Julian Lange

Ralf Takors

Bastian Blombach

Institute of Biochemical
Engineering, University of
Stuttgart, Stuttgart, Germany

Review

Zero-growth bioprocesses: A challenge for microbial production strains and bioprocess engineering

Microbial fermentation of renewable feedstocks is an established technology in industrial biotechnology. Besides strict aerobic or anaerobic modes of operation, novel innovative and industrially applicable fermentation processes were developed connecting the advantages of aerobic and anaerobic conditions in a combined production approach. As a consequence, rapid aerobic biomass formation to high cell densities and subsequent anaerobic high-yield and zero-growth production is realized. Following this strategy, bioprocesses operating with substantial overall yield and productivity can be obtained. Here, we summarize the current knowledge and achievements in such microbial zero-growth production processes and pinpoint to challenges due to the complex adaptation of the cellular metabolism during the cell's passage from aerobiosis to anaerobiosis.

Keywords: Anaerobic production / *Corynebacterium glutamicum* / *Escherichia coli* / (micro)Aerobic growth / Oxygen deprivation

Received: April 15, 2016; *revised:* August 18, 2016; *accepted:* September 19, 2016

DOI: 10.1002/elsc.201600108

1 Introduction

The shortage of fossil energy resources, accompanied by the strong impact of their use on the environment raises the demand for sustainable, safe, and efficient substitution of petrochemical production processes. Hitherto crude oil-derived chemicals and chemical building blocks are to be replaced by production from renewable resources. Besides chemical manufacturing of renewable feedstocks to valuable compounds, biotechnological processes offer more and more opportunities to produce fuels, chemicals, or pharmaceuticals in a cost effective way from biomass [1]. The cost efficient bio-based production of such products is a most relevant goal for the future and has to meet economic and environmental requirements. Therefore, the microbial production systems have to operate with maximal yield and productivity.

Current microbial fermentation processes are either anaerobic allowing efficient product formation or aerobic typically operating with a lower product yield [2]. Novel innovative and industrially applicable fermentation processes with facultative anaerobic organisms such as *Escherichia coli* or *Corynebacterium glutamicum* combine the advantages of aerobic and anaerobic

conditions in a bioprocess. Such zero-growth approaches consist of an aerobic phase for rapid biomass formation, followed by an anaerobic phase, where the target molecule is synthesized with maximal yield at high cell densities. The final process should perform with substantial overall yield and productivity. Moreover, nongrowth conditions render bacteria more resistive toward toxic entities or products [3–5] and lower the energy input due to a reduced mixing and aeration requirement.

Three variants of zero-growth approaches are so far described in literature: (i) two-stage processes, where biomass formation and zero-growth production is performed in two separated vessels (e.g. [6]), (ii) dual-phase fermentations, in which growth and production is accomplished in a single reactor with an abrupt shift from aerobiosis to anaerobiosis (e.g. [7]), and (iii) triple-phase approaches in a single reactor with an intermediate microaerobic phase that bridges aerobic biomass formation and zero-growth production (e.g. [8]) (see Fig. 1). A widespread phenomenon of dual-phase approaches is that yield and productivity in the production phase are significantly reduced compared to two-stage processes, where growth and production is performed in separated simple flasks or bottles [9, 10]. The reason for this observation can be attributed to a transition phase that cells pass in weakly aerated vessels such as shaking flasks at higher biomass concentrations. This so called microaerobic phase is so far neither exactly defined nor clearly distinguishable from the aerobic and anaerobic condition. However, the microaerobic environment obviously plays an essential role in the adaptation and preparation of the enzymatic machinery to complete anaerobic

Correspondence: Dr. Bastian Blombach (blombach@ibvt.uni-stuttgart.de), Institute of Biochemical Engineering, University of Stuttgart, Allmandring 31, D-70569 Stuttgart, Germany

Abbreviations: DO, dissolved oxygen; FLC, fuzzy logic control

Figure 1. Schematic overview of the three zero-growth production processes and respective courses of biomass, substrate, and product concentrations. (A) The two-stage mode of operation involves two separate reaction batches for: aerobic growth, and anaerobic production at high biomass. (B) A dual-phase process realizes both phases in a single bioreactor with an abrupt transition from aerobiosis to anaerobiosis. (C) The triple-phase mode of operation combines three distinct phases ranging from aerobiosis via microaerobiosis to anaerobiosis in a single bioreactor. Black line: semilogarithmic description of the biomass concentration; dotted line: substrate concentration; dashed line: product formation.

conditions and therefore has a severe impact on the overall performance of the producer strain [10–12]. Based on this knowledge, triple-phase processes were developed, that comprise an aerobic phase being separated from the anaerobic production phase by a low aerated microaerobic interface [8, 12–16]. Interestingly, there is a deep lack in the systematic understanding of the molecular events during this interface. By analogy, cells in large-scale bioreactors are frequently exposed to changing oxygen availability due to insufficient power input and mixing [17–19], which often comes along with reduced product yields [20–22].

2 Oxygen-related physiological properties of facultative microbes

Facultative bacteria in contrast to strict aerobic or anaerobic bacteria inherit the ability to master oxygen fluctuations and maintain vitality or even growth upon facing a transfer to anaerobiosis. These bacteria can shift from aerobic to anaerobic respiration, where an inorganic alternative entity functions as the terminal electron acceptor (e.g. nitrate), or even fermentation, where the cells have to secrete reduced metabolites to maintain a balanced redox state [23]. The respiratory chain of industrially relevant facultative bacteria such as *E. coli* or *C. glutamicum* has been intensely studied and typically comprises a *bo*-type terminal oxidase besides a high oxygen affine *bd*-type oxidase that is essentially active under microaerobic conditions (for reviews see [24–26]).

Following the general strategy of organisms to maximize energy conservation, strict aerobic respiration is preferred ($\Delta G^0 = -2870$ kJ/mol glucose), over anaerobic respiration ($\Delta G^0 = -858$ kJ/mol glucose with nitrate) and fermentation (e.g. *E. coli*: $\Delta G^0 = -218$ kJ/mol glucose) [27, 28] with the latter yielding only 2 mol ATP per mol of glucose through substrate level phosphorylation. Consequently, in the absence of alternative electron acceptors the growth rate, the carbon dioxide evolution rate, and the biomass yield decreases with increasing oxygen limitation, whereas the NADH/NAD⁺ ratio steadily increases (e.g. *E. coli* MC4100: 0.03 (aerobic), 0.72 (anaerobic) [29],

data withdrawn from graphic). This engenders transition states, in which growth and the formation of fermentation products are coupled [30]. Finally, the complete lack of oxygen initiates the fermentation phase characterized by minimal biomass formation and maximal product yield. Noteworthy, in some microbes such as *E. coli*, *C. glutamicum*, or *Saccharomyces cerevisiae* oxygen limitation results in significant higher specific glucose uptake rates compared to strict aerobic conditions [29, 31–34], enabling anaerobic production with high productivity.

3 Zero-growth bioprocesses with facultative anaerobes

For low energy demanding, reduced and growth-uncoupled products a suitable strategy is to set up a bioprocess with an aerobic phase for rapid biomass formation (ideally without production), followed by anaerobic zero-growth conditions to allow a maximal product yield. However, facultative anaerobic and industrial relevant microbes such as *E. coli* and *C. glutamicum* can promote growth under anaerobic conditions using anaerobic respiration or fermentation (*E. coli*: [24, 35], *C. glutamicum*: [26, 36, 37]). As a consequence, to obtain a highly efficient anaerobic production phase, growth has to be prevented by omitting terminal electron acceptors from the culture broth or manipulating the central metabolism of respective producer strains. In *C. glutamicum* it was shown, that growth in the absence of nitrate as alternative electron acceptor is only marginally promoted by fermentation of particular carbon sources [37]. In contrast, wild-type *E. coli* promotes growth under anaerobic conditions, but engineered strains such as the succinic acid producer strain *E. coli* AFP111 [38] are unable to grow under anaerobic conditions due to an inactivated pyruvate formate lyase and lactate dehydrogenase [39]. Such strains are therefore quite suitable for bioprocesses combining aerobic growth and anaerobic zero-growth production. Prominent examples of such approaches, namely two-stage, dual-phase, and triple-phase processes (see Fig. 1) are presented and discussed in the following paragraphs.

3.1 Two-stage process

A two-stage bioprocess consists of an aerobic stage for biomass formation and an anaerobic stage for production both separated into two vessels [40] (see Fig. 1A). The facultative anaerobic production strain is cultivated aerobically, subsequently harvested by centrifugation and transferred aseptically to high cell densities in anaerobic batches [6, 41]. The anaerobic environment is established in sealed flasks or nonaerated bioreactors, which may additionally be purged with N_2 or CO_2 to strip out residual oxygen in the medium and in the headspace of the reactor [6, 41–44]. Especially for *C. glutamicum* strains, two-stage processes were successfully applied for the efficient production of a broad product spectrum of metabolites including L-alanine [45, 46], L-valine [47, 48], ethanol [5, 49], L-lactate [6], D-lactate [50], isobutanol [10, 51], succinic acid [6, 44, 52–54], or xylitol [55] (see Table 1). Notable examples are *C. glutamicum* BOL-3/pAN6-gap, which produced 1134 mM (134 g/L) succinic acid with a product yield of 1.67 mol succinic acid per mol of glucose (1.09 g/g) and a volumetric productivity of 21 mM/h (2.5 g $L^{-1} h^{-1}$) [54] and *C. glutamicum* R BN^{GE}CTMDLD/ Δ LDH, which produced 1940 mM (227 g/L) L-valine with a yield of 0.63 mol L-valine acid per mol of glucose (0.41 g/g) and a volumetric productivity of 40 mM/h (4.7 g $L^{-1} h^{-1}$) [47] (see Table 1). Also, several engineered *E. coli* strains were successfully applied for succinic acid production in two-stage processes (see Table 1) and further used to identify the optimal state of transition from the first culture to yield highly active and long-time producing bacteria in the second process stage. For a two-stage process with the succinic acid producer *E. coli* BS002 it was shown that cells from the early in contrast to cells from late exponential phase of the aerobic growth stage showed reduced glucose consumption and succinic acid formation in the anaerobic production stage [56]. Wu et al. [42] conducted growth of *E. coli* NZN111 in the aerobic stage on various gluconeogenic substrates (pyruvate, glycerol, succinate, malate, α -ketoglutarate) and found that growth on malate led to a yield of 0.87 mol succinate per mol glucose (see Table 1), which is about seven times higher compared to conditions with glucose as growth substrate. Extended anaerobic production was presented for *C. glutamicum* strain 534, which was shown to maintain its volumetric productivity for 267 h as immobilized cells in a repetitive batch approach for succinic acid production [43] or even 360 h with a constant volumetric productivity of acetate, L-lactate, and succinate [52]. Recently, Jojima et al. [57] presented stable zero-growth succinic acid production for 576 h with *C. glutamicum* R (JCM18229), which was achieved by a cell recycling approach. However, for most approaches the physiological state of the growing culture in the first stage of the process is insufficiently defined in particular with regard to the oxygen availability.

These two-stage processes with facultatively anaerobic producer strains demonstrate that aerobic growth followed by anaerobic zero-growth production has the potential to obtain high yields and productivities. Undeniably, the presented two-stage process is simple and straightforward in lab-scale and offers the opportunity to change or adapt the medium for optimal production conditions. Yet, a scale-up to a large industrial process is hardly economical due to the intermediate biomass harvest and the laborious reinoculation procedure.

3.2 Dual-phase process

In order to adapt the two-stage principle to feasibility in industrial single reactor scale, dual-phase processes were developed. These comprise an aerobic phase for biomass formation, which is directly followed by an anaerobic production phase (see Fig. 1B). The shift from aerobiosis to anaerobiosis is immediate and initiated by stop of aeration or optionally promoted by N_2 or CO_2 gassing [10, 13, 58]. Dual-phase processes were applied for succinic acid [9, 12, 16, 41, 59, 60] and D,L-alanine [61] production with tailored *E. coli* strains, but also conducted with *C. glutamicum* strains engineered for isobutanol [10] or succinic acid [16] production. Most approaches performed the shift to anaerobiosis at a certain biomass concentration or point in process time but often without considering the cell's history or the physiological state of the culture [56, 58, 59, 61–64].

Vemuri et al. [7] systematically identified relevant states for the process shift based on selected physiological properties (enzyme activities, growth behavior, respiratory coefficient (RQ)) of *E. coli* AFP111 and found that depending on the transition state, productivities, and yields varied dramatically. Most effective shifts were achieved at a moderate oxygen transfer coefficient $k_L a$ of 52 h^{-1} , where an about 3.4 times higher specific productivity and 2.5 times higher yield in the anaerobic phase was achieved compared to the most unsuitable transition point. In their final process 99.2 g/L succinic acid was produced with a yield of 1.17 g succinate per g glucose and a specific production rate of 135 mg $g^{-1} h^{-1}$ in the anaerobic phase [7] (see Table 1). This work highlighted the relevance to optimally adjust the enzymatic set up of the producer strain during aerobic growth for high performance in the anaerobic production stage. Wu et al. [9] investigated the effect of an acetate feed on the cultivation of the succinic acid producer *E. coli* NZN111 [65]. In two-stage shaking flasks a four-times higher glucose consumption rate of 1.34 g $L^{-1} h^{-1}$ and a 10-fold elevated yield of 1.28 mol succinate per mol glucose was shown, when the strain was aerobically grown on acetate compared to flasks, where the strain was solely cultivated on glucose in the aerobic phase [9]. Derived thereof, a dual-phase process was installed, where an aerobic glucose batch was followed by an intermediate aerobic acetate fed-batch prior to a glucose-fed anaerobic succinic acid production phase [9, 66]. The interposed acetate feed activated the glyoxylate pathway and gluconeogenesis and finally resulted in a yield of 1.13 mol succinate per mol glucose (see Table 1).

There are few examples available, where a two-stage process is directly comparable to a dual-phase mode of operation with the same producer strain. As described above, Wu et al. [9] conducted two-stage and dual-phase cultivations with the *E. coli* NZN111 succinic acid producer and showed that the volumetric productivity was about 40% lower in the anaerobic phase of the dual-phase process compared to the two-stage approach. Blombach et al. [10] compared the performance of the engineered *C. glutamicum* isobutanol producer Iso7 in a two-stage process and in a dual-phase fed-batch process. In the latter, growth and production were performed in a single bioreactor and anaerobic production was induced by an immediate stop of the aeration. The dual-phase approach showed a yield of 0.48 mol isobutanol per mol of glucose in the production phase, which is 38% lower compared to the applied two-stage cultivation.

Table 1. Current literature status concerning relevant zero-growth production

Product	Substrate ^{a)}	Organism	Strain	Titer, mM	Y_{PS} , mol/mol	Q_P , mmol L ⁻¹ h ⁻¹ anaerobic	Ref.
A Two-stage							
Succinic acid	D-glucose, yeast extract, tryptone	<i>E. coli</i>	AFP111 pTrc99A- <i>pyc</i>	—	1.46	—	[41]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	SBS550MG (pHL413)	129	1.6	5.4 ^{b)}	[69]
	D-glucose, acetate	<i>E. coli</i>	NZN111	95	1.28	9.6	[9]
	D-glucose	<i>C. glutamicum</i> R	$\Delta ldhA$ -pCRA717	1240	1.40	27.1	[53]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	NZN111	65	0.87	0.7	[42]
	D-glucose, formate	<i>C. glutamicum</i>	BOL-3/pAN6- <i>gap</i>	1134	1.67	21.4	[54]
	D-glucose	<i>C. glutamicum</i>	NC-3-1	957	1.43	19.9 ^{b)}	[44]
	D-glucose	<i>C. glutamicum</i> R	$\Delta ldhA$ /pCRB204	1336	1.73	44.5 ^{b)}	[50]
D-Lactic acid	D-glucose	<i>C. glutamicum</i> R	$\Delta ldhA$ /pCRB204	1336	1.73	44.5 ^{b)}	[50]
Xylitol	D-glucose, xylose	<i>C. glutamicum</i> R	CtXR7	1091	—	51.9	[55]
L-Alanine	D-glucose	<i>C. glutamicum</i> R	$\Delta ldhA \Delta ppc \Delta alr + alaD + gapA$	1097	1.68	34.3 ^{b)}	[45]
	D-glucose	<i>C. glutamicum</i> R	GLY3/pCRD500	2430	1.84 ^{b)}	50.6 ^{b)}	[46]
L-Valine	D-glucose	<i>C. glutamicum</i> R	BN ^{GE} C TM DLD/ Δ LDH	1940	0.63	40.4 ^{b)}	[47]
	D-glucose	<i>C. glutamicum</i> R	Val-9	1280	0.88	53.3 ^{b)}	[48]
Isobutanol	D-glucose, yeast extract, L-valine, L-leucine, L-isoleucine	<i>C. glutamicum</i>	Iso7	82	0.77	2.7 ^{b)}	[10]
	D-glucose	<i>C. glutamicum</i> R	IBU8	505	0.78	8.4 ^{b)}	[51]
Ethanol	D-glucose, pyruvate	<i>C. glutamicum</i> R	<i>ldhA-ppc</i> -pCRA723	146 ^{b)}	1.48 ^{b)}	48.6	[49]
B Dual-phase							
Succinic acid	D-glucose, yeast extract, tryptone	<i>E. coli</i>	AFP-111/pTrc99A- <i>pyc</i>	840	1.73	11.2 ^{b)} / -	[7]
	D-glucose, acetate, yeast extract	<i>E. coli</i>	NZN111	239	1.13	3.7 ^{b)} / 5.9	[9]
	D-glucose	<i>E. coli</i>	AFP111	857	1.63	10.1 ^{b)} / 16.0	[59]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	SBS550MG (pHL413)	115	1.25	- / 7.6	[12]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	SD111	844	1.67	13.2 ^{b)} / 14.1	[60]
D,L-Alanine	D-glucose, yeast extract, casaminoacids, L-isoleucine	<i>E. coli</i>	<i>pfl pps poxB ldhA aceEF</i> pTrc99A- <i>alaD</i>	988	2.02	20.6 ^{b)} / 44.9	[61]
Isobutanol	D-glucose, acetate, yeast extract, L-valine, L-leucine, L-isoleucine	<i>C. glutamicum</i>	Iso7	175	0.48	3.6 ^{b)} / 4.4	[10]
C Triple-phase							
Succinic acid	D-glucose, yeast extract, tryptone	<i>E. coli</i>	NZN111(pTrcML)	24	0.47 ^{b)}	0.6 ^{b)} / 0.8 ^{b)}	[13]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	SBS550MG (pHL413)	167	1.55	- / 11.0	[12]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	SBS550MG (pHL413)	538	1.60	- / 21.5 ^{b)}	[14]
	Sucrose, yeast extract, tryptone	<i>E. coli</i>	SBS550MG pHL413 pUR400	178	3.33	3.6 / 3.9 ^{b)}	[15]
	D-glucose, yeast extract, tryptone	<i>E. coli</i>	YL106/pSCsfCA	722	1.14	18.0 / 8.5 ^{b)}	[8]
	D-glucose, acetate, L-alanine	<i>C. glutamicum</i>	ELB-P	330	1.02	- / 5.5 ^{b)}	[16]

^{a)}Substrates applied in the anaerobic production phase.^{b)}Calculated from given data within the reference.

Publications were sorted based on the mode of operation (A: two-stage, B: dual-phase, C: triple-phase) and grouped for identical products. The substrate column lists all carbon sources without taking CO₂ or bicarbonate supplementation into account. Substrate specific product yields (Y_{PS}) are given for the anaerobic production phase. The volumetric productivity (Q_P) is either given for the anaerobic production phase for the two-stage process (A) or for the overall process/anaerobic production phase for the dual-phase (B) and triple-phase (C) processes.

Taken together, these results pinpoint to the relevance of the physiological state of the cells before the shift to the anaerobic production phase to yield an efficiently operating dual-phase process.

3.3 Triple-phase processes

Consequently, triple-phase processes were developed, which offer an oxygen limited interface between the aerobic biomass formation and the zero-growth production phase to stimulate the microbial adaptation and adjustment of the enzymatic machinery toward anaerobiosis. Hong and Lee [13] developed a triple-phase approach with the succinic acid producer *E. coli* NZN111, which was firstly subjected to aerobic growth until an OD₆₀₀ of 5. Then, the agitation was gradually lowered causing a limited oxygen transfer and a steadily decreasing growth rate. Complete anaerobiosis was initiated at an OD₆₀₀ of 11 by a stop of aeration. Similar strategies reduced the oxygen supply at certain cultivation conditions to install a microaerobic phase [8] or simply set a constant aeration at a defined process point to achieve self-induced microaerobic conditions [12, 14–16, 67, 68]. A systematic approach of Zhu et al. [14] investigated the effect of various lengths of the microaerobic phase, culture temperature, and pH regulation prior to the anaerobic succinic acid production phase with *E. coli* SBS550MG (pHL413). The authors showed that a microaerobic phase of 2–5 h length without pH regulation in the aerobic phase promoted the specific productivity and cell viability.

Sánchez et al. [69] performed a two-stage process with *E. coli* SBS550MG (pHL413) yielding 1.6 mol succinate per mol of glucose, which was about 30% higher compared to a dual-phase process [12] with the same strain (1.25 mol succinate per mol of glucose). The implementation of a triple-phase process with a 3 h microaerobic interphase led again to a product yield of 1.55 mol succinate per mol of glucose in the anaerobic phase [12] or in a similar approach even up to 1.60 mol succinate per mol of glucose [14]. A comparison of the two-stage, dual-phase, and triple-phase process was also performed with the succinic acid producer *C. glutamicum* ELB-P [16, 70] resulting in yields of 0.92, 0.46, and 1.02 mol succinate per mol in the anaerobic production stage or phase, respectively. Again, the dual-phase approach with a direct switch to anaerobiosis led to an about 50% decreased product yield compared to the other processes. These examples emphasize that a microaerobic phase for metabolic adjustment is highly favorable for an optimal performance of the strain during the production phase.

Yet, microaerobic conditions are not clearly defined and are per se metrologically difficult to access. Mostly, microaerobiosis is referred to as low dissolved oxygen (DO) conditions or DO tensions between 0 and 5% [12, 14, 67, 71–75]. Nakano et al. [76] developed a process control to maintain constant microaerobic conditions with a DO concentration between 1 and 0.1 ppm by a fuzzy logic control (FLC). The FLC integrates the input signals DO and CO₂ exhaust gas concentration and thus controls the air flow rate. With this system it was possible to maintain so defined microaerobic conditions for 830 h in a xylitol production process with *Candida magnolia* TISTR5663 [76]. Characterization of the metabolic state under microaerobic conditions was also

investigated by in situ determination of the online fluorescence of NAD(P)H of denitrifying *Pseudomonas aeruginosa* [30]. Additionally, wide-spread in microaerobic wastewater treatment is the measurement of the oxygen-reduction potential by respective electrodes. This potentiometric signal indirectly monitors the oxygen availability to control the processes [77–79] and was already applied in a two-stage [32] and dual-phase processes [80].

Still, clear and unbiased physiological signals or biosensors are currently not available but would be of great value to optimize triple-phase approaches.

4 The regulatory response toward oxygen deprivation

Given the importance that oxygen plays for the energy conservation, sophisticated mechanisms for oxygen and redox sensing and related regulation have evolved in living organisms [25, 81]. These mechanisms have been intensively studied for an oxygenic photosynthesis of nonsulfur bacteria, nitrogen fixation of symbiotic, or free-living microbes and also in the infection mechanism and biofilm formation of pathogens [82–90].

For industrially relevant microbes a holistic understanding of these mechanisms will be mandatory to further optimize zero-growth production strategies. In *E. coli* the general adaptation to anaerobiosis is studied well and coordinately regulated by the direct oxygen sensing iron-sulfur cluster protein FNR [91, 92], the two-component systems ArcBA [93] and DipB/DipA [94] and the chemotaxis system Aer [95, 96]. The FNR protein is a dual-regulator activating genes relevant for anaerobic metabolism, while inhibiting genes involved in aerobic respiration [97]. The membrane bound kinase ArcB senses the redox state of the quinone pool and phosphorylates in the absence of oxygen the cognate response regulator ArcA, which in turn represses genes of the aerobic respiration [98]. FNR and/or ArcBA represent master regulators in several microbes and fine-tune the transcriptional response to altering oxygen concentrations [82, 99]. Besides this transcriptional regulation, adjustment of optimal metabolic flux distributions is complemented by intracellular metabolite concentrations and cofactor (e.g. NADH/NAD) levels or ratios [100]. Also in *C. glutamicum* hypoxia results in an upregulation of genes encoding key enzymes of the glycolytic and organic acid production pathways [32]. However, the observed transcriptional pattern only partly explains the flux distributions in the nongrowing cells [32, 101] and pleiotropic transcriptional regulators or other regulatory mechanisms await to be identified. A comprehensive understanding of the regulatory regime and its mechanisms might allow metabolic engineering to imprint the cellular metabolism toward anaerobiosis already during aerobic growth. This could circumvent the tricky installation of microaerobic interfaces as implemented in triple-phase modes of operation. In accordance, Portnoy et al. [102] removed three terminal cytochrome oxidase genes and a quinol monooxygenase gene from *E. coli* MG1655. This already led to an identical physiological behavior under aerobic and anaerobic conditions characterized by lactate secretion and an increased glucose uptake rate. For *C. glutamicum* it was shown that deletion of the cytochrome *bc₁-aa₃* and *bd* oxidase resulted in an aerobic

fermenting phenotype [103]. However, these strains have not been evaluated for their use to improve zero-growth production so far.

5 Concluding remarks

Technically, dual-phase bioprocesses offer the advantage to be readily feasible in industrial scale. An immediate switch to the anaerobic production phase by a stop of aeration is more convenient for large bioreactors than laborious two-stage or sensitive triple-phase fermentations. However, due to the applied high cell densities in dual-phase processes, the production strain consumes residual oxygen within seconds during the shift to the production phase. This displays a virtually short timeframe for the microbe to readjust the entire metabolism in this rapidly changing environment. Poorly adapted cells show decreased product yields and reduced cell viability in the anaerobic production phase [9, 14, 47, 53, 63, 69, 104] or even end up in an at least temporary metabolic dead-end as described for *Brettanomyces intermedius* [105]. Consequently, for optimal process performance an imprinting of the microbial metabolism in the microaerobic interphase is obligatory. On the one hand, installing microaerobic conditions is technically challenging, on the other hand gradients of dissolved oxygen and heterogeneities are commonly found in large-scale bioreactors [17–19]. These might already represent a microaerobic environment to adapt the microbial metabolism for the production phase. However, a systematic understanding of key regulatory events during the transitions from aerobic to anaerobic via microaerobic conditions is essential to obtain a comprehensive picture of the complex and multilayered adaptation and will finally provide instructions to optimize production strains and zero-growth production processes.

This work was supported by a grant from the Ministry of Science, Research and the Arts of Baden-Württemberg (Az: 33-7533-10-5/84) as part of the BBW ForWerts Graduate Program.

The authors have declared no conflict of interest.

6 References

- [1] Bozell, J. J., Petersen, G. R., Technology development for the production of biobased products from biorefinery carbohydrates—the US Department of Energy’s “Top 10” revisited. *Green Chem.* 2010, 12, 539–554.
- [2] Weusthuis, R. A., Lamot, I., van der Oost, J., Sanders, J. P. M., Microbial production of bulk chemicals: Development of anaerobic processes. *Trends Biotechnol.* 2011, 29, 153–158.
- [3] Jenkins, D. E., Schultz, J. E., Matin, A., Starvation-induced cross protection against heat or H₂O₂ challenge in *Escherichia coli*. *J. Bacteriol.* 1988, 170, 3910–3914.
- [4] Steels, E. L., Learmonth, R. P., Watson, K., Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically. *Microbiology* 1994, 140, 569–576.
- [5] Sakai, S., Tsuchida, Y., Nakamoto, H., Okino, S. et al., Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested *Corynebacterium glutamicum* R. *Appl. Environ. Microbiol.* 2007, 73, 2349–2353.
- [6] Inui, M., Murakami, S., Okino, S., Kawaguchi, H. et al., Metabolic analysis of *Corynebacterium glutamicum* during lactate and succinate productions under oxygen deprivation conditions. *J. Mol. Microbiol. Biotechnol.* 2004, 7, 182–196.
- [7] Vemuri, G. N., Eiteman, M. A., Altman, E., Succinate production in dual-phase *Escherichia coli* fermentations depends on the time of transition from aerobic to anaerobic conditions. *J. Ind. Microbiol. Biotechnol.* 2002, 28, 325–332.
- [8] Li, Y., Li, M., Zhang, X., Yang, P. et al., A novel whole-phase succinate fermentation strategy with high volumetric productivity in engineered *Escherichia coli*. *Bioresour. Technol.* 2013, 149, 333–340.
- [9] Wu, H., Li, Z.-M., Zhou, L., Ye, Q., Improved succinic acid production in the anaerobic culture of an *Escherichia coli* *pflB* *ldhA* double mutant as a result of enhanced anaplerotic activities in the preceding aerobic culture. *Appl. Environ. Microbiol.* 2007, 73, 7837–7843.
- [10] Blombach, B., Riester, T., Wieschalka, S., Ziert, C. et al., *Corynebacterium glutamicum* tailored for efficient isobutanol production. *Appl. Environ. Microbiol.* 2011, 77, 3300–3310.
- [11] Blombach, B., Eikmanns, B. J., Current knowledge on isobutanol production with *Escherichia coli*, *Bacillus subtilis* and *Corynebacterium glutamicum*. *Bioeng. Bugs* 2011, 2, 346–350.
- [12] Martínez, I., Bennett, G. N., San, K.-Y., Metabolic impact of the level of aeration during cell growth on anaerobic succinate production by an engineered *Escherichia coli* strain. *Metab. Eng.* 2010, 12, 499–509.
- [13] Hong, S. H., Lee, S. Y., Metabolic flux distribution in a metabolically engineered *Escherichia coli* strain producing succinic acid. *J. Microbiol. Biotechnol.* 2000, 10, 496–501.
- [14] Zhu, J., Thakker, C., San, K.-Y., Bennett, G., Effect of culture operating conditions on succinate production in a multi-phase fed-batch bioreactor using an engineered *Escherichia coli* strain. *Appl. Microbiol. Biotechnol.* 2011, 92, 499–508.
- [15] Wang, J., Zhu, J., Bennett, G. N., San, K.-Y., Succinate production from different carbon sources under anaerobic conditions by metabolic engineered *Escherichia coli* strains. *Metab. Eng.* 2011, 13, 328–335.
- [16] Wieschalka, S., Blombach, B., Bott, M., Eikmanns, B. J., Bio-based production of organic acids with *Corynebacterium glutamicum*. *Microb. Biotechnol.* 2013, 6, 87–102.
- [17] Lara, A. R., Leal, L., Flores, N., Gosset, G. et al., Transcriptional and metabolic response of recombinant *Escherichia coli* to spatial dissolved oxygen tension gradients simulated in a scale-down system. *Biotechnol. Bioeng.* 2006, 93, 372–385.
- [18] Buchholz, J., Graf, M., Freund, A., Busche, T. et al., CO₂/HCO₃[−] perturbations of simulated large scale gradients in a scale-down device cause fast transcriptional responses in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2014, 98, 8563–8572.
- [19] Lemoine, A., Maya Martínez-Iturralde, N., Spann, R., Neubauer, P. et al., Response of *Corynebacterium glutamicum* exposed to oscillating cultivation conditions in a two- and a novel three-compartment scale-down bioreactor. *Biotechnol. Bioeng.* 2015, 112, 1220–1231.

- [20] Oosterhuis, N. M., Kossen, N. W., Dissolved oxygen concentration profiles in a production-scale bioreactor. *Biotechnol. Bioeng.* 1984, 26, 546–550.
- [21] Bylund, F., Collet, E., Enfors, S.-O., Larsson, G., Substrate gradient formation in the large-scale bioreactor lowers cell yield and increases by-product formation. *Bioprocess Eng.* 1998, 18, 171.
- [22] Sandoval-Basurto, E. A., Gosset, G., Bolívar, F., Ramírez, O. T., Culture of *Escherichia coli* under dissolved oxygen gradients simulated in a two-compartment scale-down system: Metabolic response and production of recombinant protein. *Biotechnol. Bioeng.* 2005, 89, 453–463.
- [23] Uden, G., Becker, S., Bongaerts, J., Schirawski, J. et al., Oxygen regulated gene expression in facultatively anaerobic bacteria. *Antonie Van Leeuwenhoek* 1994, 66, 3–22.
- [24] Ingledew, W. J., Poole, R. K., The respiratory chains of *Escherichia coli*. *Microbiol. Rev.* 1984, 48, 222–271.
- [25] Bunn, H. F., Poyton, R. O., Oxygen sensing and molecular adaptation to hypoxia. *Physiol. Rev.* 1996, 76, 839–885.
- [26] Bott, M., Niebisch, A., Metabolism, R. E., in: L., Eggeling, M., Bott (Eds.), *Handbook of Corynebacterium glutamicum* (1st ed.), CRC Press, Boca Raton, FL, USA 2005, pp. 305–332.
- [27] Uden, G., Bongaerts, J., Alternative respiratory pathways of *Escherichia coli*: Energetics and transcriptional regulation in response to electron acceptors. *Biochim. Biophys. Acta* 1997, 1320, 217–234.
- [28] Tran, Q. H., Uden, G., Changes in the proton potential and the cellular energetics of *Escherichia coli* during growth by aerobic and anaerobic respiration or by fermentation. *Eur. J. Biochem.* 1998, 251, 538–543.
- [29] de Graef, M. R., Alexeeva, S., Snoep, J. L., Teixeira de Mattos, M. J., The steady-state internal redox state (NADH/NAD) reflects the external redox state and is correlated with catabolic adaptation in *Escherichia coli*. *J. Bacteriol.* 1999, 181, 2351–2357.
- [30] Ju, L.-K., Chen, F., Xia, Q., Monitoring microaerobic denitrification of *Pseudomonas aeruginosa* by online NAD(P)H fluorescence. *J. Ind. Microbiol. Biotechnol.* 2005, 32, 622–628.
- [31] Varma, A., Palsson, B. O., Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type *Escherichia coli* W3110. *Appl. Environ. Microbiol.* 1994, 60, 3724–3731.
- [32] Inui, M., Suda, M., Okino, S., Nonaka, H. et al., Transcriptional profiling of *Corynebacterium glutamicum* metabolism during organic acid production under oxygen deprivation conditions. *Microbiology* 2007, 153, 2491–2504.
- [33] Van Maris, A. J. A., Winkler, A. A., Porro, D., Van Dijken, J. P. et al., Homofermentative lactate production cannot sustain anaerobic growth of engineered *Saccharomyces cerevisiae*: Possible consequence of energy-dependent lactate export. *Appl. Environ. Microbiol.* 2004, 70, 2898–2905.
- [34] Racker, E., History of the Pasteur effect and its pathobiology. *Mol. Cell. Biochem.* 1974, 5, 17–23.
- [35] Clark, D. P., The fermentation pathways of *Escherichia coli*. *FEMS Microbiol. Lett.* 1989, 63, 223–234.
- [36] Nishimura, T., Vertès, A. A., Shinoda, Y., Inui, M. et al., Anaerobic growth of *Corynebacterium glutamicum* using nitrate as a terminal electron acceptor. *Appl. Microbiol. Biotechnol.* 2007, 75, 889–897.
- [37] Michel, A., Koch-Koerfges, A., Krumbach, K., Brocker, M. et al., Anaerobic growth of *Corynebacterium glutamicum* by mixed-acid fermentation. *Appl. Environ. Microbiol.* 2015, 81, 7496–7508.
- [38] Donnelly, M. I., Millard, C. S., Clark, D. P., Chen, M. J. et al., A novel fermentation pathway in an *Escherichia coli* mutant producing succinic acid, acetic acid, and ethanol. *Appl. Biochem. Biotechnol.* 1998, 70–72, 187–198.
- [39] Singh, A., Cher Soh, K., Hatzimanikatis, V., Gill, R. T., Manipulating redox and ATP balancing for improved production of succinate in *E. coli*. *Metab. Eng.* 2011, 13, 76–81.
- [40] Sánchez, A. M., Bennett, G. N., San, K.-Y., Efficient succinic acid production from glucose through overexpression of pyruvate carboxylase in an *Escherichia coli* alcohol dehydrogenase and lactate dehydrogenase mutant. *Biotechnol. Prog.* 2005, 21, 358–365.
- [41] Vemuri, G. N., Eiteman, M. A., Altman, E., Effects of growth mode and pyruvate carboxylase on succinic acid production by metabolically engineered strains of *Escherichia coli*. *Appl. Environ. Microbiol.* 2002, 68, 1715–1727.
- [42] Wu, H., Li, Z., Zhou, L., Xie, J. et al., Enhanced anaerobic succinic acid production by *Escherichia coli* NZN111 aerobically grown on gluconeogenic carbon sources. *Enzyme Microb. Technol.* 2009, 44, 165–169.
- [43] Shi, X., Chen, Y., Ren, H., Liu, D. et al., Economically enhanced succinic acid fermentation from cassava bagasse hydrolysate using *Corynebacterium glutamicum* immobilized in porous polyurethane filler. *Bioresour. Technol.* 2014, 174, 190–197.
- [44] Xu, H., Zhou, Z., Wang, C., Chen, Z. et al., Enhanced succinic acid production in *Corynebacterium glutamicum* with increasing the available NADH supply and glucose consumption rate by decreasing H(+)-ATPase activity. *Biotechnol. Lett.* 2016, 38, 1181–1186.
- [45] Jojima, T., Fujii, M., Mori, E., Inui, M. et al., Engineering of sugar metabolism of *Corynebacterium glutamicum* for production of amino acid L-alanine under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2010, 87, 159–165.
- [46] Yamamoto, S., Gunji, W., Suzuki, H., Toda, H. et al., Overexpression of genes encoding glycolytic enzymes in *Corynebacterium glutamicum* enhances glucose metabolism and alanine production under oxygen deprivation conditions. *Appl. Environ. Microbiol.* 2012, 78, 4447–4457.
- [47] Hasegawa, S., Uematsu, K., Natsuma, Y., Suda, M. et al., Improvement of the redox balance increases L-valine production by *Corynebacterium glutamicum* under oxygen deprivation conditions. *Appl. Environ. Microbiol.* 2012, 78, 865–875.
- [48] Hasegawa, S., Suda, M., Uematsu, K., Natsuma, Y. et al., Engineering of *Corynebacterium glutamicum* for high-yield L-valine production under oxygen deprivation conditions. *Appl. Environ. Microbiol.* 2013, 79, 1250–1257.
- [49] Inui, M., Kawaguchi, H., Murakami, S., Vertès, A. A. et al., Metabolic engineering of *Corynebacterium glutamicum* for fuel ethanol production under oxygen-deprivation conditions. *J. Mol. Microbiol. Biotechnol.* 2004, 8, 243–254.

- [50] Okino, S., Suda, M., Fujikura, K., Inui, M. et al., Production of D-lactic acid by *Corynebacterium glutamicum* under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2008, 78, 449–454.
- [51] Yamamoto, S., Suda, M., Niimi, S., Inui, M. et al., Strain optimization for efficient isobutanol production using *Corynebacterium glutamicum* under oxygen deprivation. *Biotechnol. Bioeng.* 2013, 110, 2938–2948.
- [52] Okino, S., Inui, M., Yukawa, H., Production of organic acids by *Corynebacterium glutamicum* under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2005, 68, 475–480.
- [53] Okino, S., Noburyu, R., Suda, M., Jojima, T. et al., An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. *Appl. Microbiol. Biotechnol.* 2008, 81, 459–464.
- [54] Litsanov, B., Brocker, M., Bott, M., Toward homosuccinate fermentation: Metabolic engineering of *Corynebacterium glutamicum* for anaerobic production of succinate from glucose and formate. *Appl. Environ. Microbiol.* 2012, 78, 3325–3337.
- [55] Sasaki, M., Jojima, T., Inui, M., Yukawa, H., Xylitol production by recombinant *Corynebacterium glutamicum* under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2010, 86, 1057–1066.
- [56] Bai, B., Zhou, J., Yang, M., Liu, Y. et al., Efficient production of succinic acid from macroalgae hydrolysate by metabolically engineered *Escherichia coli*. *Bioresour. Technol.* 2015, 185, 56–61.
- [57] Jojima, T., Noburyu, R., Suda, M., Okino, S. et al., Improving process yield in succinic acid production by cell recycling of recombinant *Corynebacterium glutamicum*. *Fermentation* 2016, 2, 5.
- [58] Lu, S., Eiteman, M. A., Altman, E., Effect of CO₂ on succinate production in dual-phase *Escherichia coli* fermentations. *J. Biotechnol.* 2009, 143, 213–223.
- [59] Jiang, M., Liu, S.-W., Ma, J.-F., Chen, K.-Q. et al., Effect of growth phase feeding strategies on succinate production by metabolically engineered *Escherichia coli*. *Appl. Environ. Microbiol.* 2010, 76, 1298–1300.
- [60] Wang, D., Li, Q., Mao, Y., Xing, J. et al., High-level succinic acid production and yield by lactose-induced expression of phosphoenolpyruvate carboxylase in *ptsG* mutant *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 2010, 87, 2025–2035.
- [61] Smith, G. M., Lee, S. A., Reilly, K. C., Eiteman, M. A. et al., Fed-batch two-phase production of alanine by a metabolically engineered *Escherichia coli*. *Biotechnol. Lett.* 2006, 28, 1695–1700.
- [62] Nghiem, N. P., Donnelly, M., Millard, C. S., Stols, L. et al., Method for the production of dicarboxylic acids. US Patent 5,869,301. 1999.
- [63] Andersson, C., Petrova, E., Berglund, K., Rova, U., Maintaining high anaerobic succinic acid productivity by product removal. *Bioprocess Biosyst. Eng.* 2010, 33, 711–718.
- [64] Liang, L.-Y., Liu, R., Ma, J., Chen, K. et al., Increased production of succinic acid in *Escherichia coli* by overexpression of malate dehydrogenase. *Biotechnol. Lett.* 2011, 33, 2439–2444.
- [65] Bunch, P. K., Mat-Jan, F., Lee, N., Clark, D. P., The *ldhA* gene encoding the fermentative lactate dehydrogenase of *Escherichia coli*. *Microbiology* 1997, 143, 187–195.
- [66] Wu, H., Li, Q., Li, Z., Ye, Q., Succinic acid production and CO₂ fixation using a metabolically engineered *Escherichia coli* in a bioreactor equipped with a self-inducing agitator. *Bioresour. Technol.* 2012, 107, 376–384.
- [67] Biswas, R., Yamaoka, M., Nakayama, H., Kondo, T. et al., Enhanced production of 2,3-butanediol by engineered *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* 2012, 94, 651–658.
- [68] Cheng, K.-K., Wu, J., Lin, Z.-N., Zhang, J.-A., Aerobic and sequential anaerobic fermentation to produce xylitol and ethanol using non-detoxified acid pretreated corncob. *Biotechnol. Biofuels* 2014, 7, 166.
- [69] Sánchez, A. M., Bennett, G. N., San, K.-Y., Batch culture characterization and metabolic flux analysis of succinate-producing *Escherichia coli* strains. *Metab. Eng.* 2006, 8, 209–226.
- [70] Wieschalka, S., Engineering *Corynebacterium glutamicum* as a designer-bug for the bio-based production of chemical building blocks and biofuel, University of Ulm, 2012.
- [71] Chen, X., Xiu, Z., Wang, J., Zhang, D. et al., Stoichiometric analysis and experimental investigation of glycerol bioconversion to 1,3-propanediol by *Klebsiella pneumoniae* under microaerobic conditions. *Enzyme Microb. Technol.* 2003, 33, 386–394.
- [72] Alfenore, S., Cameleyre, X., Benbadis, L., Bideaux, C. et al., Aeration strategy: A need for very high ethanol performance in *Saccharomyces cerevisiae* fed-batch process. *Appl. Microbiol. Biotechnol.* 2004, 63, 537–542.
- [73] Li, S., Huang, D., Li, Y., Wen, J. et al., Rational improvement of the engineered isobutanol-producing *Bacillus subtilis* by elementary mode analysis. *Microb. Cell Fact.* 2012, 11, 101.
- [74] Wieschalka, S., Blombach, B., Eikmanns, B. J., Engineering *Corynebacterium glutamicum* for the production of pyruvate. *Appl. Microbiol. Biotechnol.* 2012, 94, 449–459.
- [75] Yamauchi, Y., Hirasawa, T., Nishii, M., Furusawa, C. et al., Enhanced acetic acid and succinic acid production under microaerobic conditions by *Corynebacterium glutamicum* harboring *Escherichia coli* transhydrogenase gene *pntAB*. *J. Gen. Appl. Microbiol.* 2014, 60, 112–118.
- [76] Nakano, K., Katsu, R., Tada, K., Matsumura, M., Production of highly concentrated xylitol by *Candida magnoliae* under a microaerobic condition maintained by simple fuzzy control. *J. Biosci. Bioeng.* 2000, 89, 372–376.
- [77] Hewitt, L. F., *Oxidation-Reduction Potentials in Bacteriology and biochemistry* (6th ed.), Baltimore, Williams and Wilkins, Edinburgh 1950.
- [78] Oktyabrsky, O. N., Smirnova, G. V., Dynamics of redox potential in bacterial cultures growing on media containing different sources of carbon, energy and nitrogen. *Acta Biotechnol.* 1989, 9, 203–209.
- [79] Goncharuk, V. V., Bagrii, V. A., Mel'nik, L. A., Chebotareva, R. D. et al., The use of redox potential in water treatment processes. *J. Water Chem. Technol.* 2010, 32, 1–9.
- [80] Wimpenny, J. W., Firth, A., Levels of nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide in facultative bacteria and the effect of oxygen. *J. Bacteriol.* 1972, 111, 24–32.
- [81] Green, J., Paget, M. S., Bacterial redox sensors. *Nat. Rev. Microbiol.* 2004, 2, 954–966.
- [82] Patschkowski, T., Bates, D. M., Kiley, P. J., Mechanisms for sensing and responding to oxygen deprivation, in: G.,

- Storz, R., Hengge-Aronis (Eds.), *Bacterial Stress Responses*, ASM Press, Washington, D.C. 2000, pp. 61–78.
- [83] Starck, J., Källénus, G., Marklund, B.-I., Andersson, D. I. et al., Comparative proteome analysis of *Mycobacterium tuberculosis* grown under aerobic and anaerobic conditions. *Microbiology* 2004, 150, 3821–3829.
- [84] Fuchs, S., Pané-Farré, J., Kohler, C., Hecker, M. et al., Anaerobic gene expression in *Staphylococcus aureus*. *J. Bacteriol.* 2007, 189, 4275–4289.
- [85] Grahl, N., Cramer, R. A., Regulation of hypoxia adaptation: An overlooked virulence attribute of pathogenic fungi? *Med. Mycol.* 2010, 48, 1–15.
- [86] Trunk, K., Benkert, B., Quäck, N., Münch, R. et al., Anaerobic adaptation in *Pseudomonas aeruginosa*: Definition of the Anr and Dnr regulons. *Environ. Microbiol.* 2010, 12, 1719–1733.
- [87] Fuchs, T. M., Eisenreich, W., Heesemann, J., Goebel, W., Metabolic adaptation of human pathogenic and related non-pathogenic bacteria to extra- and intracellular habitats. *FEMS Microbiol. Rev.* 2012, 36, 435–462.
- [88] Potzkei, J., Kunze, M., Drepper, T., Gensch, T. et al., Real-time determination of intracellular oxygen in bacteria using a genetically encoded FRET-based biosensor. *BMC Biol.* 2012, 10, 28.
- [89] Fei, F., Mendonca, M. L., McCarry, B. E., Bowdish, D. M. E. et al., Metabolic and transcriptomic profiling of *Streptococcus intermedius* during aerobic and anaerobic growth. *Metabolomics* 2016, 12, 46.
- [90] Sousa, A. M., Pereira, M. O., *Pseudomonas aeruginosa* diversification during infection development in cystic fibrosis lungs—a review. *Pathogen* 2014, 3, 680–703.
- [91] Khoroshilova, N., Popescu, C., Münck, E., Beinert, H. et al., Iron-sulfur cluster disassembly in the FNR protein of *Escherichia coli* by O₂: [4Fe-4S] to [2Fe-2S] conversion with loss of biological activity. *Proc. Natl. Acad. Sci. USA* 1997, 94, 6087–6092.
- [92] Jordan, P. A., Thomson, A. J., Ralph, E. T., Guest, J. R. et al., FNR is a direct oxygen sensor having a biphasic response curve. *FEBS Lett.* 1997, 416, 349–352.
- [93] Bekker, M., Alexeeva, S., Laan, W., Sawers, G. et al., The ArcBA two-component system of *Escherichia coli* is regulated by the redox state of both the ubiquinone and the menaquinone pool. *J. Bacteriol.* 2010, 192, 746–754.
- [94] Sawers, G., The aerobic/anaerobic interface. *Curr. Opin. Microbiol.* 1999, 2, 181–187.
- [95] Bibikov, S. I., Biran, R., Rudd, K. E., Parkinson, J. S., A signal transducer for aerotaxis in *Escherichia coli*. *J. Bacteriol.* 1997, 179, 4075–4079.
- [96] Gosink, K. K., Burón-Barral, M., del, C., Parkinson, J. S., Signaling interactions between the aerotaxis transducer Aer and heterologous chemoreceptors in *Escherichia coli*. *J. Bacteriol.* 2006, 188, 3487–3493.
- [97] Kang, Y., Weber, K. D., Qiu, Y., Kiley, P. J. et al., Genome-wide expression analysis indicates that FNR of *Escherichia coli* K-12 regulates a large number of genes of unknown function. *J. Bacteriol.* 2005, 187, 1135–1160.
- [98] Georgellis, D., Kwon, O., Lin, E. C., Quinones as the redox signal for the *arc* two-component system of bacteria. *Science* 2001, 292, 2314–2316.
- [99] Partridge, J. D., Sanguinetti, G., Dibden, D. P., Roberts, R. E. et al., Transition of *Escherichia coli* from aerobic to micro-aerobic conditions involves fast and slow reacting regulatory components. *J. Biol. Chem.* 2007, 282, 11230–11237.
- [100] Berríos-Rivera, S. J., Bennett, G. N., San, K.-Y., The effect of increasing NADH availability on the redistribution of metabolic fluxes in *Escherichia coli* chemostat cultures. *Metab. Eng.* 2002, 4, 230–237.
- [101] Radoš, D., Turner, D. L., Fonseca, L. L., Carvalho, A. L. et al., Carbon flux analysis by ¹³C nuclear magnetic resonance to determine the effect of CO₂ on anaerobic succinate production by *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2014, 80, 3015–3024.
- [102] Portnoy, V. A., Scott, D. A., Lewis, N. E., Tarasova, Y. et al., Deletion of genes encoding cytochrome oxidases and quinol monooxygenase blocks the aerobic-anaerobic shift in *Escherichia coli* K-12 MG1655. *Appl. Environ. Microbiol.* 2010, 76, 6529–6540.
- [103] Koch-Koerfges, A., Pfler, N., Platzen, L., Oldiges, M. et al., Conversion of *Corynebacterium glutamicum* from an aerobic respiring to an aerobic fermenting bacterium by inactivation of the respiratory chain. *Biochim. Biophys. Acta* 2013, 1827, 699–708.
- [104] Soellner, S., Rahnert, M., Siemann-Herzberg, M., Takors, R. et al., Evolution of pyruvate kinase-deficient *Escherichia coli* mutants enables glycerol-based cell growth and succinate production. *J. Appl. Microbiol.* 2013, 115, 1368–1378.
- [105] Wijsman, M. R., van Dijken, J. P., van Kleeff, B. H., Scheffers, W. A., Inhibition of fermentation and growth in batch cultures of the yeast *Brettanomyces intermedius* upon a shift from aerobic to anaerobic conditions (Custers effect). *Antonie Van Leeuwenhoek* 1984, 50, 183–192.