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Anodic Electro-Fermentation: Anaerobic production of L-Lysine by recombinant *Corynebacterium glutamicum*†

Running title: Anodic Electro-Fermentation with *C. glutamicum*

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Abstract

Microbial electrochemical technologies (MET) are promising to drive metabolic processes for the production of chemicals of interest. They provide microorganisms with an electrode as an electron sink or an electron source to stabilize their redox and/or energy state. Here, we applied an anode as additional electron sink to enhance the anoxic metabolism of the industrial bacterium *Corynebacterium glutamicum* through an anodic electro-fermentation. In using ferricyanide as extracellular electron carrier, anaerobic growth was enabled and the feedback-deregulated mutant *Corynebacterium glutamicum lysC* further accumulated L-lysine. Under such oxidising conditions we achieved L-lysine titers of 2.9 mM at rates of 0.2 mmol/L/h. That titer is comparable to recently reported L-lysine concentrations achieved by anaerobic production under reductive conditions (cathodic electro-fermentation). However unlike other studies, our oxidative conditions allowed anaerobic cell growth, indicating an improved cellular energy supply during anodic electrofermentation. In that light, we propose anodic electro-fermentation as the right choice to support *C. glutamicum* stabilizing its redox and energy state and empower a stable anaerobic production of L-lysine.

Keywords: Anodic electro fermentation; lysine; anaerobic growth; *Corynebacterium glutamicum*

Introduction

A key sector of industrial biotechnology today is the amino acid market. As world's leading feed amino acid, used as additive in animal nutrition, L-lysine has an annual production of 2.2 million tons with an estimated increase of 7% per year due to increasing population and growing demand for meat (Ajinomoto Co. 2014; Eggeling and Bott 2015).

The Gram-positive soil bacterium *Corynebacterium glutamicum* is the major industrial producer of lysine. For over 50 years *C. glutamicum* has asserted its place in industrial biotechnology as a safe production host and has become a model organism in industrial microbiology (Eggeling and Bott 2005; Tatsumi and Inui 2012; Wittmann and Becker 2007). Apart from the production of amino acids, *C. glutamicum* has recently also been developed into a producer for organic acids, diamines, and biofuels (Becker and Wittmann 2012; Connor and Liao 2009; Wendisch et al. 2006), thus underlining its importance in biotechnology.

Growing aerobically, *C. glutamicum* relies on oxygen as terminal electron acceptor, which limits product yields through substrate loss (Weusthuis et al. 2011). Oxygen transfer rates in bioreactors limit process scale up (Gill et al. 2008; Hannon et al. 2007), resulting in higher capital costs compared to anaerobic systems (Garcia-Ochoa and Gomez 2009). Despite the potential benefits of anaerobic fermentation, the anoxic metabolism of *C. glutamicum* has been studied poorly over the past 50 years. Obviously, the microorganism was regarded as obligate aerobe for many years (Bott and Niebisch 2003), and just in 2004 it was realised that *C. glutamicum* can survive under oxygen deprivation by fermenting glucose into organic acids, mainly to lactate, succinate and acetate (Inui et al. 2004). Anoxic growth of *C. glutamicum* is enabled by using nitrate as a final electron acceptor, but the growth is limited due to accumulation of toxic nitrite (Takeno et al. 2007). An alternative way to promote

growth and metabolism under anoxic conditions is to use a bioelectrochemical system (BES). In such a configuration, an electrochemical potential is applied to an anode or a cathode to support microorganisms able to perform extracellular electron transfer to balance their redox state (Rabaey et al. 2009). Most proposed applications focus on cathodic processes to enhance the reducing power of a microbial cell to support the reduction of a substrate to a target product. For example, the reduction of CO_2 (microbial electrosynthesis) (Ganigué et al. 2015; Jourdin et al. 2014) or of energy rich carbohydrates (cathodic electro-fermentation) (Choi et al. 2012; Emde and Schink 1990; Zhou et al. 2013) to organic acids and/or alcohols was studied. The availability of an anode as electron acceptor enables the microbes to oxidise NADH and recover NAD^+ using the electrode as an extracellular electron sink (anodic respiration). This allows to stabilize the microbial redox state and/or redirect the metabolic carbon flow towards a product of interest (Kracke and Krömer 2014). In *C. glutamicum* this should in principle allow anaerobic growth and production (Kracke et al. 2015). A recent study describes the incubation of *C. glutamicum* in a BES under reductive conditions to enhance anaerobic lysine production (Xafenias et al. 2017), however, under these electron 'feeding' conditions no growth could be observed. Here we provide the L-lysine producing strain *C. glutamicum lys*C (Kim et al. 2006) with the extracellular electron acceptor ferricyanide, a mediator with a positive redox potential (+0.44V vs standard hydrogen electrode). In order to drive re-oxidation of the mediator, the anode was poised at a more positive potential. This should provide of an inexhaustible electron acceptor for the microbe. The anodic process enabled anaerobic growth, glucose consumption and production of organic acids and L-lysine, showing the potential of anodic electro-fermentation.

Material and methods

Strain and cultivation conditions. In this study, the lysine-producing strain *C. glutamicum lys*C was used. This strain has a single nucleotide exchange (S301Y) in the gene encoding the aspartokinase (Kim et al. 2006). This results in lysine overproduction due to release of the feedback inhibition of the aspartokinase.

Colonies were grown on lysogeny broth agar plates at 30 °C and were transferred afterwards into 500-mL baffled shake flasks, containing 100 mL of cultivation medium (minimal medium, for composition see supplementary material), for aerobic overnight cultivation in an orbital shaking incubator (Multitron, Infors, Bottmingen, Switzerland) at 200 rpm and 30 °C. When cells had reached an optical density (OD_{660}) between 5 and 6 (log phase), cells were harvested by centrifugation (10000 × *g*, room temperature, 3 min), washed and resuspended in fresh cultivation medium. The BESs were inoculated to a start OD_{660} of 0.55 or 3.65, respectively, to study the effect of higher inoculum, respectively.

Bioelectrochemical system and operation. The BES design was adopted from a previous work (Lai et al. 2016). A detailed description is given in the supplementary material (Fig. S1). The potential of the working electrode (a carbon cloth with a projected surface area of 25 cm^2) was set to a value of +0.697 V vs. standard hydrogen electrode (SHE) using a potentiostat (Potentiostat/Galvanostat VSP, BioLogic Science Instruments, France). All potentials in the following work are reported with respect to the SHE. An Ag/AgCl electrode in saturated KCl (+0.197 V vs SHE) was used as reference electrode. Furthermore, in abiotic experiments, cyclic voltammetry (CV) was used to characterise the mediator potassium ferricyanide, $K_3[Fe(CN)_6]$ at the working concentration used in this study (1.5 mM). The details of the setup and procedure are described in the supplementary material. In order to

provide sufficient CO_2 to support anaplerotic synthesis of oxaloacetate under anaerobic conditions (Inui et al. 2004), a sterile solution containing saturated sodium bicarbonate was added to the anodic chamber (7.5 mM) before inoculation with a 2 mL pre-culture.

Analytics. Cell density was measured photometrically at a wavelength of 660 nm (OD_{660}), using water as a blank. Optical density was converted into cell dry weight (CDW) by the following empirically determined conversion factor: CDW [g/L] = 0.353 × OD_{660} (Krömer et al. 2004). Each sample for the analysis of sugars, organic acids, alcohols and amino acids via high pressure liquid chromatography (HPLC) was centrifuged (15,000 ×*g*, 4 °C, 8 min) followed by 0.22 µm filtration of the supernatant. The ^{13}C labelling enrichment of secreted alanine was analysed using gas chromatography mass spectrometry (GC-MS). Each sample for GC-MS analysis was centrifuged and filtered as described above, followed by drying the sample under a nitrogen stream and derivatisation with a mixture of 0.1% pyridine in dimethylformamide and *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide. The details of the used HPLC and GC-MS methods are described in the supplementary material.

Results

Mediator enhances anoxic respiration of the anode. Under anaerobic conditions, glucose as the sole carbon source provides an electron excess and *C. glutamicum lys*C is forced to perform mixed-acid fermentation with limited energy generation (Michel et al. 2015). Therefore, we cultivated *C. glutamicum lys*C in a BES providing the microorganism an anode as an alternative electron sink. However, the microbial interaction with the anode was very limited (Fig. 1A). After 120 h a current of only 0.022 mA/cm^2 was reached, which is equivalent to 4.09 mM transferred electrons (equivalent to a consumption of 170 µM glucose assuming complete oxidation to CO_2). The approach to enhance the extracellular electron

transfer was to provide the cells with a redox mediator, which can be reversibly reduced and oxidised and can catalyse the transfer of electrons between the anode and cells. The chosen mediator ferricyanide, $K_3[Fe(CN)_6]$ was characterised by cyclic voltammetry (CV) (Fig. 1B). The CV profile of $K_3[Fe(CN)_6]$ showing clear oxidative and reductive peaks confirming that the chosen working electrode material (carbon cloth) was suitable to cycle the redox reaction at the electrode. The midpoint potential was calculated to be 0.438 ± 0.002 V.

Figure 1A shows that $K_3[Fe(CN)_6]$ mediated the microbial interaction with the anode in a BES at an applied potential of +0.697 V. *C. glutamicum lys*C reduced ferricyanide ($[Fe(CN)_6]^{3-}$) to ferrocyanide ($[Fe(CN)_6]^{4-}$), which was then reoxidised by the anode. This resulted in transfer of electrons to the anode and the development of a catalytic current due to the oxidation of the metabolic substrate. The anodic current increased during the fermentation and reached two maxima of 89.5 µA/cm^2 and 86.8 µA/cm^2 after 29 h and after 80 h, respectively, followed by a slow continuous current decrease afterwards (Fig. 1A). After 80 h and 119 h, 19.67 mM and 31.28 mM electrons were transferred to the anode, respectively (calculations are explained in supplementary material).

As glucose was the only carbon source, we hypothesized that the electrons, which were transferred to the anode, are cellular surplus electrons originating from the sugar being oxidised in the metabolism.

Anodic enhancement of growth and glucose consumption. After demonstrating that *C. glutamicum lys*C can utilize the anode via a redox mediator as an electron sink, we compared the anodic microbial growth and glucose consumption under anaerobic conditions to open circuit controls with mediator (control A), and without mediator (control B) (Fig. 2).

By using the anode as a final electron acceptor the bacteria achieved an enhanced growth compared to the controls. After 72 hours the biomass had more than doubled and reached a

CDW of 0.42 ± 0.06 g/L. Afterwards, the cell concentration started to decrease steadily. At that time point (72 – 78 h, start of the death phase), more than 96% of the glucose was consumed. The volumetric glucose consumption rate was calculated as 0.86 ± 0.10 mmol/L/h. The behaviour of the two control experimental sets were similar, but they differed strongly from the anodic supported fermentation experiments. In fact, we only observed a growth in the first 29 h, reaching a lower maximal CDW of 0.31 ± 0.03g/L (control A) and 0.27 ± 0.03 g/L (control B). After 29 h the cell density started to decrease slowly. The volumetric glucose consumption was also significantly lower: 0.46 ± 0.15 and 0.43 ± 0.03 mmol/L/h for control A and control B, respectively. Same trends were also observed for the biomass-specific glucose consumption rates (mmol/g CDW/h) (see Table SII, and statistic tests proving the significant differences, see Table SIII). Furthermore, after 77 h less than 49% of the glucose were consumed and after 120 h more than 36% remained as residual glucose.

Anodic enhancement of organic acids, L-lysine and L-alanine production. Besides glucose consumption, the production of organic acids and amino acids were analysed to investigate the effect of the artificial electron sink on the product spectrum. In general, carbon and redox balances were determined higher than 84% (Table I). During the fermentation mainly three organic acids (lactate, succinate and acetate) and three L-amino acids (lysine, alanine and glycine) were accumulated in the medium (Fig. 3). Further, small concentrations of trehalose were also detected (Fig. S2), which *C. glutamicum* uses as an osmoprotectant (Wolf et al. 2003).

The anodic electro-fermentation showed faster production rates and higher titers than the control experiments. After 78 h, when the glucose was consumed, lactate, succinate and acetate concentrations were measured as 68.24 ± 11.17, 12.22 ± 1.58 and 6.14 ± 1.07 mM,

respectively, that was approximately 1.6, 2.2 and 2.1 times, higher than in the control experiments, respectively (Fig. 3A, B, and C). Similar trends were observed for the production of lysine (2.18 ± 0.28 mM) and alanine (0.89 ± 0.12 mM) after 78 h (Fig. 3D and C), hence, approximately 2.1 and 2.2 times higher compared to the control experiments respectively. The measured amount of glycine was relatively similar in all three experimental sets to that time point (Fig. 3F).

The yields of all produced organic acids in the mediated BES experiments were observed to be similar to the controls (Table I). However, the lysine yield was lower in the anodic experiments compared to control A, but similar to control B. The alanine and glycine yields were similar to the control A, but lower than in control B (for statistics see Table SIII). Furthermore, the anode as an additional electron sink enhanced significantly the glucose consumption rates and volumetric production rates of all products except of lysine and glycine (for statistics see Table SIII).

^{13}C tracer study points towards a limitation of the pentose phosphate pathway. In order to get an insight into the metabolism of *C. glutamicum* during growth in the BES, a tracer study with [1-^{13}C] glucose was conducted. The limited cell growth did not provide appropriately ^{13}C labelled cell protein to infer flux information, so this information was obtained from secreted products, as successfully applied in previous work (Kiefer et al. 2004). Here, the accumulation of alanine was used to estimate the activity of the oxidative pentose phosphate (PP) pathway (Wittmann et al. 2004). In the oxidative PP pathway, the labelled carbon of [1-^{13}C] glucose is cleaved off as carbon dioxide. The higher the PP pathway flux, the lower the labelling enrichment in alanine, i.e. the lower the ratio of ^{13}C labelled (M+1) to non-labelled (M) alanine mass isotopomers, formed downstream of the pathway. The labelling information of alanine (Table SIV) could be used to estimate the

relative flux into the PP pathway for the BES (29%) (for calculations, see supplementary material). In comparison, the same strain exhibits a relative PP pathway flux of 53% under aerobic conditions, almost twice as high (Kim et al. 2006).

Effect of higher biomass inoculum on anodic electro-fermentation. We tested the impact of a 6.6 times higher concentrated inoculum on the anodic electro-fermentation (Fig. 4). By providing more biomass, the process was accelerated significantly, with more than 97% of the glucose being consumed after 13.4 h instead of 78 h. The CDW doubled from 1.29 ± 0.07 g/L to 2.67 ± 0.16 g/L. Here, we reached a lysine concentration of 2.94 ± 0.02 mM at a volumetric production rate of 202.23 ± 2.27 µmol/h (Table I). This demonstrates that the process can be expedited by high biomass inoculum, and that at cell densities up to OD_{660} of 7.6 ± 0.5 electron transfer in the BES is not limiting.

Discussion

The presence of the anode as an artificial electron sink supported the microorganism in staying longer in the growth phase and reaching a higher biomass. In addition, the anodic fermentation enhanced glucose uptake and allowed a complete conversion of the sugar into products after 78 h (at low inoculum), while achieving higher product concentrations at faster volumetric production rates.

How does the anode influence the energy balance in *C. glutamicum*? In initial experiments, *C. glutamicum lys*C was provided with a polarized anode at +0.697 V. On the one hand, that voltage is sufficiently high enough to drive the transfer of electrons from the components of the cellular electron transport chain (Kracke et al. 2015) to the anode, but on the other hand, sufficiently low enough to prevent the abiotic oxygen evolution through

electrolysis of water. However, the microbial interaction with the anode was very limited, and thereby enhanced by providing the microorganism with a mediator, ferricyanide. The measured redox potential of the mediator was +0.44 V, which is similar to the values reported in the literature (O'Reilly 1973), and which makes it suitable to support an anodic oxidation process (Kracke et al. 2015) as recently demonstrated for the anodic respiration of *Pseudomonas putida* (Lai et al. 2016). Since ferricyanide is a hydrophilic oxidant, we assume that it is not able to cross the cell membrane (Ertl et al. 2000; Pasco et al. 2004). Therefore, a possibility is that the mediator interacts with components of the cellular electron transport chain located in the cell membrane. Here, it is thought that membrane-bound primary dehydrogenases in the cytoplasm, soluble lipophilic molecules in the membrane like quinones, and membrane-localized (multi-) protein complexes such as cytochromes and terminal oxidases (reductases) play an important role to enable the extracellular electron transfer (Hernandez and Newman 2001; Kracke et al. 2015). Therefore, we discuss the potential sites for interaction of ferricyanide with the respiratory pathway of *C. glutamicum*.

The branched electron transport chain of *C. glutamicum* consists of at least six primary dehydrogenases (DHs): succinate DH, NADH II DH, malate:quinone oxidoreductase (OR), pyruvate:quinone OR, L-lactate DH and D-lactate DH. All these dehydrogenases contain a flavin cofactor and reduce menaquinone (MQ) to menaquinol (MQH_2), a mobile electron carrier in the membrane. MQH_2, transfers the electrons either to the cytochrome *bd* oxidase or to the super complex consisting of cytochrome bc_1c complex and a cytochrome aa_3 complex. Afterwards, the oxygen reductases pass the electrons to a final electron acceptor and couple this process with a creation of an electrochemical proton gradient across the membrane (Bott and Niebisch 2003; Eggeling and Bott 2005; Matsushita 2013).

If we assume the lipid bilayer membrane as a barrier, the mediator could only exchange electrons with the terminal cytochrome complexes, MQH_2 and/or potentially with the succinate DH, which possesses a cytochrome *b* in the membrane anchoring subunit (Kurokawa and Sakamoto 2005). Thermodynamically all three options would be feasible (Kracke et al. 2015). Kinetic respiratory experiments have demonstrated that all the mentioned candidates for reduction of ferricyanide would be in principle capable of doing so (Hederstedt and Rutberg 1981; Kabus et al. 2007; Nantapong et al. 2005; Niebisch and Bott 2003) supporting the proposed mechanisms in Figure 5.

Irrespective of the terminal electron donor in the respiratory chain, there is a need for charge balance in a BES. To maintain electroneutrality, for each electron transferred to the anode, one positive charge (*i.e.*, a proton) has to migrate to the cathode. If succinate DH would directly reduce the mediator no protons would be translocated and the available proton motive force to drive ATP synthase would shrink. It is unlikely that such a process could support the observed growth. Likewise, would the direct oxidation of MQH_2 by ferricyanide or via cytochrome *bd* oxidase not lead to a useable proton motive force. The only scenario that could deliver additional protons for the synthesis of ATP would be the oxidation of the cytochrome super complex by ferricyanide. This would transport six protons per electron couple across the membrane. It is estimated that synthesis of one ATP from ADP by F_1F_0-ATP synthase requires the transfer of three or four protons (Bott and Niebisch 2003) from the extracellular to the intracellular side of the membrane. In the BES that would mean 1-1.3 mol ATP per mol of electrons, or 0.33 mol_{ATP} / $mol_{Glucose}$ delivered via the anode. The production of lactate and acetate would contribute 2.14 and 0.48 mol_{ATP} / $mol_{Glucose}$, respectively (net production of 2 mol ATP per mol lactate and 4 mol ATP per mol acetate from glucose) (Fig.5). The anodic respirationadds about 12.6% of energy. Although it was reported that

Corynebacterium is energy limited under anaerobic conditions (Michel et al. 2015), adding around 13% of energy is a relatively small contribution of the anodic respiration to the energy balance. The observed doubling of the biomass, might be indicating that a regulatory process could be at play causing the improved growth.

How does the anodic fermentation influence regulation of metabolism? By providing an artificial electron sink to *C. glutamicum lys*C we enhanced the cellular glucose consumption, the cell growth and the biosynthesis of products, but as discussed above, the production of additional ATP could possibly not explain the phenotype. Inui *et al*. proposed that the anaerobic glycolytic pathway of *Corynebacterium* is regulated by the intracellular NAD^+/NADH ratio (Inui et al. 2004). Glucose is metabolized to pyruvate via the Embden-Meyerhof-Parnas (EMP) pathway, whereby NAD^+ is reduced to NADH by the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Fig. 6). But GAPDH is inhibited by NADH, which can be recycled to NAD^+ via lactate dehydrogenase (LDH) by converting pyruvate to lactate. Therefore, the main product of *Corynebacterium* under anaerobic conditions is lactate, which drives the glucose uptake and the glycolytic pathway (Inui et al. 2004) and reduces the oxidative PP flux compared to aerobic conditions. We assume that the anode can also recover NAD^+ via NADH DH II, which potentially could transfer reducing equivalents via the respiratory pathway and the mediator to the anode. This assumption is supported by the observation that glucose was faster consumed (Fig. 2) while the specific rate of lactate production did not increase significantly (Table SIII) when providing the mediator and the anode. Likewise, the anode as an external electron sink could also support the enzymes LDH, PQO, MQO and SDH ultimately oxidising the NADH pool (Fig. 6). If this causes a shift in the NAD+/NADH ratio remains to be tested, but it seems more plausible than the additional energy delivered by the BES.

Are reductive or oxidative BES conditions better for lysine production? In contrast to an aerobic bioprocess, where higher rates and yields have been demonstrated (Becker and Wittmann 2012), the anoxic fermentation faces the problem of an excess of reducing equivalents (Inui et al. 2004). Therefore, providing an external electron acceptor supports the bacteria achieving redox balance and enhances glucose consumption, biomass production and biosynthesis of products, including also lysine production. Lysine is more reduced than glucose and four mol NADPH per mol of lysine are needed in glucose-based production. When using an anodic electrode as electron sink, reducing equivalents will be removed from the system. While this may seem to be contradictory to production of an amino acid from glucose, but the oxidation of the NADH pool will allow anaerobic ATP production, which under electron feeding conditions could not be achieved.

Xafenias and co-workers recently tested anthraquinone-2-sulfonate (AQ2S) as a mediator to increase the production of lysine under oxidative and reductive conditions in a BES (Xafenias et al. 2017). In contrast to our study, no growth could be observed but different effects on different metabolic products were described. We consider AQ2S as a suboptimal mediator, because due to the relatively high redox potential (-0.25 V) it is not able to support thermodynamically the reduction of $NADP^+$ under reductive conditions, which is critical for lysine biosynthesis (Kracke et al. 2015). On the other hand, under oxidative conditions its redox potential is too negative to allow acceptance of electrons from the terminal components of the cellular electron transport chain of *C. glutamicum* (Kracke et al. 2015). Interestingly lysine concentrations and yields observed by us were comparable to those reported in the same study but under reductive conditions (Xafenias et al. 2017), in spite of the fact that in that study a further optimised production strain was used. This strain carried two additional

beneficial mutations for lysine production (Ohnishi et al. 2002), yet no better outcome was achieved. This might indicate that the reductive conditions with AQ2S in fact do not lead to better supply with NADPH. Our tracer study points towards a reduction in the oxidative PP flux causing potentially a NADPH limitation during Lysine production. At this point it seems that regulatory control of the central flux partitioning between glycolysis and oxidative PP is limiting production rather than the available redox equivalents in the system.

Conclusions

In this work, we used microbial electrochemical technology to improve bioproduction of amino acids. Using an anode instead of oxygen as the final electron acceptor can make it possible to turn an aerobic production process into an anoxic production process. We demonstrate a ferricyanide mediated anodic electro-fermentation using *C. glutamicum lys*C for the anoxic production of L-lysine. We achieved titers up to 2.89 mM, which are, to our knowledge, the highest reported under anaerobic conditions. This proof-of-concept research demonstrates the great power of anodic electro-fermentation for improving biochemical production processes and highlights the need to study electron transfer mechanism under anoxic conditions and the effect on the cellular regulation.

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Table I: Main parameters of anaerobic glucose fermentation of *C. glutamicum lys*C in a BES under different conditions: 1) With addition of $K_3[Fe(CN)_6]$ and poised potential at +0.697 V vs SHE. 2) With addition of $K_3[Fe(CN)_6]$ but operated under an open circuit. 3) No addition of $K_3[Fe(CN)_6]$ and operated under an open circuit. 4) With addition of $K_3[Fe(CN)_6]$, poised potential at +0.697 V vs SHE and high start inoculum. Averages and standard deviation are provided for biological replicates (n = 4).

		1.5 mM $K_3[Fe(CN)_6]$, +0.5 V	**1.5 mM $K_3[Fe(CN)_6]$, open circuit**	**no mediator, open circuit**	**1.5 mM $K_3[Fe(CN)_6]$, +0.5 V, high inoculum**
Carbon balance (%)		84.46 ± 6.55	96.95 ± 9.22	93.06 ± 8.52	96.27 ± 7.12
Redox balance (%)		84.55 ± 6.49	96.68 ± 9.58	90.86 ± 7.16	96.08 ± 6.74
Yields [$mol_{product}/mol_{glucose}$]	lactate	1.07 ± 0.05	1.59 ± 0.49	1.28 ± 0.18	1.14 ± 0.04
	succinate	0.22 ± 0.01	0.24 ± 0.04	0.26 ± 0.05	0.20 ± 0.05
	acetate	0.12 ± 0.04	0.11 ± 0.02	0.09 ± 0.03	0.03 ± 0.003
	electrons	0.25 ±0.02	-	-	0.16 ± 0.08
Yields [$mmol_{product}/mol_{glucose}$]	lysine	41.06 ± 1.97	51.90 ± 8.79	51.80 ± 14.11	55.79 ± 2.83
	alanine	9.34 ± 0.97	9.68 ± 2.87	11.07 ± 2.16	29.03 ± 0.54
	glycine	0.59 ± 0.05	1.83 ± 1.11	1.83 ± 0.51	4.59 ± 0.19
Volumetric glucose consumption rates		0.86 ± 0.10	0.46 ± 0.15	0.43 ± 0.03	3.51 ± 0.01

[mmol/L/h]					
Volumetric production rates [mmol/L/h]	lactate	0.92 ± 0.11	0.63 ± 0.08	0.55 ± 0.04	4.02 ± 0.19
	succinate	0.19 ± 0.02	0.11 ± 0.02	0.11 ± 0.01	0.67 ± 0.19
	acetate	0.11 ± 0.04	0.05 ± 0.01	0.04 ± 0.01	0.11 ± 0.01
	electrons	0.22 ± 0.04	-	-	0.56± 0.27
Volumetric production rates [µmol/L/h]	lysine	35.29 ± 4.77	23.78 ± 4.10	21.93 ± 4.93	202.23 ± 2.27
	alanine	8.11 ± 0.05	4.09 ± 1.02	4.72 ± 0.58	108.73 ± 4.61
	glycine	0.50 ± 0.04	0.77 ± 0.40	0.77 ± 0.19	20.75 ± 2.45

Figures

Figure 1: (A) Microbial electrochemical characterisation of the interaction with the anode via $K_3[Fe(CN)_6]$ acting as a mediator (*blue line*) and lacking the mediator (*red line*). The *dash lines* represent the anodic current density and the *solid lines* the charge. Data have been averaged from 4 biological replicates and the standard deviations are represented as yellow and grey areas, respectively. **(B) Abiotic electrochemical characterisation** of the mediator (1.5 mM $K_3[Fe(CN)_6]$) via cyclic voltammetry (CV) using carbon cloth as the working electrode. CVs were done at scan rate of 0.5 mV/s within a potential window between 0 and 0.8 V vs. SHE at pH 7.2 and 30 °C.

Figure 2: Anodic enhancement of growth and glucose consumption. Time courses of **(A)** anaerobic bacterial growth and **(B)** glucose consumption of *C. glutamicum lys*C in a BES under 3 different conditions: 1) With addition of $K_3[Fe(CN)_6]$ and poised potential at +0.697 V vs SHE (*blue circles*). 2) With addition of $K_3[Fe(CN)_6]$ but operated under an open circuit (*orange squares*). 3) No addition of $K_3[Fe(CN)_6]$ and operated under an open circuit (*green triangles*). Grey area indicates time frame when glucose was consumed under condition 1). Data have been averaged from 4 biological replicates for each condition.

Figure 3: Anodic enhancement of organic acid and amino acid production. Time course of main organic acid **(A-C)** and amino acid **(D-F)** produced during anaerobic fermentation of *C. glutamicum lys*C in a BES under 3 different conditions: 1) With addition of $K_3[Fe(CN)_6]$ and poised potential at +0.697 V vs SHE (*blue circles*). 2) With addition of $K_3[Fe(CN)_6]$ but operated under an open circuit (*orange squares*). 3) No addition of $K_3[Fe(CN)_6]$ and operated under an open circuit (*green triangles*). Grey area indicates time frame when glucose was

consumed under condition 1). Data have been averaged from 4 biological replicates for each condition.

Figure 4: Effect of higher biomass inoculum on anodic electro-fermentation. Time courses of **(A)** bacterial growth and glucose consumption, **(B)** main organic acid and amino acid production of *C. glutamicum lys*C in a BES under anaerobic conditions and poised potential at +0.697 V vs SHE with addition of $K_3[Fe(CN)_6]$. The BES was inoculated with a OD_{660} of 3.65. Grey area indicates time frame when glucose was consumed. Data have been averaged from 2 biological replicates.

Figure 5: Schematic image of potential interaction sides of the terminal electron transport chain of *C. glutamicum* with $K_3[Fe(CN)_6]$ acting as an external electron acceptor (mediator). By oxidising substrates primary dehydrogenases (DHs) and/or oxidoreductases (ORs) could pass the obtained reducing equivalents straight to the mediator like succinate DH or to menaquinone (MQ). The reduced MQ, menaquinol (MQH_2) could shuttle the electrons straight to the mediator or to terminal oxygen reductases, which could transfer the electrons further to the mediator. The super complex could couple this process with a creation of an electrochemical proton gradient across the membrane for energy conservation in form of ATP (adenosine triphosphate; ADP, adenosine diphosphate). The mediator is reoxidised by the anode (ox, oxidised; red, reduced). Red dashed lines indicate hypothetical electron and proton flow.

Figure 6: Catabolism of glucose by *C. glutamicum* during anodic Electro-fermentation in a BES and its central metabolism. Enzymes are shown, which could potential contribute to the transport of reducing equivalents to the mediator (*red lines*). Abbreviations: *ADP* adenosine diphosphate, *ATP* adenosine triphosphate, *CoA* coenzyme A, e^- reducing equivalents, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *ICD* isocitrate dehydrogenase, *LDH* lactate dehydrogenase, *MDH* malate dehydrogenase, *MQO* malate:quinone oxidoreductase, $NAD^+/NADH$ nicotinamide adenine dinucleotide (oxidised / reduced), $NADP^+/NADPH$ nicotinamide adenine dinucleotide phosphate (oxidised / reduced), *NDH DH II* NADH dehydrogenase II, *P* phosphate, *PDH* pyruvate dehydrogenase, *PEP* phosphoenolpyruvate, *PQO* pyruvate:quinone oxidoreductase, *SDH* succinate dehydrogenase, *SQO* succinate:menaquinone oxidoreductase.

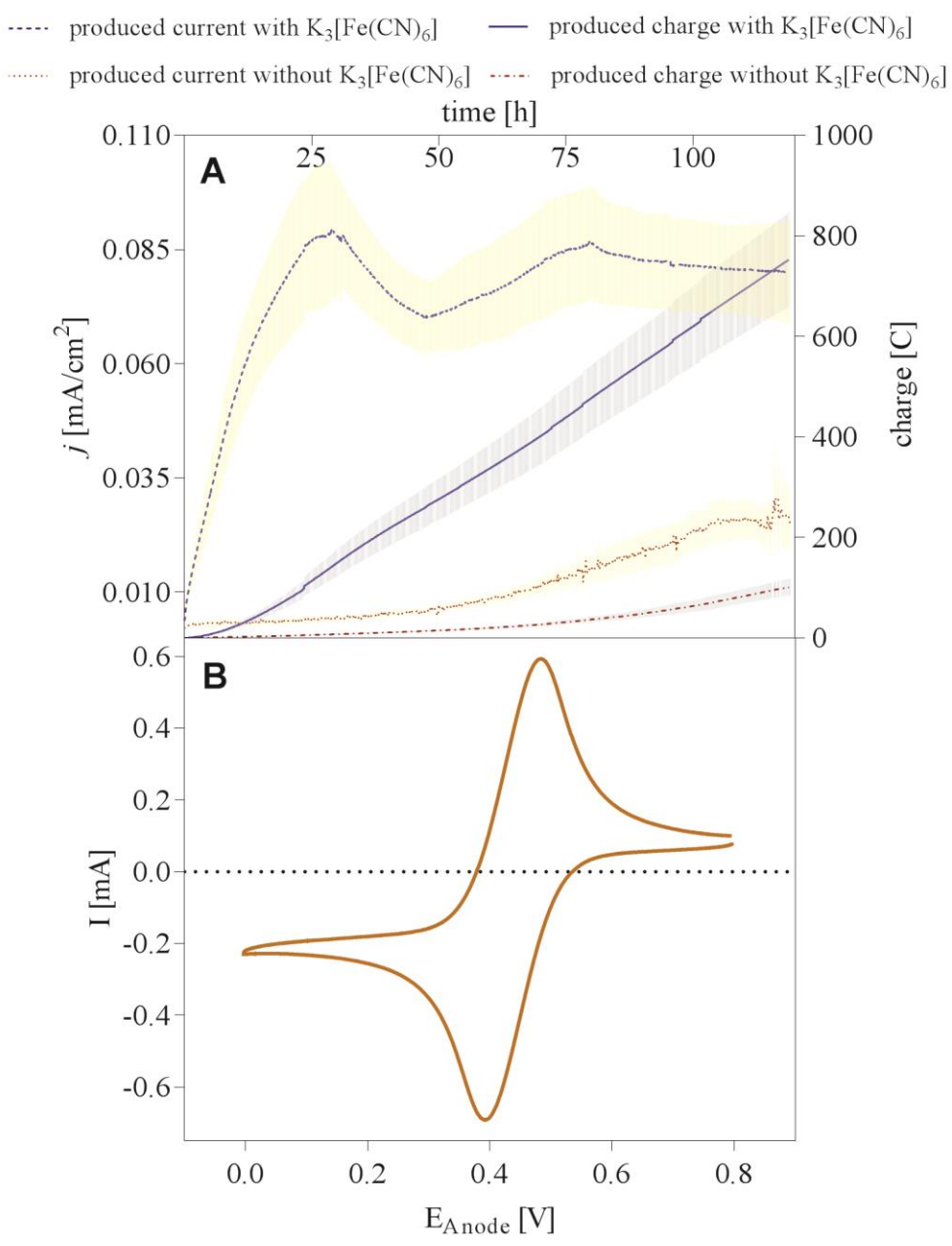

Figure 1

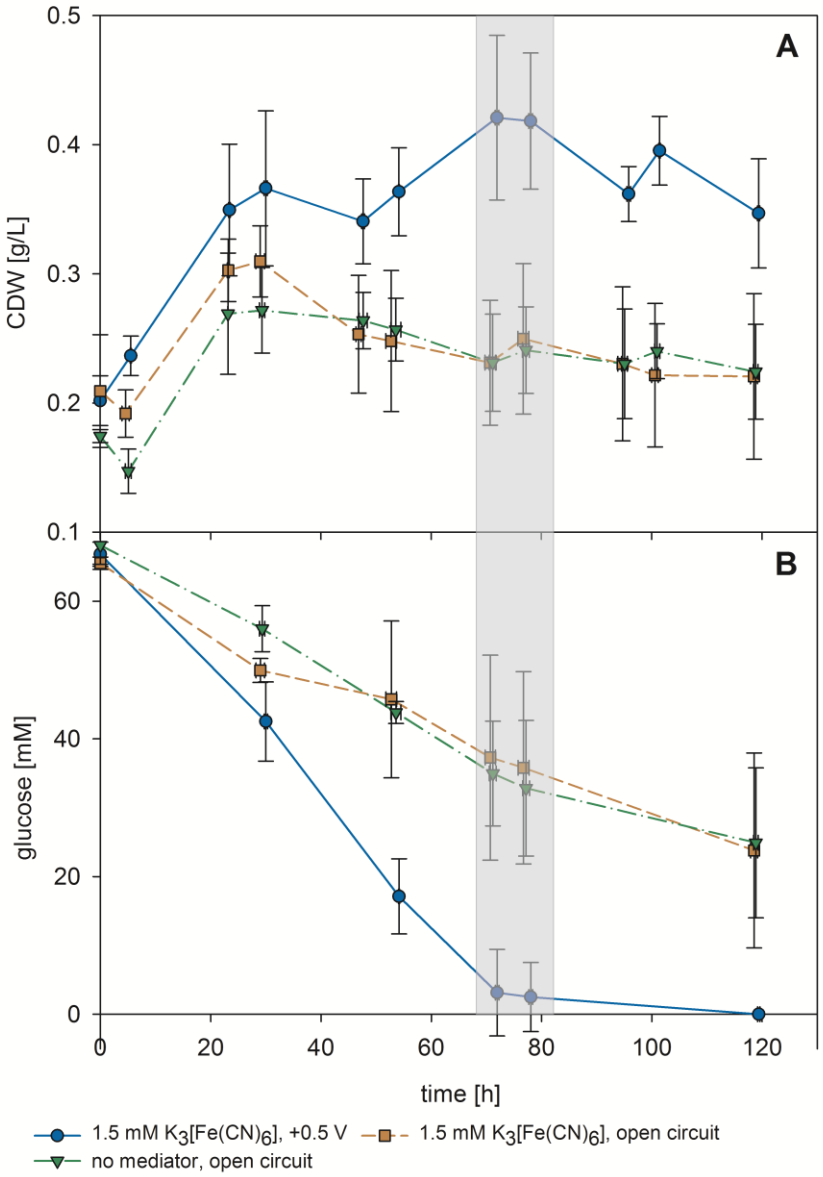

Figure 2

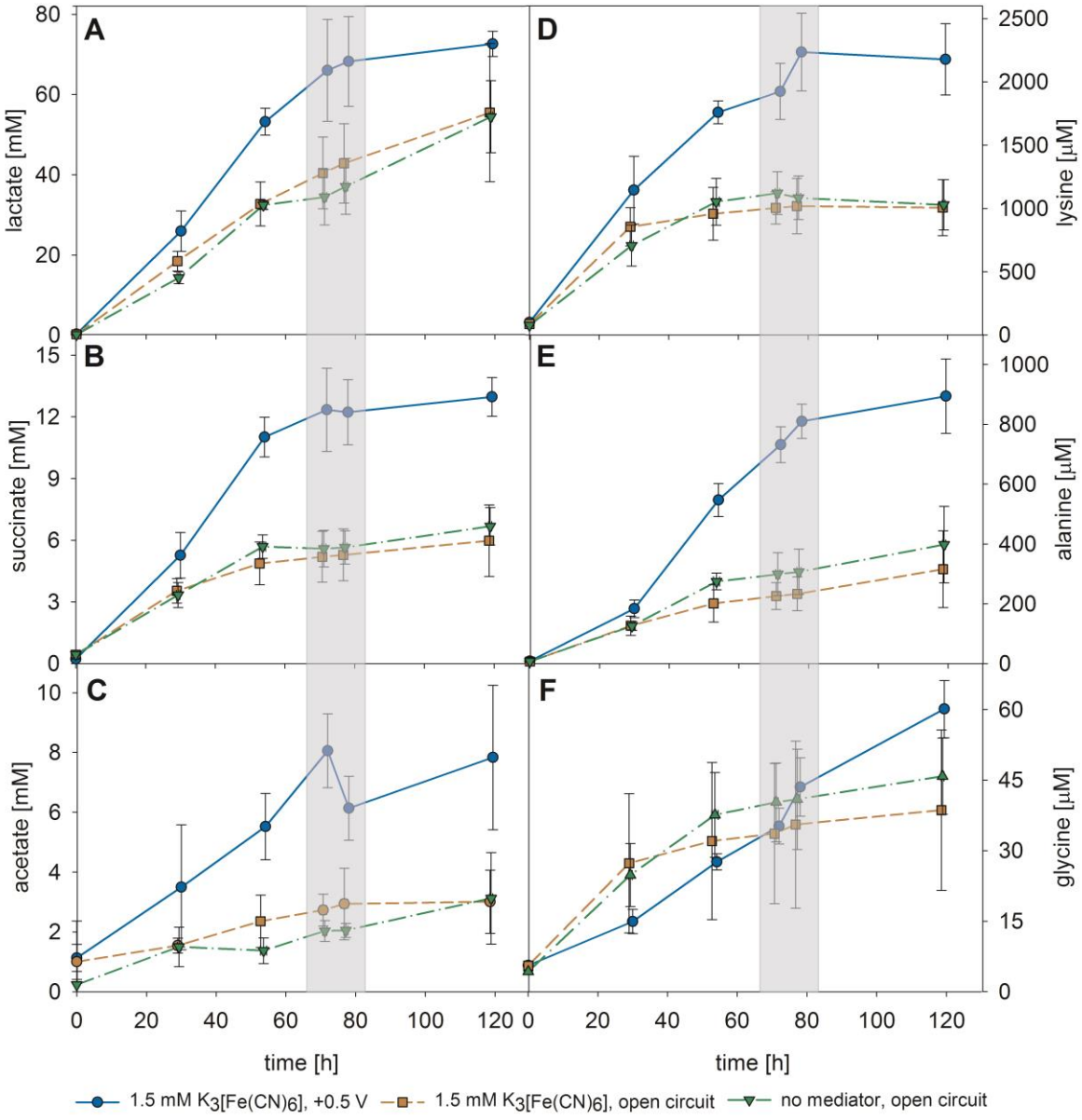

Figure 3

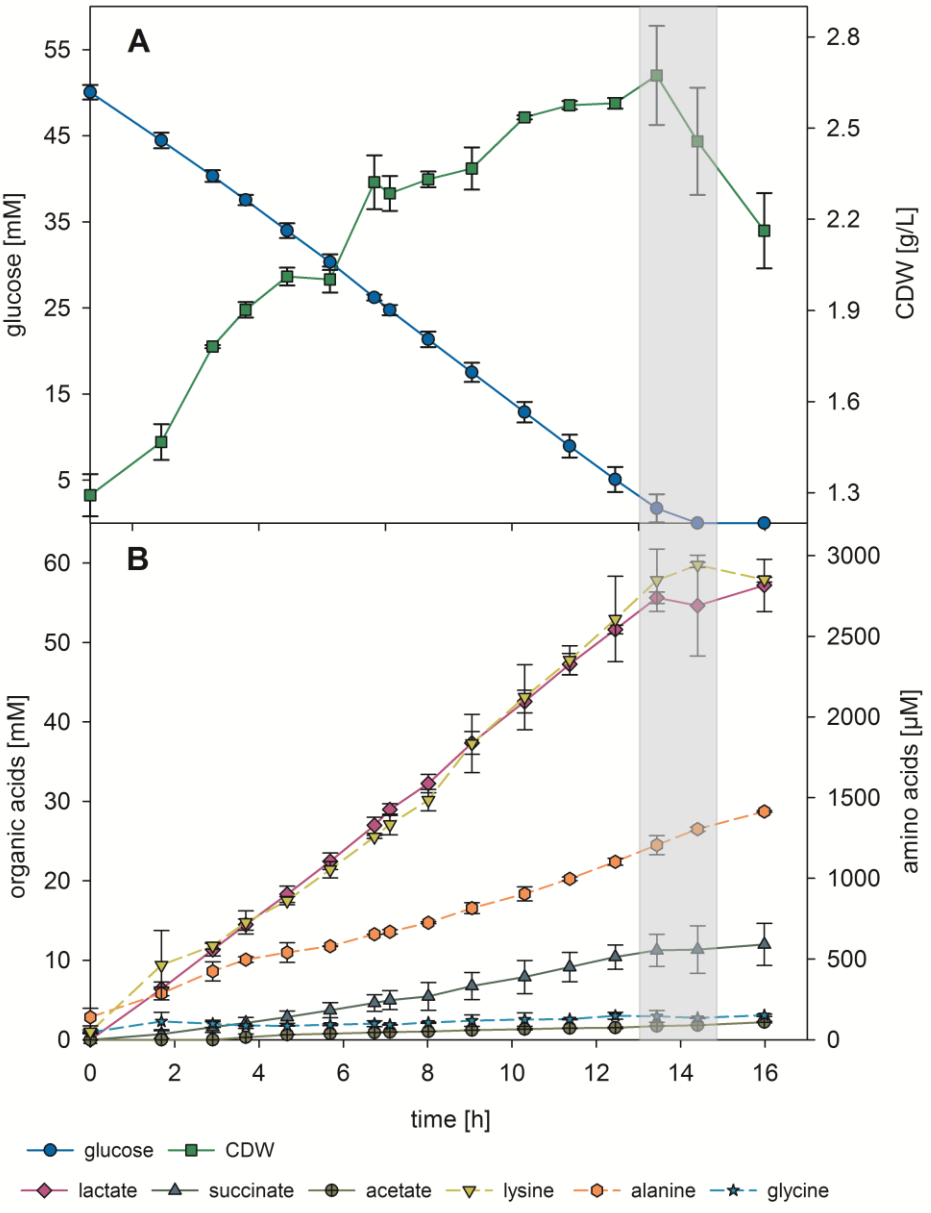

Figure 4

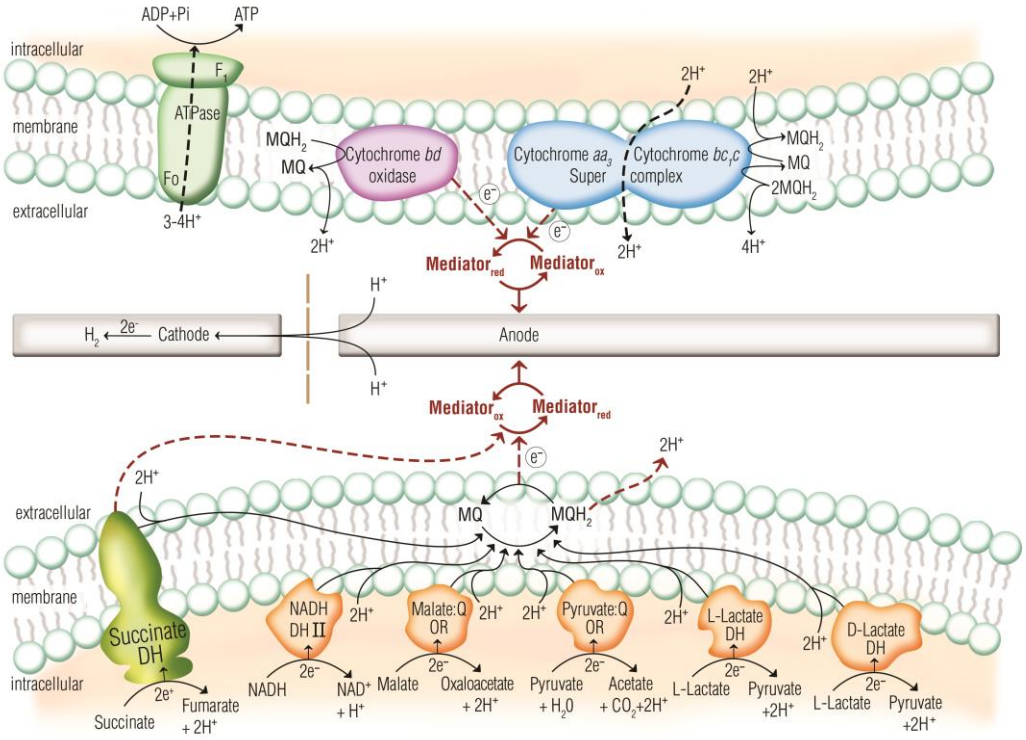

Figure 5

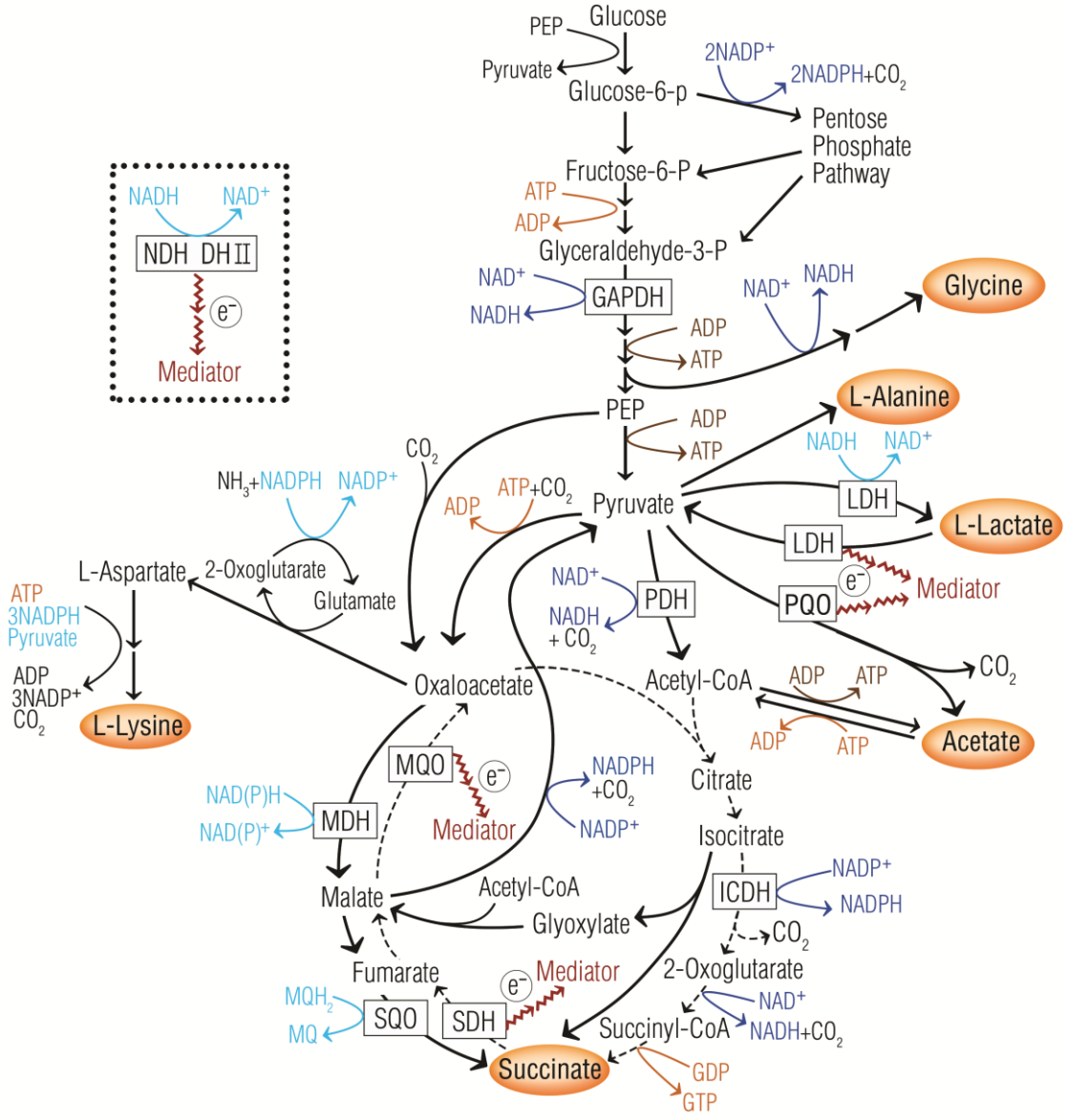

Figure 6