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Production of polyhydroxybutyrate from industrial flue gas by microbial electrosynthesis

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ABSTRACT

The aim of this work was to produce the biopolymer polyhydroxybutyrate (PHB) from industrial flue gas as CO_2 source and electrolysis originating hydrogen via microbial electrosynthesis. Besides the laboratory experiments, the experiments were carried out under industrial conditions directly on-site in a cogeneration plant. We were able to demonstrate that the use of flue gas as a CO_2 source has no detectable negative effect on bacterial growth and PHB production in comparison to a pure gas mixture. In an electrochemical H-cell 333 ± 44 mg L^{-1} PHB were obtained, which corresponds to a PHB content of 43 ± 3 % of the cell dry weight. By using flue gas for the production of the biopolymer PHB, not only CO_2 emissions are reduced, but also possible environmental pollution from non-biodegradable plastics. To the best of our knowledge, this is the first time that the production of PHB from flue gas has been demonstrated using *Cupriavidus necator*.

1. Introduction

Reducing anthropogenic CO_2 emissions to combat climate change is one of the greatest challenges of our time. By recycling CO_2 from exhaust gases as a resource for the industry, these emissions can be lowered. At the same time, this can reduce the use of fossil resources. For example, CO_2 can be a resource for the microbial production of the biodegradable polymer polyhydroxybutyrate (PHB). This not only allows us to move away from petroleum-based polymers but also to make the disposal of polymers more sustainable. Biopolymers are becoming increasingly important as a biodegradable alternative due to the growing environmental impact of plastics [1]. Therefore, the electro-biotechnological conversion of CO_2 could help to achieve the UN Sustainable Development Goals, in particular SDG 13 “Climate Action” [2]. The production of PHB as a biodegradable plastic alternative also contributes to SDGs 12 "Responsible Consumption and Production", 14 "Life below Water", and 15 "Life on Land".

PHB belongs to the short-chain length polyhydroxyalkanoates [3]. Due to their biodegradability and biocompatibility, polyhydroxyalkanoates are a promising alternative to conventional polymers [3]. Unfortunately, PHB is not yet cost-competitive with conventional polymers. The use of waste materials can help to make PHB production less expensive and more sustainable. Various waste materials have already been used for the microbial production of PHB [4]. Recently, a review article has been published outlining the state of the art in microbial PHB production from CO_2 [5]. Polyhydroxyalkanoates can be produced by a range of bacteria including *Cupriavidus*, *Alcaligenes*, *Pseudomonas*, recombinant *E. coli,* and methylotrophs [6]. *Cupriavidus necator* is considered a promising aerobic chemoautotrophic organism for third-generation biorefineries due to its ability to store CO_2 in the form of polyhydroxybutyrate [7]. Under lack of essential macroelements such as nitrogen as well as an excess of a carbon source, *C. necator* produces PHB as an energy reserve [8]. The organism is known for its broad substrate spectrum. Amongst others, PHB production has already been demonstrated on various carbon sources such as acetic acid [9], lactic acid [10], oleic acid [11], glycerol [12], or formate [13,14]. In terms of circular economy, *C. necator* is also capable of producing PHB from different carbon-containing waste

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streams [15]. Sequencing of the genome of *C. necator* H16 enables genetic engineering to further exploit the potential of the organism [16] and as a result the production of other higher-value products such as terpenes [17–19] or fuels and solvents [20] from CO_2 in perspective. *C. necator* is able to grow autotrophically with CO_2 as carbon source, hydrogen as electron donor, and oxygen as terminal electron acceptor. Hydrogen and oxygen can be produced *in situ* using water electrolysis. For microbial CO_2 fixation, H_2 is a promising electron donor because it can be produced electrochemically efficiently and has a low reduction potential [21]. The electrochemical process of water electrolysis and microbial production can be combined in microbial electrosynthesis (MES). When CO_2 is used from an exhaust stream and hydrogen and oxygen are produced by water electrolysis using renewable energy (e.g. photovoltaics), an overall "green" process can be created. While PHB production with *C. necator* under autotrophic conditions has been extensively studied, research on electroautotrophic cultivation, especially with a real exhaust CO_2 source, is still lacking. Harmful substances such as nitrogen oxides or sulfur oxides are often present in these exhaust gas streams and might affect the process. Garcia-Gonzalez and De Wever investigated the impact of real off-gases on autotrophic PHB production with *C. necator* [22]. They observed that impurities of real off-gases did not affect the bacterial performance or the properties of the produced PHB in comparison to pure CO_2. Wu et al. demonstrated the microbial electrosynthesis of lycopene from flue gas using a genetically modified *C. necator* strain [23]. Previously, we have shown microbial PHB production based on CO_2 by *C. necator* using drop-in electrolysis [13,14]. In this process, CO_2 was electrochemically reduced to formate, which served in a second step as the sole substrate for *C. necator*. However, for the direct electrochemical conversion of flue gasses, a gas purification is usually required since components other than CO_2 (such as O_2) lead to significant decrease of CO_2 electrolysis. In contrast, the direct microbial utilization of CO_2 in flue gas is a rather robust approach, as microbial growth and synthesis are generally less sensitive towards impurities or toxic substances in the flue gas. Especially for the application of aerobic microorganisms, the oxygen in the flue gas stream can even be beneficial.

The objective of this study was to produce PHB using a bioelectrochemical process with *C. necator* based on CO_2 from a coal-fired cogeneration plant. Data from the industrial plant experiments are compared with analogous investigations from laboratory experiments.

2. Materials and methods

2.1. Precultures for microbial electrosynthesis

A seed train of three precultures was used to inoculate the microbial electrosynthesis. From a cryo-culture of *Cupriavidus necator* H16 (DSM-428, DSMZ, Braunschweig, Germany), a preculture was inoculated in LB medium. The LB medium consisted of 5 g L^{-1} NaCl, 5 g L^{-1} yeast extract, and 10 g L^{-1} trypticase peptone and had a pH value of 7.1. This was followed by heterotrophic and autotrophic preculture in the minimal medium used in the bioelectrochemical system. The minimal medium for the cultivation of *C. necator* in bioelectrochemical systems was developed in a previous work [24]. It contains 2.895 g L^{-1} Na_2HPO_4, 2.707 g L^{-1} NaH_2PO_4*H_2O, 0.17 g L^{-1} K_2SO_4, 0.097 g L^{-1} $CaSO_4$*$2H_2O$, 0.8 g L^{-1} $MgSO_4$*$7H_2O$, and 0.934 g L^{-1} $(NH_4)_2SO_4$ as well as trace elements: 750 µg L^{-1} $FeSO_4$*$7H_2O$, 120 µg L^{-1} $MnSO_4$*H_2O, 120 µg L^{-1} $ZnSO_4$*$7H_2O$, 24 µg L^{-1} $CuSO_4$*$5H_2O$, 90 µg L^{-1} Na_2MoO_4*$2H_2O$, 75 µg L^{-1} $NiSO_4$*$6H_2O$, and 2 µg L^{-1} $CoSO_4$*$7H_2O$. The pH value of the minimal medium was 6.6. For heterotrophic preculture, 4 g L^{-1} fructose was added to the medium. For autotrophic preculture, a gas atmosphere of $H_2/CO_2/O_2$ (64:16:20) at 1.5 bar was applied. Heterotrophic and autotrophic preculture in the minimal medium were inoculated from the previous culture in late exponential or early stationary phase to an optical density (OD_{600}) of 0.1. All precultures were incubated at 30 °C and 180 rpm.

2.2. Microbial electrosynthesis with C. necator

Microbial electrosynthesis was performed in an undivided electrochemical reactor with a 3-electrode setup. Platinized titanium expanded metal electrodes (50 g Pt per m^2, Metakem GmbH, Usingen, Germany) with a geometric area of 22.5 cm^2 (45 mm×25 mm) each were used as working and counter electrodes. The working electrode was contacted with a platinum wire with a diameter of 0.5 mm and the counter electrode with a stainless steel wire with a diameter of 0.5 mm. The working and counter electrodes were held at a distance of 2 mm and were separated from each other using a PTFE mesh. A photograph of the electrodes is provided in Figure S1. An Ag/AgCl electrode (Xylem Analytics, Weilheim, Germany) was used as the reference electrode, which was introduced into the cells through a Haber-Luggin capillary filled with saturated KCl solution. The reactors were autoclaved and filled to a total volume of 110 mL with the minimal medium described in the previous chapter. A constant current of −15 mA (-0.67 mA cm^{-2}) was applied for water electrolysis via a potentiostat (Interface 1010B, Gamry Instruments, Warminster, PA, USA). The MES took place in an incubation hood (CERTOMAT® HK, Sartorius, Göttingen, Germany) at 30 °C. A stir bar was used for mixing at 300 rpm. In the laboratory experiments, a pure gas mixture consisting of 85 % N_2, 10 % CO_2, and 5 % O_2 (Nippon Gases Deutschland, Düsseldorf, Germany) was continuously supplied via a cannula at a gas flow rate of 25 mL min^{-1}. In addition, a laboratory experiment was carried out with 100 % CO_2 and a gas flow rate of 2.5 mL min^{-1}. In the experiments with flue gas from the cogeneration plant, the gas flow rate was also 25 mL min^{-1} to keep the supplied volume of CO_2 approximately identical. The gas supply was passed through a sterile filter. The flue gas was continuously withdrawn after flue gas desulfurization from a sampling line intended for exhaust gas analysis in the cogeneration plant using an internal pump and fed directly into the reactors. Besides nitrogen, the flue gas consisted mainly of oxygen (7 – 11 %) and carbon dioxide (approx. 10 %). It also contained traces of NO (39 – 302 mg m^{-3}), SO_2 (3 – 279 mg m^{-3}), and CO (2 – 32 mg m^{-3}) The gas concentration data are given under standard conditions and were provided by Mainova AG. Figure S2 shows photos of the experimental setup in the cogeneration plant of Mainova AG. The cogeneration plant is located in Frankfurt am Main, Germany. It is operated with hard coal or natural gas, whereby the experiments have only taken place on the units for hard coal. Annually, about 350,000 tons of hard coal are consumed in the cogeneration plant, which together with the combustion of natural gas leads to CO_2 emissions of the cogeneration plant of about 800,000 tons (for the year 2022) [25].

MES was inoculated from the autotrophic preculture in late exponential or early stationary phase to an OD_{600} of 0.2. The process is divided into a microbial growth phase (first phase) and a PHB formation phase (second phase). The growth phase each lasted 4 days in the laboratory experiments and in the cogeneration plant experiment, whereby an additional experiment with a growth phase of 5 days was carried out in the cogeneration plant. In the second phase, the production of PHB is initiated by switching to an ammonium-free medium. For this purpose, the entire reaction volume was centrifuged at 3000xg for 30 min and the pellet was resuspended in 110 mL of ammonium-free minimal medium. For the determination of OD_{600} and pH value, 1 mL of sample was taken daily. The terminal voltage was also measured daily with a digital multimeter (VC165 VOLTCRAFT®, Conrad Electronic SE, Hirschau, Germany). All experiments were performed in triplicates.

2.3. Determination of cell dry weight and polyhydroxybutyrate concentration

For the determination of the cell dry weight and the PHB concentration, 5 mL of sample were taken daily and stored at −20 °C until analysis. The 5 mL sample was first centrifuged at 3000xg for 15 min. The pellet was then resuspended in 1 mL ddH_2O and centrifuged again in a smaller reaction tube at 16000xg for 5 min. The supernatant was

discarded, and the cell pellet was dried at 100 °C for 24 h. The difference in weight of the reaction vessels with and without samples was determined. After determination of the cell dry weight, 1 mL of 96 % sulfuric acid was added to the dried cell pellet. The mixture was incubated at 99 °C and 500 rpm for one hour to convert the PHB to crotonic acid. After conversion, samples were diluted 1:50 with ddH_2O and filtered (0.22 μm). The crotonic acid was quantified by HPLC with an Aminex HPX-87 H (Bio-Rad Laboratories, Hercules, CA, USA). The mobile phase was 5 mM H_2SO_4 with a flow rate of 0.6 mL min^{-1}. The column was heated to 60 °C and detection was performed at 210 nm. For calibration, standards from commercial PHB (Sigma-Aldrich, St. Louis, MO, USA) were prepared and quantified in the same way. This resulted in the following calibration curve: PHB [mg] = $4.0707*10^{-6}$ * $Area_{crotonic\ acid}$ [mAU].

3. Results and discussion

To evaluate the performance of microbial electrosynthesis in an industrial environment with flue gas as the CO_2 source, the identical experiment was first performed in the laboratory under controlled and optimized conditions (e.g. by using an artificial gas mixture with a constant composition of 85 % N_2, 10 % CO_2, and 5 % O_2). As described above, MES was divided into a microbial growth phase and a PHB formation phase. The course of the OD_{600} as well as the PHB concentration in the laboratory experiment is shown in Fig. 1a. The OD_{600} increases continuously from 0.19 ± 0.00–1.6 ± 0.1 by the end of the growth phase on day four. After the medium change to ammonium-free medium, the OD_{600} decreases to 1.1 ± 0.1 due to the dilution of the cell pellet with the initial medium volume (volume decrease during the experiment by sampling) and increases again up to an average maximum OD_{600} of 2.7 ± 0.5 after 240 h. During the growth phase, only small amounts of PHB of <10 mg L^{-1} are detectable. In the second phase, the PHB concentration follows the OD_{600}, suggesting that the increase in OD_{600} is mainly due to PHB production and not cell multiplication. It was previously shown that the actual cell mass without PHB, expressed as residual cell concentration, remains more or less constant in the PHB production phase [26]. PHB concentration increases from 2.7 ± 0.2 mg L^{-1} to a value as high as 347 ± 65 mg L^{-1} on average after six days following medium exchange. This corresponds to an PHB content of 59 ± 1 % of cell dry weight. The PHB concentration then decreases in proportion to the OD_{600}. In addition, we carried out a laboratory experiment with 100 % CO_2 at a gassing rate of 2.5 mL min^{-1} (Figure S3). The experiment showed that without additional gassing with O_2, PHB production already sets in during the growth phase due to oxygen limitation. As a result, a lower maximum PHB concentration of 275 ± 81 mg L^{-1} is achieved on average at a maximum OD_{600} of 2.2 ± 0.4.

In the literature, PHB concentrations of 23.9 g L^{-1} [27] and 61.9 g L^{-1} [28] could be achieved in autotrophic processes. In heterotrophic fed-batch processes, up to 121 g L^{-1} [29] and 125 g L^{-1} [30] of PHB could be obtained. The highest content of PHB in cell mass was reported by Tanaka and Ishizaki with a PHB content of 82.1 % [27], although it is usually lower. The PHB concentrations achieved with the electroautotrophic setup are far below the presented values, as it only serves as a proof of concept and still has plenty of room for optimization (e.g., electrode-to-volume ratio, residence time of the gasses in the reactor, gas pressure, and process mode).

After the successful microbial electrosynthesis in the laboratory, experiments with the same setup were performed in a cogeneration plant with flue gas as CO_2 source. Fig. 1b shows the course of OD_{600} and PHB concentration in the cogeneration plant experiment. The additional experiment with a growth phase of 5 days can be found in the supplementary materials (Figure S4), which will also be discussed below. In the presented results, the OD_{600} increases from 0.19 ± 0.02–2.0 ± 0.2 during the growth phase in the first 4 days. Thus, we achieved an even faster growth than in the laboratory experiment with the final OD_{600} being 0.4 higher. It should be noted that there is no evidence of growth inhibition due to contaminants in the flue gas. In the experiment with a growth phase of 5 days, an OD_{600} value of 1.7 ± 0.0 was achieved at the end of the growth phase (Figure S4a). Therefore, the extension of the growth phase from 4 to 5 days did not lead to an increase in microbial growth. As in the laboratory experiments, dilution occurs due to the medium exchange. As a result, the OD_{600} in the shown experiment drops to 1.2 ± 0.1 before rising to 3.3 ± 0.4 in the PHB formation phase after another 7 days and showing evidence of reaching a plateau. No PHB formation is detectable during the growth phase of both experiments at the cogeneration plant. After switching to ammonium-free medium, PHB is produced continuously. Over the 7 days, a maximum of 333 ± 44 mg L^{-1} PHB was formed on average in the shown experiment, which corresponds to a PHB content of 43 ± 3 % of the cell dry weight. Therefore, the PHB concentration is in a comparable range to the laboratory experiment employing the artificial gas mixture since the standard deviation of both experiments is considerably higher than the difference in the PHB concentration. In the experiment with a growth phase of 5 days, only a maximum PHB concentration of 265 ± 46 mg L^{-1} was formed with a PHB content of 51 ± 4 % of the cell dry

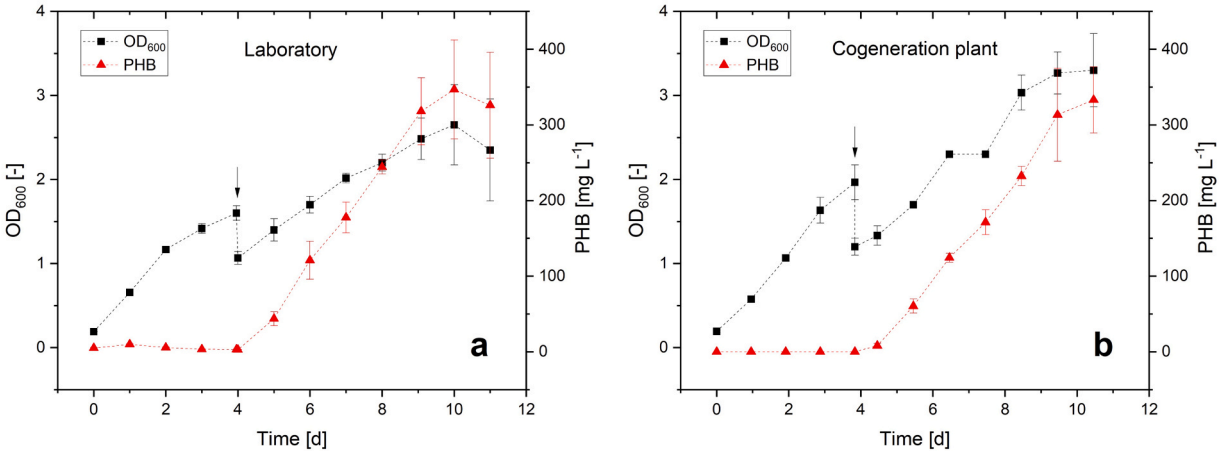

Fig. 1. Optical density and PHB concentration of the MES in the laboratory with a pure gas mixture consisting of 85 % N_2, 10 % CO_2, and 5 % O_2 (a) and the experiment in the cogeneration plant with flue gas (b) (n=3). Reaction parameters: 30 °C, mixing at 300 rpm, minimal medium with an initial volume of 110 mL, gassing rate of 25 mL min^{-1}, constant current density of 0.67 mA cm^{-2} for electrolysis, platinized titanium expanded metal electrodes as working and counter electrodes, Ag/AgCl reference electrode. The arrow indicates the change to ammonium-free medium after 4 days and the start of the PHB production phase. Dashed lines serve as a guide to the eye.

weight

In contrast to the setup of Wu et al. who fed gas into a reactor every 24 h for 30 min [23], we continuously supplied both the pure gas mixture and the flue gas into the bioelectrochemical reactor. Nevertheless, Wu et al. also found no influence of the use of flue gas as a CO_2 source on the growth of *C. necator*. The composition of the flue gas was comparable to the flue gas used in this work and thus, it can be concluded that even continuous exposure to the flue gas does not result in growth inhibition. A similar example with different microorganisms was also shown by Rovira-Alsina et al., in which they demonstrated that the use of real exhaust gas had no relevant effect on the bioelectrochemical conversion of CO_2 to acetate with a microbial community from an anaerobic digester [31].

Besides the OD_{600} and the PHB concentration, the course of pH value as well as the resulting electrode potential *vs.* Ag/AgCl of the laboratory experiment with the pure gas mixture and the experiment in the cogeneration plant with flue gas were monitored. The respective data are presented in Fig. 2. In the laboratory experiment, the pH value slightly decreases from 6.61 ± 0.02–6.33 ± 0.03 during the growth phase and remains relatively constant during the PHB formation phase. The laboratory experiment with 100 % CO_2 (Figure S3b) shows a similar course of the pH value. Accordingly, the pH value cannot be a limiting factor in the laboratory experiments. The pH value of the experiment with flue gas decreases continuously until the end of the growth phase from 6.70 ± 0.01 to about 5.90 ± 0.09. After the medium change, the pH value starts at about 6.77 ± 0.05 and decreases again continuously. After approximately 179 h the pH value drops considerably faster to a pH value of 3.39 ± 0.28 after 251 h. Apparently, a component of the flue gas is responsible for the drop in pH value either directly, through electrochemical conversion, or the metabolism of the microorganism. A similar course of the pH value can also be observed in the other experiment with flue gas (Figure S4b). Thus, regulating the pH value appears to be an important aspect in the further optimization of the process when using flue gas. In contrast to the pH value, the resulting courses of the electrode potentials of the laboratory and the industrial experiments are described by rather similar patterns. On average, the potential in the laboratory experiment is around −1.0 to −1.1 V *vs.* Ag/AgCl, which is slightly lower than the potential in the cogeneration plant at around −0.8 to −1.0 V *vs.* Ag/AgCl, respectively. However, the potential of the laboratory experiment with 100 % CO_2 (Figure S3b) lies in a similar range as the experiments with flue gas.

Based on the average terminal voltage (Figure S5) and the applied current of −15 mA, the consumed electrical energy can be calculated for the experiments (Equation S1). The period up to the maximum PHB concentration is taken into account, thus up to 240 h in the laboratory and up to 251 h in the cogeneration plant. This amounts to an electrical energy input of 9.67 ± 0.25 Wh for the laboratory experiment and 9.35 ± 0.24 Wh for the experiment in the cogeneration plant. Based on these results, the electrical energy required to produce 1 kg of PHB in the laboratory and the cogeneration plant is 410 ± 97 and 444 ± 67 kWh, respectively (Equation S2). Assuming an electricity price of 0.182 € per kilowatt hour in industry [32], the electricity costs amount to around 75 ± 18 or 81 ± 12 € per kg of PHB (Equation S3). Although the values between the laboratory and the cogeneration plant hardly differ, at this stage the energy costs for PHB production alone are far too high for possible commercialization.

The use of CO_2 as a carbon source in combination with electrochemically produced H_2 is considered to have a high technology readiness level since electrochemical hydrogen production is well advanced [33]. The bottleneck now lies in microbial conversion, which can probably be significantly improved above all by genetic engineering of suitable autotrophic microorganisms. Kim et al. were able to increase biomass accumulation by 11 % and PHB production by 28 % through genetic modifications [34]. In addition to the modification of the biocatalyst, the parameters on the process side are also crucial. The low gas solubility and the resulting limitation of gas transfer restrict microbial growth and PHB production in autotrophic cultivation. It has been reported that oxygen transfer can limit PHB accumulation at high cell mass concentrations [26]. Gas mass transfer can be enhanced through higher operation pressure, which can increase productivity [35]. In our case, the experiments were performed at ambient pressure. Designing a suitable bioreactor, for example, an airlift reactor or a bubble column can improve limitations such as gas transfer [36]. Furthermore, our results have also shown that the continuous use of flue gas can lead to a drop in the pH value. This phenomenon can be avoided by using an appropriate bioreactor with pH control. Concerning the gaseous substrates, both the ratio of the individual gases and the gas flow can be optimized to ensure optimal utilization of gases. However, one challenge in aerobic cultivation using hydrogen as an electron donor is the existing risk of explosion. Lambauer and Kratzer have shown how to design a laboratory-scale explosion-proof reactor for PHB production from CO_2 using *C. necator* [37]. It has also been suggested that the growth phase

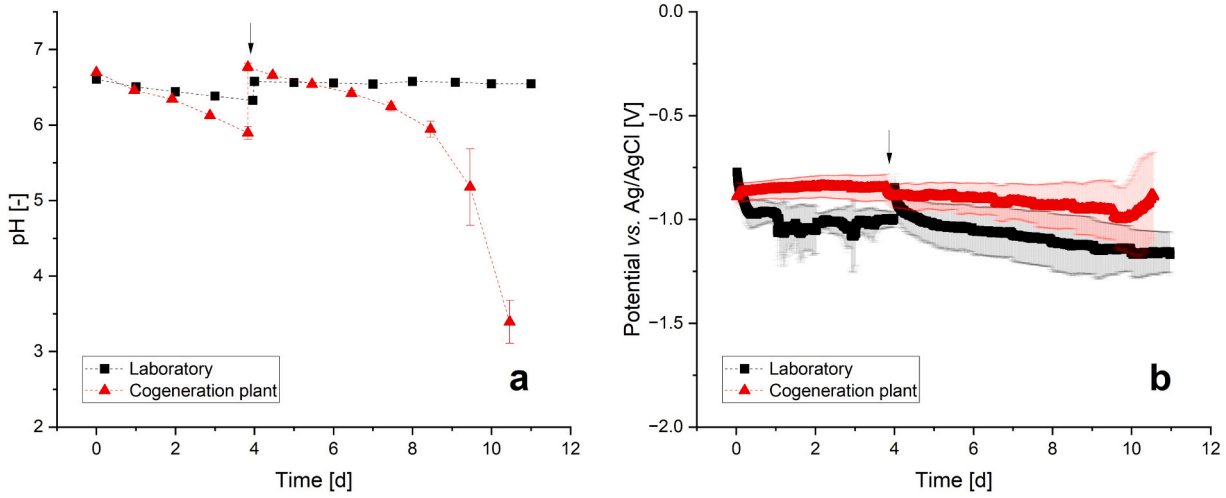

Fig. 2. Course of pH value (a) and potential *vs.* Ag/AgCl (b) of the MES in the laboratory with a pure gas mixture consisting of 85 % N_2, 10 % CO_2, and 5 % O_2 and the experiment in the cogeneration plant with flue gas (n=3). Reaction parameters: 30 °C, mixing at 300 rpm, minimal medium with an initial volume of 110 mL, gassing rate of 25 mL min^{-1}, constant current density of 0.67 mA cm^{-2} for electrolysis, platinized titanium expanded metal electrodes as working and counter electrodes, Ag/AgCl reference electrode. The arrow indicates the change to ammonium-free medium after 4 days and the start of the PHB production phase. Dashed lines serve as a guide to the eye.

should first be carried out heterotrophically with, for example, fructose as substrate and for PHB production to switch to autotrophic cultivation [27,38]. To make such a process more sustainable, substrates from waste materials can be used in the growth phase as we have shown with a growth medium from grass clippings for the cultivation of *C. necator* [39]. A heterotrophic growth phase may result in higher cell mass in less time and thus presumably generate more PHB from CO_2. However, it has also been reported that high cell dry weight at the beginning of the second phase can negatively affect PHB productivity [22]. The efficiency of water electrolysis can be further increased by reducing the internal resistance, for example through an optimized electrode setup or increased conductivity of the medium. A key parameter is to match the rate of hydrogen generation and hydrogen consumption by the microorganisms. In the future, an enhanced gas analysis of the gas outlet could be used to determine how much hydrogen is left to optimize the efficiency of water electrolysis. This could also provide information on CO_2 consumption. In addition, it is likely to be advantageous to separate water electrolysis and fermentation spatially in order to make both processes individually more efficient and thus achieve higher productivity. The combination of both processes requires a compromise between electrolyte and growth medium. A similar approach is the electrochemical reduction of CO_2 to one-carbon compounds such as carbon monoxide, formic acid, or methanol, which can be used subsequently as a simultaneous carbon source and electron donor for microorganisms [33]. However, direct utilization of CO_2 by microorganisms and *in-situ* water electrolysis can bypass this additional step.

4. Conclusions

We successfully realized the microbial electrosynthesis of PHB with *Cupriavidus necator* by using real flue gas directly on-site in a cogeneration plant. In addition, no detectable inhibitory effects on bacterial growth or PHB production were observed compared to the same experiments carried out in the laboratory with a pure gas mixture. However, the results presented here are only a proof of concept that still leaves room for optimization. Limitations lie primarily in microbial conversion, which can be addressed by genetically modifying the biocatalyst and optimizing the process regarding gas transfer. Further consideration should also be given to practical implementation on a larger scale in the industry. To realize such a concept in the future, a corresponding bioreactor with all necessary infrastructure would have to be implemented in power plants. A detailed analysis of the CO2 reduction effects and the associated costs must also be carried out. Nevertheless, the results show a promising possibility of using exhaust gas CO_2 as an alternative resource and reducing emissions at the same time.

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CRediT authorship contribution statement

Julian Philipp Schütz: Writing – review & editing, Validation, Methodology, Investigation. **Alexander Langsdorf:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Dirk Holtmann:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Markus Stöckl:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Roland Ulber:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jcou.2024.102800.

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