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Key factors of brewery wastewater influencing treatment efficiency and power output of microbial fuel cells

Brewery wastewater treatment with microbial fuel cells (MFC) offers an alternative to conventional biological treatment that could make a wastewater treatment plant profitable even for smaller breweries. The great potential of MFCs lies in their ability to treat wastewater and generate electrical energy simultaneously. However, the efficiency of MFCs strongly depends on the wastewater composition. Therefore, identifying the main factors in brewery wastewater affecting the degradation of organic substances and the power density of MFCs is necessary. For this purpose, identical MFCs were operated with brewery wastewater and diluted beer wort for ~180 days. The fermentable sugars in beer wort were first degraded to acetate and the absence of cleaning and disinfecting agents had no influence. However, the high proportion of easily degradable organic acids, influencing the biofilm composition, and higher conductivity of brewery wastewater resulted in an almost twice as high mean degradation rate of 82 ± 8 % and mean power density of 58 ± 24 mW/m³. The change from beer wort to brewery wastewater indicated that the process conditions, not the inoculum, determine the microbial composition of the biofilm. Inhibiting competing methanogens will be mandatory to enhance the energy efficiency needed to become competitive with conventional biological treatment.

Descriptors: microbial fuel cell, brewery wastewater, different compositions, influence factors

1 Introduction

Microbial fuel cells (MFCs) have received considerable attention owing to their dual function of wastewater treatment and simultaneous energy generation from wastewater [7]. In the context of wastewater treatment, the typical principle of MFCs consists of oxidation at the anode, exoelectrogenic microorganisms can degrade wastewater constituents, and oxygen reduction at the air cathode, to which the electrons and protons generated on the anode side are transferred [8].

Wastewater concentrations and their compositions are among the most important factors influencing chemical oxygen demand (COD) removal and power densities in MFCs [37]. Investigations on single substrate-fed MFCs showed that the substrate type affects power generation, as it is a crucial parameter for performance and the microbial community [59]. In terms of molecular complexity and conductivity-dependent resistance, the selection of suitable effluents remains a major challenge [37]. Moreover, the use of mixed microbial communities can lead to undesirable biomass growth, incomplete biodegradation of substrates, hydrogen production, methanogenesis, or aerobic degradation processes [37]. However, the stability, robustness through nutrient adaptation and stress resistance of mixed microbial communities in MFCs compared to pure cultures provides a significant advantage in wastewater treatment [14]. Therefore, achieving high performance with MFCs using an anaerobic sludge is challenging, as methanogenesis, precisely, cannot be easily avoided [5].

One possible way of increasing MFC performance is by identifying the process parameters through which typical exoelectrogenic microorganisms, including model organisms (e.g. *Geobacter, Shewanella* species), can be enriched on anodes and, methanogens can also be inhibited [5, 29]. Thus, the problem in anaerobic digesters with high sensitivity of methanogens to various inhibitory compounds, in contrast to the exoelectrogens in MFCs with higher resistance to perturbation by shocks in the organic load, could be exploited [47]. Furthermore, synthetic metabolic relationships between different microbial communities (fermenters and exoelectrogens) will probably benefit brewery wastewater degradation [47].

The energy efficiency and treatment performance of MFCs generally need to be improved to be comparable with conventional biological wastewater treatment approaches [3]. In addition to organic removal, other nutrients must be considered because the nitrogen and phosphate removal and the oxidation of odour-intensive sulphur and nitrogen compounds occur in aerobic wastewater treatment

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[15]. This trend is crucial for identifying potential MFCs' applications.

Therefore, the following questions arise. Is the pre-treatment of brewery wastewater necessary to be competitive? What are the main influencing components in the wastewater? Do the methanogens have to be explicitly suppressed? Does a pre-grown biofilm remain stable after feeding with brewery wastewater?

Consequently, the treatment of two media, occurring in breweries, using MFCs was investigated in parallel for ~180 days. The differences in the degradation of brewery wastewater and beer wort, which can be used as an ideal alternative matrix in the brewery sector due to the richness of nutrients and the absence of cleaning and disinfection agents, were analysed. Beer wort contains fermentable sugars, assimilable nitrogen, minerals and vitamins as well as middle chain fatty acids [35, 48], allowing ingredients affecting the degradation of brewery wastewater and beer wort to be compared. Brewery wastewater at the end of the beer wort-fed MFC was added to investigate biofilm stability and adaptability. By analysing the efficiency of the organic acid content of the different compositions and the amount as well as the composition of the gas produced in the MFCs, conclusions can be drawn about the microbial composition and the resulting organic degradation. Moreover, nitrate and nitrite, phosphorus and sulphate contents were considered. Finally, the effects on the power output were investigated, and the benchmarks of conventional brewery wastewater treatment were classified.

2 Material & Methods

2.1 Microbial fuel cell design

In the cylindrical single-chamber MFC, brewery wastewater was treated in the anode chamber using 1-L (Fig. 1). The anode consists of one carbon fibre brush, screwed into an impregnated, gas-tight graphite plate to increase the surface area developing a biofilm. The graphite plates on the anode and cathode sides serve as current collectors through which the produced voltage was measured.

A so-called membrane cathode assembly (MCA) was used on the cathode side, fixed between the current collector and the membrane support plate. The MCA consists of a perfluorosulfonic acid (PFSA) membrane (Fumatech BWT Group, Germany), coated on one side (facing the cathode current collector) with 0.5 mg/cm² activated carbon by the Fumatech BWT Group. The coating catalyses the cathode-side oxygen reduction. Mechanically milled channels in the cathode current collector enable air supply, and the PFSA membrane facilitates the transport of the required protons from the anode to the cathode. The projected surface area of the cathode was calculated as 113 cm². The carbon fibre brush (d = 10 cm and l = 6 cm; Bürsten-Baumgartner, Germany) has a significantly larger surface area with 0.3 mm diameter fibres and a fibre count of ~1800. Thus, the cylinder equivalent and total anode areas were calculated as 0.018 and 0.085 m², respectively.

2.2 Process design and operation

The process was operated in a closed circuit (Fig. 2), with a circulation provided during a batch cycle (hydraulic retention time (HRT): minimum of 48 h and mean of 60 h) by a membrane pump (SHURflo 8015-114-111, Pentair, Ireland) with ~500 mL/min flow rate. The treated wastewater after each batch cycle was entirely replaced using a valve system (V1 and V2), opened and closed in parallel in a time-controlled manner using a microcontroller (Arduino™ Mega 2560). Hence, anaerobic conditions could also be guaranteed during wastewater replacement.

The gas produced was collected by collection bags via a gas wash bottle and a gas volume counter (MilliGascounter, Ritter, Germany) using hand valves (V3 and V4). To maintain anaerobic conditions during sampling, V3 was opened in front of the nitrogen bag allowing nitrogen, instead of oxygen, to balance the vacuum.

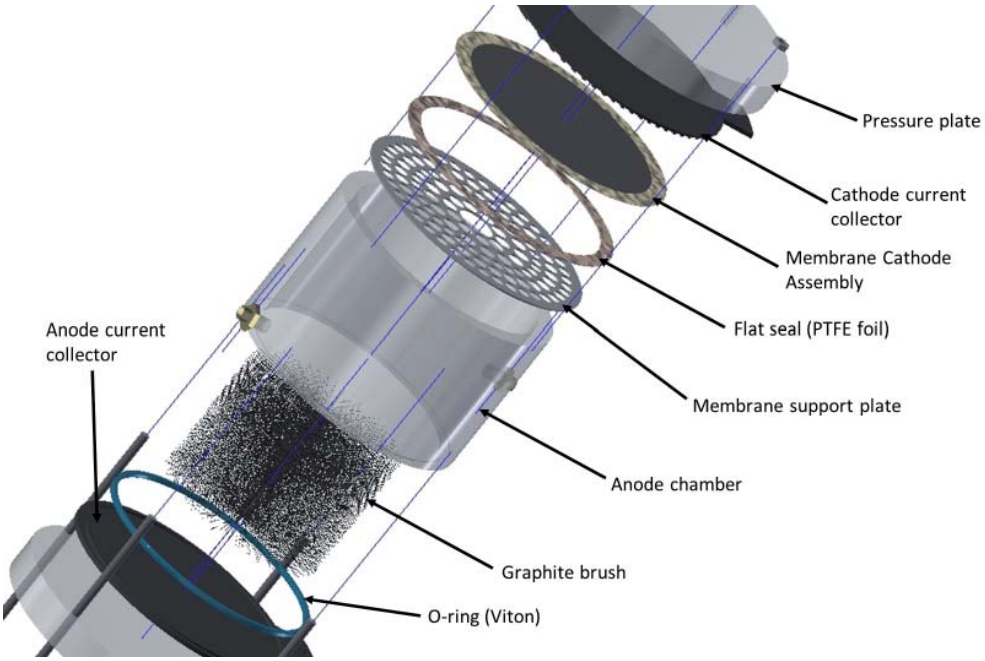

Fig. 1 Schematic representation of the MFC

The voltage produced was measured using an external circuit through a 200 Ω resistor (setting based on preliminary tests for determining the internal resistances of this MFC configuration) of a resistor box (RBox01, Voltcraft, Germany).

Anaerobic sludge from the wastewater treatment plant of a brewery served as the inoculum for both MFCs. The inoculum-to-medium ratio was set as 1:20 based on a study by *Riedl* et al. [42].

The duration of the experiment was approximately 180 days, at least 66 batch cycles were divided into three periods. In the initial period (16 batch cycles), sodium acetate (4.78

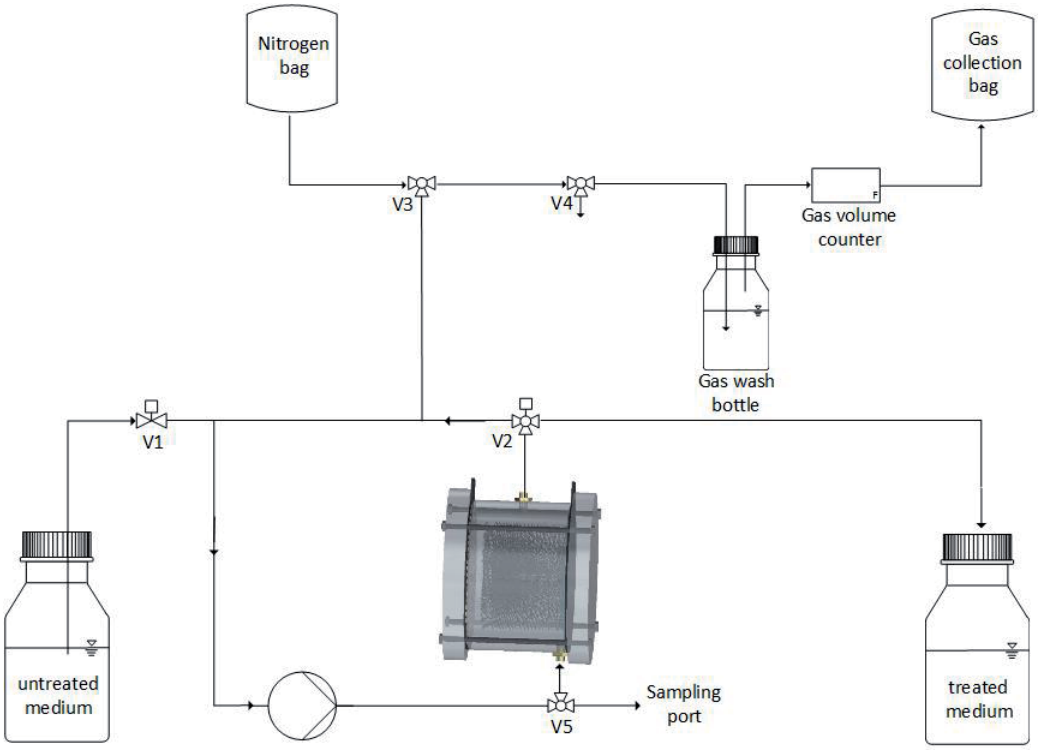

Fig. 2 P&ID of the process in A1 and A2

Table 1 Composition of brewery wastewater and beer wort before pH adjustment and dilution, respectively

Parameter	Unit	Beer wort (A1)	Brewery wastewater (A2)
COD	mg/L	178,408 ± 15,458	3,380 ± 306
DOC	mg/L	62,706 ± 1,892	866 ± 136
Organic acids	mg/L	5053 ± 947	723 ± 289
pH	-	5.7 ± 0.0	10.8 ± 0.4
Conductivity	mS/cm	2.0 ± 0.1	3.7 ± 0.6
COD:DOC	-	2.8	3.9
COD:Organic acids	%	2.8	21.4

g/L) was used as a simply degradable carbon source spiked by atres c-plus™ (0.1 mL/L), which is a complex trace element solution consisting of cobalt, nickel, selenium and iron. In the second transfer period (25 batch cycles), the change from sodium acetate to diluted beer wort (A1) or brewery wastewater (A2) as a carbon source was initiated in both cases. The amount of beer wort or brewery wastewater was increased by ~30 % after each sixth batch cycle. In the last six batch cycles, the increase was ~10 %, initiating the last operating period (25 batch cycles) with 100 % brewery wastewater or diluted beer wort. At the end of approach A1, the medium was changed from beer wort to brewery wastewater for further five batch cycles.

Table 1 shows the characteristics of the composition of the unadjusted brewery wastewater and beer wort, which provides a rich source of nutrients with fermentable sugars and medium-chain fatty acids as carbon source. The pH of the brewery wastewater was adjusted by phosphoric acid, and the beer wort was diluted to obtain comparable initial dissolved organic carbon (DOC) concentrations, and in both cases, atres c-plus™ (0.1 mL/L) was added.

2.3 Data evaluation and analytical methods

From the inflow and outflow of each batch cycle, pH, redox potential, conductivity, DOC and anion concentrations were determined. The multimeter (Multi3320, WTW, GER), with SenTix® 41, SenTix® ORP and TetraCon® 325 electrodes, was used to measure the pH, redox potential and conductivity. DOC and anion concentrations, including organic acids, were determined using the TOC analyser (liquiTOC II, Elementar, GER) and the ion chromatography system (Thermo Scientific Dionex™ ICS-1100; anion separator column IonPac™ AS22, carbonate–bicarbonate eluent), respectively. Before measuring DOC and anion concentrations, samples were filtered with a 0.45-μm syringe filter.

The unadjusted brewery wastewater and beer wort were additionally analysed using photometric tests (Machery Nagel, Germany) for COD (Nanocolor® COD 1500), organic acids (Nanocolor® Organic Acids 3000), NO_3-N (Nanocolor® Nitrat 50) and P_{ges} (Nanocolor® ortho-and total Phosphate 50).

Online volume measurement of the produced gas was conducted using a gas volume counter (MilliGascounter, Ritter, Germany), and the composition of the collection bags was analysed using a gas analysis system (AwiFLEX, Awite, Germany).

To determine the power densities, MFCs' voltages were continuously recorded via external resistance, transformed and recorded on a PC using a data logger (PicoLog ADC-20, Pico Technology, UK) and the associated software (PicoLog 6). The internal resistance of the MFC was calculated by taking the slope of the Linear Sweep Voltammetry (LSV) measured with a potentiostat (SP-150 Z, BioLogic, France) at steady-state conditions. A scan rate of 1 mV/s within a two electrode set-up was chosen for the LSV measurements.

For evaluating DOC degradation, organic acid degradation, gas quantity and composition, and the power densities, the data of the operating period were used since the medium was completely

different and stable data could be generated.

MFC performance was described with the power density normalised to the treated volume and DOC treatment efficiency (ΔDOC [%]). The power output was calculated using the measured voltage (E_{MFC} [V]) and external resistance (R_{ext} [Ω]) [8, 27].

$$PD_{vol} = \frac{E_{MFC}^2}{R_{Ext} \times V_{MFC}} \quad \text{(Eq. 1)}$$

$$\Delta DOC = \frac{DOC_0 - DOC_t}{DOC_0} \times 100\ \% \quad \text{(Eq. 2)}$$

PD_{vol} Power density normalised to the volume (W/m³)
V_{MFC} Liquid volume of the MFC (L)
DOC_0 DOC concentration (g/L) in the initial composition
DOC_t DOC concentration (g/L) after time t (final composition)

To compare with the conventional brewery wastewater treatment, the normalised energy recovery (NER) values of the MFCs was determined. The power generated by MFCs is normalised to the degraded COD concentration (NER_{kgCOD} [kWh/kg_{COD}]) or the treated wastewater volume (NER_{vol} [kWh/m³]) [13].

$$NER_{vol} = PD_{vol} \times HRT \quad \text{(Eq. 3)}$$

$$NER_{kgCOD} = \frac{PD_{vol} \times HRT}{\Delta COD} \quad \text{(Eq. 4)}$$

HRT Hydraulic retention time (h)
ΔCOD Degraded COD concentration (g/L), calculated from the determined COD-DOC factor

Furthermore, the overall efficiency (η_{MFC} [%]) of MFCs can be described based on the Coulombic and voltage efficiencies (CE [%] and VE [%]) [18, 46].

$$\eta_{MFC} = CE \times VE \quad \text{(Eq. 5)}$$

VE is the ratio between the generated voltage (E_{MFC}) and theoretical maximum voltage (E_{emf}). The standard potential E_{emf}, taken as 1.1 V, was determined using the Nernst equation by assuming that the MFC is operated with acetate as the substrate and oxygen as the electron acceptor [27, 50].

$$VE = \frac{E_{MFC}}{E_{emf}} \quad \text{(Eq. 6)}$$

The fraction of the degraded substrate effectively converted into electrons can be described using CE [27], which is influenced by fermentative or methanogenic metabolism.

$$CE = \frac{M \times \int_{t0}^{t} I(t)dt}{F \times n \times V_{MFC} \times \Delta COD} \times 100\ \% \quad \text{(Eq. 7)}$$

M Molar mass of oxygen (32 g/mol)
I Produced current (A) integrated over time t (s)
F Faraday's constant (96,485 C/mol)
n Number of electrons exchanged per molecule of oxygen (4)

3 Results and Discussion

3.1 Composition effect on the degradation efficiency

For the analysis of different DOC degradation efficiencies of A1 and A2, the operation period with 25 batch cycles was considered, where the medium differed completely, and a certain consistency was to be expected.

In the first few batch cycles, the initial DOC concentration of A1 exceeds that of A2 (Fig. 3A), owing to the dilution factor (20 and 50), which was increased to have initial DOC concentrations in the same range as A2. Although the initial DOC concentration of A2 also fluctuates, the end concentration of A2 (73 ± 20 mg/L) is much more stable and lower in contrast than the end DOC concentration of A1 (665 ± 187 mg/L).

Figure 3B shows similar median DOC removal of A1 and A2 with 323 mg/L and 393 mg/L for A1 and A2, respectively. However, the mean DOC removal differed significantly between A1 (537 ± 408 mg/L) and A2 (390 ± 154 mg/L). Also, due to the different initial DOC concentrations, the relative DOC removal of A2 (82 ± 8 %) was twice that of A1 (40 ± 17 %).

Even though the direct comparison of different MFC studies is generally impossible due to various influencing parameters (such as type of inocula, electrode materials, configuration, operating conditions), at least the feasibility and classification of the degradation efficiency can be presented. For industrial wastewater treatment (such as brewery, winery, dairy), a median COD removal efficiency of 81 % has been reported within a median HRT of 60 h considering 36 laboratory-scale MFC studies on industrial wastewater treatment [2]. In some studies where brewery wastewater was treated under similar conditions, COD removal efficiencies of 30 % in 24 h [25], > 96 % in 144 h [17] and 85 % in 60 h [52] were achieved in a laboratory scale, indicating the direct correlation between HRT and COD removal. Besides, COD removal of 87 % in 48 h was completed during the treatment of 10-L of brewery wastewater using a stacked MFC [61].

Since a correlation factor between DOC and COD was determined, the same percentage degradation rate can be used for comparison with the literature. Thus, the COD degradation rates of A2 (82 ± 8 %) compared to that of A1 (40 ± 17 %) in 48 h were similar to the COD degradation range in other studies on industrial wastewater treatment using MFCs. Consequently, the expectation of A1 for higher DOC degradation owing to a lack of cleaning and disinfecting agents in the medium was not confirmed. Therefore, the proportion of organic acids (acetate and lactate) and the amount and composition of the gas produced by both approaches have also been reflected.

A considerably different initial composition of A1 and A2 is evident in Fig. 4 by splitting the sum parameter DOC into faster degradable acetate and lactate and other organic components (DOC-rest). The initial proportion of acetate and lactate (Figs. 4A and 4B) was significantly higher in A2 (acetate: 25 %, lactate: 12 %) than in A1 (acetate: 11%, lactate: 1 %). Accordingly, the final composition of A2 also differed from A1 (Figs. 4C and 4D). In A2, acetate and lactate

were completely degraded (< 0.2 %) or partly converted into methane (72 %) and/or carbon dioxide (15 %). However, at the end of a batch cycle in A1 a high proportion of acetate (29 %) was detected. Only little concentrations of methane (2 %) and carbon dioxide (1 %) were produced. The unknown parts result from balancing the sum parameter DOC and the resulting average of the concentrations over the batch cycles, and calculation of the produced gas concentrations and carbon content converted into biomass.

Thus, the different initial compositions were most likely responsible for the significantly different DOC degradation rates of A1 and A2 since the two approaches were operated in identical MFCs under the same conditions. Although both MFCs were initially inoculated with identical anaerobic sludge, different microbial diversity develop due to different available organic ingredients. *Chae* et al. showed that different bacterial diversity developed in MFCs when different monosubstrates (acetate, butyrate, propionate and glucose) were available despite inoculation with the same anaerobic sludge [6]. The same effect was also demonstrated by *Kiely* et al., where significant variations among the dominant microbial consortia in anode biofilms were analysed when fed with different organic acids (acetic, lactic, formic or lactic acid) or ethanol [24]. For treating low-strength wastewater using MFCs, the phylogenetic diversity in the microbial communities of the anodic biofilms differed completely from real domestic wastewater to synthetic wastewater, justified by the type and amount of fermentable substrates and by the fermentation products [57].

Therefore, the microbial composition is not dependent on the original inoculum but is determined mainly by the organic acids added to the system [11].

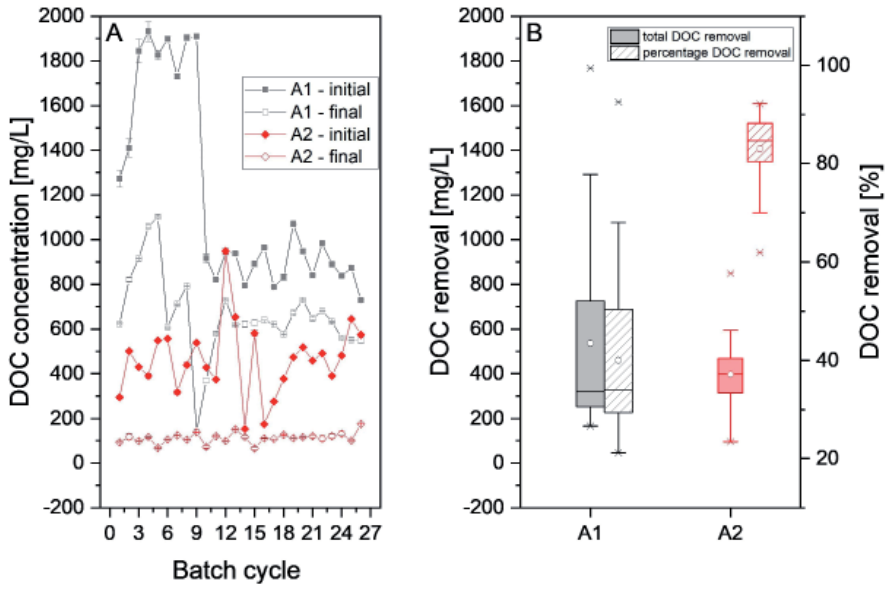

Fig. 3 **DOC concentration in the (A) initial and final compositions and (B) DOC degradation efficiencies of A1 and A2**

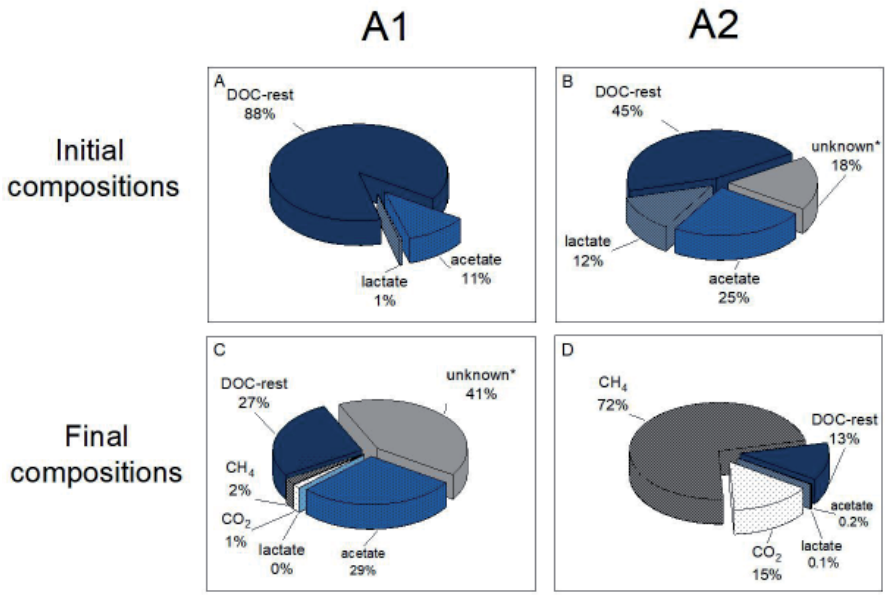

Fig. 4 **DOC concentration in the (A) initial and final compositions and (B) DOC degradation efficiencies of A1 and A2**

Furthermore, the formation of organic acids by the degradation of sugars can result from various metabolic processes, through which by-products can be formed [49]. The formation of acetate and propionate by the degradation of glucose or xylose was observed by Thygesen et al. during the operation of MFCs, assuming the presence of glucolytic or acidogenic bacteria [49]. Moreover, the difference in the initial conversion of glucose to propionate and acetate using lactate for power generation, as opposed to acetate-fed MFCs where acetate is directly used for power generation [55], confirms the different degradation patterns of A1 and A2. In general, beer wort consists of fermentable sugar (e.g. glucose, fructose) and middle-chain fatty acids in contrast to the used brewery wastewater, in which more short-chain fatty acids were present.

Yu et al. demonstrated the same pattern in the degradation of brewery wastewater by metabolizing ethanol via lactate, propionate and butyrate to finally acetate [56]. Besides, in this study, a similar degradation pattern of organic acids was observed in all MFCs, regardless of the starting substrate (glucose, butyrate, propionate, acetate, mixture of these substrates or brewery wastewater) [56]. By comparing different wastewater types, a temporary increase in organic acids was observed in brewery wastewater treatment, followed by its consumption [52]. Also, in the same study, direct consumption was observed in dairy, bakery and paper wastewaters, although thesewastewaters contained initial organic acids [52].

In summary, the lower organic degradation rates in A1 compared to A2 were caused by a lower short-chain organic acid content in the original composition. Consequently, different microbial diversity will develop in the anode biofilms. Due to the high methane concentrations produced in A2, the known concurrence of exoelectrogens

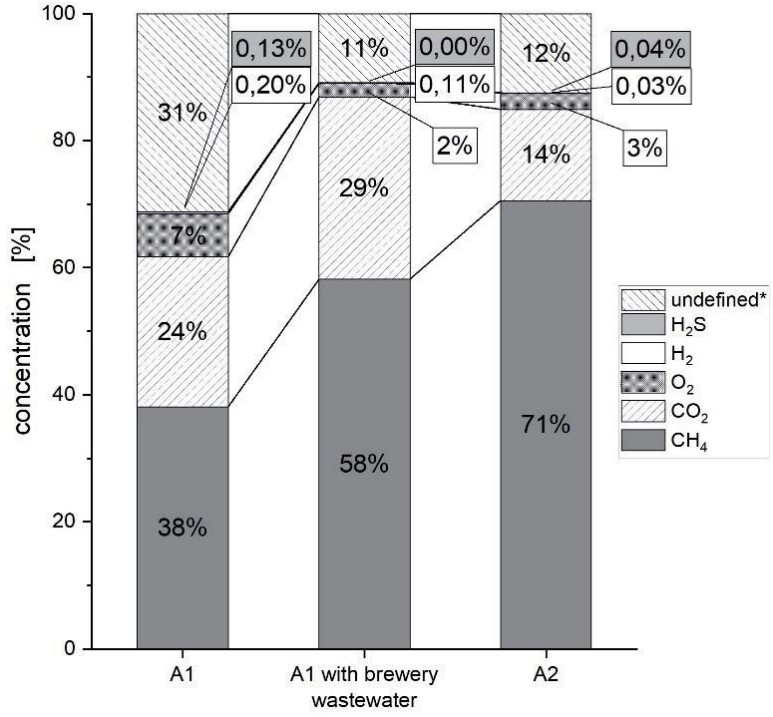

Fig. 5 Gas compositions of A1, A1 fed with brewery wastewater and A2. *Undefined indicates that nitrogen was undetermined with the measurement system.

and methanogens in A1 and A2 was analysed in detail.

Figure 5 illustrates the main difference in terms of gas composition–the methane concentration between A1 and A2, which was ~twice as high in A2 (71 %) as in A1 (38 %). However, the carbon dioxide concentration in A1 (24 %) is higher that in A2 (14 %). The other gas components (O_2, H_2 and H_2S) were in the same range in both MFCs. Since the MFCs were operated within a completely closed system, the oxygen input could be due to sampling or handling of the gas system, where oxygen could have entered the gas collection bags. A possible inhibiting influence by H_2S was not expected, as the concentrations in both cases were below the critical H_2S concentration (3 %) described in conventional anaerobic treatment [44].

In addition to the difference in gas composition between A1 and A2, the volume rate of gas production was 2.3 mL/h (A1) and 19.3 mL/h (A2), consistent with the difference in DOC removal rate, which was significantly higher in A2.

At the end of the experiment, the same brewery wastewater as in A2 was used for five batch cycles in A1 to investigate the adaptability of microbiology. With the increasing volume rate of gas production, having an average value of 11.8 mL/h, the gas composition changes into a similar composition than in A2 (Fig. 5). This trend induces an adaptation of microbiology in A1 within a short time. Depending on the substrate type, according to Chae et al., an anode biofilm enriched for a specific substrate can acclimatise to other substrates within a short time [6].

Generally, the presence of methanogens in MFCs inoculated with anaerobic sludge is common, as the growth conditions for exoelectrogens are like those of methanogens [5]. The possible substrates of methanogenesis in microbial fuel cell anodes are limited to acetate (acetoclastic methanogenesis) and hydrogen (hydrogenotrophic methanogenesis) [41]. Therefore, it was expected that in A2, with a significantly higher acetate concentration, the methane concentration produced was also considerably higher than in A1. In summary, the presence of more fermentable sugars in A1 leads first to degradation to acetate and in a second step to methane or carbon dioxide. The conversion of any substrate into methane, instead of electrons, negatively affects the power output [41]. Consequently, the competition between methanogens and exoelectrogens becomes apparent.

In single substrate-fed MFCs, the produced methane concentration out of glucose was highest compared with acetate, lactate, propionate or butyrate. However the produced methane concentration was only around 10 % [55] and a decrease was observed over the operating time. Thus, it was assumed that the exoelectrogens out-compete the methanogens [21]. Both the hydrogenotrophic [38, 43, 45] and acetoclastic [5, 22] methanogens were identified in MFCs, so no strict competition exists with exoelectrogens. Possible different niches within the biofilm with exoelectrogens and methanogens in the inner (connection to the electrode) and in the outer layers, respectively, confirm their coexistence rather than their strict competition [41, 47].

For treating brewery wastewater using MFCs in a previous study, a decrease in CE with increasing organic loading rates (OLR) was reported, attributed to the out-competition of methanogens [47]. Therefore, a low OLR helps reduce methane production [46] and shocks to the organic load can be used to reduce the proportion of sensitive methanogens by starving them out [23].

Hence, the methane concentration in A1 and A2 was probably caused by high OLR and thicker biofilms, where the exoelectrogens cannot compete directly with the methanogens due to the different niches. Similar high methane concentrations as in A2 were also found by *Park* et al., where ~71 % methane concentration was measured, with a possible reduction to ~31 % by selective inhibition using sodium 2-bromoethanesulfonate (BES) [39]. Such selective inhibition experiments were successfully investigated using BES [5, 16, 53], acetylene [53] or oxygen stress [5]. However, these methods are cost-ineffective for large systems [20, 43].

Finding optimal conditions that inhibit methanogens without using the necessary costly additives, without affecting the exoelectrogens, would be the ideal way to improve the efficiency of MFCs [5].

3.2 Nitrogen and sulphate removal

The discharge limits for wastewater treatment in breweries also consider nitrogen and phosphorus compounds. Thus, reducing these compounds with MFCs will be beneficial. Additionally, a high proportion of sulphur components can cause inhibitory effects. Besides, organic degradation, nitrogen and sulphur compounds have

already been reduced in some MFC wastewater treatment studies, whereas phosphorus reduction is rather unlikely [2] and did not occur in A1 and A2.

Nitrogen appears in wastewater in various compounds, which are removed using conventional biological wastewater treatment by biological nitrification and denitrification [1]. The nitrification of ammonium to nitrate occurs in the presence of oxygen, in contrast to denitrification, through which nitrate and nitrite are reduced to nitrogen gas under anaerobic conditions, the reduction requiring electrons produced by the removal of organic compounds [1].

In the anaerobic anode chamber of A1 and A2, nitrate and nitrite could be almost entirely reduced in both MFCs (nitrate > 98 % and nitrite > 99 %), even if only low initial concentrations were detected (Nitrate removal: A1: 17 ± 12 mg/L, A2: 11 ± 6 mg/L; Nitrite removal: A1: 4 ± 3 mg/L, A2: 10 ± 5 mg/L).

In many MFC studies on wastewater treatment [2], as against denitrification, nitrification of ammonia to nitrate or nitrite, requiring oxygen, was observed, not investigated in A1 and A2. E.g. NH_4-N removal of > 85 % has been reported in brewery wastewater treatment during long-term operation of a 10-L MFC [61]. Thus, instead of nitrite/nitrate removal, some studies have measured the increase in nitrite/nitrate with a simultaneous decrease in ammonia [9, 12, 28, 34, 60]. The oxygen required for the nitrification process was attributed to its possible diffusion through the cathode to the anode chamber [34, 60]. Consequently, in contrast to many MFC studies on wastewater treatment in A1 and A2, almost complete denitrification was achieved at low initial nitrite/nitrate concentrations.

Sulphur compounds are generally undesirable in wastewater, as possible sulphide toxicity has inhibitory effects in anaerobic treatment and corrosion in the system [44]. In A1 and A2, the initial sulphate concentration was ~50 mg/L (A1: 53 ± 22 mg/L, A2: 56 ± 17 mg/L). Almost all sulphate content was degraded in A1 (96 ± 6 %) and a mean sulphate degradation of 87 ± 10 % was achieved in A2. In both cases, only a minor part of the sulphate was converted to H_2S, resulting in a final H_2S concentration in the collection bag of 0.125 % in A2 and 0.043 % in A1, which was well below the critical H_2S concentration of 3 % [44]. Few studies have reported sulphate reduction within wastewater treatment using MFCs. Maximum removals of 39 % and 30 % have been reported for dairy wastewater treatment [30, 31] and up to 70 % removal for swine wastewater, attributed to the sulphate-reducing bacteria [1].

3.3 Influence factors on the power density

In addition to the priority of wastewater treatment efficiency, power output is also considered to evaluate MFCs for brewery wastewater treatment, completely. During the operating phase consisting of 25 batch cycles, a significantly constant higher mean power density (58 ± 24 mW/m³) was achieved in A2 compared to that in A1 (36 ± 20 mW/m³), which agrees with their internal resistance trend of 152 ± 45 Ω in A2 and 444 ± 26 Ω in A1.

For both MFCs, a general downward trend in power densities was observed over the operating time, possibly attributed to increasing biofouling and scaling of the membrane over time, clearly visible at the end of the experiments. Therefore, proton transfer to the cathode was prevented, which increases the ohmic and charge transfer resistances on the cathode side [36], causing the described downward trend.

Table 2 Operation parameters of A1 and A2

Operation parameter	unit	A1	A2
Conductivity	mS/cm	2.0 ± 0.4	4.4 ± 0.6
pH	-	6.3 ± 0.4	7.3 ± 0.3
Redox potential	mV	-111 ± 54	-208 ± 101
Average power density	mW/m³	36 ± 20	58 ± 24

The significant differences in power densities between A1 and A2 could be attributed to (1) the different physicochemical properties (pH, conductivity, redox potential) of the media, (2) the different organic compositions and (3) the resulting different consortia in the biofilm. Since the MFCs were identically constructed and use the same electrode material, these possible construction related influencing factors could be neglected when comparing the power densities of A1 and A2.

The significant difference in conductivity between A1 and A2 was particularly noticeable considering the physicochemical properties in table 2, where the conductivity in A1 (2.0 ± 0.4 mS/cm) is half that in A2 (4.4 ± 0.6 mS/cm) because of the dilution of beer wort in A1. Lower conductivities could negatively affect power densities in MFCs, with a linear correlation shown in defined systems [10, 26]. Although no clear correlation could be determined for the data, the general trend in practice can be confirmed that higher power densities can be achieved during the treatment of conductive wastewater [4, 19, 52].

In both cases, the pH values were or adjusted to neutral before use, as the optimal pH for most MFC studies was limited to neutral or near-neutral (6-8) ranges [51]. However, the pH of the electrolyte directly affect the growth and development of bacterial communities and their structure [40]. Therefore, slight pH difference could also indirectly influence the power density, depending on the sensitivity of the developed biofilms.

The influence of the redox potential in wastewater has not been investigated for MFCs, but is known for co-occuring methanogens in conventional anaerobic treatment. Since they are strictly anaerobic bacteria, methanogens require a negative redox potential of about –200 to –400 mV, indicating a reductive environment [44]. Thus, at least the presence of anaerobic conditions in these two MFC systems could be described. The redox potential may vary due to the offline measurements, which also explain the presence of methanogens in both systems although the corresponding range was not observed.

The different initial compositions of A1 and A2 not only affect the DOC degradation rates, but can also influence the power output. Generally, acetate-fed MFCs have higher power outputs than MFCs fed with other monosubstrates (e.g., lactate, butyrate, xylose glucose) [24, 32, 49, 58]. However, investigating substrate switching

in MFCs, which did not only affect power generation, showed that the biofilms in the glucose-fed MFCs had a much wider substrate usage and the acclimatisation time to other substrates was much shorter than in the acetate-fed MFCs [6, 59].

The main reason for the influence of different substrates [6, 24, 59] or different wastewater types [33, 52] on the power output can be attributed to the development of different consortia in the biofilm on the anode. Thus, a relatively diverse bacterial community was established in glucose-fed MFCs, but simultaneously, a low CE value was determined [6, 59]. Moreover, the acetate-fed MFCs showed the highest CE value, indicating a high proportion of exoelectrogens in the biofilm, possibly partly attributed to the *Geobacter* species [24, 59].

In A1 and A2, the mean CE values were below 1.5 % (Fig. 6); thus, for both cases, a low proportion of exoelectrogens could be assumed in the system. Surprisingly, the large difference in methane content between A1 and A2 was not reflected in CE values. However, methane producers will be the main cause for the low CE values and the associated comparatively low power densities.

Hence, the main influence on the power output of A1 and A2 was caused by the difference in conductivity. However, the other parameters also influenced the biofilm composition, which could be decisive for the power output performance.

3.4 Classification with conventional biological treatment and improvement strategies or possible applications

Based on the degradation rates and power densities, the energy recoveries (NER_{vol} and NER_{COD}) of A1 and A2 can be calculated using equations 3 and 4, which allows the overall performance of the MFCs to be classified in the existing literature and conventional biological wastewater treatment.

In both MFCs, the mean energy recoveries (NER_{vol} and NER_{COD}) were in the same range (Fig.6) of 0.002 ± 0.001 kWh/m³ (A1) and 0.003 ± 0.001 kWh/m³ (A2). The almost twice as high power output in A2 was not decisive for these key figures, as both energy recoveries were comparably low. In a previous study, median energy recoveries of 0.097 kWh/m³ and 0.058 kWh/kg_{COD}, respectively, were reported for industrial wastewater treatment using MFCs at pilot scale [2] and 0.10 kWh/m³ and 0.04 kWh/kg_{COD}, respectively, regardless of scale [13]. While energy recovery does not decrease with increasing volume under certain conditions, which would be beneficial for scaling MFCs, HRT and organic loading rate (OLR), in particular, have been identified as key factors for energy recovery [54]. Therefore, the short HRT and high OLRs in A1 and A2 probably negatively affected the energy recovery. Furthermore, the low CE values remain an additional major reason.

Compared to conventional brewery wastewater treatment (anaerobic-aerobic treatment) with positive energy balance values of 1.964 kWh/m³ (indirect discharge) and 0.511 kWh/m³ (direct discharge) [3], A1 and A2 achieved significantly lower energy recoveries. However, the degradation performance of A2 (82 %) was in a similar range with the anaerobic treatment efficiency (89 % in 8 h indirect discharge and 84 % in 19 h direct discharge) [3], although the median HRT of 60 h must be reduced. Achieving a shorter HRT would be possible, as the degradation curves (data not shown) indicated that the main degradation occured in the first 24 h. Furthermore, no pre-treatment and heating as usually preformed before the anaerobic treatment were conducted, improving the degradation rates.

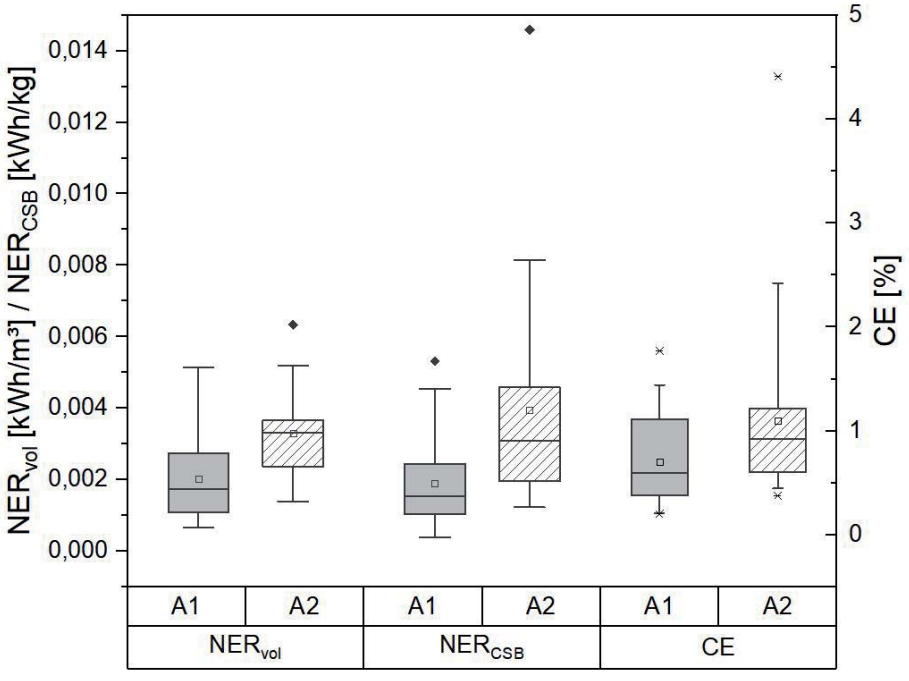

Fig. 6 **Normalised energy recoveries (NER_{vol} and NER_{kgCOD}) and Coulombic efficiencies (CE) of A1 and A2.**

Concerning comparable energy recovery in conventional brewery wastewater treatment; the negative energy balance of the aerobic stage becomes interesting. Thus, for its replacement, the energy balance of these MFCs should not be lower than –0.114 kWh/m³ (indirect discharge) or –1.020 kWh/m³ (direct discharge) [3]. The challenge here is the further nutrient removal, which can be partially covered with MFCs in terms of nitrogen or sulphur compound removal.

Considering COD removal and energy recovery, an overall efficiency of 4.5 % should be achieved with MFCs for a theoretical replacement of a conventional brewery wastewater treatment plant [3]. Using equation 7, an overall efficiency of A2 (VE = 18 % and CE = 1 %), less than 1 % (0.18 %) was achieved without any pre-treating or optimising the brewery wastewater. Therefore, improving the CE value would be mandatory to be a comparable treatment technology in terms of energy efficiency.

4 Conclusion

The composition of brewery wastewater, especially the proportion of organic acids, strongly influences the microbial composition of the biocoenosis and consequently the COD degradation performance and power density, which is also strongly influenced by the conductivity. The change in medium showed that inoculum is not the decisive factor, but the organic source. Furthermore, a gradual degradation of fermentable sugars first leads to an increase in short-chain fatty acids before complete fermentation. The process parameters should be adjusted throughout the treatment process (e.g., pre-acidification as in conventional anaerobic treatment). In addition to organic degradation, the successful removal of nitrogen and sulphur components was demonstrated.

In summary, untreated brewery wastewater could be degraded with MFCs in long-term operation. However, the energy efficiency was low due to losses in CE, so methanogens, in particular, must be inhibited to achieve higher power densities. Scale-up experiments (factor 100) are currently running at a brewery to gain more knowledge regarding the transferability of the results.

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