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Adaptive Laboratory Evolution of *Cupriavidus necator* to Improve Energy Demand in Bioelectrochemical Cultivations

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ABSTRACT

This study investigates adaptive laboratory evolution (ALE) to improve the tolerance of *Cupriavidus necator* H16 ΔPHB to high electrolyte concentrations, enabling more energy-efficient electroautotrophic cultivation. Over 3 months, strains were gradually adapted to up to 400 mM Na_2SO_4. Compared to the wild type, adapted strains exhibited higher growth under elevated salt conditions, maintaining viability at concentrations up to 300 mM. In bioelectrochemical systems, the addition of Na_2SO_4 significantly increased medium conductivity, reducing the cell voltage required under galvanostatic conditions. As a result, energy demand for cultivation decreased. Experiments demonstrated that the adapted strain grew comparably to the wild type under standard conditions but performed markedly better under high-salt conditions, shortening lag phases and reaching higher optical densities. Calculations revealed an energy saving of approximately 11% during 204 hr of cultivation when using the adapted variant in electrolyte-supplemented media. This work highlights the potential of combining biological robustness with optimized electrochemical conditions to reduce energy input in microbial electrosynthesis. Unlike purely technical optimizations, ALE provides a straightforward, natural, and transferable strategy to adapt production hosts to electrochemical process conditions. The findings demonstrate a practical route toward more sustainable bioelectrochemical processes by lowering energy consumption without compromising microbial performance.

1 | Introduction

Modern life is characterized by high level consumption, not only of foods, cloths, and other material goods, but also energy, which still largely derives from fossil sources and is often treated as inexhaustible. In the last decades, global awareness has increasingly focused on climate change, to which such consumption contributes significantly. While changes in personal lifestyle alone are insufficient to counteract this challenge, industry and the energy sector have a rather big impact thus are the ones that should lead the way to reduce climate change as they are responsible for a great deal of emissions of greenhouse gasses causing it [1]. Consequently, research aims to make production processes more energy efficient, achieving equivalent performance at reduced cost and environmental impact. Production hosts such as *Cupriavidus necator* are already industrially established, particularly for the production of polyhydroxybutyrate PHB [2]. First described by Schlegel et al. (1961) *C. necator* is recognized as a metabolically versatile production host, capable of heterotrophic, chemolithoautotrophic, and mixotrophic growth [3]. It's broad substrate and product spectrum includes different sugars, amino acids, organic acids, off-gas, and syngas. Especially relevant for sustainable biotechnology is its ability to convert waste streams into valuable compounds such as ethanol, acetone, butanol, succinic acid, biodiesel but also the bioplastic PHB and many more [4–13]. Especially electro-autotrophic approaches gain interest for the production of basic chemicals while simultaneously utilizing CO_2, a major greenhouse gas [14]. Within the field of bioelectrochemical systems (BES), microorganisms can access electrical energy in several ways [15, 16]. Electrons can be transferred directly if the organisms have membrane complexes or pili enabling them to transfer electrons over the membrane (direct electron transfer – DET) [17, 18]. Mediated electron transfer (MET) employs redox mediators that are reduced by the electrode and subsequently oxidized by the microorganism; this requires that the host tolerate often-toxic mediators and interact effectively with them [19, 20]. Indirect electron transfer (IET) involves electrochemically generated intermediates, such as hydrogen and oxygen from water splitting, and can be metabolized by microorganisms without direct electrode contact or interaction with mediators. These principles find application in already common ways like wastewater treatment where electroactive bacteria degrade pollutants while generating electrical energy [21]. A similar concept is demonstrated in microbial "urine batteries," in which bacteria metabolize urine, producing a surplus of electrons that are harvested as electrical energy [22]. For efficient application of electrical energy in aqueous medium however, sufficient conductivity is required. Electrochemical reactions depend on the presence of electrolytes, but high salt concentrations impose osmotic stress on most bacteria, leading to water efflux, desiccation, and in severe cases plasmolysis [23]. Nevertheless, many bacteria have evolved mechanisms to withstand osmotic stress. Bacteria from all kinds of environments like soil-dwelling *C. necator* but also *Escherichia coli* from the gut or *Vibrio fischeri* isolated from marine environments are able to accumulate compounds called compatible solutes, uncharged organic molecules synthesized de novo, or acquired from the environment, to counteract hyperosmotic conditions [24–27]. Compatible solutes thus present a natural way to support bacterial growth in electrolyte-rich environments suitable for electrochemical processes. However, the regulation of compatible soulute production is very complex with several transporters and metabolic pathways which makes genetic engineering less suitable to enhance the bacterial stress response to osmotic pressure, as no single gene provides a universal solution [28, 29]. Adaptive laboratory evolution (ALE) offers a promising alternative. With this method it's possible to adapt organisms to conditions and change them genotypically without interfering genetically [30]. By slight changes of the culture medium and transfer of cells in periodical steps, a medium without electrolytes is transformed over time to a medium with a high concentration of electrolytes which cells do tolerate. For ALE, random mutations which occur regularly are inevitable as cells with beneficial mutations for current environmental conditions have an advantage compared to wildtype cells and are able to overgrow them [31].

In a previous publication we showed that in electrochemical experiments the electrolyte can be optimized – here we want to improve the microbial strain [23]. Accordingly, ALE was used to adapt *C. necator* H16 ΔPHB to higher concentrations of the sodium salt Na_2SO_4 to be able to use those strains in electro-autotrophic growth experiments with high electrolyte medium and reduce the needed energy.

2 | Material and Methods

2.1 | Culture Conditions

For electro-autotrophic cultivation, all strains (*C. necator* H16 DSM 541 and adapted variants) were cultivated in a seed train of three precultures. From a cryo-conserved glycerin-stock stored at -80°C, cells were spread on LB agar plates (5 g/L NaCl, 5 g/L yeast extract, 10 g/L tryptone, 1.8 % agar, pH 7) and incubated at 30°C for 24 h. 5 mL LB-medium (5 g/L NaCl, 5 g/L yeast extract, 10 g/L tryptone, pH 7) were inoculated from a single colony [32]. This preculture was followed by precultures in minimal medium. The minimal medium was composed as described before [23]. The overall second preculture was heterotrophic by the addition of 4 g/L fructose. From this preculture either heterotrophic growth curves or autotrophic precultures were inoculated. The autotrophic preculture was supplied with a gas atmosphere of H_2, CO_2, and O_2 (8:1:1). The cultures were incubated at 30°C and 180 rpm. Growth was measured photometrically via optical density at a wavelength of 600 nm (OD_{600}).

2.2 | Adaptive Laboratory Evolution (ALE)

ALE was performed in 100 mL baffled Erlenmeyer flasks using heterotrophic minimal medium [23] supplemented with 4 g/L fructose as carbon source. Over a period of 3 months, Na_2SO_4 concentrations were gradually increased from 0 to 400 mM. Cultures were transferred daily to fresh medium by diluting to an OD_{600} of 0.1 in 20 mL medium. Growth was monitored photometrically, and adapted populations were cryopreserved for subsequent analysis. Characterization of adapted strains was carried out in a Stratus microplate reader (Cerillo, Charlottesville, VA, USA) across a Na_2SO_4 gradient from 0 to 300 mM. A schematic overview of the ALE process is shown in Figure 1.

2.3 | Setup of the Bioelectrochemical System (BES)

The BES was composed of an undivided electrochemical reactor with a working volume of 110 mL and a two-electrode setup. Platinized titanium expanded metal with a surface factor of 1.7 (50 g Pt per m^2, Metakem GmbH, Usingen, Germany) and a geometrical surface area of 6 cm^2 was used as anode for water splitting, and a graphite rod with a geometrical surface area of 6.48 cm^2 was used as cathode for the electrochemical production of H_2 [33, 34]. Both electrodes were contacted with a platinum wire with a diameter of 0.5 mm. The contact of the graphite rod was additionally secured with Teflon tape. The electrodes were held at a distance of 1 cm with a 3D-printed separator, printed

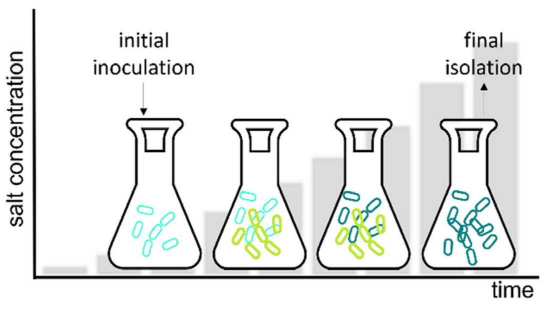

FIGURE 1 | ALE – scheme. During the ALE process the salt concentration is increased (gray bars) over time, causing the continuous adaptation of the bacteria from initial inoculation till final isolation to high salt concentrations. ALE is performed by periodically transferring small amounts of culture to fresh medium with an increased amount of salt.

with a heat-resistant poly amid fiber (Fiberthree F3 PA-GF30 Pro, Fiberthree, Darmstadt, Hessen, Germany) in the Prusa 3D printer (Original Prusa i3 MK3S+ printer, Prusa Research a.s., Holešovice, Czech Republic). A current of 15 mA was applied constantly for water electrolysis via a potentiostat (Gamry Interface 1010B, Gamry Instruments, Warminster, PA, USA). For gas supply PTFE tubing was mounted to the lid. For mixture at 300 rpm a stir bar was added to the reactor. After sterilizing the reactor by autoclaving, they were filled with a sterile autotrophic minimal medium as described previously [14, 23] to a total volume of 110 mL; sterilized PTFE tubes were connected to autoclaved silicone tubes and sterile filters for continuous gas supply consisting of N_2, O_2, and CO_2 (85:5:10) at a flow rate of 25 mL/min. The reactors were placed in an incubator at 30°C and after polarization at given conditions over night they were inoculated with autotrophic precultures at an OD_{600} of 0.2. Composition of the gas phase was measured daily with a gas analyzer (Gas Analyzer Micro GC Fusion, Inficon, Bad Ragaz, Switzerland), growth was measured via OD_{600} and pH with a pH meter (pH-meter Lab 845, SI Analytics – Xylem Analytics Germany, Mainz, Germany). A scheme of the setup is shown in Figure 2.

2.4 | Conductivity Measurement

The conductivity of prepared media was measured at room temperature of 20°C and after incubating closed containers (50 mL falcon tubes) at 30°C overnight. Conductivity was measured with an electrical conductivity (EC) meter (WTW cond 340i with TetraCon 325, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) by merging the sensor area of the EC meter into the medium for about 10 seconds.

2.5 | Calculations

For the evaluation of energy needed for the experiments, formula (1) was used, multiplying the voltage with the power over the elapsed period of time. In formula (2) the unit of the consumed power was changed from Wh to J.

$$P[Wh] = \Delta U[V] * \Delta t[h] * I[A] \quad (1)$$

$$Wh * 3600 = Ws = J \quad (2)$$

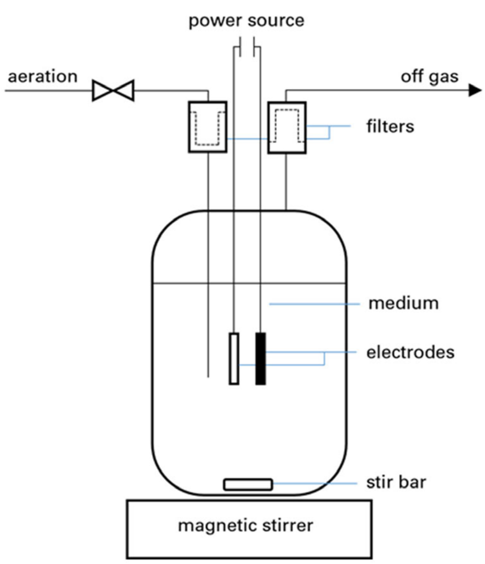

FIGURE 2 | Setup of the BES (detailed description in the main text).

3 | Results and Discussion

3.1 | Adaptive Laboratory Evolution – ALE

During and after the adaptation process, culture samples were periodically collected and cryo-conserved for analysis. The adaptation was conducted in 50 mM increments of Na_2SO_4 every 5 – 7 days beginning with initial cultivation with 100 mM of Na_2SO_4. Conserved strains that were analyzed are for enhanced growth in minimal medium containing higher concentrations of Na_2SO_4 are listed in Table 1.

The adapted strains were compared to the unadapted wildtype in minimal medium according to Sydow et al. [23] under heterotrophic conditions with 4 g/L of fructose as carbon and energy source. To evaluate the state of the adaptation, the strains were cultivated under the influence of 0, 100, 200, and 300 mM of Na_2SO_4 and compared to the wildtype. Not only strains adapted to sodium sulfate were tested but also the sodium chloride-adapted strains (Figure S1, S2). As the adapted strains performed mostly similar, a variant from an earlier adaptation phase was selected for subsequent experiments, as these strains exhibited higher overall viability and were less negatively affected by the prolonged adaptation to extreme osmolarities. Although the ad5 variant showed the highest OD_{600} in the screening experiment (Figure S1, S2) in the following electro-autotrophic BES experiments, no growth worth mentioning could be observed here (Figure S3, S4). Thus, further experiments were conducted with the strain *C. necator* H16 ΔPHB adNa_2SO_4 1 (ad1).

To assess suitability of the strains for electro-autotrophic experiments more closely, they were grown under autotrophic conditions with 0, 300, and 500 mM of Na_2SO_4. Even without additional electrolyte in the medium it was visible that strain ad1 grew to higher optical densities than the wildtype H16 ΔPHB which only reached about 1.4 whereas the adapted strain reached an OD_{600} of 2 (Figure 3). Under the influence

TABLE 1 | *C. necator* H16 ΔPHB Strains at Different Stages of Adaptation.

t (d)	Strain	Abbreviation	Adapted to X mM Na_2SO_4
0	*C. necator* H16 ΔPHB	WT	0
23	*C. necator* H16 ΔPHB adNa_2SO_4 1	ad1	200
36	*C. necator* H16 ΔPHB adNa_2SO_4 3	ad3	300
61	*C. necator* H16 ΔPHB adNa_2SO_4 5	ad5	400

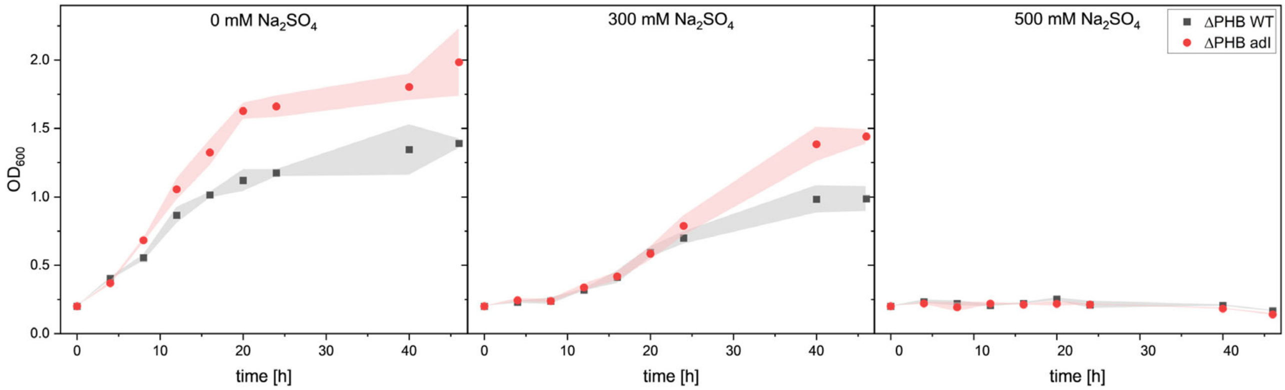

FIGURE 3 | Growth of *C. necator* H16 ΔPHB WT and ad1 under autotrophic conditions with 0, 300, and 500 mM Na_2SO_4. Cultures were grown in minimal medium according to Sydow [23] under autotrophic conditions with a gas atmosphere of H_2, CO_2, and O_2 (8:1:1). From left to right 0, 300, or 500 mM of Na_2SO_4 were added. The wildtype (black) and the ad1 variant (red) were tested. N = 3.

of 300 mM the wildtype grew to an OD_{max} of 1 after 46 h and the ad1 variant reached an OD_{600} of 1.5. For both strains additional sodium sulfate of a concentration of 500 mM was too much to show any sign of growth (Figure 3). Clearly, the adapted strain showed improved growth as measured by OD_{600} compared to the wildtype with any salt concentration under electro-autotrophic conditions. Even when the strains behaved very similar to each other in pre-experiments in the cultivation system ALE was conducted in (Figure S1), in the transfer to another cultivation system like autotrophic cultivation shown here or later electro-autotrophic cultivation, strains visibly behaved differently.

3.2 | Bioelectrochemical Experiments and Calculations

To measure the influence of higher osmolarities in the minimal medium according to Sydow et al. [23] by the addition of varying concentrations of sodium sulfate, conductivity was measured at different temperatures with an EC meter. Therefore medium was produced as usual and incubated at room temperature (20°C) or at the temperature all growth experiments were conducted in (30°C). The sensor area of the EC meter was merged into the medium for about 10 seconds. With rising temperature, a small difference of 0.2 – 0.7 mS/cm was measurable, and more influence was seen regarding the concentration of the osmolyte. The minimal medium according to Sydow [23] shows a conductivity of 6.5 mS/cm in both temperatures, by the addition of only 100 mM of sodium sulfate the conductivity could be increased by 13.6 mS/cm to a value of 20.1 mS/cm. At 200 mM Na_2SO_4 the conductivity reached 31.5 mS/cm, at 300 mM, 41.3 mS/cm, at 400 mM, 50.1 mS/cm, and the best conductivity was reached at 500 mM with 58.7 mS/cm (Figure S2). As the conductivity depends on the number of soluble particles and sodium sulfate dissociates into two sodium and one sulfate ion. As the concentration is enhanced gradually, the sodium sulfate can't dissociate fully; therefore, the conductivity enhances nonlinearly at higher concentrations. The effect of the temperature difference on conductivity was small but measurable; therefore, one factor having an impact could be the increased temperature, as solubility of conductivity-enhancing gasses in aqueous medium decreases with higher temperature [35, 36].

To evaluate the effect of addition electrolytes in the medium on the wildtype strains as well as the adapted variant, both strains were cultivated electro-autotrophically. Therefore, minimal medium according to Sydow et al. [23] was used with a graphite rod as a working electrode and a platinized titanium electrode as counter electrode. The system was gassed with a gas mixture of N_2:Air:CO_2 (65:25:10), leading to a distribution of N_2:CO_2:O_2 of 85:10:5. After an overnight polarization at 15 mA a steady voltage of around 3 V was reached in the medium without extra electrolytes and around 2.5 – 2.6 V for the medium with 300 mM of sodium sulfate. After inoculation to an OD_{600} of 0.2 both strains grew similarly in the first 4 days in the medium without electrolytes to an optical density of about 2.5, from day 5 on the adapted strain stagnated, the wildtype grew further and reached a maximal OD_{600} of about 3.3, whereas the maximal reached OD_{600} of the adapted strain lay around 2.7. Therefore, regarding both strains in minimal medium with 300 mM of sodium sulfate, the wildtype showed an extended lag phase of 3 days before it grew steady to an OD_{600} of about 2. Compared to this, the adapted strain reached OD_{600} after just 1 day of lag phase, just like in the medium without electrolyte, of nearly 3. So, regarding growth of the adapted variant, even when challenged with high electrolyte concentrations, compared to the wildtype under standard conditions, no noteworthy difference could be observed. Following the observation of growth, the voltage of both strains was analyzed and compared depending mostly on the amount of electrolyte added to the medium. Thus, it was much lower in cultures with 300 mM of sodium sulfate compared to the original medium. For further calculations the time was given in hours (Figure 4). By integration of the graph of voltage over time the area under the curve could be calculated. A duration of 204 h was taken as well as the voltage of the wildtype in the usual minimal medium (black squares) compared to the voltage of the culture of the adapted variant in minimal medium with extra electrolyte (blue triangles). Even when it's lower in the beginning the growth rate of the ad1 strain in 300 mM medium enhances strongly and is comparable or higher after 100 h of cultivation than the growth rate of both strains growing in the basic minimal medium without salt (Figure 5). Whereas the growth rate of the wildtype strain in 300 mM is low the whole time.

Following Equations (1) and (2), the electrical energy needed to cultivate strains for 204 hours in the given system could be calculated for the wildtype in unmodified medium

The energy difference between the two modi was accordingly

$$\Delta \mathbf{J} = 32315.22\,\mathrm{J} - 28824.66\,\mathrm{J} = \mathbf{3490.56\,J}$$

The difference of energy that was required for the cultivation of *C. necator* H16 ΔPHB strains in the electro-autotrophic growth amounted to 3490.56 J for a cultivation duration of 204 h (8.5 days). In percentage, the difference of 3490.56 J is 10.8% of the initially needed power. So, by using adapted strains and by the addition of salt to the medium around 11% of energy could be saved while about the same OD_{600} values were reached.

The optical density of *C. necator* can be correlated to the yielding biomass as shown by Grunewald et al. [37, 38]. It was shown that the OD_{600} multiplied by 0.363 results in the produced biomass in [g/l]. So, the resulting biomass of the strains can be used to see how much energy per biomass was needed. In Table 2, the results of these calculations are shown. It can be pointed out that the variant ad 1 in 300 mM (27 193.08 J*l/*g* ± 2.7%) is nearly as efficient as the original process with the unadapted strain (26 929.35 J*l/*g* ± 5.5%) without additional salt in the medium. Just saving energy by the addition of salt to the medium with the wildtype would even double the energy needed to yield the same biomass, whereas only ALE without media alteration also enhances the energy needed per biomass, due to a decreased performance of the adapted strain. As the strains were adapted in a heterotrophic shake flask culture and transferred to a BES, it was not predictable how the strains would behave in this new environment. Further ALE or a more BES-fitting ALE process would in this case be beneficial, but as mentioned before, ALE in a culture system that takes 8 – 9 days per cultivation is absolutely impractical. These results state, that, if possible, ALE should always be conducted in the later desired system. In the case of BES an appropriate system for ALE still needs to be developed. Nevertheless, the combination of ALE and media alteration could result already in a system where energy could be saved for similar biomass yield (Figure 4).

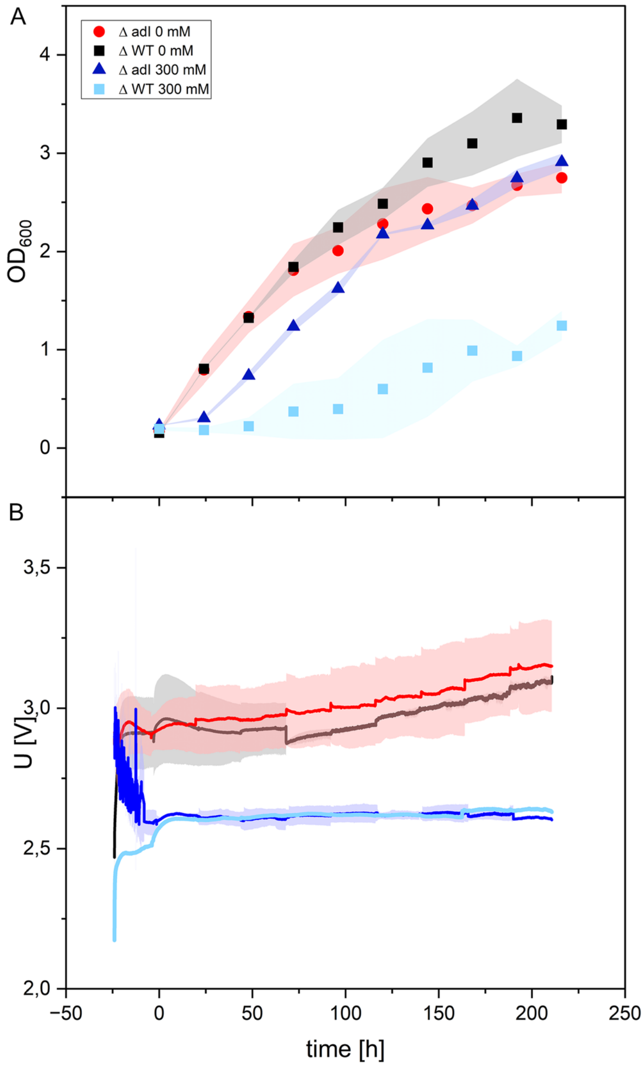

FIGURE 4 | Growth and voltage of *C. necator* H16 ΔPHB wildtype and variant ad1 with and without 300 mM Na_2SO_4 in minimal medium, N = 2 (A) Growth of *C. necator* H16 ΔPHB in minimal medium (square, black) and minimal medium with 300 mM Na_2SO_4 (square, light blue) and variant ad1 in in minimal medium (circle, red) and minimal medium with 300 mM Na_2SO_4 (triangle, blue). Cultures were grown in minimal medium for 210 hours (8.5 days) at 30°C, 300 rpm and a constant current of 15 mA. They were gassed with N_2, O_2, and CO_2 (85:5:10) at a flow rate of 25 mL/min. (B) Voltage of H16 ΔPHB in minimal medium (black) and minimal medium with 300 mM Na_2SO_4 (light blue) and variant ad1 in minimal medium (red) and minimal medium with 300 mM Na_2SO_4 (blue).

$$P = 598.43\,\mathrm{V\ h} * 0.015\,\mathrm{A} = 8.976\,\mathrm{Wh}$$

$$8.976\,\mathrm{Wh} * 3600 = 32315.22\,\mathrm{Ws} = \mathbf{32315.22\,J}$$

According to former calculations, for the adapted variant in minimal medium with additional 300 mM Na_2SO_4, the required energy was lower.

$$P = 533.79\,\mathrm{V\ h} * 0.015\,\mathrm{A} = 8.007\,\mathrm{Wh}$$

$$8.007\,\mathrm{Wh} * 3600 = 28824.66\,\mathrm{Ws} = \mathbf{28824.66\,J}$$

4 | Conclusion

A combination of supplementary electrolytes in the medium and natural strain optimization was shown to reduce the needed energy for electro-autotrophic cultivation of an adapted *C. necator* H16 ΔPHB in optimized medium. It's always the goal to enhance the energy efficiency in each experiment, this could be therefore achieved here with salt adaptation and high-electrolyte medium by around 11% less energy needed.

Conventional targets for optimization in electrochemical or electrobiological experiments typically focus on technical or chemical parameters, e.g., the electrolytes, the medium, the material of the electrodes, or their distance. In contrast, this work shows that enhancing the biological robustness of the production host itself, through a natural, nongenetic approach, can yield significant improvements in process efficiency. Media optimization alone achieves the energy savings but only in combination with ALE usual biomass yields could be maintained. An advantage of the herein-shown biological and chemical optimization is that it is easy and applicable, compared to technical optimizations. Staying in the field of bioelectrochemistry, there are not many processes that can be used as example but a great one is the

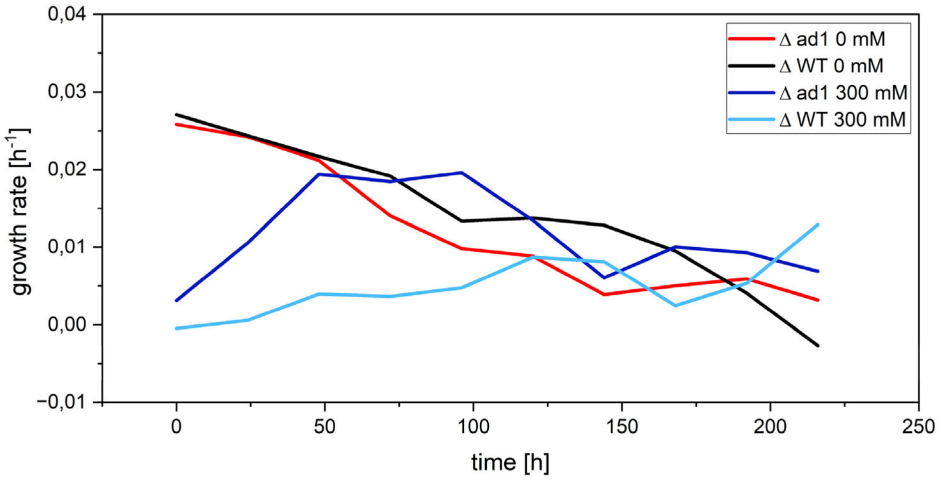

FIGURE 5 | Growth rate of *C. necator* H16 ΔPHB wildtype and variant ad1 with and without 300 mM Na_2SO_4 in minimal medium. Growth rate of *C. necator* H16 ΔPHB in minimal medium (square, black) and minimal medium with 300 mM Na_2SO_4 (square, light blue) and variant ad1 in in minimal medium (circle, red) and minimal medium with 300 mM Na_2SO_4 (triangle, blue). Cultures were grown in minimal medium for 210 h (8.5 days) at 30°C, 300 rpm, and a constant current of 15 mA. They were gassed with N_2, O_2, and CO_2 (85:5:10) at a flow rate of 25 mL/min.

TABLE 2 | *C. necator* H16 ΔPHB strains at different stages of adaptation.

Strain & Condition	OD_{600} at final measurement	Biomass at final measurement (g/l)	Consumed energy (J)	Energy in biomass (J*l/g)
C. necator H16 ΔPHB ad1 0 mM	2.75 ± 0.16	1.0 ± 0.06	33 275.31	33 275.31 ± 6%
C. necator H16 ΔPHB 0 mM	3.30 ± 0.19	1.2 ± 0.08	32 315.22	26 929.35 ± 5.5%
C. necator H16 ΔPHB ad1 300 mM	2.91 ± 0.09	1.06 ± 0.03	28 824.66	27 193.08 ± 2.7%
C. necator H16 ΔPHB 300 mM	1.25 ± 0.15	0.45 ± 0.03	29 426.15	65 391.44 ± 14.82%

Rheticus project by Evonik. It builds upon the concept of technical photosynthesis of Haas et al. (2018). Following the example in this study bacteria used in the Rheticus project could be adapted to process conditions that are ideal for electrochemistry presenting unadapted strains with an unsolvable challenge to achieve energy savings as electricity is the most crucial point concerning greenhouse gas emission worldwide [1].

Achieving more sustainable production processes is becoming increasingly important in modern research and industry. The results presented here provide a simple yet effective route toward this goal, demonstrating how biological adaptation can contribute directly to improving the energy demand and sustainability of electrobiological processes.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. H. Ritchie, P. Rosado, and M. Roser, "Breakdown of Carbon Dioxide, Methane and Nitrous Oxide Emissions by Sector," *Our World in Data* 11 (2020): 2025, https://ourworldindata.org/emissions-by-sector.

2. E. Vlaeminck, et al., "Pressure Fermentation to Boost CO2-Based Poly(3-Hydroxybutyrate) Production Using Cupriavidus Necator," *Bioresource Technology* 408 (2024): 131162, https://doi.org/10.1016/J.BIORTECH.2024.131162.

3. H. G. Schlegel, G. Gottschalk, and R. Von Bartha, "Formation and Utilization of Poly-Beta-Hydroxybutyric Acid by Knallgas Bacteria (Hydrogenomonas," *Nature* 191, no. 4787 (1961): 463–465, https://doi.org/10.1038/191463A0.

4. S. Bellini, T. Tommasi, and D. Fino, "Poly(3-Hydroxybutyrate) Biosynthesis by Cupriavidus Necator: A Review on Waste Substrates Utilization for a Circular Economy Approach," *Bioresource Technology Reports* 17 (2022): 1–19, https://doi.org/10.1016/j.biteb.2022.100985.

5. E. J. Bormann and M. Roth, "The Production of Polyhydroxybutyrate by Methylobacterium Rhodesianum and Ralstonia Eutropha in Media Containing Glycerol and Casein Hydrolysates," *Biotechnology Letters* 21, no. 12 (1999): 1059–1063, https://doi.org/10.1023/A:1005640712329/METRICS.

6. I. Dinges, I. Depentori, L. Gans, D. Holtmann, S. R. Waldvogel, and M. Stöckl, "Coupling of CO2 Electrolysis with Parallel and Semi-

Automated Biopolymer Synthesis – Ex-Cell and without Downstream Processing," *ChemSusChem* 17, no. 8 (2024): e202301721, https://doi.org/10.1002/CSSC.202301721.

7. M. Stöckl, S. Harms, I. Dinges, S. Dimitrova, and D. Holtmann, "From CO2 to Bioplastic – Coupling the Electrochemical CO2 Reduction with a Microbial Product Generation by Drop-in Electrolysis," *ChemSusChem* 13, no. 16 (2020): 4086–4093, https://doi.org/10.1002/CSSC.202001235.

8. T. Krieg, A. Sydow, S. Faust, I. Huth, and D. Holtmann, "CO2 to Terpenes: Autotrophic and Electroautotrophic α-Humulene Production with Cupriavidus Necator," *Angewandte Chemie International Edition in English* 57, no. 7 (2018): 1879–1882, https://doi.org/10.1002/ANIE.201711302.

9. S. Milker and D. Holtmann, "First Time β-Farnesene Production by the Versatile Bacterium Cupriavidus Necator," *Microbial Cell Factories* 20, no. 1 (2021): 1–7, https://doi.org/10.1186/S12934-021-01562-X/FIGURES/3.

10. A. Pohlmann, et al., "Genome Sequence of the Bioplastic-Producing 'Knallgas' Bacterium Ralstonia Eutropha H16," *Nature Biotechnology* 24, no. 10 (2006): 1257–1262, https://doi.org/10.1038/nbt1244.

11. A. Sydow, L. Becker, E. Lombard, R. Ulber, S. E. Guillouet, and D. Holtmann, "Autotrophic Production of the Sesquiterpene α-Humulene with Cupriavidus Necator in a Controlled Bioreactor," *Bioengineering* 10, no. 10 (2023): 1194, https://doi.org/10.3390/BIOENGINEERING10101194.

12. N. Teetz, D. Holtmann, F. Harnisch, and M. Stöckl, "Upgrading Kolbe Electrolysis—Highly Efficient Production of Green Fuels and Solvents by Coupling Biosynthesis and Electrosynthesis," *Angewandte Chemie International Edition* 61, no. 50 (2022): e202210596, https://doi.org/10.1002/ANIE.202210596.

13. J. Wang and J. Yu, "Kinetic Analysis on Formation of Poly(3-Hydroxybutyrate) from Acetic Acid by Ralstonia Eutropha under Chemically Defined Conditions," *Journal of Industrial Microbiology & Biotechnology* 26, no. 3 (2001): 121–126, https://doi.org/10.1038/SJ.JIM.7000097.

14. A. Langsdorf, J. P. Schütz, R. Ulber, M. Stöckl, and D. Holtmann, "Production of Polyhydroxybutyrate from Industrial Flue Gas by Microbial Electrosynthesis," *Journal of CO2 Utilization* 83 (2024): 102800, https://doi.org/10.1016/J.JCOU.2024.102800.

15. A. Sydow, T. Krieg, F. Mayer, J. Schrader, and D. Holtmann, "Electroactive Bacteria—molecular Mechanisms and Genetic Tools," *Applied Microbiology and Biotechnology* 98, no. 20 (2014): 8481–8495, https://doi.org/10.1007/S00253-014-6005-Z/FIGURES/3.

16. J. Gizewski, L. v.d. Sande, and D. Holtmann, "Contribution of Electrobiotechnology to Sustainable Development Goals," *Trends in Biotechnology* 41, no. 9 (2023): 1106–1108, https://doi.org/10.1016/J.TIBTECH.2023.02.009.

17. D. R. Bond and D. R. Lovley, "Electricity Production by Geobacter Sulfurreducens Attached to Electrodes," *Applied and Environmental Microbiology* 69, no. 3 (2003): 1548–1555, https://doi.org/10.1128/AEM.69.3.1548-1555.2003.

18. N. Kanojia, J. Poitras, T. Jones, B. Virdis, G. Southam, and L. E. Doyle, "Resolving Shewanella Vesicular Nanowire Structure during Microbial Extracellular Electron Transfer to a Poised Electrode," *Bioelectrochemistry* 166 (2025): 109039, https://doi.org/10.1016/J.BIOELECHEM.2025.109039.

19. A. Gemünde, E. Rossini, O. Lenz, S. Frielingsdorf, and D. Holtmann, "Chemoorganotrophic Electrofermentation by Cupriavidus Necator Using Redox Mediators," *Bioelectrochemistry* 158 (2024): 108694, https://doi.org/10.1016/J.BIOELECHEM.2024.108694.

20. A. Gemünde, J. Gail, and D. Holtmann, "Redox Mediator Interaction with Cupriavidus Necator – Spectroelectrochemical Online Analysis," *Electrochemistry Communications* 162 (2024): 107705, https://doi.org/10.1016/J.ELECOM.2024.107705.

21. T. Mkilima, et al., "Bioelectrochemical Degradation of Pollutants in Wastewater Using a Dual-Chamber Microbial Fuel Cell with Graphene-Modified Electrodes and Electroactive Bacteria," *Case Studies in Chemical and Environmental Engineering* 11 (2025): 101184, https://doi.org/10.1016/J.CSCEE.2025.101184.

22. R. Sharma, R. Kumari, D. Pant, and P. Malaviya, "Bioelectricity Generation from Human Urine and Simultaneous Nutrient Recovery: Role of Microbial Fuel Cells," *Chemosphere* 292 (2022): 133437, https://doi.org/10.1016/J.CHEMOSPHERE.2021.133437.

23. A. Sydow, T. Krieg, R. Ulber, and D. Holtmann, "Growth Medium and Electrolyte-How to Combine the Different Requirements on the Reaction Solution in Bioelectrochemical Systems Using Cupriavidus Necator," *Engineering in Life Sciences* 17, no. 7 (2017): 781–791, https://doi.org/10.1002/ELSC.201600252.

24. G. J. Gregory and E. F. Boyd, "Stressed Out: Bacterial Response to High Salinity Using Compatible Solute Biosynthesis and Uptake Systems, Lessons from Vibrionaceae," *Computational and Structural Biotechnology Journal* 19 (2021): 1014–1027, https://doi.org/10.1016/J.CSBJ.2021.01.030.

25. P. Baumann, L. Baumann, M. Mandel, "Taxonomy of Marine Bacteria: the Genus Beneckea",*Journal of Bacteriology* 107 (1971) 268–294, https://10.1128/jb.107.1.268-294.1971.

26. A. Pleitner, Y. Zhai, R. Winter, L. Ruan, L. M. Mcmullen, and M. G. Gänzle, "Compatible Solutes Contribute to Heat Resistance and Ribosome Stability in Escherichia Coli AW1.7," *Biochimica et Biophysica Acta* 1824 (2012): 1351–1357, https://doi.org/10.1016/j.bbapap.2012.07.007.

27. J. D. Adams, K. B. Sander, C. S. Criddle, A. P. Arkin, and D. S. Clark, "Engineering Osmolysis Susceptibility in Cupriavidus Necator and Escherichia Coli for Recovery of Intracellular Products," *Microbial Cell Factories* 22, no. 1 (2023): 1–16, https://doi.org/10.1186/S12934-023-02064-8/FIGURES/5.

28. B. Kempf and E. Bremer, "Uptake and Synthesis of Compatible Solutes as Microbial Stress Responses to High-Osmolality Environments," *Archives of Microbiology* 170, no. 5 (1998): 319–330, https://doi.org/10.1007/S002030050649.

29. P. König, B. Averhoff, and V. Müller, "A First Response to Osmostress in Acinetobacter Baumannii: Transient Accumulation of K+ and Its Replacement by Compatible Solutes," *Environmental Microbiology Reports* 12, no. 4 (2020): 419–423, https://doi.org/10.1111/1758-2229.12857.

30. C. H. Calvey, et al., "Improving Growth of Cupriavidus Necator H16 on Formate Using Adaptive Laboratory Evolution-Informed Engineering," *Metabolic Engineering* 75 (2023): 78–90, https://doi.org/10.1016/J.YMBEN.2022.10.016.

31. T. Hirasawa and T. Maeda, "Adaptive Laboratory Evolution of Microorganisms: Methodology and Application for Bioproduction," *Microorganisms* 11, no. 1 (2023): 92, https://doi.org/10.3390/MICROORGANISMS11010092.

32. G. Bertani, "STUDIES ON LYSOGENESIS I," *Journal of Bacteriology* 62, no. 3 (1951): 293–300, https://doi.org/10.1128/JB.62.3.293-300.1951.

33. S. Sai Tak Chu, et al., "As Featured in: Showcasing the Study on Electrochemical Activation of Carbon-Based Cathodes for Hydrogen Evolution Reaction by the Group of Dr Title: Insight into the Electrochemical Activation of Carbon-Based Cathodes for Hydrogen Evolution Reaction Insight into the Electrochemical Activation of Carbon-Based Cathodes for Hydrogen Evolution Reaction †," *Journal of Materials Chemistry A* 3 (2015): 13080-13086, https://doi.org/10.1039/c5ta02551f.

34. D. W. Lima, F. Fiegenbaum, F. Trombetta, M. O. de Souza, and E. M. A. Martini, "Influence of Graphitic Materials Microstructure in the Hydrogen Evolution in Aqueous Solution of Tetra-Alkylammonium-Sulfonic Acid Ionic Liquid," *International Journal of*

Hydrogen Energy 43, no. 3 (2018): 1239–1250, https://doi.org/10.1016/J.IJHYDENE.2017.11.097.

35. G. Schulze and J. M. Prausnitz, "Solubilities of Gases in Water at High Temperatures," *Industrial and Engineering Chemistry Fundamentals* 20, no. 2 (1981): 175–177, https://doi.org/10.1021/I100002A013/ASSET/I100002A013.FP.PNG_V03.

36. T. Umecky, M. Kanakubo, T. Makino, T. Aizawa, and A. Suzuki, "Effect of CO2 Dissolution on Electrical Conductivity and Self-Diffusion Coefficients of 1-Butyl-3-Methylimidazolium Hexafluorophosphate Ionic Liquid," *Fluid Phase Equilibria* 357 (2013): 76–79, https://doi.org/10.1016/J.FLUID.2013.05.023.

37. S. Grunwald, et al., "*Kinetic and Stoichiometric Characterization of Organoautotrophic Growth of Ralstonia Eutropha on Formic Acid in Fed-Batch and Continuous Cultures*,“ 8 (2014): 155–163, https://doi.org/10.1111/1751-7915.12149.

38. T. Haas, R. Krause, R. Weber, M. Demler, and G. Schmid, "Technical Photosynthesis Involving CO2 Electrolysis and Fermentation," *Nature Catalysis* 1, no. 1 (2018): 32–39, https://doi.org/10.1038/s41929-017-0005-1.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Figure S1:** Overview of Na_2SO_4 and NaCl-adapted strains compared to the Wildtype H16 ΔPHB cultivated in MM with 0 – 300 mM Na_2SO_4. The wildtype (light green) was compared to adapted variants from timestamp 1 (green), 3 (turquoise), and 5 (purple) in minimal medium containing (from left to right) 0 – 300 mM Na_2SO_4; the first row shows the Na_2SO_4-adapted variants, the second row the NaCl-adapted variants. The cultivation was performed in baffled shake flasks at 180 rpm and 30°C. **Supporting Figure S2:** Growth rate overview of Na_2SO_4 and NaCl-adapted *C. necator* H16 ΔPHB strains compared to the Wildtype cultivated in MM with 0 – 300 mM Na_2SO_4**.** The wildtype (light green) was compared to adapted variants from timestamp 1 (green), 3 (turquoise), and 5 (purple) in minimal medium containing (from left to right) 0 – 300 mM Na_2SO_4; the first row shows the Na_2SO_4-adapted variants, the second row the NaCl-adapted variants. The cultivation was performed in baffled shake flasks at 180 rpm and 30°C. **Supporting Figure S3:** Growth (black squares), pH (line), and potential (black triangles) of *C. necator* H16 in MES in autotrophic minimal medium ($n = 4$), 30°C, 300 rpm, a constant current of 15 mA, and a gas mixture of 8% N_2, 10% CO_2, 6% O_2 (gassing rate 25ml/min). An OD_{600max} of 3.2 is reached after 7 days with a pH drop from 6.7 to 6 and a potential of 2.9 V. **Supporting Figure S4:** Growth (white squares), pH (line), and potential (white triangles) of Na_2SO_4-adapted *C. necator* H16 in MES in autotrophic minimal medium with 250 mM Na_2SO_4 ($n = 3$), 30°C, 300 rpm, a constant current of 15 mA and a gas mixture of 84% N_2, 10% CO_2, 6% O_2 (gassing rate 25ml/min). An OD_{600max}.of 3.2 is reached after 7 days with a pH drop from 6.7 to 6 and a potential of 2.9 V. **Supporting Figure S5:** Conductivity of minimal medium according to Sydow et al. [22] with rising amounts of Na_2SO_4 in 20 and 30°C. Conductivity was measured with an EC meter by emerging the sensor in medium with 20°C room temperature and after incubation of media in closed falcon tubes over night at 30°C, $n = 3$.