

Scalable Unseparated Bioelectrochemical Reactors by Using a Carbon Fiber Brush as Stirrer and Working Electrode

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The concept of energy conversion into platform chemicals using bioelectrochemical systems (BES) has gained increasing attention in recent years, as the technology simultaneously provides an opportunity for sustainable chemical production and tackles the challenge of Power-to-X technologies. There are many approaches to realize the industrial scale of BES. One concept is to equip standard bioreactors with static electrodes. However, large installations resulted in a negative influence on various reactor parameters. In this study, we present a new

single-chamber BES based on a stirred tank reactor in which the stirrer was replaced by a carbon fiber brush, performing the functions of the working electrode and the stirrer. The reactor is characterized in abiotic studies and electro-fermentations with *Clostridium acetobutylicum*. Compared to standard reactors an increase in butanol production of $20.14 \pm 3.66\%$ shows that the new BES can be efficiently used for bioelectrochemical processes.

Introduction

Since fossil resources for fuel and platform chemicals will become limited in the near future, it is important to develop new concepts to reduce our dependence on the petrochemical industry. One promising alternative could be the electricity-driven bioproduction in bioelectrochemical systems (BES). On the one hand, these systems enable the generation of electricity through fermentative processes and on the other hand, metabolic pathways can be influenced by applying a potential during fermentation. In BES, electroactive microorganisms or enzymes acts as biocatalyst.^[1] The cell metabolism can benefit from the applied voltage or current by using various electron transfer mechanisms. This ability leads to a potential increase in product yields, but also offers the possibility of converting

electricity into platform chemicals (Power-to-X), which is another step towards future bioeconomy.^[2]


In general, BES with whole-cell catalysts are used for three different fields of application. They are used for electricity generation in microbial fuel cells, for the production of methane or hydrogen in microbial electrolysis cells and for the production of higher value products such as butanol,^[3] succinate,^[4] terpenes^[5] and bioplastics^[6] in electrochemical driven or influenced microbial electrosynthesis (MES).^[7–9] The term MES is either understood as the entirety of microbially catalyzed electrochemical reactions for the conversion of substances into a desired product, whereby anodic and cathodic processes are equally included, or it is divided into the terms MES and electro-fermentation.^[10] In this context, the term MES covers all microbially catalyzed electrochemical reactions based on the reduction of CO₂ with electrons as the driving force. Instead, the term electro-fermentation is used for self-driven fermentations on organic carbon compounds, in which electrons are used to influence the metabolic pathway by influencing the intercellular oxidation-reduction potential. The electrons are therefore not the driving force of the reaction, but a trigger that influences the metabolism.^[11] For all three fields of application, the main reactor system used in research is a two-chamber glass reactor system (H-cell reactor).^[12] It is suitable for most processes thanks to the simple design and easy handling of electrode and membrane installation. The H-cell reactor has also several disadvantages, such as high internal electrical resistance due to the barely mixed zones around the membrane, wide distances between the electrodes and no possibility of scaling up to a pilot or industrial scale.^[13]


Some of the disadvantages of H-cells are caused or amplified by the built-in membrane. Nevertheless, various separated and unseparated reactor systems for electro-biotechnological applications are described in literature. These, in turn, differ in various parameters such as the choice of physical separation, the ratio of electrode surface area to volume, the

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electrode spacing and the mode of operation.^[9,14,15] The advantage of a separate BES is, that it allows the use of different electrolyte solutions for each half-cell and the separation of the anodic and cathodic reactions, preventing interactions between products or organisms with the counter electrode.^[9] However, it is known that the use of physical separation methods can generate concentration and pH gradients, as well as a high ohmic voltage drop between the electrodes.^[16,17] Furthermore, the separation membranes represent a high-cost factor, which would stand in the way of a potential scale-up.^[17] In contrast, in unseparated BES the oxidation of water and with it the formation of oxygen at the anode is a challenge in anaerobic processes.^[15]

One potential BES setup that has already been investigated is based on the integration of electrochemical installations into commercially available stirred tank bioreactors. These can potentially be upgraded to non-separated BES or to separated BES via a separate chamber with a counter electrode, a membrane window and a working electrode in the main chamber.^[18–20] This would have the great advantage that existing infrastructures and systems could be used, which would enable a more cost-effective upgrade. In addition, advantages can be drawn from the measurement and control units already very well integrated in bioreactors with regard to factors such as gassing, foam formation and pH regulation.^[20] Unfortunately, the large installations show a negative effect on the mixing time and fluid dynamics in the reactor.^[18]

The electrodes, which are usually statically integrated in bioelectrochemical reactors, account for the largest part of the installation and an additional stirrer is responsible for mixing the microbial culture. Only a few authors use rotating electrodes, although rotating ring-disk electrodes are widely used in electrochemical analysis and electrochemical reactors have already been equipped with passive mixer electrodes.^[21,22] In this context, rotating electrodes offer the possibility to enhance the mass transport of electroactive components in the electrolyte to the electrode.^[23] In BES, the use of rotating electrodes has been rare to date. Liao et al. used a rotating electrode incorporated into a tubular reactor to enhance mass transport from organic substrate to the electrode.^[24] Other studies used rotating electrodes to increase the gas input into the system.^[25,26] All these investigations were performed in the anodic compartment of a BES. To our best knowledge, rotating electrodes have not been used for cathodic processes in standard stirred bioreactors.

This study focuses on the application of a rotating carbon brush electrode as a working electrode (WE) and stirrer in a cathodic electro-fermentation. For this purpose, a new single-chamber BES was constructed, based on the standard stirred bioreactor. In the first step the BES was characterized abiotically. Parameters such as the electrode resistance, the influence on the pH measurement and electrochemical characteristics via cyclic voltammetry with a redox active compound (potassium hexacyanoferrate(III)) are performed. As biotic proof of concept, the reactor system was used for butanol production in an electro-fermentation with *Clostridium acetobutylicum* without the addition of a mediator.

Results and Discussion

Reactor concept and design

The reactor constructed in this study for application in electro-fermentation, should simultaneously allow the integration into stirred tank reactors and the use of large area electrodes to increase the electrochemical conversion. To achieve this goal, both the use of a carbon fiber electrode and the establishment of a rotating WE were envisaged. The latter has the advantage that a component could be spared by combining the stirrer with the WE, so that more space is available overall for the electrodes in the reactor chamber. The reactor was designed for a working volume of up to 1.5 liters and can thus be compared to the typical volume of commercially available benchtop bioreactors. The single-chamber system is made of borosilicate 3.3, has an inner diameter of 100 mm and a height of 265 mm. For temperature control a double jacket heating system was used. The temperature probe and other regularly used bioreactor components, such as the pH probe, sample pipe and gas supply were inserted via the connections in the lid (Figure 1A).

A three-electrode system is used for bioelectrochemical applications. The core piece of the new designed reactor is the WE, which has been specially designed for use in BES. It is a custom-made rotating carbon fiber brush with a titanium bridge, a diameter of 21 mm, a length of 110 mm and a surface

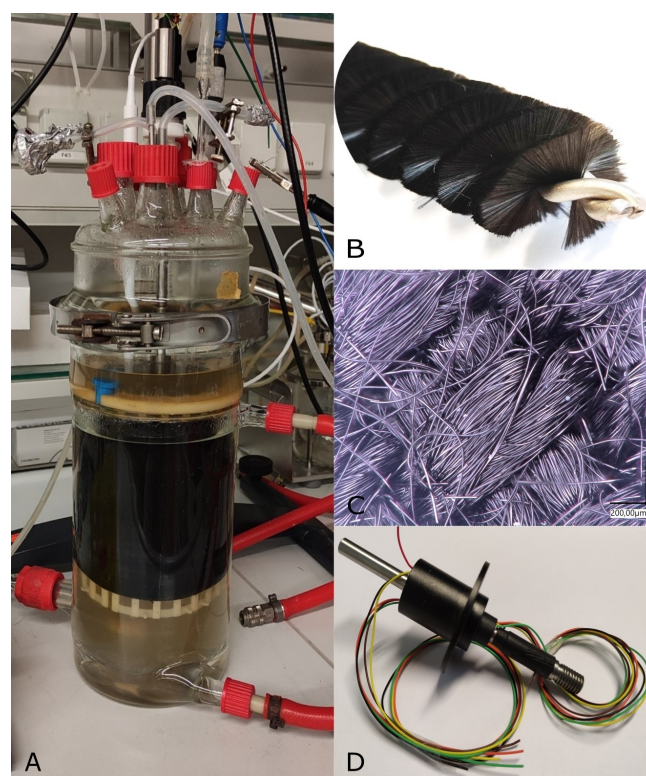


Figure 1. Pictures of the main reactor components with (A) the completely assembled reactor vessel (B) the installed carbon fiber brush as working electrode and stirrer, (C) a microscopic image of the counter electrode material and (D) showing the used slip ring.

area of approximately 90 cm² (Figure 1B). The carbon fiber electrode material was selected based on several promising results reported in literature. Among others, good performances have already been observed with organisms such as *Shewanella oneidensis*,^[27] *Sporomusa ovata*^[28] and *Methanococcus maripaludis*^[29] on carbon fiber or carbon cloth electrodes. For the simultaneous use of the carbon brush as a WE and as a stirrer, a slip ring was installed in the system to ensure contact with the potentiostat during the stirring motion. (Figure 1D, Figure S1).

As counter electrode (CE), a 28 x 13 cm carbon felt is inserted into the reactor (Figure 1C). For this purpose, autoclavable brackets made of polyamide 12 are used to place the CE in a cylindrical shape around the WE which results in a distance between CE and WE of 3.65 cm (Figure 2). Like a reactor wall,

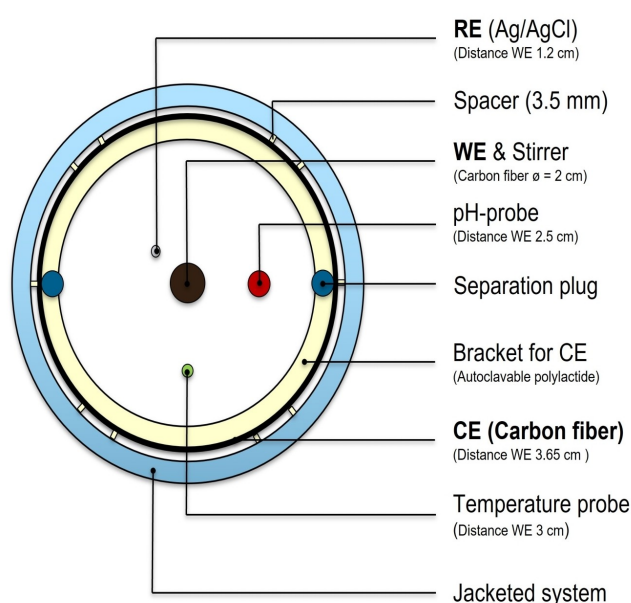


Figure 2. Sketch of a horizontal cross section of the reactor. Beside the single components of the reactor, the distances to the working electrode (WE) are shown. Abbreviations: counter electrode (CE), reference electrode (RE).

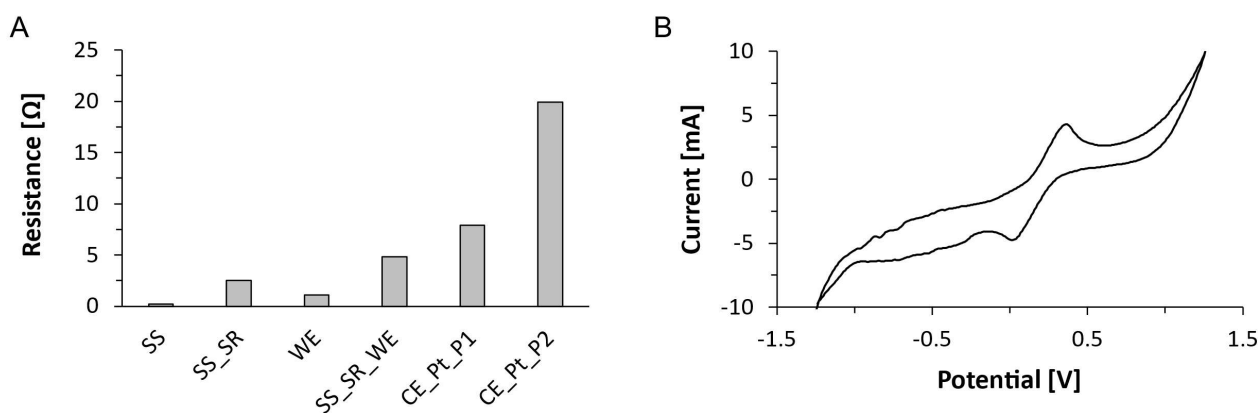


Figure 3. Abiotic electrochemical characterization of the reactor. (A) Resistances of the reactor components stirrer shaft (SS), slip ring (SR), working electrode (WE) and counter electrode (CE Pt), with two measuring positions for the counter electrode (P1/P2). (B) Cyclic voltammogram using 0.5 mM potassium hexacyanoferrate(III) in 0.1 mM potassium phosphate buffer against Ag/AgCl electrode with 3 M KCl electrolyte ($v = 0.005$ V s⁻¹).

the cylindrical installation should have a small effect on typical process parameters, such as the flow regime in the reactor, which could have been a problem in previous tested commercially available reactors with large electrochemical installations.^[18–20] The resulting geometric surface area of the CE is several times larger than the WE, which should avoid electrochemical limitations of the electro-fermentation process due to the CE.^[12] The CE itself is connected to a potentiostat via a platinum wire ($\phi = 0.4$ mm), which is inserted into the reactor through a septum on the lid. The three-electrode system is closed with an Ag/AgCl electrode with 3 M KCl electrolyte, which is used as a reference and is also inserted over the reactor lid. The distance to the WE is approx. 1.2 cm (Figure 2).

Bioelectrochemical reactor characterization

Prior to the bioelectrochemical experiments for proof of concept, an abiotic reactor characterization was performed. First, the electrical resistances in the system were measured to ensure that the self-constructed components of the WE and CE did not have too high resistances for the operation of a BES. The values illustrated in Figure 3A, show the resistances of the electrodes and their individual components. Thereby, the fully assembled WE, consisting of the stirrer shaft, the slip ring and the carbon fiber brush, shows a very low resistance of 4.81 Ω . This matches well with the values of the individual components, which show that the resistance is primarily caused by the built-in slip ring and not by the brush part of the electrode. The highest resistance, on the other hand, is introduced into the system by the CE. Position P1 measured from the beginning of the platinum wire to the bottom of the carbon felt at the point where the platinum wire was woven into the felt gives a resistance of 7.9 Ω . For P2, the measurement contact on the cylindrical CE was moved 180 degrees, giving the maximum measured value of up to 19.9 Ω . Comparing these values to values from other bioelectrochemical systems with carbon fiber electrodes, such as H-cells (approx. 165 Ω ,^[12] 30 Ω)^[30] or bubble

columns (approx. $140 \Omega^{[12]}$), the 4.81Ω of the WE measured in this study is clearly lower. In addition, the new reactor system was constructed without membrane, which should also lead to the reduction of the overall cell resistance.^[31,32] The stirrer shaft, the slip ring and the carbon brush electrode thus have a sufficient conductivity to carry out electro-fermentation with the rotating brush as WE.

Cyclic voltammetry (CV) measurements with potassium hexa-cyanoferrate(III) $[\text{Fe}(\text{CN})_6]^{3-}$ were performed for an electrochemical characterization of the reactor system. The result of a CV measurement with $0.5 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-}$ in a 0.1 M potassium phosphate buffer (PPB) is shown in Figure 3B. The voltammogram shows an oxidation peak at 359 mV and a reduction peak at 19 mV . The curve profile of the already very well-known electrochemically redox system shows similarities to other work carried out in potassium phosphate or potassium nitrate buffer systems.^[33,34] Calculating the formal potential from the two peaks yields a value of 170 mV for $E^{\circ}_{[\text{Fe}(\text{CN})_6]^{3-}}$. This value deviates slightly from the value of 225 mV determined by O'Reilly^[35] in a 0.1 M phosphate buffer at $\text{pH } 7$. Nevertheless, in the work of O'Reilly a 20-fold higher $[\text{Fe}(\text{CN})_6]^{3-}$ concentration was used. Moreover, the curve profile shows that the oxidation and reduction peaks are farther apart from each other than the theoretical 59 mV . In an ideal case of an electrochemical reversible reaction at 298 K , which is only diffusion limited, the difference of the peak potentials $E_p^{\text{red}} - E_p^{\text{ox}}$ should result in $59 \text{ mV}/z$, whereby z is defined by the electrons transferred in the reaction.^[36] A peak distance higher than 59 mV indicates either a quasi- or irreversible electrochemical process or an increased ohmic voltage drop (IR drop). In case of the redox pair $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ the reaction is usually considered reversible, although quasi-reversibility was observed in some studies due to the formation of prussian blue on the electrode surfaces.^[37] In our study, it can be assumed that there is no quasi-reversibility but rather that the IR drop leads to the shift of the peaks. Several factors can be responsible for an IR drop. First, the solution resistance, which is the resistance of the ionic electrolyte between the tip of the reference electrode and the surface of the working electrode, plays a central role.^[38] This solution resistance is favored by a large distance between the RE and the WE (1.2 cm). Thus, it can be assumed that the PPB (0.1 M) used as electrolyte and the distance of the RE to the WE in the new reactor system led to a high IR drop, which caused the shift of the peaks during the CV measurement. This problem was also observed in 2020 by Muhammad et al. in a potassium chloride buffered system with $5 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-}$.^[39] Here, a peak difference of 183 mV was observed, which was explained by a slowed electron transfer through the electrode and solution resistance. The usage of a higher concentrated electrolyte solution would have been possible for the abiotic study. However, a 0.1 M buffer is more comparable to a biological growth medium than a 1 M buffer with salt concentrations higher than 100 g L^{-1} . In addition to the peak distance, the ratio of the peak currents can also be used to draw conclusions about the reversibility of the reaction. For a reversible reaction the following applies: $\Delta I_p = 1$.^[36] In this case, the value for ΔI_p is 0.92 . This strengthens the thesis that the peak shift is caused by

an IR drop. The results of the electrochemical investigation of the reactor indicate that an increased overvoltage in the system can be assumed.

Based on the results of the abiotic experiments, it is concluded that more negative potential should be adjusted for an initial microbial electro-fermentation for solvent production. This conclusion is supported by a first preliminary experiment in the reactor with a voltage of -600 mV , as no effect on the metabolism of clostridia was detected with the used potential (Figure S5). Consequently, a potential of -800 mV is used in comparison to the potentials of -600 mV to -700 mV known from the literature.^[3,40,41] In this way, sufficiently reducing conditions should be present at the WE to enable an influence on the metabolism of Clostridia. Also, this is important due to the larger electrode surface of the WE compared to H-cell reactors, since a specific potential can only be applied locally using the RE. Finally, for a future improvement of the reactor system, the closer positioning of the RE to the WE would be beneficial. Also, the positioning of several RE at different points of the WE would be conceivable to reduce the influence of a potential gradient across the length and width of the electrode.

Since an influence of the applied voltage on the measured pH was observed in other BES, the influence of different voltages on the pH measurements in the new reactor system was abiotically investigated.^[27] This was especially important considering the fermentation of Clostridia, since the controlled adjustment of pH is essential for an efficient acetone, butanol and ethanol fermentation (ABE). Figure 4 shows the measurements of pH in a voltage range from -2000 mV to 2000 mV . For each voltage, the previously measured pH without applied voltage (OCP) was used as a control. The results show a clear pH shift compared to the OCP when the voltage is too low or too high. In addition, a 180°C rotation of the pH probe also results in a different orientation to the electric field present in the reactor leading to an exactly opposite trend in the separation of the pH shift. Consequently, measurements outside the voltage range of -1000 mV to 700 mV against Ag/AgCl

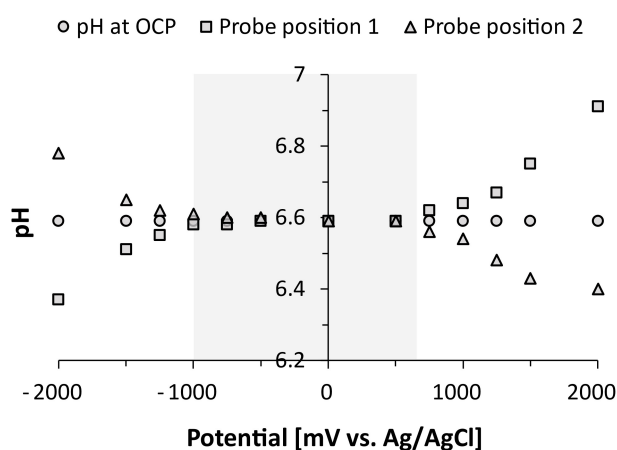


Figure 4. Influence of the applied voltage on the pH measurement in the reactor. As a control, the pH values during open circuit potential (OCP) are shown. Two electrode positions were tested (180°C rotated). The optimum operating range is highlighted in gray.

were not possible without interferences. The operating range identified in this way overlaps with the -800 mV selected for electro-fermentation, which should not cause any problems in pH measurement. Moreover, the operating range determined is similar to the values of Krieg et al., who determined an operating range of -1000 mV to 600 mV against Ag/AgCl for a further single-chamber system.^[27] Thus, the constructed reactor can also be used for typical electroactive organism such as *Sporomusa ovata*, which are often fermented in a voltage range of -1000 to -500 mV against Ag/AgCl.^[42–44] For the application as BES that uses higher or lower potentials, other pH measurement methods must be considered, such as sensors based on optical measurement techniques.^[45]

Electro-fermentation with *Clostridium acetobutylicum*

To verify whether the developed reactor system is in suitable for application in electro-fermentation, a proof of concept was performed in a parallel approach in two reactors. One reactor was operated under electro-fermentation conditions and the other as a control fermentation. As biological system, *Clostridium acetobutylicum* was cultivated in the reactor without the addition of mediators, as this organism had already been used successfully in previous electro-fermentation studies and the ability of direct electron transfer was already shown.^[3,46] Figure 5A illustrates the substrate and product concentrations for electro-fermentation with a WE polarized at -800 mV against Ag/AgCl electrode with 3 M KCl electrolyte during the entire fermentation period. In comparison, the results of the control

experiment without the application of an electric potential are shown in Figure 5B.

The product profiles are in line with the typical biphasic product formation described for *C. acetobutylicum* in literature. Initial acidogenesis leads to the formation of acetate (values not shown) and butyrate. From acidogenesis, the organism changes to the solventogenesis phase in which ABE products are synthesized and the previously produced butyric acid is assimilated.^[47,48] In the control experiment, first butyric acid concentrations could be detected after approx. 25 h. Correspondingly, in the electro-fermentation the first butyric acid values were measured between 30 and 40 hours. The highest butyric acid concentration in the control experiment reached 4.36 g L⁻¹ after approximately 44 h. Maximum butyric acid concentration of 5.02 g L⁻¹ were measured after approx. 47 h in the bioelectrochemical experiment. Both experiments show a clear assimilation of butyric acid during solventogenesis, which is reflected in the pH profile and shown exemplarily for the fermentation with an applied voltage in Figure 5D. Thereby, the acid values drop to 2.40 g L⁻¹ for control and to 2.01 g L⁻¹ for the electro-fermentation. The change of metabolism into solventogenesis and the resulting ABE production was detected after 41 h (control) and after 44 h (electro-fermentation). The target product butanol could be produced at a maximum concentration of 9.22 g L⁻¹ with an applied voltage. Therefore, the maximum production titer is 20% higher than the 7.66 g L⁻¹ of the control fermentation. The maximum yield of all ABE products was increased by electro-fermentation. In case of butanol, the yield is 0.204 g g⁻¹ compared to 0.170 g g⁻¹ measured in the control experiment. Furthermore, no large difference is observed in the substrate consumption curve of

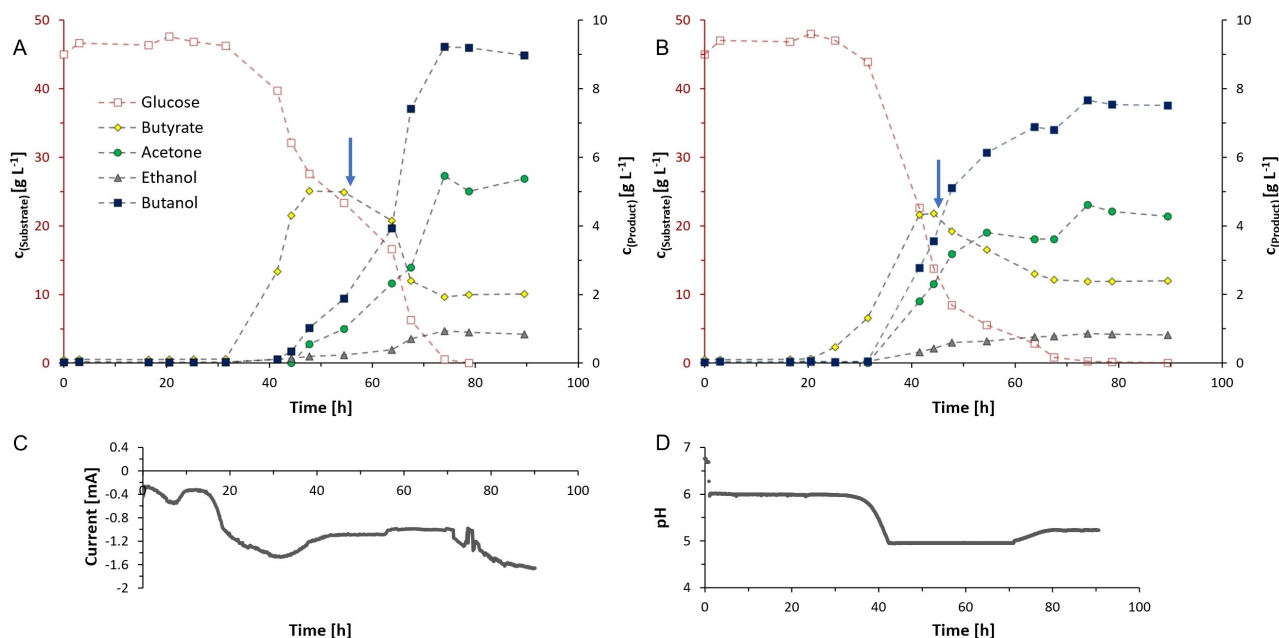


Figure 5. Substrate and product concentrations over time for (A) fermentation of *C. acetobutylicum* with -800 mV versus Ag/AgCl (3 M KCl) and (B) control experiment without applied potential in the new reactor system. (C) and (D) show the corresponding current profile over time and the pH profile of the fermentation with an applied potential. The blue arrows mark the beginning of butyric acid assimilation which is an indicator for the initial phase of solventogenesis. Fermentation parameters: MP2opt-medium, $T = 37$ °C, $V = 1.5$ L, 50 rpm, $n = 1$.

the experiments. In both approaches, glucose was completely metabolized by the end of the fermentation. In addition to the glucose decrease, Figure 5C shows a decrease in current response over the fermentation period which correlates strongly with the change from lag phase to exponential growth. Despite the installed exhaust gas cooler, the evaporation of the solvents could not be prevented 100%, as slight solvent losses can be observed at the end of the fermentations.

However, when the maximum ABE titers of this study are compared with literature, it becomes clear that the butanol concentration (9.22 g L^{-1}) and yield ($0.207 \pm 0.018 \text{ g g}^{-1}$) ob-

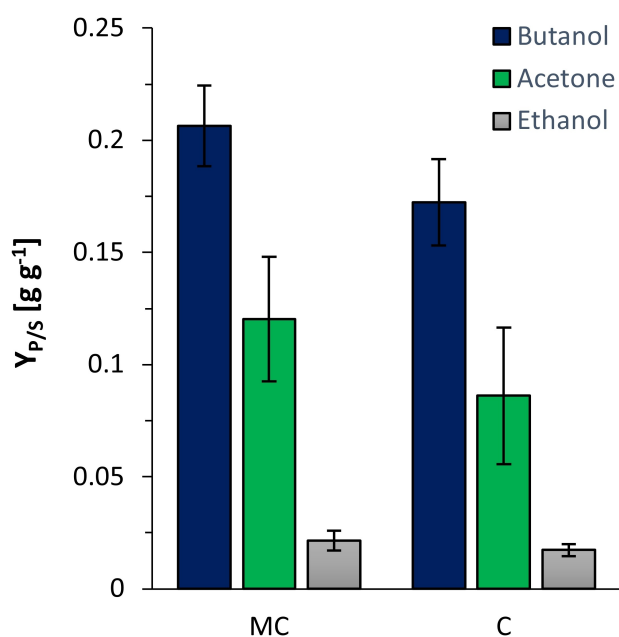


Figure 6. Mean solvent yield ($Y_{P/S}$, $S = \text{Glucose}$) of the main cultures (MC) and controls (C) in the reactor. Fermentation was performed with a potential of -800 mV or without an applied potential. Fermentation parameters: MP2opt-medium, $T = 37^\circ\text{C}$, $V = 1.5 \text{ L}$, 50 rpm . The results are means and standard deviations from four independent experiments ($n = 4$).

tained do not approach yields of up to 0.330 g g^{-1} and concentrations of about 13 to 15 g L^{-1} .^[49,50] Most likely, this is due to the medium optimized for electro-fermentation. The mineral salt medium itself was not designed to achieve maximum butanol yields, but for a good balance between its properties as a growth medium and as an electrolyte for electrochemical processes.^[3,51] Moreover, a high yield is not crucial in terms of the proof of concept experiment and can be further increased by media optimization in future studies. To proof replicability of results, four identical experiments were performed each with main culture (MC) and control (C). Average yields and standard deviations were calculated (Figure 6). As already observed in the stand-alone experiment, all solvent yields were increased compared to the control. For the target product butanol an average yield of $0.207 \pm 0.018 \text{ g g}^{-1}$ was obtained in the MC, which represents an increase of $20.14 \pm 3.66\%$ compared to the $0.172 \pm 0.019 \text{ g g}^{-1}$ of the control experiment. The results show that the experimental setting with the developed BES provides replicable results.

Table 1 summarizes the results of this study compared to results of an electro-fermentation with *C. acetobutylicum* performed in conventional H-cell reactors.^[3] In addition to the yield of the different solvents, the carbon recovery for the target product butanol and important reactor parameters are shown. In both experiments, the tendency towards increased solvent production by application of a potential of -800 mV or -600 mV versus Ag/AgCl is evident. In general, the yield $0.207 \pm 0.018 \text{ g g}^{-1}$ from the reactor based on a stirred tank reactor is closer to the maximum yields of up to 0.330 g g^{-1} reported in literature. Compared to the values of Engel et al.,^[3] the total solvent yield in the reactor increased by 0.146 g g^{-1} , which results in a difference of 72% . This fact is also reflected in the carbon recovery in butanol which amounts $33.46 \pm 2.91\%$ in the new reactor system, about 10% higher than the value from H-cells. The poor performance of the H-cells in terms of yields has already been reported for various electrochemical fermentations. The discrepancy is most likely caused by the problem of pH-gradient formation through the membrane and

Table 1. Comparison of control and electro-fermentations between reactor and H-cell experiments.

Parameter	New Reactor		H-Cell ^[3]	
	Control	-800 mV	Control	-600 mV
$Y_{\text{Acetone/Glucose}} (\text{g g}^{-1})$	0.086 ± 0.030	0.120 ± 0.028	0.033 ± 0.002	0.060 ± 0.003
$Y_{\text{Butanol/Glucose}} (\text{g g}^{-1})$	0.172 ± 0.018	0.207 ± 0.019	0.103 ± 0.016	0.135 ± 0.005
$Y_{\text{Ethanol/Glucose}} (\text{g g}^{-1})$	0.017 ± 0.003	0.021 ± 0.004	0.006 ± 0.008	0.007 ± 0.001
Total solvents (ABE) (g g^{-1})	0.276 ± 0.043	0.348 ± 0.045	0.141 ± 0.022	0.202 ± 0.010
Carbon recovery (%) (BtOH)	27.57 ± 2.51	33.46 ± 2.91	16.74 ± 3.42	23.15 ± 0.77
Average Y increase (%) (BtOH)	20.14 ± 3.66		31.07^{a}	
WE/CE surface	90/364		8.75/8.75	
Cathode material	carbon fiber brush		carbon fabric	
RE	Ag/AgCl		Ag/AgCl	
Resistance WE (Ω)	4.81		approx. $30^{\text{[30]}}$	

Abbreviations: counter electrode (CE), reference electrode (RE), working electrode (WE), Acetone-Butanol-Ethanol (ABE), Butanol (BtOH)
[a] Calculated with values from related literature.^[30]

by high ohmic resistances due to losses in the membrane and large distances between the electrodes.^[9,17]

However, when analyzing the influence of the applied voltage between the two BES, it becomes clear that the product increase due to the applied potential is higher for the H-cells than for the new reactor system, although yields in the reactor were increased by an impressive 72%. In the reactor itself, an average butanol increase of $20.14 \pm 3.66\%$ was recorded by applying a voltage, whereas the production in H-cells with the same strain and medium increased by 31.07%.^[3] For the related strain *C. pasteurianum* (strain DSM 525) an even higher increase in butanol production has already been recorded.^[52,53] In a mediator-less cathodic electro-fermentation attempt an increase in butanol of up to 51% was achieved in a typical H-cell set-up with 50 g L^{-1} glucose.^[52] Several reasons may underlie this phenomenon. One cause for the phenomenon may be a potential gradient across the length and width of the electrode. As described before, a specific potential is applied locally in the reactor using a single RE. Since the dimension of the WE in the reactor is much larger than the $3.5 \times 2.5 \text{ cm}$ WE used in H-cells,^[3] a potential gradient across the electrode can be assumed. The gradient may result in insufficient reducing conditions at locations of the WE further away from the RE, causing an artificial reduction of the effective electrode surface area which will affect the electron uptake of clostridia. The problem with potential gradients on large electrodes has already been described in other work. Lacroix et al.,^[54] for example, showed via modeling of the potential and current distribution in a microbial electrochemical system (MES) that potential gradients can occur, whereby the whole length of the anode did not operate at the same potential.

Furthermore, the potential influence of the single-chamber system on the strictly anaerobic Clostridia must be considered. Due to the absence of the membrane, products generated by side reactions at the CE can have a negative impact on the growth and metabolism of the organisms. The main reason for including a membrane in strictly anaerobic cultivations is oxygen produced at the anode.^[9,15] It has already been shown that the oxygen produced can also diffuse through certain membranes in two-chamber systems. Since the evaluated reactor is a single-chamber system, oxygen production due to the applied voltage had to be taken into account. On the one hand, the reactor was continuously gassed with nitrogen during the lag phase to remove oxygen. On the other hand, the position of the CE in the reactor could be another factor. By positioning the cylindrical CE directly at the reactor wall, it is assumed that a large amount of produced oxygen migrated up the reactor wall behind the CE, which prevented the gas from reaching the clostridia biofilm (Figure S2) at the WE through the initial gassing and subsequent carbon dioxide production. A similar principle was previously reported for an unseparated MES reactor in which the anode was positioned at the upper end of the reactor to flush out the produced oxygen more easily with the gassing of the reactor. The results showed that no negative effect of oxygen production on the strictly anaerobic biofilm of *Sporomusa ovata* could be detected.^[32]

Another reason for the reduced efficiency of the applied voltage in contrast to the H-cell experiment may be related to the electron transfer mechanism. The performance of the direct electron transfer (DET) is closely related to biofilm formation of microorganisms on the electrode surface. This has already been shown in detail for the used strain *C. acetobutylicum* (Figure S2). The study of DET mechanisms of *C. acetobutylicum* revealed the ability of the organisms to form pili to directly contact the electrode and each other in consortium.^[46] These conductive pili are most likely essential for the DET of the organisms. Due to the fact that in the new reactor system the WE also functions as a stirrer, an increased shear stress on the biofilm can be assumed. The increased shear stress due to the selected 50 rpm probably inhibits the biofilm formation and consequently pili formation, resulting in less pili connecting to the WE in the reactor compared to the experiments in H-cells, leading to less electron transfer. This assumption is supported by several studies in which stirring or other sources of shear stress show a negative effect on biofilm formation.^[55–57] If further experiments confirm the assumption, the developed reactor would be more suitable for electroactive organisms which mainly use mediated electron transfer (MET) mechanisms or an indirect electron transfer by H_2 or formate. These types of electron transfer mechanism do not depend on biofilm formation and can be carried out by planktonic cells in the reactor solution. Among others, Song et al.^[58] reported increased acetate production rates by adding mediators such as neutral red to the conducted MES, indicating that the mediators enhance the electron transport ability between the suspended cells and the electrode.

Conclusions

In this study, we were able to present a novel scale-up concept for a bioelectrochemical reactor which, to the best of our knowledge, is the first BES for cathodic electro-fermentation to be equipped with a rotating WE which, in addition to the functioning as an electrode, also acts as a stirrer in the system. The successful electro-fermentation with *C. acetobutylicum* and further abiotic experiments using cyclic voltammetry and pH measurements demonstrated the general suitability of the reactor and especially the suitability of a rotating WE for use in BES. This opens up the possibility of using stirred tank reactors where the classical stirrer is replaced by a rotating electrode. As stirred tank reactors are widely used and well characterized in bioprocess engineering, this could avoid the need to develop completely new systems to carry out electro-fermentations. Moreover, comparison of results from the new bioelectrochemical reactor with results from H-cells show that the total solvent yield in the reactor is increased by 72%. Therefore, the reactor is more suitable for production processes and scale-up than the H-cells used for the laboratory experiments. However, results from the BES show decreased percentual butanol increases resulting from the applied voltage compared to experiments in H-cells.^[3,30] For this reason, further optimization is needed. The influence of rotation of the WE on biofilm formation and related

pili formation for DET should be further investigated. It should be considered whether there is a change in biofilm layer thickness or nutrient transport. A view on electroactive organisms with a well-studied and understood MET would also be useful in this context. In addition, the possibility of a negative influence caused by a large potential gradient across the electrode should be further investigated.

Experimental Section

Strain and cultivation conditions

The used strain in this study was the type strain of *Clostridium acetobutylicum* DSM 792. (Leibniz Institute DSMZ German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Modified PY+X medium was used as preculture medium (DSMZ 104b). Precultures were grown in 200 mL serum bottles under nitrogen atmosphere. Glucose was separately autoclaved as a 100-fold concentrated stock solution and added after the sterilization process (Systec, Linden, Germany). As Inoculum a 1 mL cryo culture was used. All serum bottles were incubated for 48 h at 37 °C and 50 rpm (Infors AG, Bottmingen, Switzerland). For the main experiments in the designed reactor (Pehl Laborbedarf, Montabaur, Germany) optimized P2-medium (MP2opt) was used.^[3] The medium optimized for electro-fermentation contains glucose (45 g L⁻¹), ammonium acetate (2.2 g L⁻¹), KH₂PO₄ (0.5 g L⁻¹), K₂HPO₄ (0.5 g L⁻¹), MgSO₄ × 7 H₂O (0.2 g L⁻¹), Fe(II)SO₄ × 7 H₂O (0.015 g L⁻¹), MnSO₄ × H₂O (0.01 g L⁻¹), p-aminobenzoic acid (2 mg L⁻¹), biotin (0.01 mg L⁻¹) and thiamine-HCl (2 mg L⁻¹). Heat-unstable vitamins were prepared in a 1000-fold concentrated stock solution, sterile filtered (0.2 μm, cellulose acetate) and then added to the autoclaved and cooled medium. Glucose was autoclaved separately as a 450-fold concentrated stock solution and added to the medium. After all components were combined, the reactor was gassed with nitrogen (0.2 vvm). After 30 min of gassing the reactor was inoculated with a 48 h grown preculture (10% (v/v)) and further gassed with nitrogen until the start of exponential phase. The pH, temperature and rpm were controlled with the BIOSTAT Q Plus system (Sartorius AG, Göttingen, Germany) and a laboratory stirrer motor RW 20 (IKA-Werke GmbH, Staufen im Breisgau, Germany).

Electrode setup

The electro-fermentations were performed in bioelectrochemical systems (BES) with a three-electrode setup. The working electrode (WE) (The Mill-Rose Company, Mentor, United States) was a custom-made carbon brush connected to the potentiostat MultiEmStat3 (PalmSens, Utrecht, Netherlands) via slip ring (B-Command, Hamburg, Germany). For the simultaneous use of the carbon brush as a WE and as a stirrer a multi-piece stainless steel stirrer shaft was manufactured. The slip ring with continuous hollow shaft provides the conductive connection of the rotating brush to the potentiostat (Figure 1D). Above the slip ring the stirrer shaft is inserted into a plastic holder and screwed in place to prevent conductive contact of the brush electrode with the stirrer motor. Activated carbon felt ACC-5092-15 was utilized as counter electrode material (CE) (KYNOL EUROPA GmbH, Hamburg, Germany) and connected to the potentiostat via a platinum wire with a diameter of 0.4 mm (Der Hedinger, Stuttgart, Germany). An Ag/AgCl electrode (saturated KCl) with NSK 7 (Sensortechnik Meinsberg, Waldheim, Germany) was installed in the reactor as reference electrode (RE). If not otherwise stated, the potentials in this study are related to the Ag/AgCl electrode (3 M KCl).

Abiotic experiment setups

For the resistance measurements of this study, the MASTERTECH MS8229 (MGL International Group, London, United Kingdom) multi-meter was used. Once a stable potential was reached between two measurement points, the resistance between both points could be calculated using Ohm's law. In addition to the resistance measurements, the electrochemical characterization of the bioreactor assembly was further advanced using cyclic voltammetry. The method was performed in a voltage range of -1.25 to 1.25 V vs Ag/AgCl with different scan rates. A voltage of -1.25 V was set as starting point and at least three cycles were measured for each setting with the MultiTrace software (PalmSens, Utrecht, Netherlands). The investigation of the effect of an applied voltage on the pH measurements in the reactor system was analyzed by an abiotic experiment with 1.5 liters of the used main culture medium MP2opt. Initially, pH was measured as a blank to confirm that pH values were constant without an applied voltage. Measurements were taken in the potential range of -2.0 V to 2.0 V.

Analytics

Samples were separated and quantified chromatographically using high performance liquid chromatography (HPLC). An Aminex HPX-87H column (Bio-Rad Laboratories GmbH, Feldkirchen, Germany) with dimensions of 300 mm × 7.8 mm was used to separate glucose and the products butyric acid, acetone, butanol, and ethanol. Sulfuric acid with a concentration of 2.5 mM served as mobile phase. The mobile phase was pumped over the column with the Merck Hitachi L-6200 intelligent pump (Merck KGaA, Darmstadt, Germany) at a flow rate of 0.6 mL min⁻¹. A sample volume of 20 μL was used. During chromatography, the column oven was controlled to a temperature of 80 °C and a pressure of approximately 40 bar was present. The Shodex RI-101 refractive index detector (Showa Denko Europe GmbH, Munich, Germany) was installed to detect the target components. For the later evaluation different concentrations (10, 7.5, 5, 2.5, 1, 0.1 g L⁻¹) of a mixed standard were measured and a calibration line was generated using Linear regression.

Microscopy

The VHX-S750E digital microscope was used to capture microscopic images of the electrode surfaces (KEYENCE DEUTSCHLAND GmbH, Siemensstraße 1, Germany).

Chemicals

All chemicals were used as supplied by the manufacturers AppliChem GmbH (A), AnalytiChem GmbH (AC), Carl Roth GmbH + Co. KG (C), Honeywell International Inc. (H) and Merck KGaA (M). Acetone ≥ 99.5% (H), ammonium acetate ≥ 98% (H), calcium chloride dihydrate ≥ 99% (H), biotin ≥ 98.5% (A), butanol ≥ 99.7% (M), butyric acid ≥ 99% (C), ethanol ≥ 99.8% (M), glucose ≥ 99.5% (M), iron(II) sulfate heptahydrate ≥ 99% (M), magnesium sulfate heptahydrate ≥ 99.5% (AC), manganese(II) sulfate monohydrate ≥ 99% (H), p-aminobenzoic acid ≥ 99% (A), peptone (C), potassium chloride ≥ 99.5% (H), potassium dihydrogen phosphate ≥ 99% (AC), potassium hexacyanoferrate(III) ≥ 99% (M), potassium phosphate dibasic ≥ 99% (M), sodium chloride ≥ 99.5% (C), sodium hydrogen carbonate ≥ 99.7% (M), sodium hydroxide ≥ 98% (M), thiamine-HCl ≥ 99% (A), tryptone (C) and yeast extract (C).

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

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

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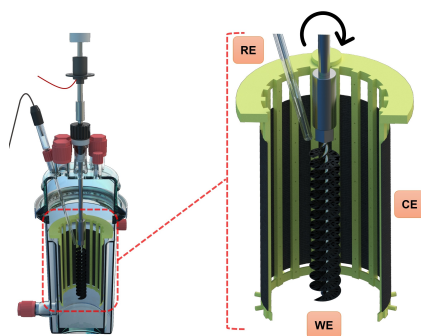
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RESEARCH ARTICLE

A bioelectrochemical system based on a stirred tank bioreactor with a rotating carbon fiber brush as working electrode (WE) and stirrer was developed. The three-electrode system was closed with a carbon felt counter electrode (CE) and an Ag/AgCl (3 M KCl) reference electrode (RE). Initial abiotic experiments and a biotic proof of concept via a cathodic electro-fermentation with *Clostridium acetobutylicum* proved the functionality of the new system.



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1 – 10

Scalable Unseparated Bioelectrochemical Reactors by Using a Carbon Fiber Brush as Stirrer and Working Electrode

