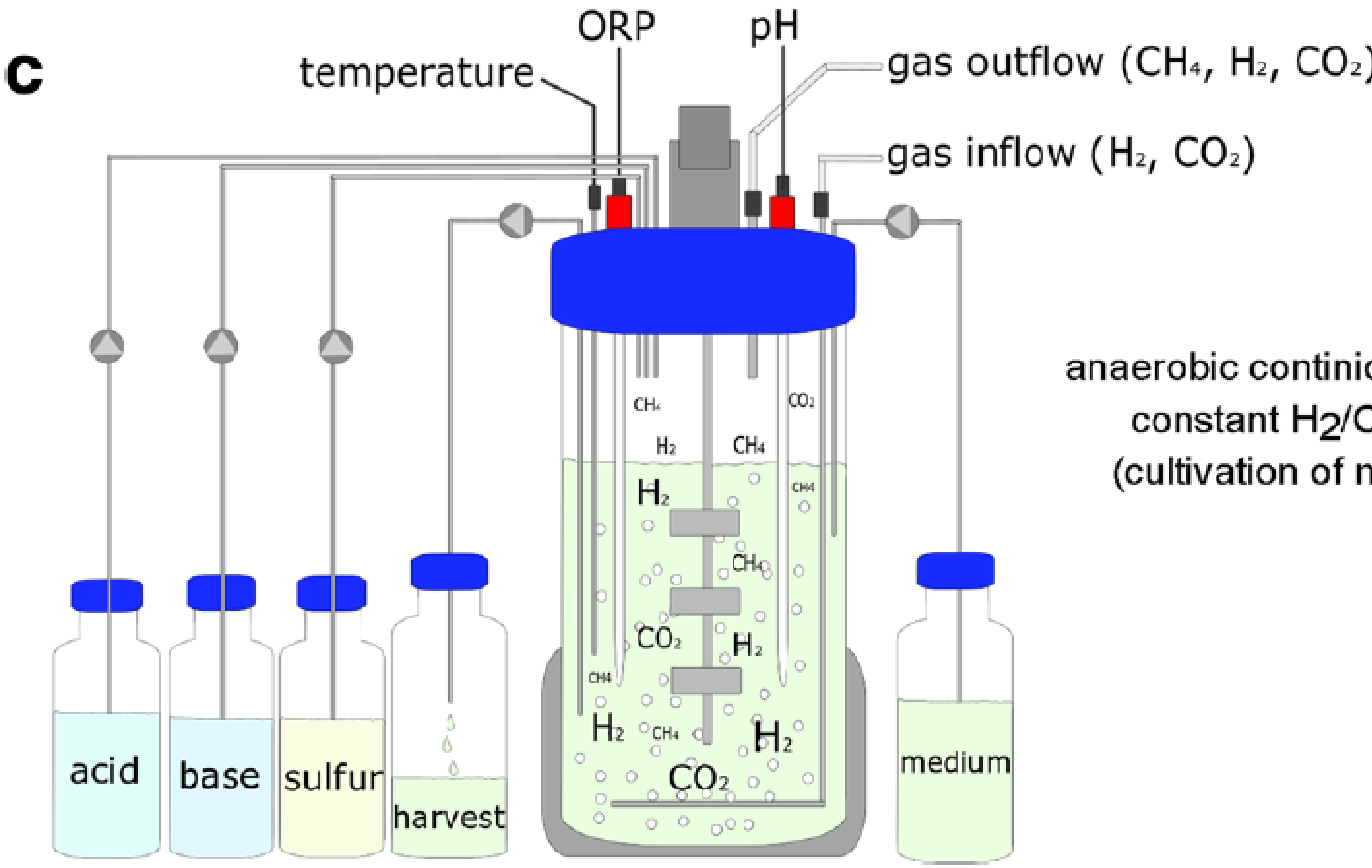
Exponential fed-batch



Model for biomass concentration (x) calculated from the exponential fed-batch data of *M. marburgensis*.

Abdel Azim *et al.*, 2017

Continuous culture



anaerobic continious bioreactor set-up with constant H_2/CO_2 and medium feed (cultivation of methanogenic archaea)

Mauerhofer *et al*. 2018

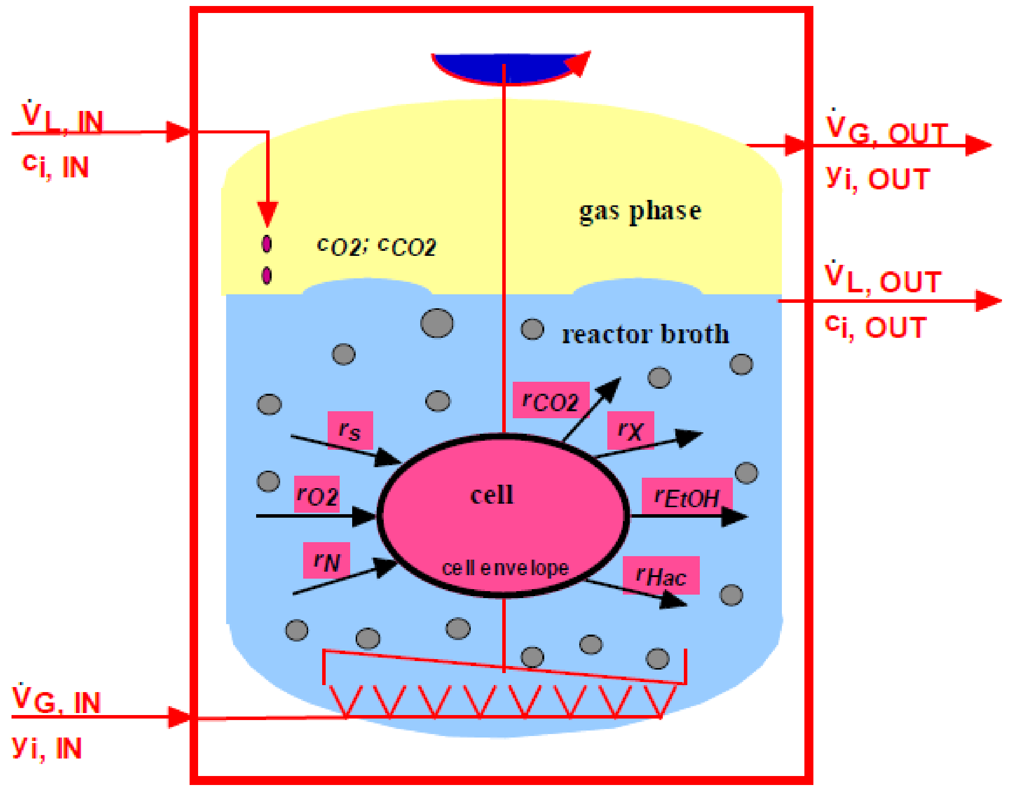
Continuous culture

Mass balance for continuous culture

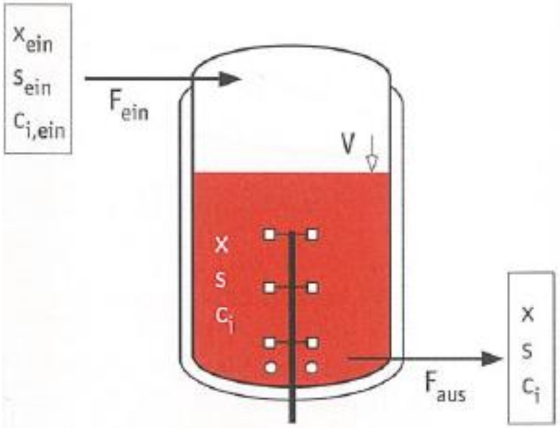
$$D = \mu = \frac{\dot{V}}{V_R} = \left[\frac{1}{h}\right]$$

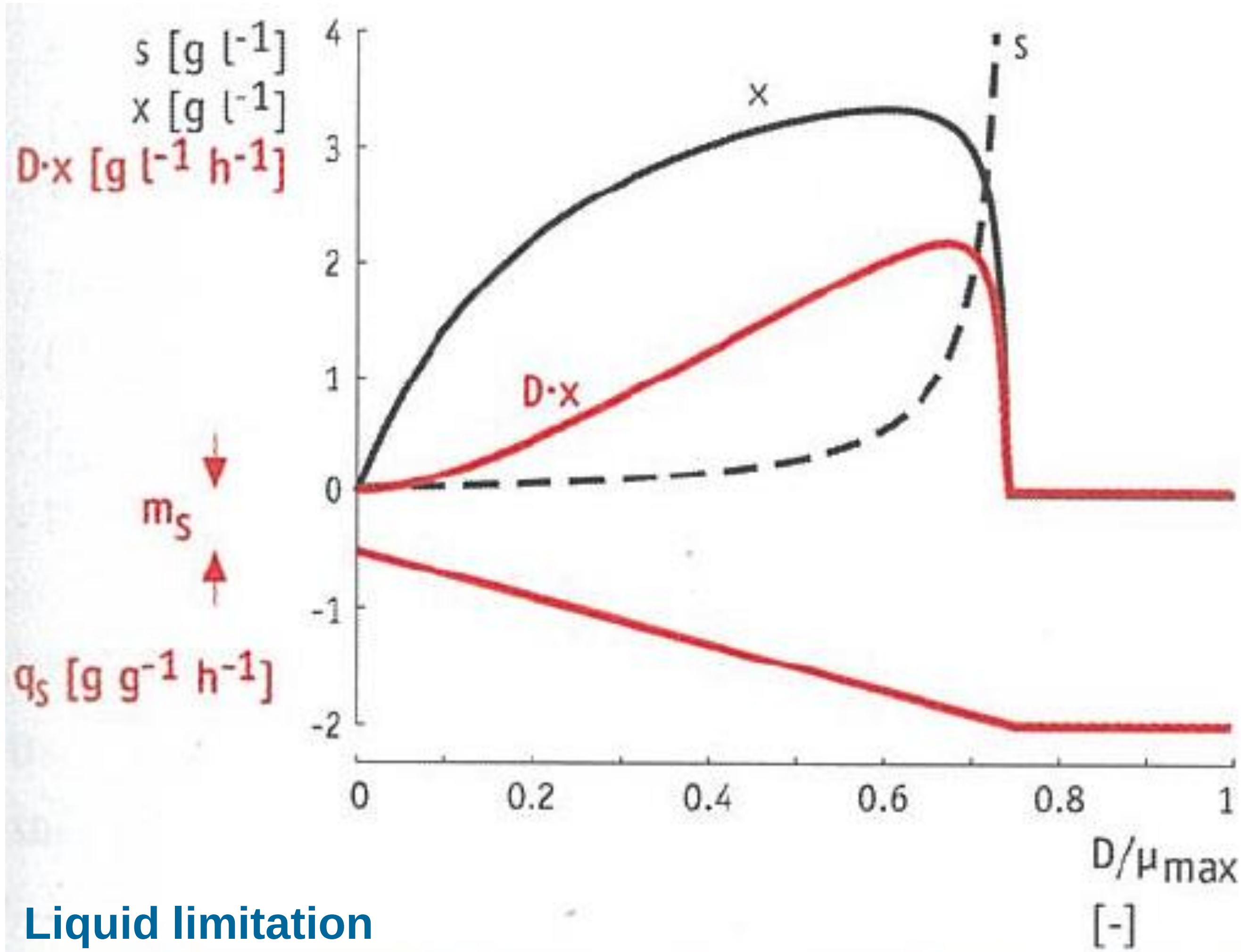
$$\dot{V}_{In} c_{i,In} - \dot{V}_{Out} c_{i,out} + V_R r_i = 0$$

$$r_i = -D(c_{i,In} - c_{i,out})$$



Continuous culture

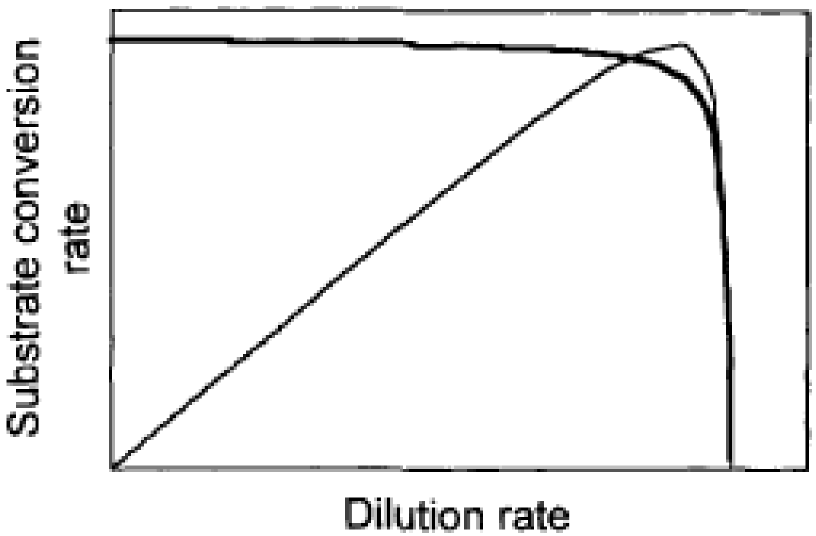


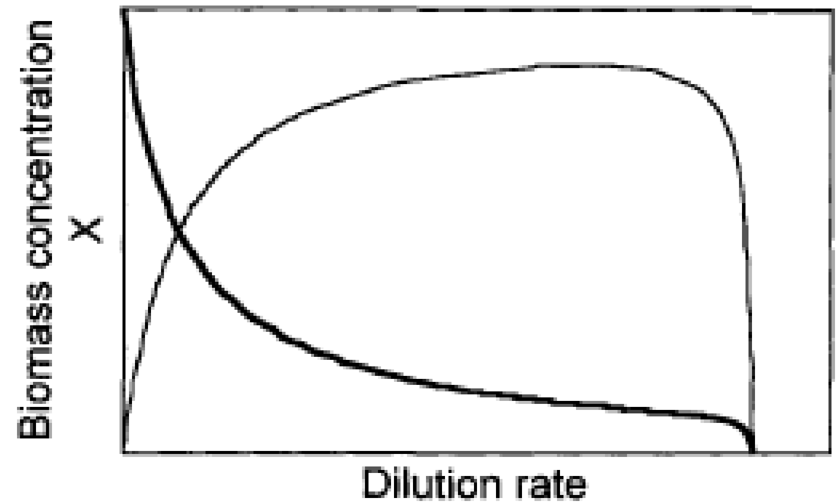


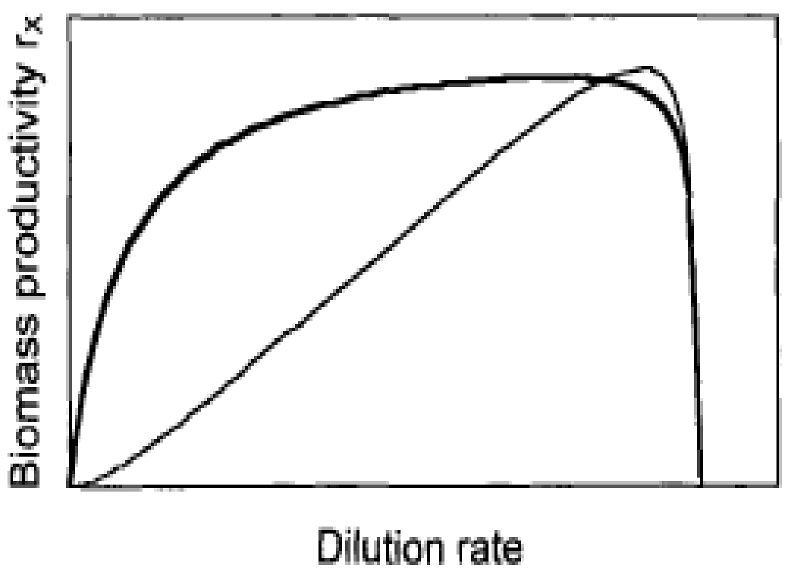
Liquid limitation

Spektrum Akademischer Verlag, 2006

Continuous culture







Principles of continuous culture bioprocessing

- Liquid limitation: thin line
- Gas limitation: bold line

Continuous Cultures Limited by a Gaseous Substrate: Development of a Simple, Unstructured Mathematical Model and Experimental Verification with *Methanobacterium thermoautotrophicum*
N. Schill, W. M. van Gulik, D. Voisard, and U. von Stockar*
Biotechnology and Bioengineering, Vol. 51, Pp. 645–658 (1996)

Continuous culture

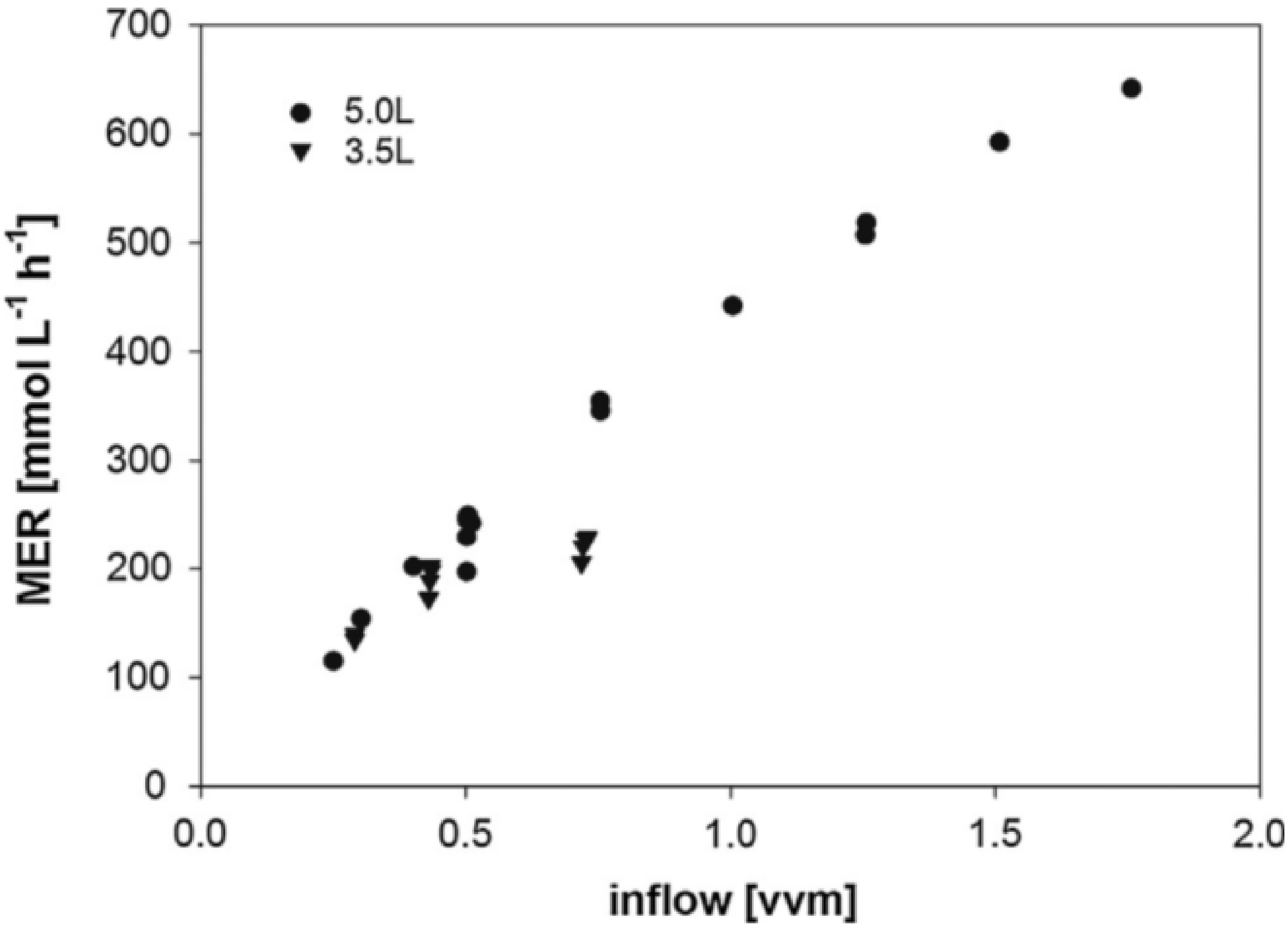


Fig. 6. Increase of MER with increased gasflow at 3.5 L and 5 L culture volume.

Seifert *et al.*, 2014

Continuous culture

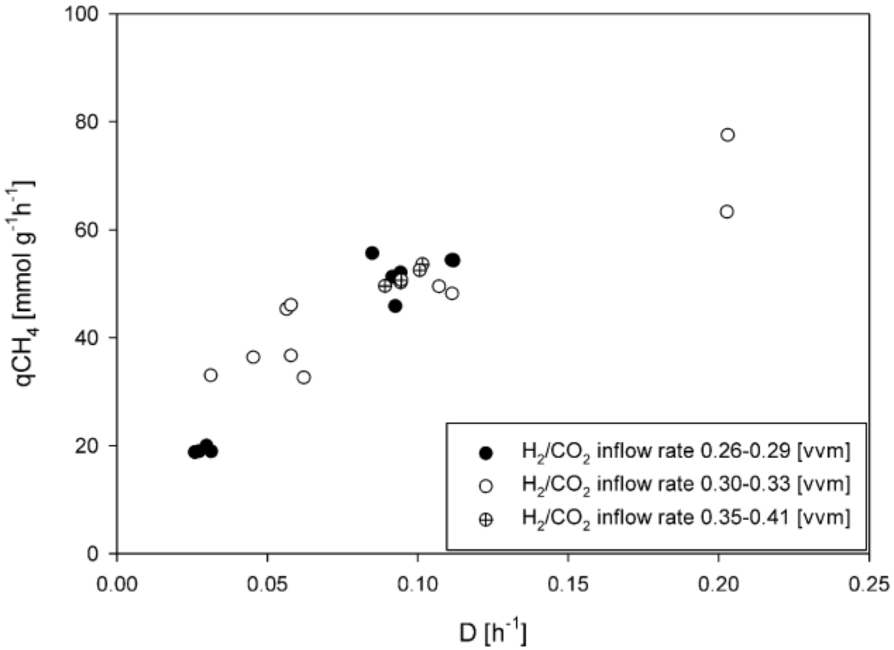


Fig. 2 — Specific methane productivity is shown as a function of the liquid dilution rate. By increasing the liquid dilution rate the specific methane productivity increased. An elevated H_2/CO_2 gassing rate ambiguously influenced qCH_4.

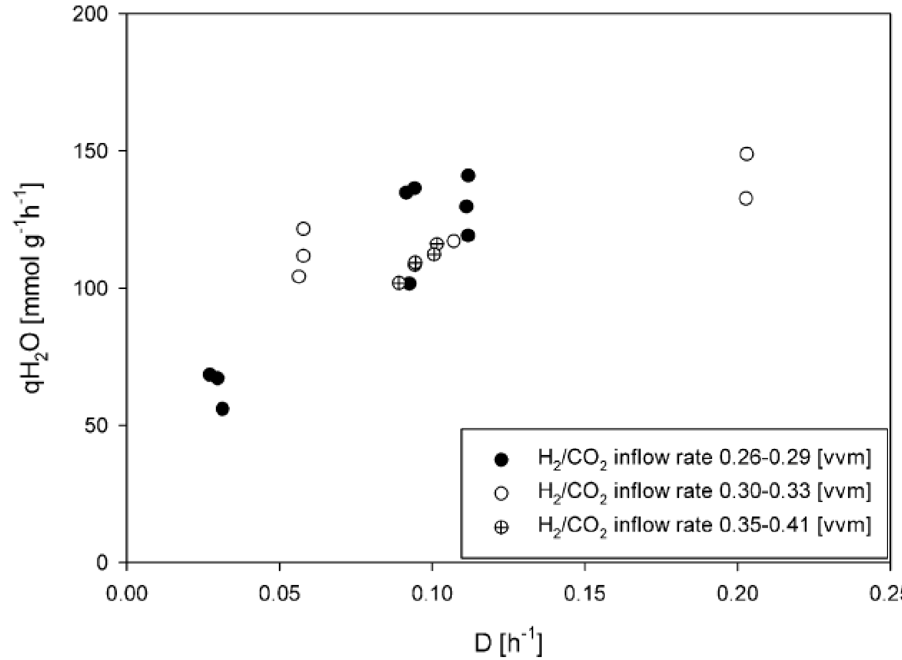
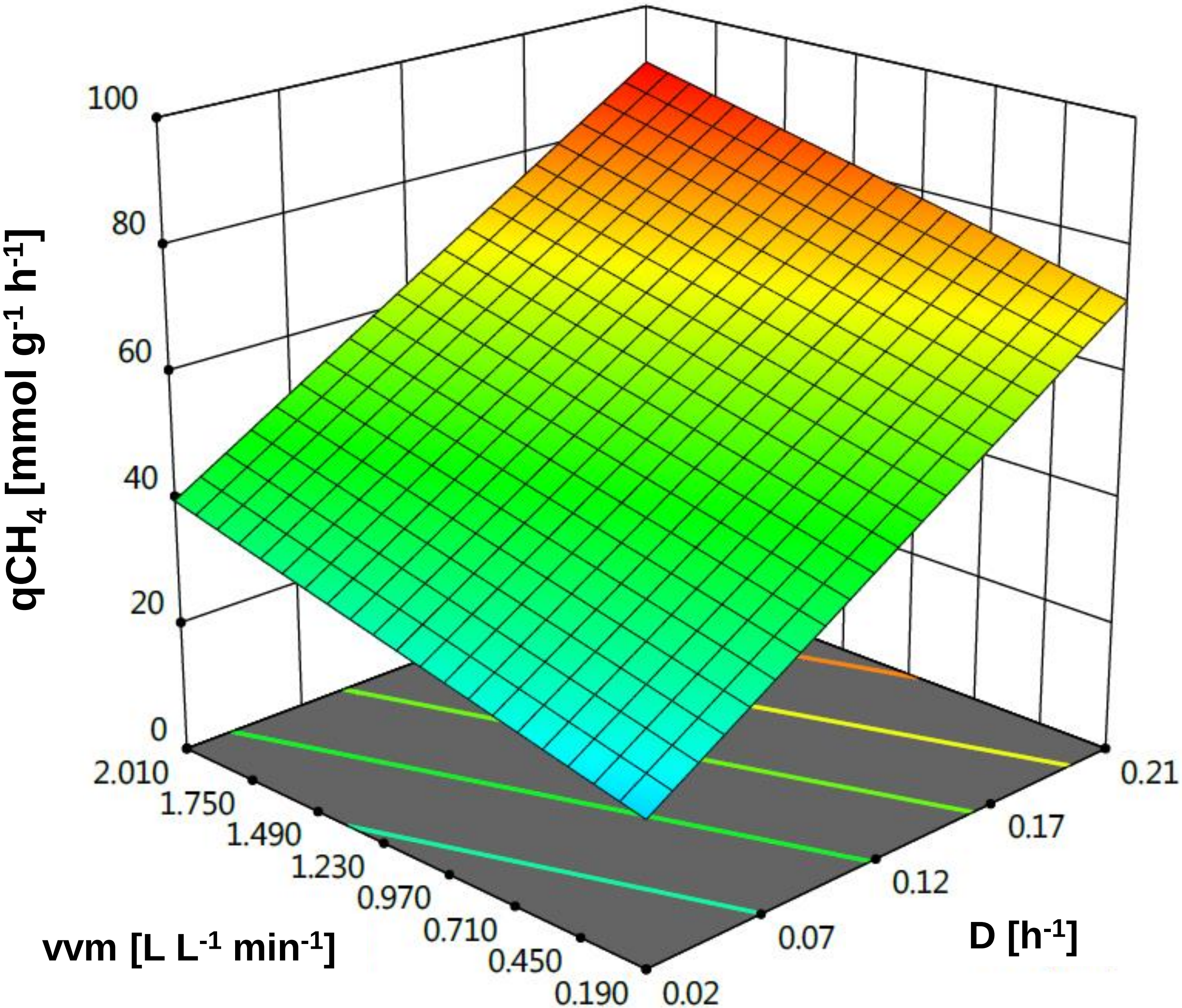


Fig. 5 — Specific water production of *M. marburgensis* is illustrated as a function of the liquid dilution rate. By increasing the liquid dilution rate the specific water production increased. An elucidation of different H_2/CO_2 gassing rates ambiguously influenced specific water productivity.

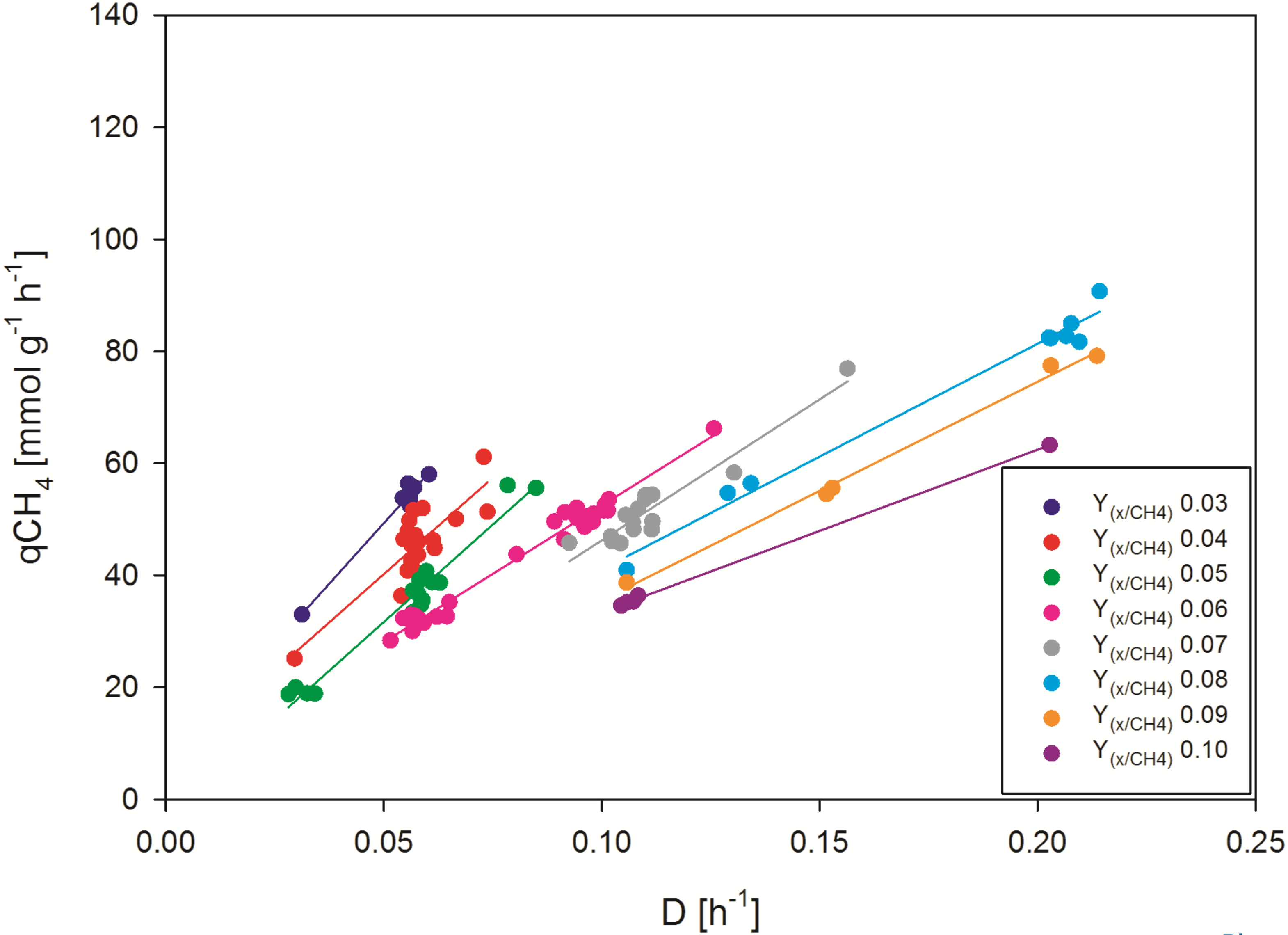
Rittmann *et al*. 2012

Continuous culture



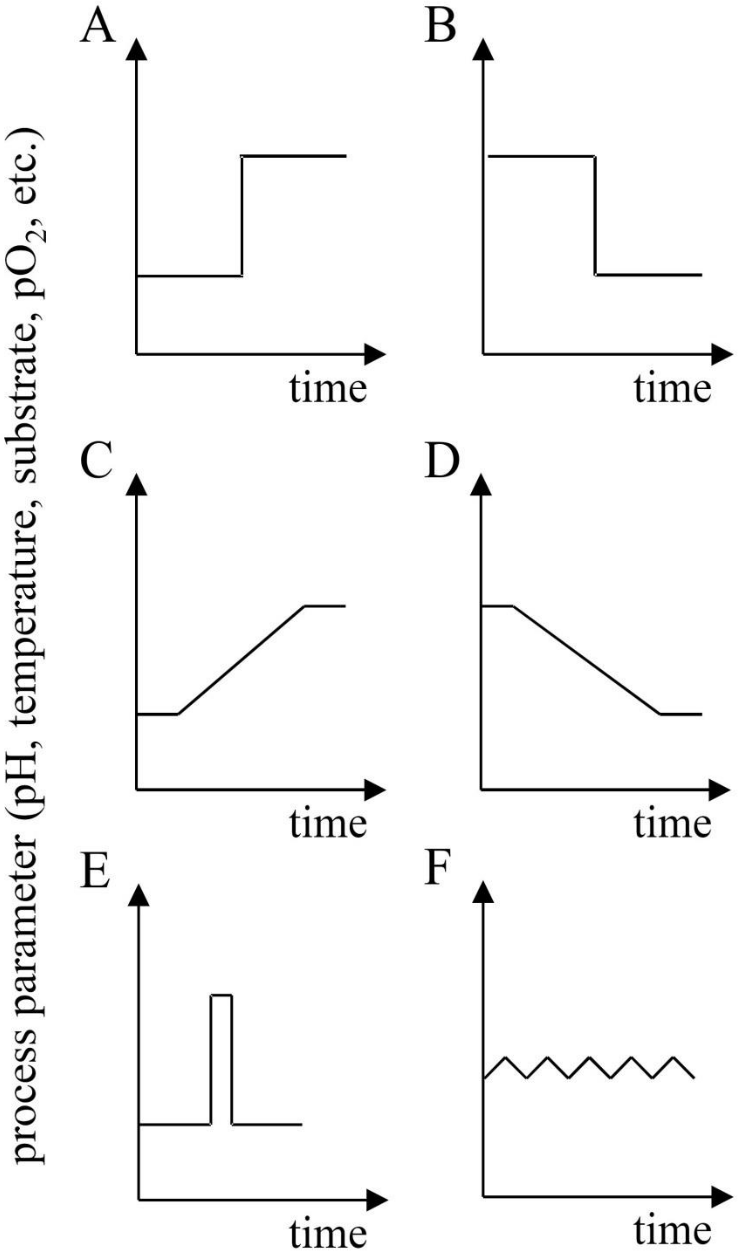
Rittmann *et al*. 2018

Continuous culture



Rittmann *et al*. 2018

Dynamic process conditions



- A: shift-up
- B: shift-down
- C: ramp-up
- D: ramp-down
- E: pulse
- F: oscillation

Figure 1. An overview of dynamic process conditions which can be used in bioprocess development.

Spadiut et al. 2013

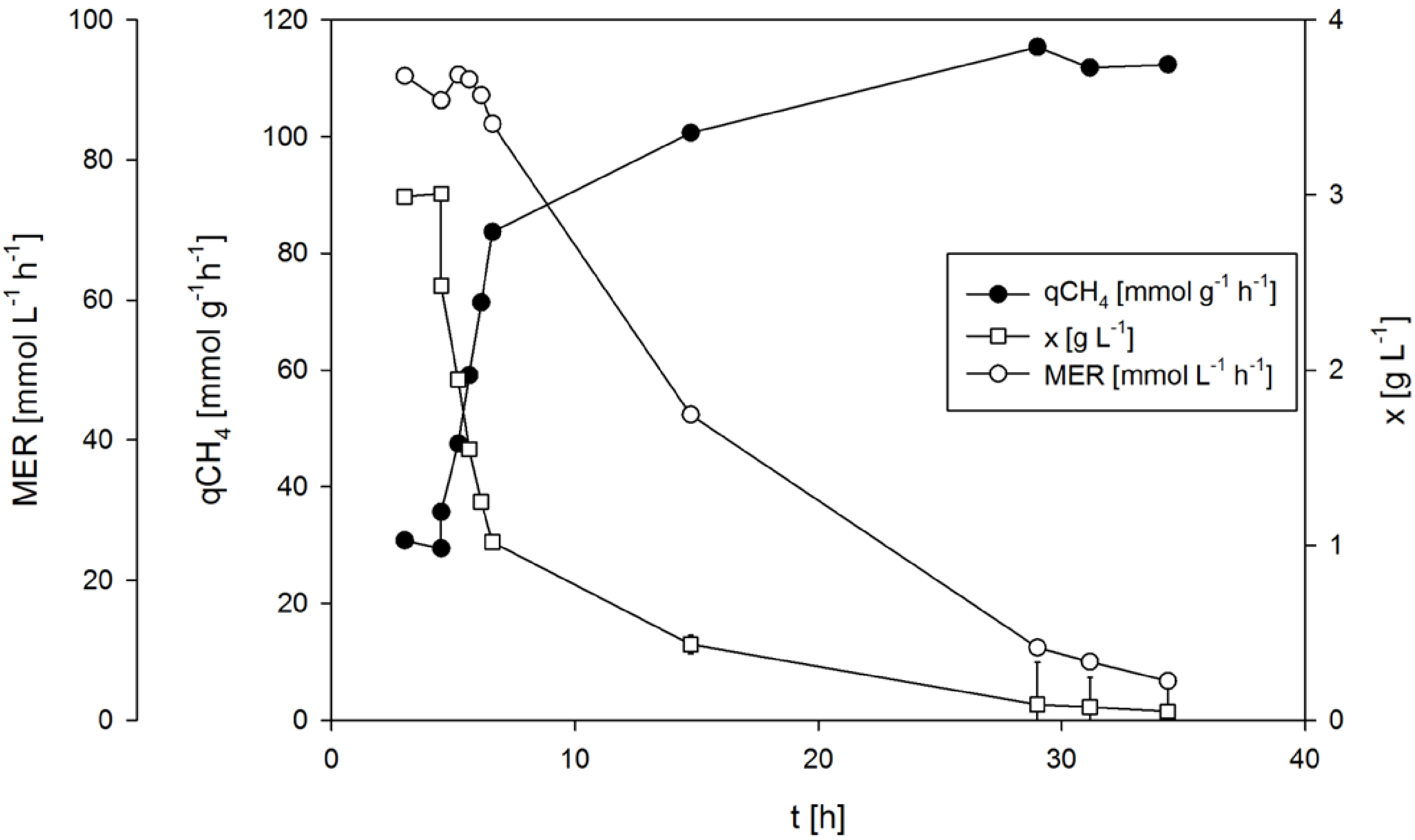
Dynamic process conditions

Dynamic condition	Modus operandi	Changed condition	Identification/Optimisation of
Shift	rapid change of parameter(s) followed by stable condition(s)	physical parameters (D, T, rpm, vvm, light intensity, feed profile) chemical parameters (pH, nutrient concentrations, osmolality) physiological parameters (μ, q_i)	maximum physiological capacity (μ_{max}, $q_{s,max}$) productivity ($q_{p,max}$) maintenance energy stress response metabolism morphology changes limitations
Ramp	continuous and slow change of parameter(s)		productivity yields growth and production kinetic morphology viability limitations physiological capacity
Pulse	sudden change of parameter(s)		productivity uptake rates yields growth kinetics short time cellular response product and metabolite release unscramble physiological and metabolic changes strain characteristic parameters (q_s, q_p)
Oscillation	controlled short up and down ramp(s) in a defined or changing frequency and/or amplitude		productivity growth kinetics metabolic and physiological optimisation heat and stress response quality improvement metabolite formation

Spadiut et al. 2013

Dynamic process conditions

Identification of the maximum specific CH_4 evolution rate ($qCH_{4,max}$).



Rittmann *et al.* 2012

Dynamic process conditions

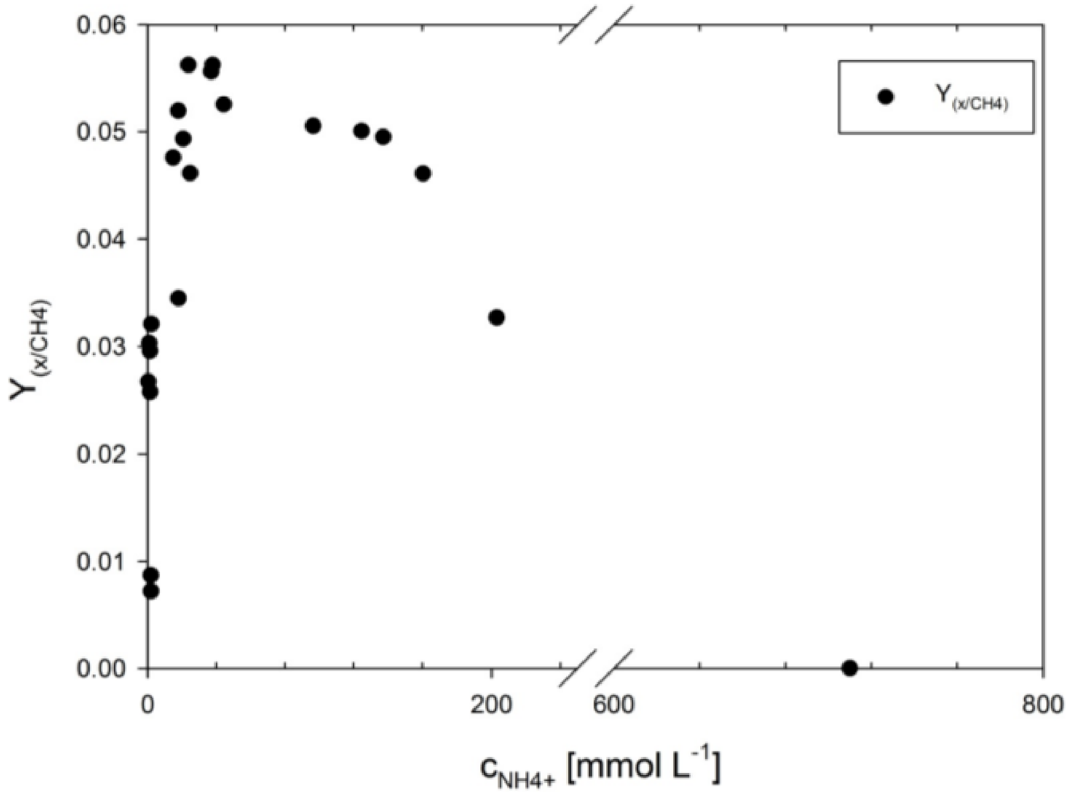


Figure 5. Analysis of $Y_{(x/CH4)}$ as function of the C_{NH4+} from dynamic experiments and chemostat cultures at fixed DM. The results indicate that a window of operation for a high $Y_{(x/CH4)}$ can be achieved if the C_{NH4+} is set to values between 30 and 60 mmol L^{-1}. C_{NH4+} lower than 30 mmol L^{-1} and higher than 60 mmol L^{-1} clearly reduces $Y_{(x/CH4)}$.

Bernacchi *et al*. 2014

Anaerobic cultivation techniques

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Reducing agent	Concentration in media	ORP (mV)	Reference
$Na_2S{\cdot}9H_2O$	0.025–0.05%	− 571	(Bast 2001a; Breznak and Costilow 2007)
Cysteine-HCl	0.025–0.05%	− 340	(Bast 2001a; Breznak and Costilow 2007)
Dithiothreitol	0.01–0.05%	− 330	(Cleland 2002; Breznak and Costilow 2007)
FeS (amorphous hydrated)	4 μg mL^{-1}	− 270	(Brock and Od'ea 1977)
Sodium thioglycolate	0.05–0.1%	− 140	(Bast 2001a; Breznak and Costilow 2007)
Ascorbic acid	0.05–0.1%	+ 58	(Bast 2001a; Breznak and Costilow 2007)
H_2 ($PdCl_2$)	Variable	− 413	(Breznak and Costilow 2007)
Titanium(III)citrate	1–4 mM	− 480	(Zehnder and Wuhrmann 1976; Jones and Pickard 1980)

Table 1 Commonly used reducing agents in anaerobic microbiology

Mauerhofer *et al*. 2018

Anaerobic cultivation techniques

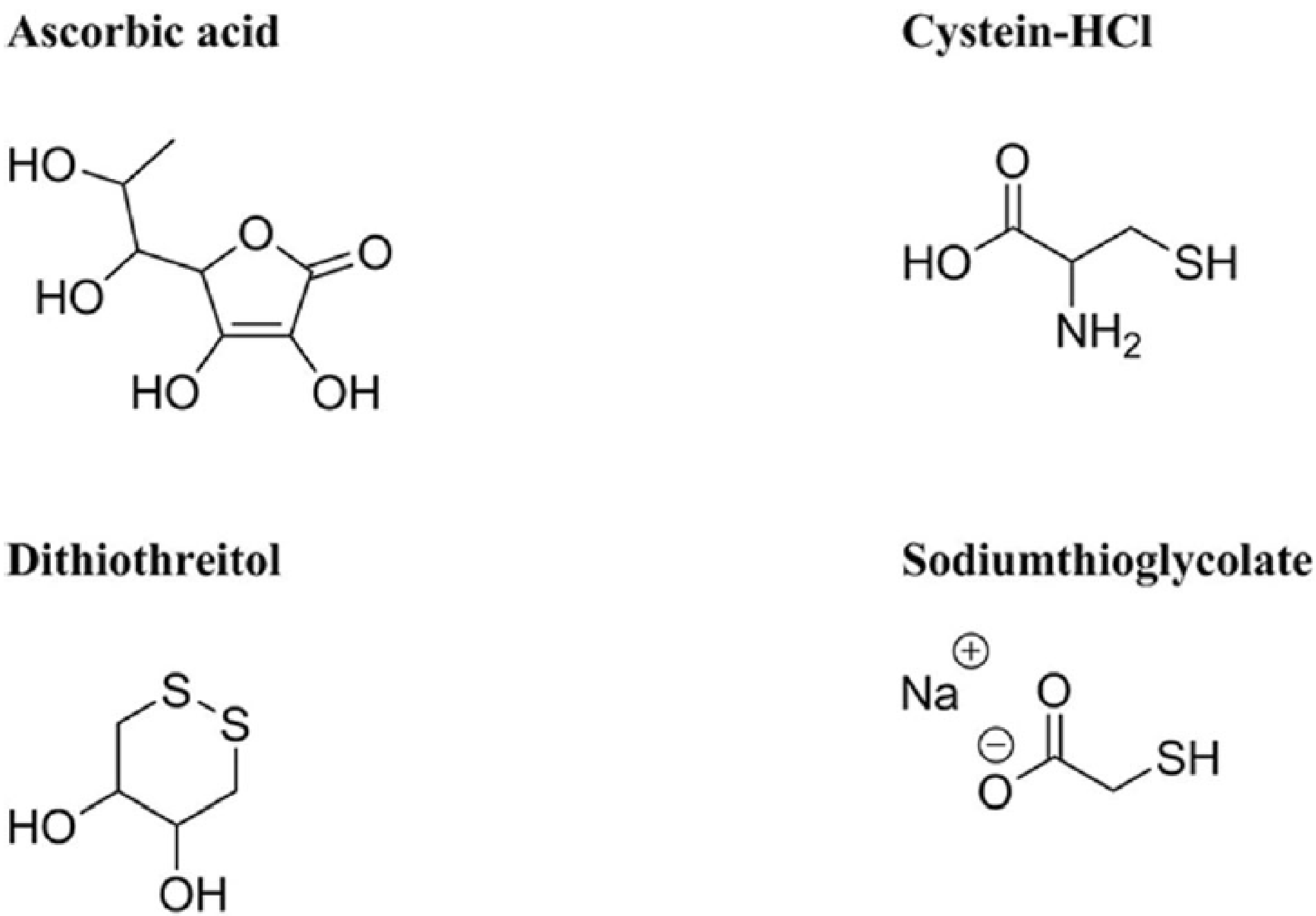

Fig. 2 Chemical structures of selected reducing agents

Mauerhofer *et al*. 2018

Anaerobic cultivation techniques

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Table 2 Redox dyes and their corresponding standard ORP values at 30 °C and pH 7.0

Redox dye	Colour			ORP [mV]	Reference
	reduced	oxidized	oxidized/ reduced		
Methylene blue	transparent	blue		+11	(Bast 2001a; Breznak and Costilow 2007)
Tolouidine blue	blue	pink		-11	(Breznak and Costilow 2007)
Resorufin	violet	pink	transparent	-51	(Bast 2001a; Tratnyek et al. 2001; Breznak and Costilow 2007)
Indigo disulfonate/ Indigo carmine	yellow	orange	green, blue	-125	(Tratnyek et al. 2001; Breznak and Costilow 2007)
Phenosafranine	transparent	red		-252	(Bast 2001a; Tratnyek et al. 2001; Breznak and Costilow 2007)
Titanium(III)citrate	violet	transparent		-480	(Zehnder and Wuhrmann 1976; Bast 2001; Collins et al. 2005)

Mauerhofer *et al.* 2018

Anaerobic cultivation techniques

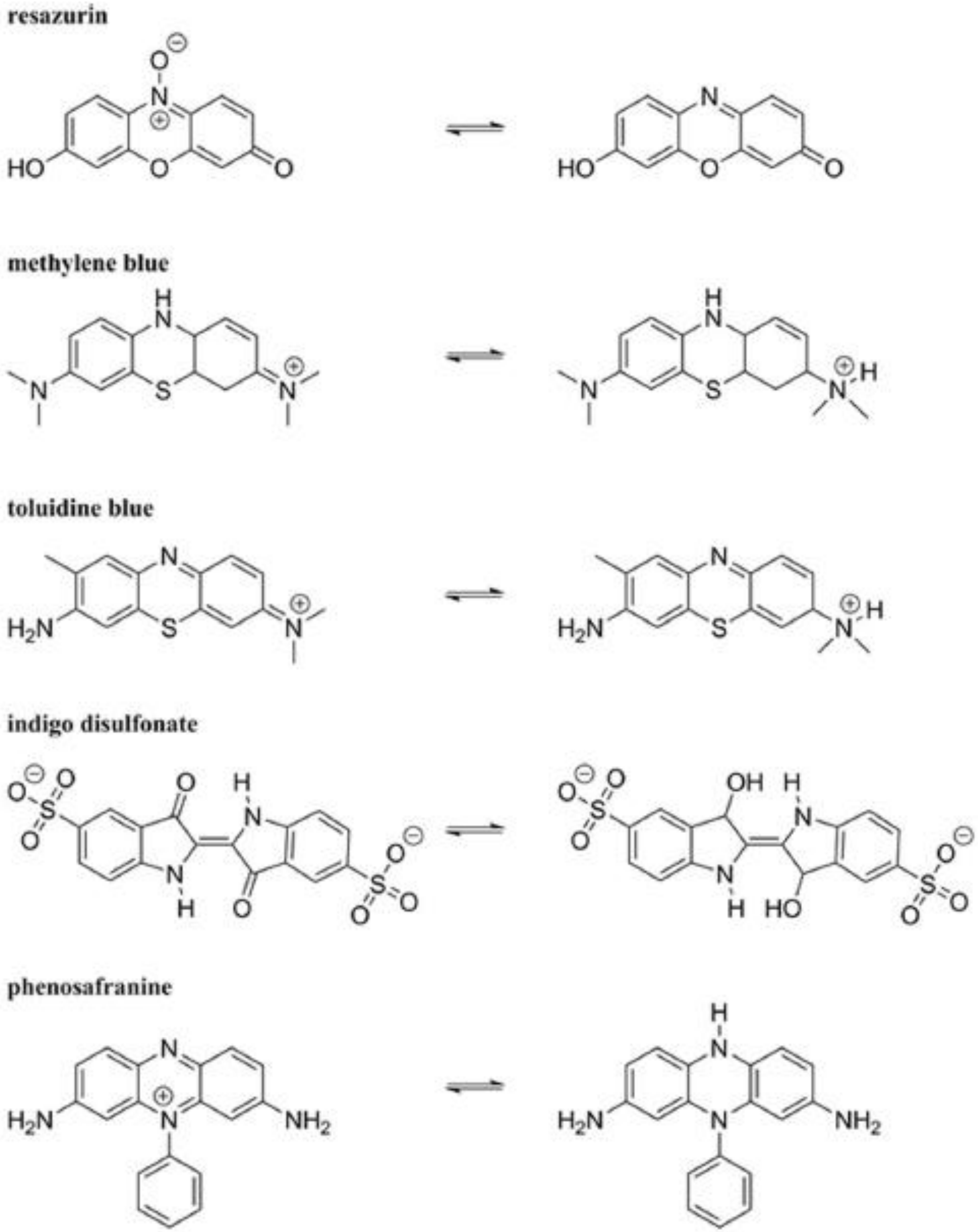
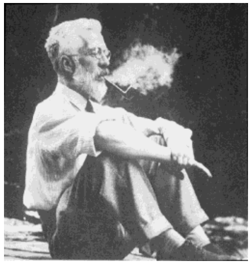


Fig. 3 Oxidized (left) and reduced (right) form of the redox dyes. The structure of titanium(III)citrate is not shown due to different forms of the oxidized form depending on the predominant pH in the medium (Collins et al. 2005)

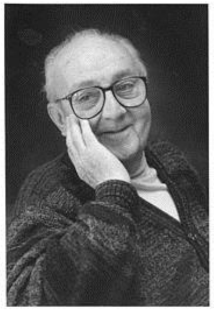
Mauerhofer *et al.* 2018

Introduction – DoE

- **DoE was fouded by Ronald Fisher (UK), who basically developed factoral experiments as well as ANalysis Of VAriance**


Ronald Fisher

- **George Box developed basis for optimisation of DoE designs (Response Surface Modeling (RSM)**
  - „To find out what happens if you change something, is necessary to change it.“
  - „Esentially all models are wrong, but some are useful“


George Box

- **Within the DoE concept Gen‘ichi Taguchi (Japan) developed a qualitative approach (Taguchi-Methodology)**


Gen‘ichi Taguchi

Introduction – DoE

Why do we need DoE?

- **Which (process/cultivation/environmental) parameters have which influence on which variables (response of an organism)?**

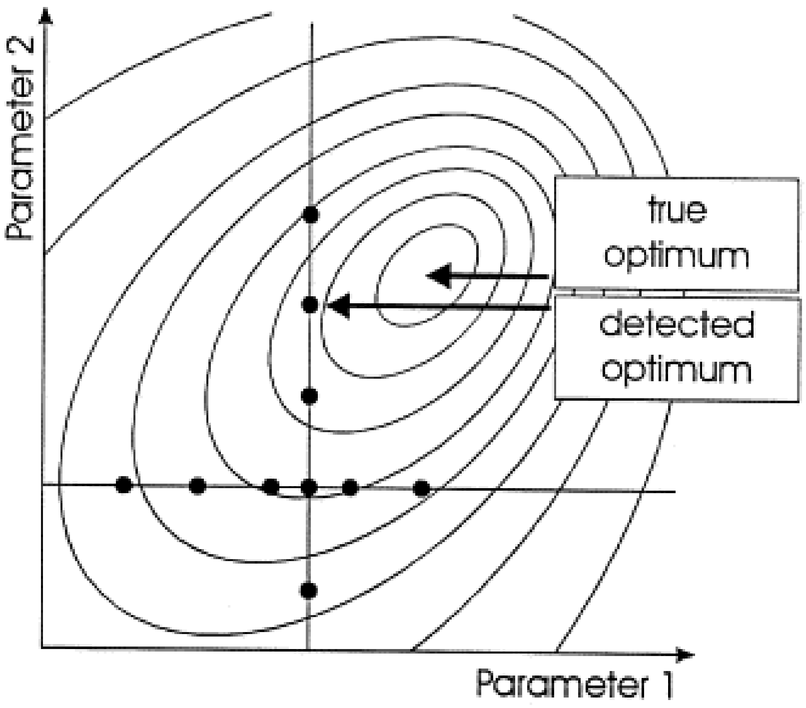
- **How can we determine with a minimum of experiments which parameters and interactions of parameters are beneficial/detrimental for the cultivation of an organism in an experimental design space?**

Introduction – DoE

Why do we need DoE?

- Classical way to perform an experiment is to vary one parameter (factor) at a time

  → OVAT (one-variable-at-a-time)



Drawbacks

- Time consuming
- Interactions
- Maybe the optimum will not be identified

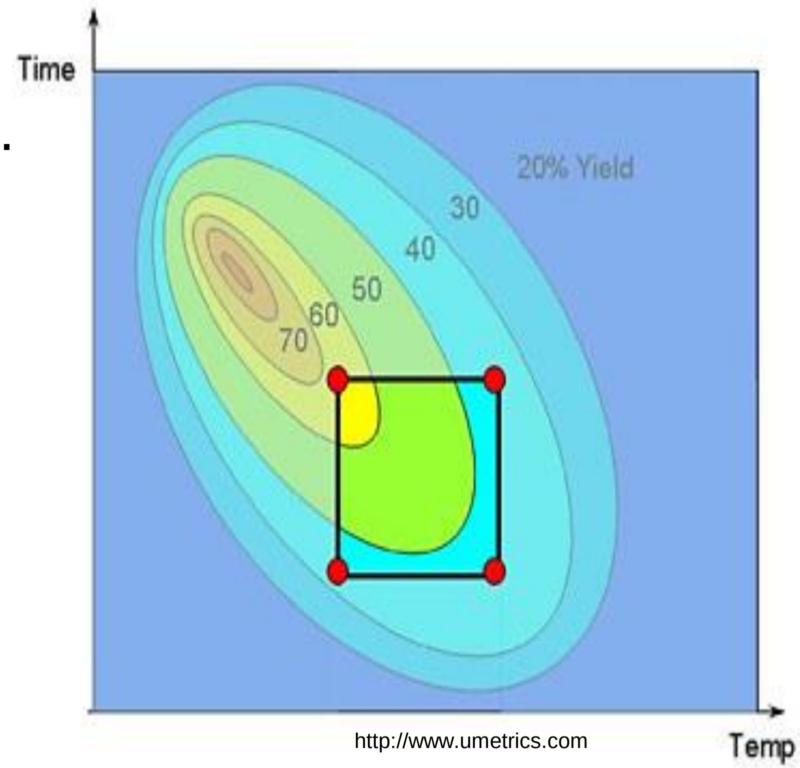
Introduction – DoE

Why do we need DoE?

- Determine parameters (independent variables), which influence responses (dependent variables).
- Optimise cultivation (process)
- Improve growth, product quality, quantity..

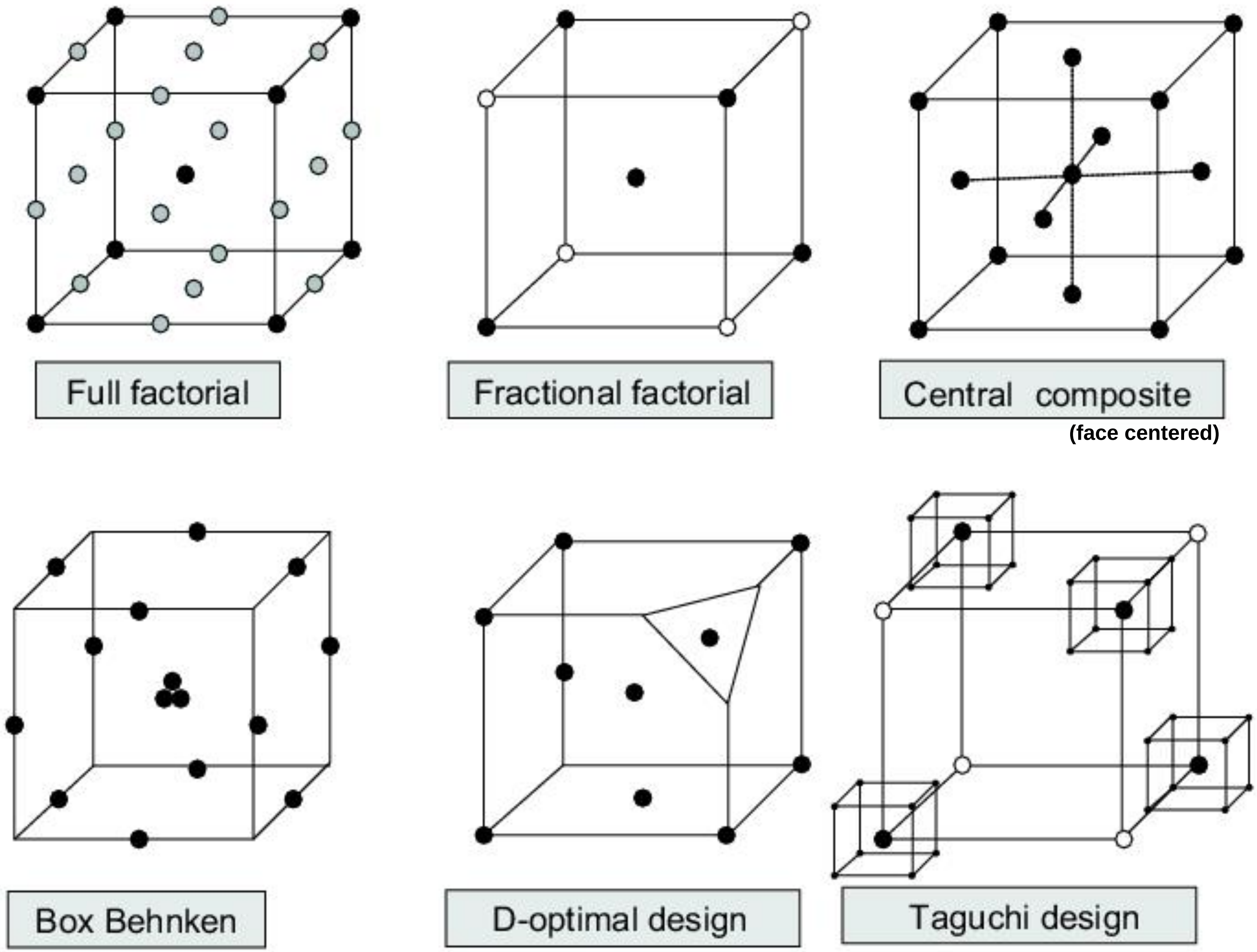
DoE requires

- Planning of randomised experiments
- Dicipline
- Application of statistics



http://www.umetrics.com

DoE designs



Source: http://www.gmpua.com/World/GMPManual/daten/autorenteil/kapitel_07/07_i.htm

Types of DoE experiments

1.) Screening

- Full or fractional factorial designs
  - Resolution V designs are best to be used, but also
  - Resolution IV designs are possible

2.) Optimization or modelling

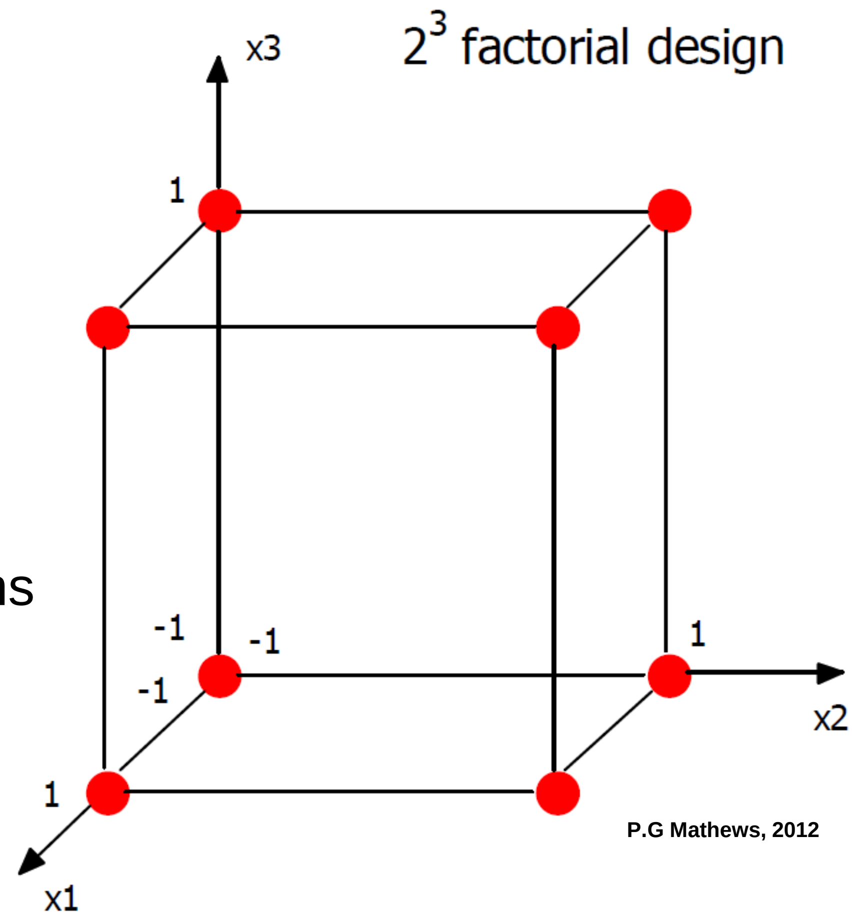
- Response Surface Model (RSM)
  - Cetral composite, Box-Behnken or Taguchi-design

3.) Verification

DoE – Screening designs

Screening

- Good for first experiment(s)
- Can consider lots of variables
- Usually only two levels of each variable
- Relatively few runs
- Limited if any ability to identify interactions
- (depending on the design)
- Risky?

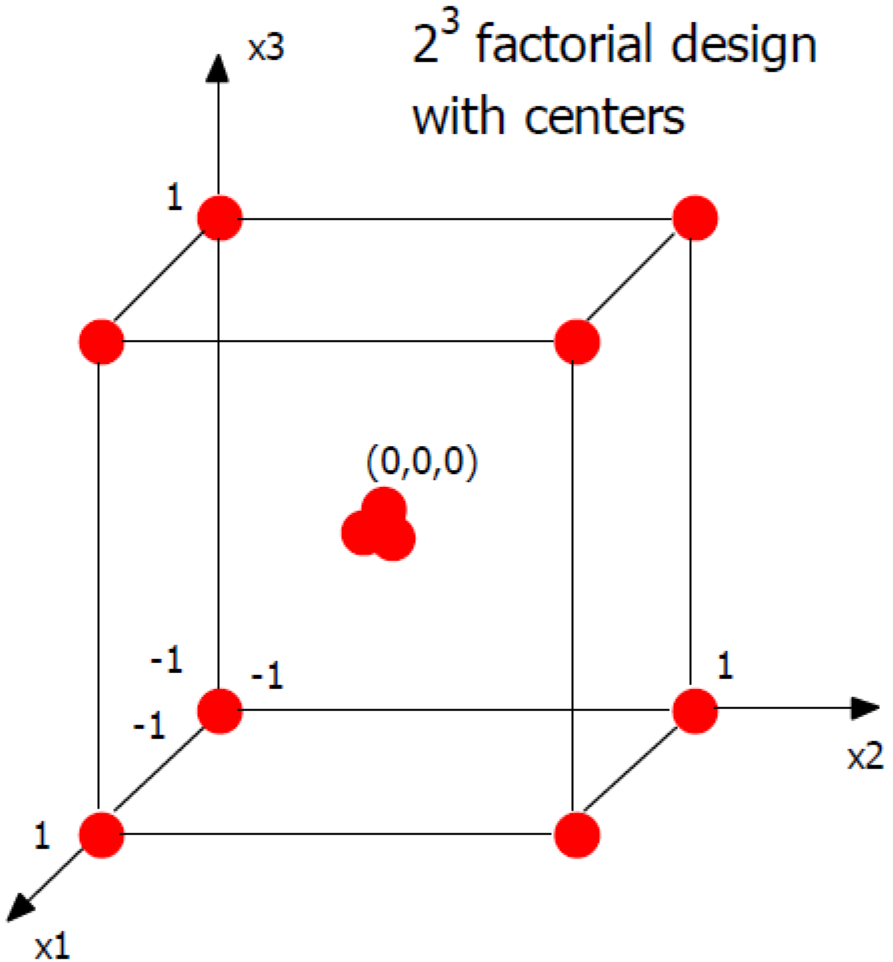


P.G Mathews, 2012

DoE – Screening designs

Screening

- Useful for estimating main effects and interactions
- Fractional factorial design can be used for screenign many factors to find the significant few



P.G Mathews, 2012

DoE – Screening designs

Number of Factors

Runs	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4	2^{2}	2^{3-1}_{III}																		
8		2^{3}	2^{4-1}_{IV}	2^{5-2}_{III}	2^{6-3}_{III}	2^{7-4}_{III}														
16			2^{4}	2^{5-1}_{V}	2^{6-2}_{IV}	2^{7-3}_{IV}	2^{8-4}_{IV}	2^{9-5}_{III}	2^{10-6}_{III}	2^{11-7}_{III}	2^{12-8}_{III}	2^{13-9}_{III}	2^{14-10}_{III}	2^{15-11}_{III}						
32				2^{5}	2^{6-1}_{VI}	2^{7-2}_{IV}	2^{8-3}_{IV}	2^{9-4}_{IV}	2^{10-5}_{IV}	2^{11-6}_{IV}	2^{12-7}_{IV}	2^{13-8}_{IV}	2^{14-9}_{IV}	2^{15-10}_{IV}	2^{16-11}_{IV}	2^{17-12}_{III}	2^{18-13}_{III}	2^{19-14}_{III}	2^{20-15}_{III}	2^{21-16}_{III}
64					2^{6}	2^{7-1}_{VII}	2^{8-2}_{V}	2^{9-3}_{IV}	2^{10-4}_{IV}	2^{11-5}_{IV}	2^{12-6}_{IV}	2^{13-7}_{IV}	2^{14-8}_{IV}	2^{15-9}_{IV}	2^{16-10}_{IV}	2^{17-11}_{IV}	2^{18-12}_{IV}	2^{19-13}_{IV}	2^{20-14}_{IV}	2^{21-15}_{IV}
128						2^{7}	2^{8-1}_{VIII}	2^{9-2}_{VI}	2^{10-3}_{V}	2^{11-4}_{V}	2^{12-5}_{IV}	2^{13-6}_{IV}	2^{14-7}_{IV}	2^{15-8}_{IV}	2^{16-9}_{IV}	2^{17-10}_{IV}	2^{18-11}_{IV}	2^{19-12}_{IV}	2^{20-13}_{IV}	2^{21-14}_{IV}
256							2^{8}	2^{9-1}_{IX}	2^{10-2}_{VI}	2^{11-3}_{VI}	2^{12-4}_{VI}	2^{13-5}_{V}	2^{14-6}_{V}	2^{15-7}_{V}	2^{16-8}_{V}	2^{17-9}_{V}	2^{18-10}_{IV}	2^{19-11}_{IV}	2^{20-12}_{IV}	2^{21-13}_{IV}
512								2^{9}	2^{10-1}_{X}	2^{11-2}_{VII}	2^{12-3}_{VI}	2^{13-4}_{VI}	2^{14-5}_{VI}	2^{15-6}_{VI}	2^{16-7}_{VI}	2^{17-8}_{VI}	2^{18-9}_{VI}	2^{19-10}_{V}	2^{20-11}_{V}	2^{21-12}_{V}

Color coding represents the design resolution:

green = resolution V design or higher, yellow = resolution IV design and red = resolution III design

Design Expert (Stat-Ease Inc., USA)

DoE – Screening designs

2^{5-1}

Factorial Effects Aliases
[Est. Terms] Aliased Terms

[Intercept] = Intercept
[A] = A
[B] = B
[C] = C
[D] = D
[E] = E
[AB] = AB + CDE
[AC] = AC + BDE
[AD] = AD + BCE
[AE] = AE + BCD
[BC] = BC + ADE
[BD] = BD + ACE
[BE] = BE + ACD
[CD] = CD + ABE
[CE] = CE + ABD
[DE] = DE + ABC

Factor Generator
E = ABCD

Factorial Effects Defining Contrast
I = ABCDE

Resolution 5 design

2^{6-2}

Factorial Effects Aliases
[Est. Terms] Aliased Terms

[Intercept] = Intercept
[A] = A + BCE + DEF
[B] = B + ACE + CDF
[C] = C + ABE + BDF
[D] = D + AEF + BCF
[E] = E + ABC + ADF
[F] = F + ADE + BCD
[AB] = AB + CE
[AC] = AC + BE
[AD] = AD + EF
[AE] = AE + BC + DF
[AF] = AF + DE
[BD] = BD + CF
[BF] = BF + CD
[ABD] = ABD + ACF + BEF + CDE
[ABF] = ABF + ACD + BDE + CEF

Factor Generator
E = ABC
F = BCD

Factorial Effects Defining Contrast
I = ABCE = ADEF = BCDF

Resolution 4 design

DoE – Optimisation designs

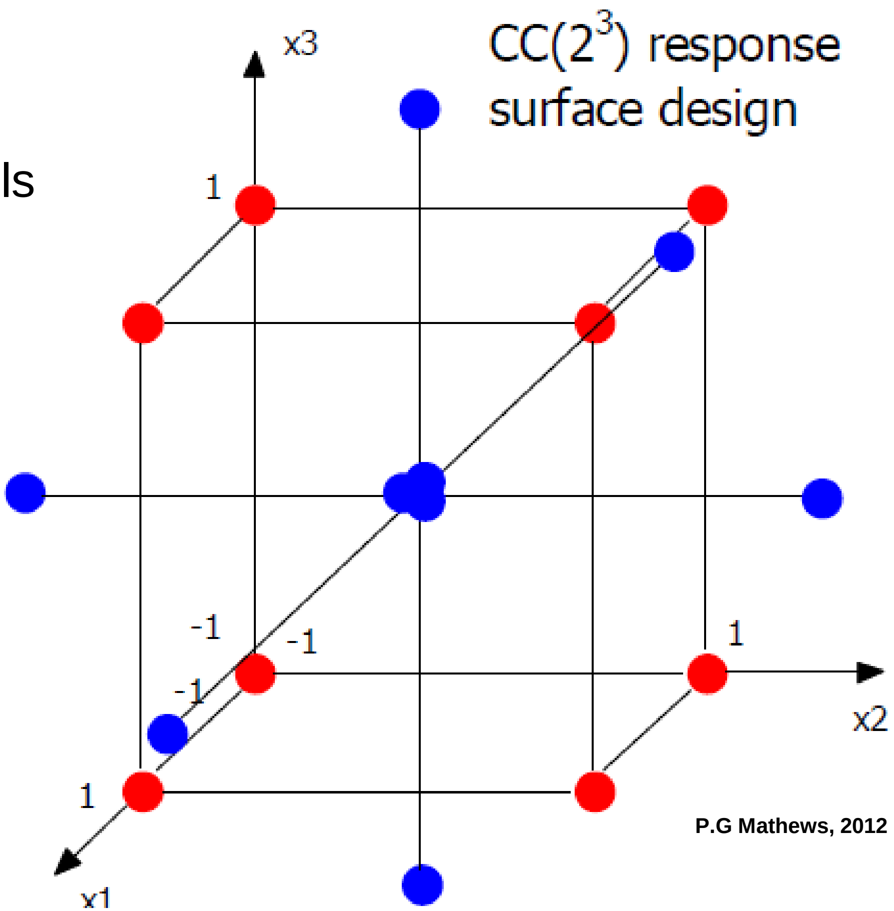
Optimization

- Good follow-up experiment to a screening experiment
- Fewer variables - generally the most important ones
- Often three or more levels of each variable
- Provide a more complex model for the process

DoE – Optimisation designs

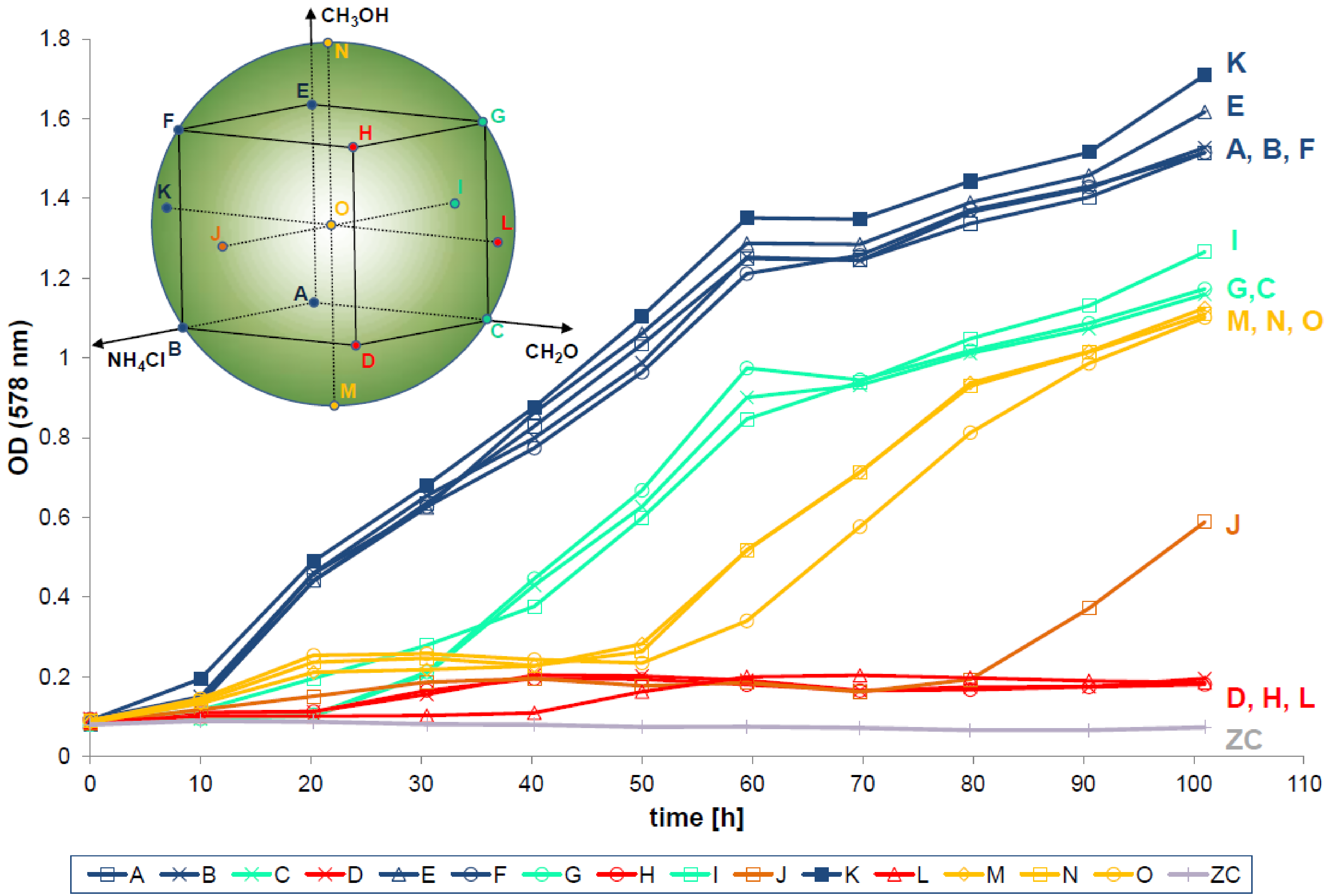
Central composite

- Each numeric factor is varied over 5 levels
- plus and minus α (axial points)
- plus and minus 1 (factorial points)
- usually three to six center points



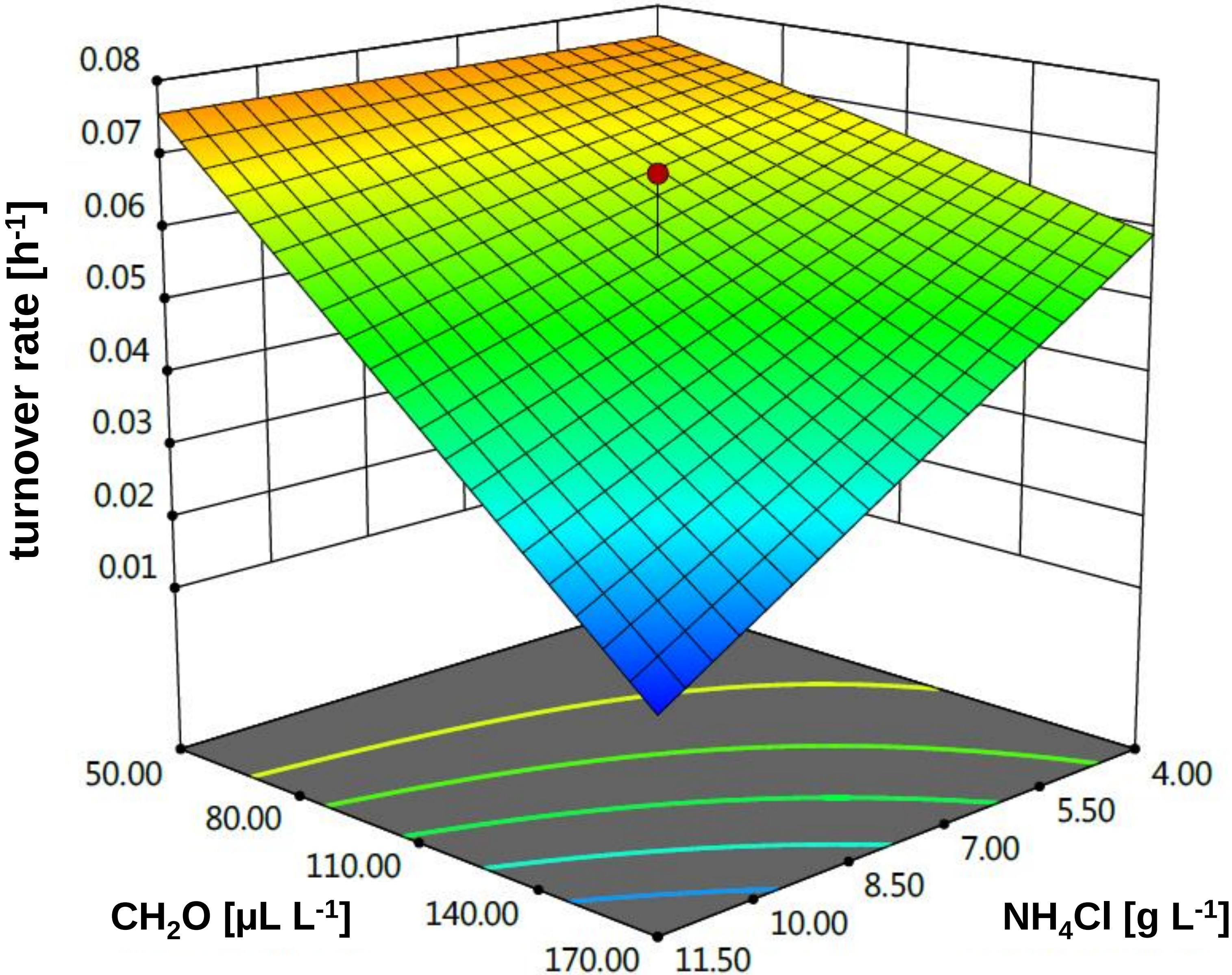
If factorial factors have to be added the central composite design will be doublicated for every combination of the categorial factor levels

DoE – Examples closed batch



Taubner *et al.* 2018

DoE – Examples closed batch



Turnover rate in [h^{-1}] as function of CH_2O and NH_4Cl concentrations. The turnover rate reached its maximum value at low CH_2O concentration. At high CH_2O concentration the turnover rate is higher for low NH_4Cl concentrations. This study was based on a DoE approach.

Taubner *et al.* 2018

DoE – Examples batch

Organism:

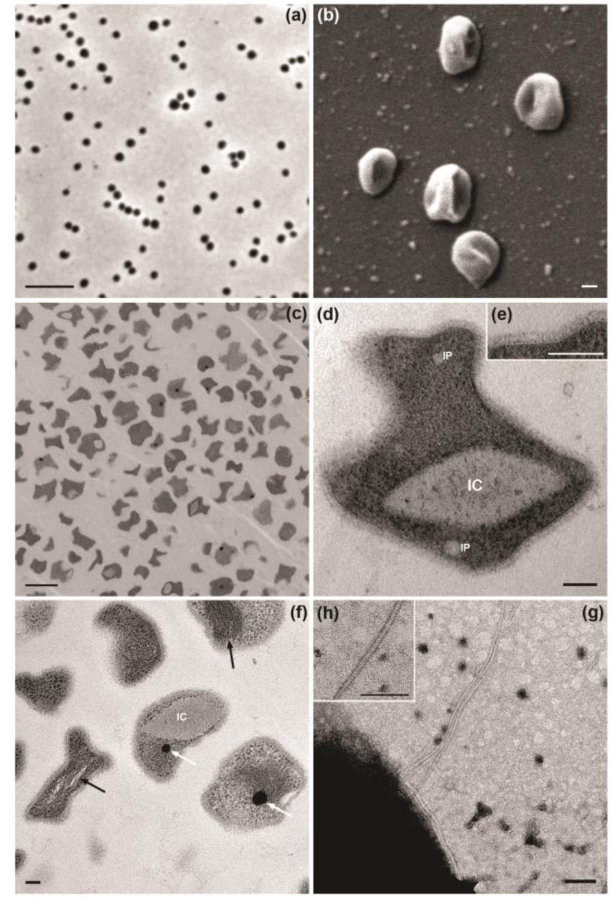
Nitrososphera viennensis

Factors:

- Ammonia concentration 1, 2.5 and 4 mM
- Pyruvate concentration 0.1, 0.8 and 1.5mM
- Temperature 37, 42 and 47 °C

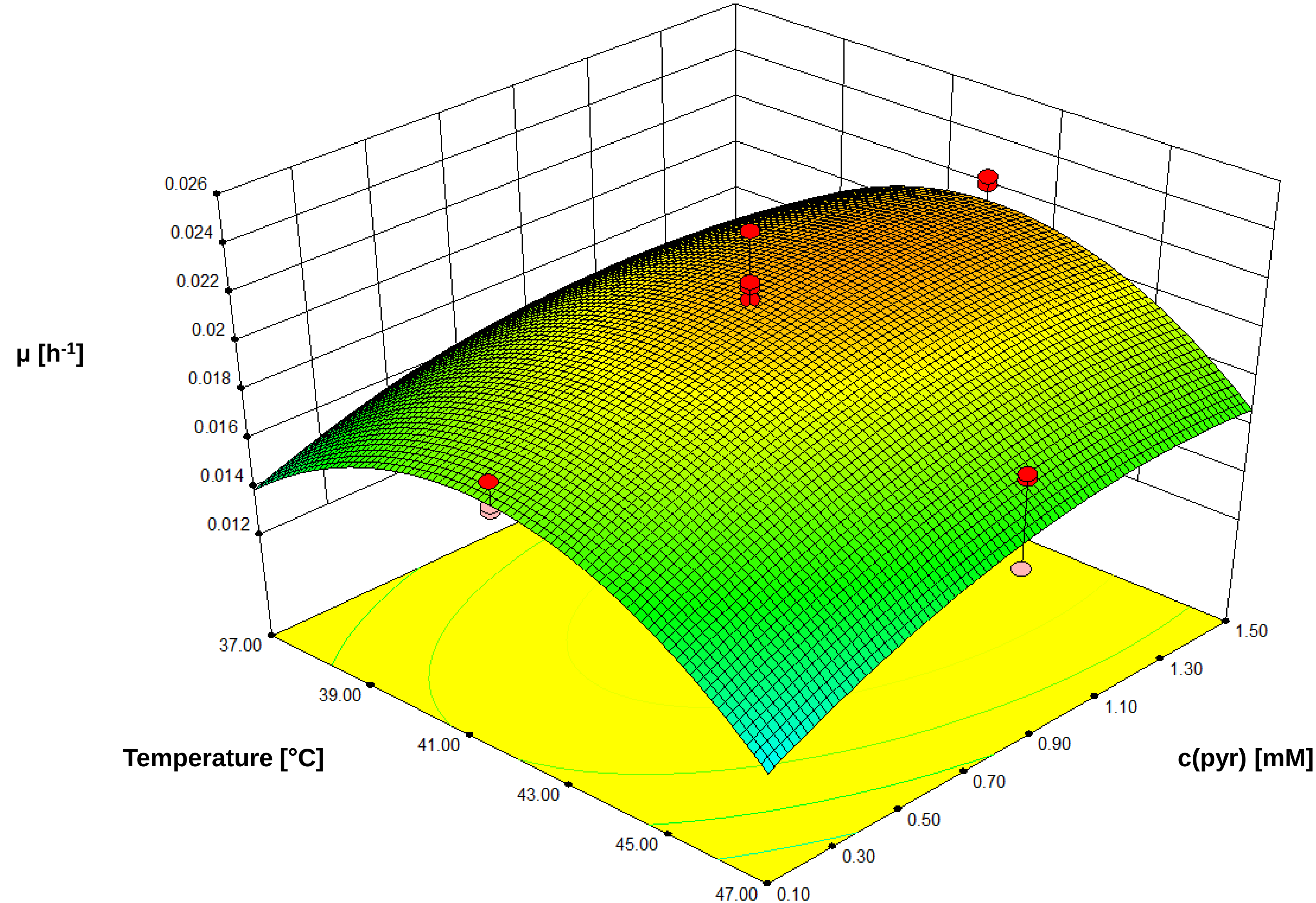
Calculation of:

- NH_4 uptake rates [mmol L^{-1} h^{-1}]
- NO^{2-} production rates [mmol L^{-1} h^{-1}]
- Cell counts
- Specific growth rate [h^{-1}] from NO^{2-} production rate



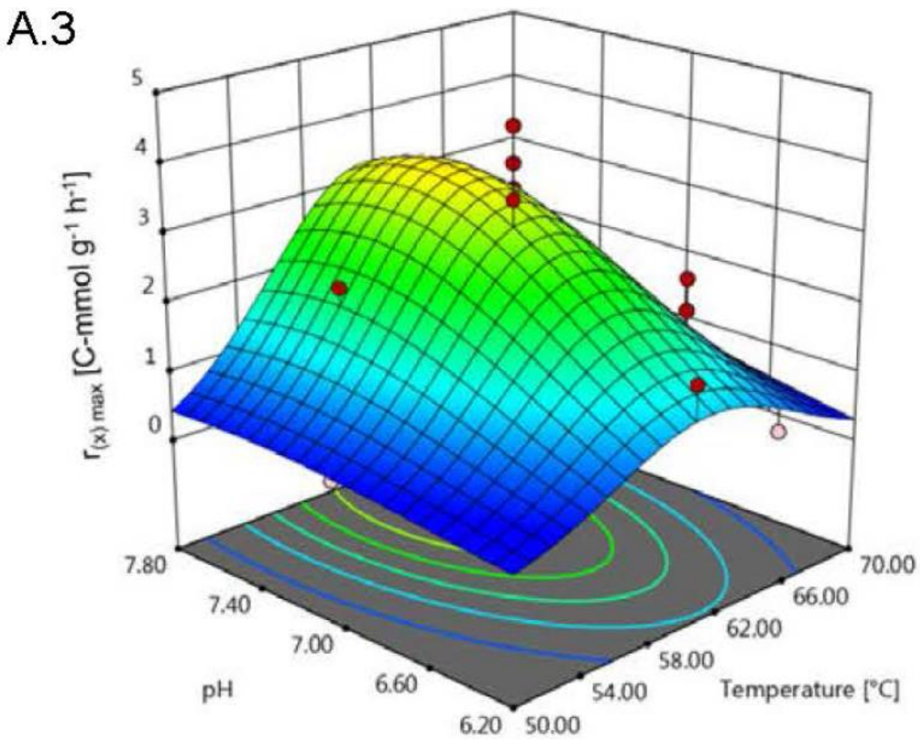
Stieglmeier *et al.*, 2014

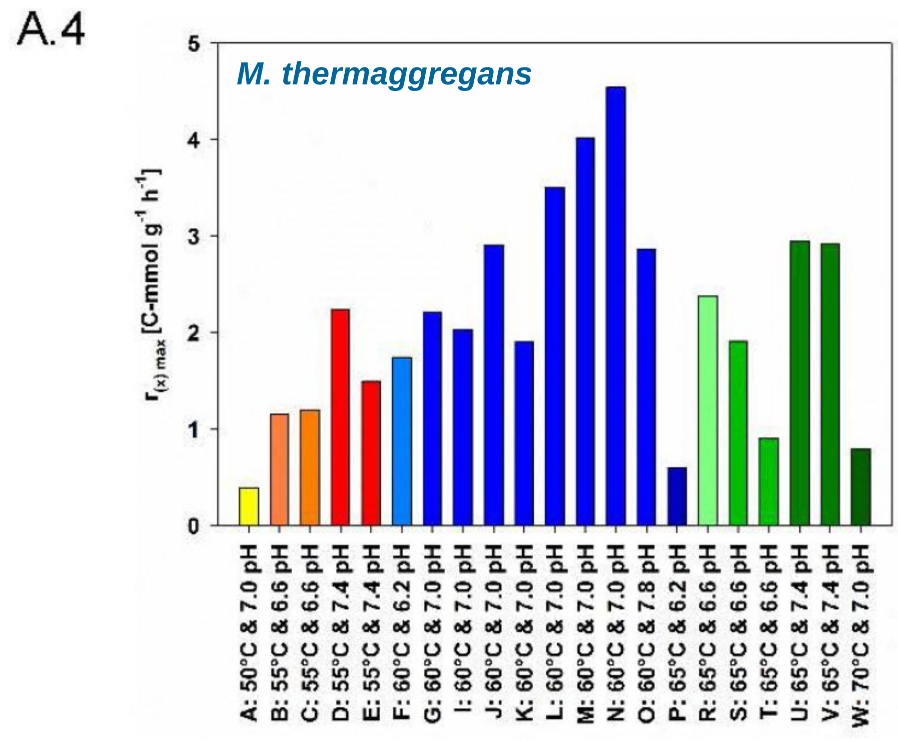
DoE – Examples batch



The graph illustrates the effect of pyruvate concentration (c(pyr)) [mM] and temperature [°C] on the growth rate (μ) [h^{-1}] of EN76^T at a fixed ammonium concentration (c(NH_4^+)) of 2.5 mM. Based on the results of the closed batch cultivation and the subsequent generated response surface model (RSM), the optimal conditions for the cultivation of EN76^T within this three-factorial design space could be retrieved. The optimal cultivation conditions using μ as target value for maximization were calculated as follows: c(pyr) = 1.15 mM, c(NH_4^+) = 2.03 mM, temperature = 42.02 °C, with a desirability of 0.854. Data points of the individual experiments are presented in red or rose colour.

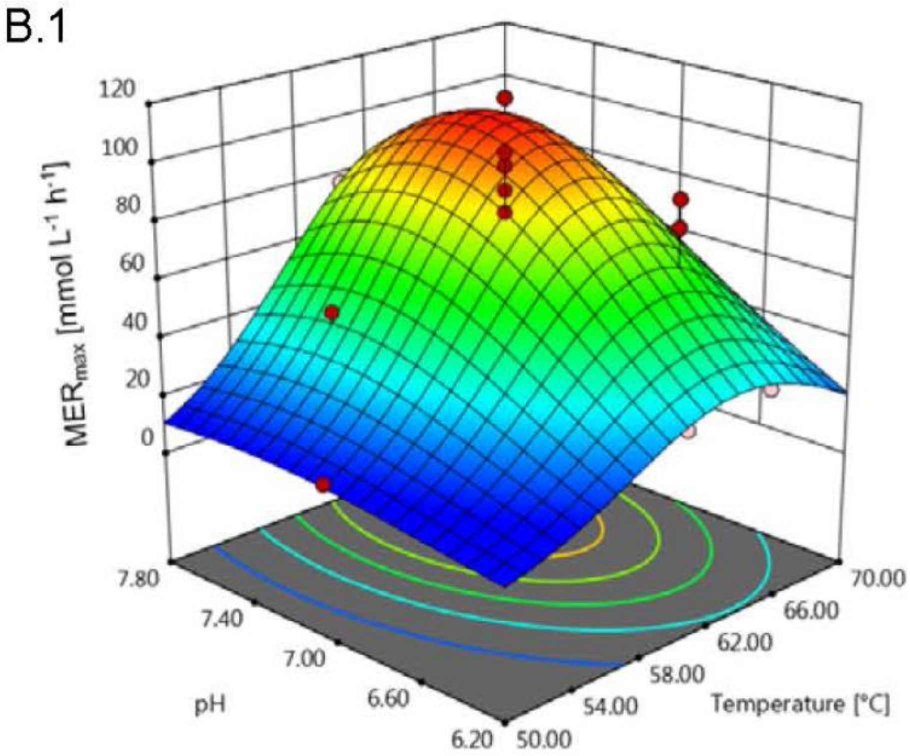
DoE – Examples fed-batch

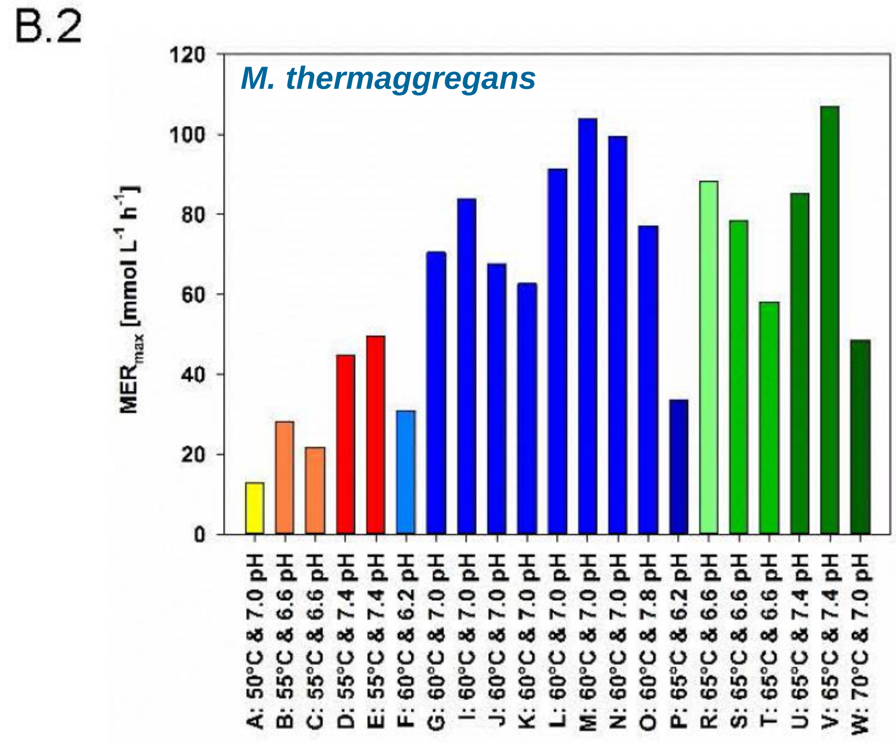




Mauerhofer *et al*. 2018

DoE – Examples fed-batch





Mauerhofer *et al*. 2018

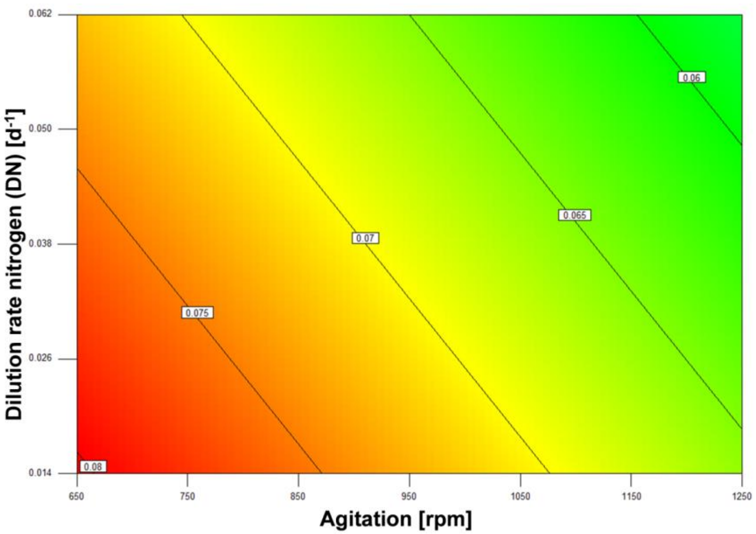
DoE – Examples conti culture

Run	DM [h^{-1}]	pH	DS [$L\ L^{-1}\ d^{-1}$]	T [°C]	rpm	vvm [$L\ L^{-1}\ min^{-1}$]	ratio (H_2/CO_2)	DN [$L\ L^{-1}\ d^{-1}$]	C_{NH4+} [$mmol\ L^{-1}$]	x [$g\ L^{-1}$]	MER [$mmol\ L^{-1}\ h^{-1}$]	q_{CH4} [$mmol\ g^{-1}\ h^{-1}$]	CH_4 offgas [Vol.%]	$Y_{(X/CH4)}$ [C-mol/mol]	r_x [C-mmol $L^{-1}\ h^{-1}$]	DoR-balance	C-balance	N-balance
1	0.043	6.16	0.012	60	654	0.16	3.0	0.014	54.2	0.92	16.4	17.8	4.5	0.07	1.21	96.8%	75.3%	84.1%
2	0.055	7.83	0.013	60	1243	0.19	4.9	0.051	111.9	1.35	45.1	33.4	13.6	0.05	2.25	95.7%	101.8%	142.3%
3	0.211	7.84	0.010	60	1242	0.49	3.0	0.019	26.6	1.14	105.0	91.9	11.6	0.07	7.35	98.5%	80.7%	84.5%
4	0.057	6.16	0.049	61	1243	0.50	5.0	0.016	37.4	3.80	114.0	30.0	12.9	0.06	6.73	94.6%	99.5%	95.0%
5	0.21	6.16	0.053	61	1245	0.20	3.0	0.042	58.5	0.88	71.4	80.9	28.4	0.08	5.72	101.9%	95.6%	103.5%
6	0.059	6.15	0.011	70	1242	0.50	3.0	0.062	128.7	2.76	99.2	36.0	10.5	0.05	4.96	97.2%	124.0%	108.3%
7	0.202	6.15	0.012	70	1245	0.20	5.1	0.016	38.2	0.79	65.2	82.8	23.8	0.08	4.89	103.3%	93.7%	82.8%
8	0.161	7.85	0.009	69	650	0.16	3.1	0.044	64.3	0.27	17.8	66.0	5.0	0.08	1.33	101.6%	96.6%	100.7%
9	0.056	7.85	0.044	70	1245	0.19	2.9	0.014	44.4	1.75	65.2	37.2	26.0	0.05	3.00	97.0%	95.1%	110.4%
10	0.207	7.84	0.051	69	1245	0.49	5.0	0.048	44.7	1.29	111.3	86.7	12.7	0.07	8.13	98.2%	110.0%	101.9%
11	0.110	6.98	0.033	63	951	0.28	4.0	0.034	47.8	1.30	60.7	46.7	11.7	0.07	4.37	101.1%	82.4%	92.3%
12	0.111	6.99	0.025	64	947	0.29	3.9	0.018	37.8	1.26	61.5	48.7	11.5	0.07	4.31	103.7%	82.1%	94.0%
13	0.114	6.99	0.026	65	950	0.30	4.0	0.017	49.0	1.16	54.6	47.0	9.4	0.07	4.04	96.7%	129.8%	109.1%
14	0.107	6.99	0.020	65	946	0.29	3.9	0.026	45.6	1.05	59.5	56.7	11.0	0.06	3.39	95.1%	106.0%	105.2%
15	0.061	6.14	0.055	70	1245	0.20	5.1	0.045	100.3	1.77	67.8	38.2	25.8	0.05	3.32	101.1%	90.9%	109.3%
16	0.065	5.53	0.010	67	1242	0.49	2.9	0.055	96.6	1.98	99.2	50.0	10.7	0.04	3.97	95.4%	71.2%	112.5%
17	0.064	8.41	0.053	68	1242	0.19	2.9	0.004	18.2	2.06	66.4	32.2	27.1	0.06	4.01	106.0%	76.0%	93.0%
18	0.212	5.6	0.059	59	1242	0.19	2.9	0.047	39.7	0.84	71.2	85.0	31.5	0.08	5.43	103.5%	85.4%	84.3%

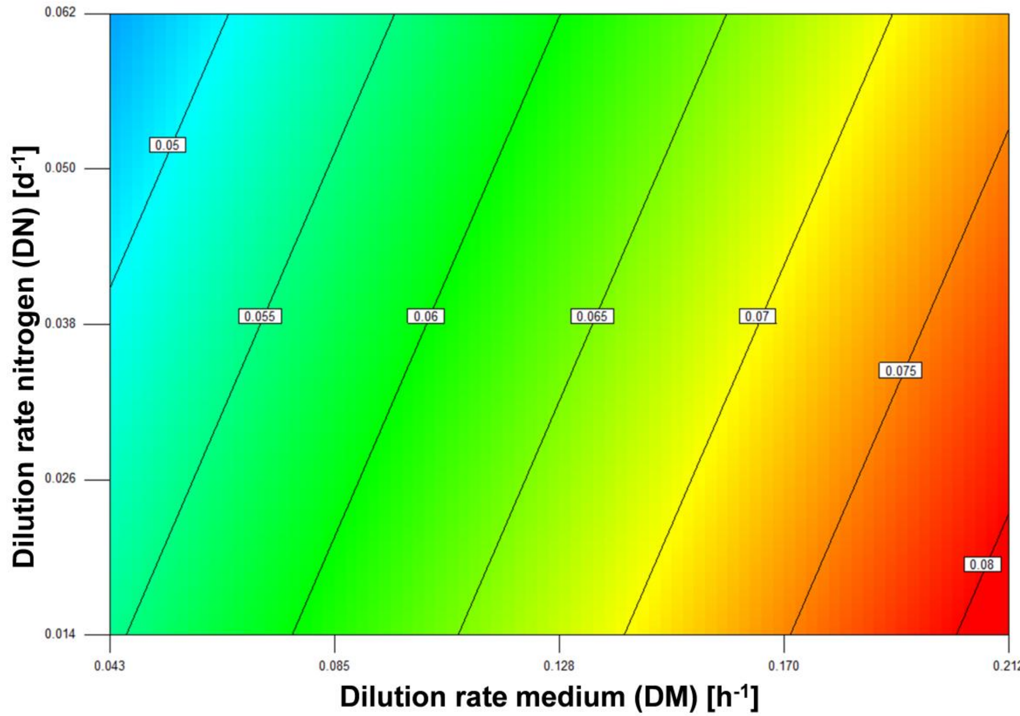
Multivariate model generation from a nona-factorial DoE

Bernacchi *et al*. 2014

DoE – Examples conti culture



Plot of nitrogen dilution rate (DN) [d^{-1}] versus medium dilution rate (DM) [h^{-1}] in order to analyze growth to product yield ($Y_{(x/CH4)}$). Individual levels of $Y_{(x/CH4)}$ are indicated through lines and boxes within the graph. The analysis shows that $Y_{(x/CH4)}$ varies by adjusting DN, DM, or both. An increase of DN reduces $Y_{(x/CH4)}$. However, an increase of DM increases $Y_{(x/CH4)}$.



Plot of the nitrogen dilution rate (DN) versus the agitation speed in order to analyze $Y_{(X/CH4)}$ [C-mol/mol]. Individual levels of $Y_{(x/CH4)}$ are indicated through lines and boxes within the graph. $Y_{(x/CH4)}$ is highest at the lowest investigated range of both factors.

Bernacchi *et al*. 2014

DoE – Software

- **Modde (Umetrics, Sweden)**
- **Design Expert (Stat-Ease Inc., USA)**
- **Statistica (StatSoft, USA)**
- **R Commander**