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Rapid start-up of a bioelectrochemical system under alkaline and saline conditions for efficient oxalate removal

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Abstract

This study examined a new approach for starting up a bioelectrochemical system (BES) for oxalate removal from an alkaline (pH >12) and saline (NaCl 25g/L) wastewater. An oxalotrophic biofilm pre-grown aerobically onto graphite carriers was used directly as both the microbial inoculum and the BES anode. At anode potential of +200 mV (Ag/AgCl) the biofilm readily switched from using oxygen to graphite as sole electron acceptor for oxalate oxidation. BES performance was characterised at various hydraulic retention times (HRTs, 3-24 h), anode potentials (-600 to +200 mV vs. Ag/AgCl) and influent oxalate (25 mM) acetate (0-30 mM) ratios. Maximum current density recorded was 363 A/m^3 at 3 h HRT with a high coulombic efficiency (CE) of 70%. The biofilm could concurrently degrade acetate and oxalate (CE 80%) without apparent preference towards acetate. Pyro-sequencing analysis revealed that known oxalate degraders *Oxalobacteraceae* became abundant signifying their role in this novel bioprocess.

Keywords: Alumina; alkaline industrial wastewater; Bayer process; microbial fuel cell; microbial electrolysis cell; oxalotrophic

1. Introduction

Smelting grade alumina (Al_2O_3) is refined from Al-containing bauxite minerals through Bayer process (Meyers, 2004). In the Bayer process, crushed bauxite is digested in a concentrated caustic solution (~ 3 M NaOH) at high temperature (140˚C – 250˚C) in a pressurised reactor (~3.5 Mpa) (Balomenos et al., 2011). After the product aluminium hydroxide (gibbsite, $Al(OH)_3$) is separated from the process liquor, the remaining caustic solution (spent liquor) is recycled to the digestion reactors to minimise caustic consumption (Hind et al., 1999; Meyers, 2004). With the continuous recycling of the spent liquor during the Bayer process, organic substances extracted from the bauxite also accumulate in the process liquor (Hind et al., 1999; Power et al., 2011b). These organics consist of various compounds ranging from very complex high molecular weight humic substances to simple organic acids (Power et al., 2012).

Among the organics present in the Bayer process liquor, sodium oxalate ($Na_2C_2O_4$) is a key organic impurity (Power et al., 2012). It causes detrimental impact to the quality and yield of the alumina products. Depending on the digestion conditions, 5-10% of the organic carbon is typically converted into sodium oxalate (Sipos et al., 1999). If not controlled, sodium oxalate affects the settling of gibbsite and scaling of pipes and tanks (Gnyra & Lever, 1979; Turhan et al., 2011). The most widely used industrial technique for oxalate removal involves crystallisation of sodium oxalate in a Bayer process side stream and disposal of the solid residues in residue areas (Brown, 1991; Rosenberg et al., 2004). Since Australian bauxite typically contains high organic content, Australian alumina refineries (particularly those in Western Australia) can produce up to 40 T/day of oxalate, which requires treatment and storage (McSweeney, 2011). The storage of oxalate in alkaline residue lakes poses significant risk on the

environment such as groundwater contamination and dusting, demanding strict handling and disposal guidelines (Power et al., 2011a).

Biological oxalate degradation has been increasingly considered as an environmentally friendly option to destroy oxalate in alumina refineries. However, microbial degradation of oxalate is challenging due to to the strict requirement of unique enzymes only present in specific microbial cultures (Allison et al., 1995; Miller & Dearing, 2013; Sahin, 2003). Three main enzymes, namely oxalate oxidase, oxalate decarboxylase and oxalyl-CoA decarboxylase present in oxalotrophic bacterial strains are known to be responsible for catalysing the cleavage of the C-C bond of oxalate, which is the crucial first step of oxalate biodegradation (Svedruzic et al., 2005). Different bacterial strains capable of using oxalate as carbon source and or energy source have been isolated from various living organisms and environmental sources such as human gastrointestinal tracts, sheep rumen, rhizosphere soil and aquatic sediments (Sahin, 2003). The most commonly found oxalate degrading bacterial genus is *Oxalobacter* within the family *Oxalobacteraceae* (Baldani et al., 2014).

Recently, Bonmati et al. (2013) have reported a successful use of a cation exchange membrane (CEM)-equipped bioelectrochemical system (BES) for the removal of oxalate from a low salinity liquor (sodium and potassium concentrations were ~ 2 g/L). Their BES was inoculated with an active anodophilic mixed microbial culture collected from a separate acetate-fed microbial fuel cell (MFC) and a mixed culture obtained from an upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater. During process start-up, acetate was used as a co-substrate to stimulate oxalate degradation. When oxalate was tested as the sole substrate, their anodic biofilm could efficiently (almost completely) remove oxalate from the anodic influent (10.3

kg/m^3.d at hydraulic retention of 7 h) (Bonmati et al., 2013). However, only low coulombic efficiency (CE) of 21±2% was achieved and the operational pH was not reported. Hence, it was unclear if their oxalotrophic biofilm would remain active under alkaline conditions.

In our previous study, the use of BES for removing oxalate under alkaline and saline conditions was investigated for the first time (Weerasinghe Mohottige et al., 2017b). Activated sludge was chosen as a microbial inoculum considering that it can be easily sourced in large quantity from domestic sewage treatment plants, and that microbes in activated sludge are generally capable of degrading different types of organics. However, start-up of the BES was unsuccessful when oxalate was used as the sole carbon and energy source. Even with acetate added as a co-substrate as practiced by Bonmati et al. (2013), the maximal oxalate removal was only marginal (<10% at a removal rate of 0.4 kg/m^3.d). Microbial community analysis of the anodic biofilm suggested that the inefficient oxalate removal was possibly associated with a paucity of microorganisms responsible for catalysing decarboxylation of oxalate into formate (Weerasinghe Mohottige et al., 2017b). Hence, it was concluded that activated sludge was not a suitable BES inoculum for oxalate removal under the alkaline and saline conditions used.

To address this microbial inoculation issue, it was postulated that microorganisms habituated in existing oxalate degrading reactors or oxalate-rich environment (e.g. soda lake or residue lake within an alumina plant) are likely having the enzymes required for catalysing the crucial first step of oxalate biodegradation (C-C bond cleavage), and are thus a more suitable inoculum for the described BES process. However, to be efficient in BES these microorganisms must also be able to readily grow

as a biofilm on the anode and be electrochemically active. One conceivable strategy to accomplish this is to first expose the electrode material to an oxalate enriched environment (e.g. an aerobic bioreactor), facilitating the formation of an active oxalate-degrading biofilm onto the electrode carrier. Subsequently, this so-called "biofilm-electrode assemblage" may directly serve as both the microbial inoculum and the anode of a BES to facilitate bioelectrochemical oxidation of oxalate.

In this work, the concept of using this "biofilm-electrode assemblage" for starting up an oxalate removing BES was studied. Such an assemblage was prepared by growing an aerobic biofilm onto graphite carriers (granules) within an aerobic bioreactor operated under both saline (NaCl 25 g/L) and alkaline (pH 9) conditions, and with oxalate as the sole carbon and energy source (> 250 days) (Weerasinghe Mohottige et al., 2017a). For the first time, the following questions were examined: (1) Can a metabolically active aerobic oxalate-degrading biofilm be readily acclimatised to generate anodic current under saline-alkaline conditions? (2) How long would it take for the aerobic biofilm to become anodically active with oxalate as the sole source of carbon and electrons under saline-alkaline conditions? (3) What would be the changes in the biofilm microbial community over the transition from aerobic- into anodic-(anaerobic) oxalate degradation? These questions were answered by operating a dual chamber BES reactor inoculated with an aerobically pre-grown active oxalotrophic biofilm (> 130 d) under alkaline-saline conditions.

2. Materials and methods

2.1 Bioelectrochemical systems and general process operation

A dual-chamber BES consisted of two identical half cells (14 cm × 12 cm × 2 cm), which were separated by a cation exchange membrane (Ultrex CMI-7000, Membrane International Inc., surface area 168 cm^2) was used in this study. The anodic chamber (working chamber) was loaded with 300 mL volume of biofilm coated graphite granules (anode material, 3-5 mm diameter, KAIYU Industrial (HK) Ltd.) collected from an aerobic bioreactor operated with oxalate as a sole carbon source for over 250 days. The start-up, acclimatisation and oxalate removal performance of the aerobic biofilm was reported in earlier study (Weerasinghe Mohottige et al., 2017a). The total biomass dry weight on the granules was 9.6 mg/mL packed volume of biofilm coated graphite granules, and the biomass had an initial aerobic oxalate degradation activity of 111 mg/h.g biomass. The cathodic chamber was loaded with similar type and quantity of graphite granules but without any biomass. After loaded with the granules, the void volume of each half cell reduced from 336 to 250 mL. Four graphite rods (5 mm diameter, length 12 cm) were used as current collectors in each half cell to enable electric connection between the graphite granules and the external circuit. The BES was operated as a three-electrode system coupled to a potentiostat (VMP3, BioLogic) (Cheng et al., 2010). The working electrode (anode) was polarized against a silver-silver chloride (Ag/AgCl) reference electrode (MF-2079 Bioanalytical Systems, USA) at a defined potential using the potentiostat. The reference electrode was inserted (*ca.* 1 cm from the top) within the granular graphite working electrode bed to minimise ohmic resistance. Total liquid volumes of 0.5 and 2.0 L were continuously recirculated (at recirculation rate of approximately 14 L/h) through the anodic and the cathodic half cells via two separate external bottles (0.25 and 2.0 L), respectively (Fig. 1). The headspace of the anodic recirculation bottle was intermittently flushed with nitrogen gas

(every 20 min for 30 second) to create an anaerobic environment in the anodic half cell. The process was operated at ambient temperature (22±2^{o}C).

Unless specified otherwise, the anodic chamber of the BES was operated predominately in continuous mode. Fresh anolyte (maintained at 4^{o}C in a refrigerator) was introduced at a specified flow rate into the external recirculation bottle and an equal volume of the old anolyte was withdrawn (and discarded) from the recirculation line using a peristaltic pump (Masterflex® Cole-Parmer L/S pump drive fitted with a Model 77202-60 Masterflex® pump head; Norprene® tubing 06404-14). Throughout the experimental period, the cathodic chamber of the BES was operated in batch mode, and the catholyte was occasionally renewed as per experimental requirements (approximately once per week). The BES process was continuously monitored and was controlled using a computer program (LabVIEW). The working electrode potential and the current of the BES were monitored via the potentiostat. All electrode potentials (mV) reported in this paper refer to values against Ag/AgCl reference electrode (ca. +197 mV vs. standard hydrogen electrode (Bard & Faulkner, 2001)). To ensure the accuracy of electrode potentials, the reference electrode was regularly checked against a new reference electrode. The pH of the working electrolyte was continuously monitored using in-line pH sensors (TPS Ltd. Co., Australia). All signals were regularly recorded to an Excel spreadsheet via the computer programme interfaced with a National Instrument^{TM} data acquisition card.

2.2 Synthetic refinery process water (anolyte) and BES catholyte

A synthetic medium, which simulated an alumina refinery process water stream in terms of its salinity and pH was used as the influent of the BES anode. Unless specified otherwise, sodium oxalate (3.35 g/L as $Na_2C_2O_4$, 25 mM) was used as the only carbon source, and NaCl (25 g/L) was added to increase the solution salinity equivalent to a typical Bayer liquor (Hind et al., 1997). Stock solution of NaOH (2 M) was used to maintained the feed solution pH (>12). The nutrient medium used for the BES anolyte consisted of (mg/L): 130 NH_4Cl; 125 $NaHCO_3$; 51 $MgSO_4 \cdot 7H_2O$; 15 $CaCl_2 \cdot 2H_2O$; and 20.5 $K_2HPO_4 \cdot 3H_2O$ and 1.25 ml/L of trace element solution which had the composition of (g/L): 0.43 $ZnSO_4 \cdot 7H_2O$; 5 $FeSO_4 \cdot 7H_2O$; 0.24 $CoCl_2 \cdot 6H_2O$; 0.99 $MnCl_2 \cdot 4H_2O$; 0.25 $CuSO_4 \cdot 5H_2O$; 0.22 $NaMoO_4 \cdot 2H_2O$; 0.19 $NiCl_2 \cdot 6H_2O$; 0.21 $NaSeO_4 \cdot 10H_2O$; 15 ethylenediaminetetraacetic acid (EDTA); 0.014 H_3BO_3; and 0.05 $NaWO_4 \cdot 2H_2O$. (Cheng et al., 2010). Unless otherwise stated, this medium was used as the electrolyte in the anodic chamber throughout the entire study. A similar NaCl concentration (25 g/L) as the anolyte was used as the catholyte medium of the BES. The catholyte recirculation bottle was exposed to air during the operation.

2.3 Experimental Procedures

2.3.1 Process start-up with the aerobic biofilm coated graphite granules

After the BES was setup, the synthetic medium was continuously fed into the anodic chamber to obtain a hydraulic retention time (HRT) of one day. Soon after loading the medium into the reactor (within an equilibration period of ~1h), the working electrode (anode) was poised at a constant potential of +200 mV, which was similar to the one used in our previous work for process start up (Weerasinghe Mohottige et al., 2017b). Over this period, sodium oxalate was used as the sole carbon and electron

source and the influent pH was maintained at 10 ± 0.2. Anodic current production and oxalate removal in the anolyte were used as the parameters to indicate the establishment of biofilm activity after initiation of the process. The following sections summarise the experimental procedures of evaluating the effects of HRT in the anodic chamber, anode potential and supplementation of various acetate concentrations as a co-substrate on the BES performance.

2.3.2 Effect of Hydraulic Retention Time (HRT) on BES performance

The effect of HRT on current production and oxalate removal rate of the biofilm was studied using different HRTs for anodic chamber ranging from 24 to 1 h (24, 12, 6, 3, 1 h) on days between 15 to 23, corresponding to COD loading rates of 1.76 kg/m^3.d to 13.7 kg/m^3.d, respectively. The BES was operated at a fixed HRT until it generated stable current over time (≥ one HRT). During this experiment, the BES was operated with an influent pH of 12.5 resulting in an in-reactor pH between 9.0 and 9.5 at a constant anode potential of -300mV, which was similar to process operation in our previous study (Weerasinghe Mohottige et al., 2017b). The CE (%) of the anodic reaction was calculated based on the electrons recovered as anodic current versus the theoretical amount of electrons liberated from the removed oxalate (assuming two moles of electrons per mole of oxalate oxidised) (Weerasinghe Mohottige et al., 2017b).

2.3.3 Effect of anode potential on BES performance

To test the performance of the established biofilm at different poised anode potentials, the following experiment was carried out after day 61. The BES anode was operated in continuous mode with 6 h HRT throughout this experiment. Initially the BES was operated in open circuit mode for more than 12 h until the open circuit voltage

became stable. Thereafter, the anode potential was increased stepwise from -300 mV to +200 mV and then was decreased from +200 mV to -600 mV. The BES was operated at a poised anode potential until the current become stable for ≥ 12 h. Throughout the experiment, pH (influent pH 12.5, in-reactor pH 9-9.5) and feed composition (25 mM oxalate as the sole carbon and electron source) remained constant.

2.3.4 Effect of increasing acetate concentration on oxalate degradation rate and BES performance

The effect of acetate, another organic compound present in Bayer liquor, on the oxalate degradation and coulombic efficiency of the BES was investigated by various concentrations of sodium acetate to the BES influent after day 95. At the beginning of the experiment, the BES was operated with 25 mM oxalate in the influent and acetate concentration was gradually increased from 0 mM to 30 mM (0 mg/L to 1500 mg/L). During the study, the BES was operated at an anode potential of -300 mV and HRT in the anodic compartment of 12 h. The influent pH was ranging from 12.5 to 13.4 resulting in in-reactor pH 9 – 9.5. Both acetate and oxalate removal were considered for CE calculation.

2.4 Chemical analyses

During these experiments, liquid samples were collected and immediately filtered through 0.22 µm syringe filters (Cat No SLGNO33NK, Merck Millipore, USA) for oxalate and acetate measurements, which were carried out using a Dionex ICS-3000

reagent free ion chromatography (RFIC) system equipped with an IonPac® AS18 4 × 250 mm column. Potassium hydroxide was used as an eluent at a flow rate of 1 ml/min. The eluent concentration was 12-45 mM from 0-5 min, 45 mM from 5-8 min, 45-60 mM from 8-10 min and 60-12 mM from 10-13 min. The temperature of the column was maintained at 30°C. Suppressed conductivity was used as the detection signal (ASRS ULTRA II 4 mm, 150 mA, AutoSuppression® recycle mode).

2.5 Biofilm samples collection and DNA extraction

Biofilm samples for microbial analysis were collected from the aerobic biofilm granules, which were used as the inoculum, and the anodic biofilm granules from the BES anodic chamber for DNA extraction and microbial community analysis. Before sampling, the biofilm inside the BES was dislodged from the granules within the reactor by forward-backward flushing with a syringe as described in Wong et al. (2014). The biomass samples were collected on days 21 (BES-21D) (HRT of 6h and anode potential of -300mV) and 75 (BES-75D) (HRT of 1d and anode potential of -300mV) after the BES had shown stable performance with oxalate as the sole substrate. Another two samples were collected at and after adding the acetate as co-substrate on days 99 (BES-99D) (1 day after addition of 5 mM acetate) and 103 (BES-103D) (5 days after addition of acetate). For comparison, two biofilm samples (BR-1 and BR2) were also collected from the aerobic bioreactor that was operated more than 250 days with oxalate as the only carbon source at pH 9 - 9.5 before taking the biofilm-coated granules to inoculate the BES.

DNA was extracted from 250 μL of suspended biomass dislodged from the graphite granules by using Power Soil DNA isolation kit from MO BIO laboratories, Inc. according to the manufacturer's instructions. The extracted DNA was stored at -20˚C prior to sending to the School of Pathology and Laboratory Medicine, University of Western Australia for 454 sequencing.

2.5.2 Microbial community analysis

The 454 sequencing was carried out as described by Nagel et al. (2016). In brief, microbial 16S rRNA genes were amplified from 1 ng aliquots of the extracted DNA using V4/5 primers (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT). A mixture of gene-specific primers and gene-specific primers tagged with Ion Torrent-specific sequencing adaptors and barcodes were used. The tagged and untagged primers were mixed at a ratio of 90:10. Using this method, the amplification of all samples was achieved using 18‑20 cycles, thus minimising primer-dimer formation and allowing streamlined downstream purification. Amplification was confirmed by agarose gel electrophoresis, and product formation was quantified by fluorometry. Up to 100 amplicons were diluted to equal concentrations and adjusted to a final concentration of 60 pM. Templated Ion Sphere Particles (ISP) were generated and loaded onto sequencing chips using an Ion Chef (Thermofisher Scientific) and sequenced on a PGM semiconductor sequencer (Thermofisher Scientific) for 650 cycles using a 400 bp sequencing kit yielding a modal read length of 309 bp. Data collection and read trimming/filtering was performed using TorrentSuite 5.0.

The open source software package QIIME (Quantitative Insights Into Microbial Ecology) was used for the post sequence analysis. The fasta, qual and mapping files were analysed using the downstream computational pipelines of QIIME. USEARCH61 was used for identification of chimeric sequences and was carried out in reference to an unaligned database (Greengene). On removal of chimeric sequences, the sequences were assigned operational taxonomic units (OTU) using the same reference database. The sequence similarity threshold was set at 97 %. Then a representative sequence was assigned from each OTU and taxonomies were assigned to each of the selected representative sequences using RDP classifier and the same Greengenes reference database. Subsequently a phylogenic tree was created on aligning all sequences against the same reference database using the Greengenes core alignment. Diversity analysis were finally carried out using the BIOM table, mapping file and the phylogenetic tree.

3. Results and Discussion

3.1. The aerobic oxalotrophic biofilm could readily switch from using oxygen to graphite as electron acceptor

Soon after loading the BES anodic chamber with the graphite granules acclimatised from the aerobic bioreactor, the BES was continuously fed with the synthetic medium (containing only oxalate as organic electron donor) and the potential of the granules were poised at +200 mV (Fig. 2). Unexpectedly, anodic current was recorded immediately, reaching a peak of approximately 20 mA (Fig. 2a, at ~6 h). Although, the current declined gradually thereafter plausibly due to a concomitant decrease in the anolyte pH (from 9.2 to ~8), it was apparent that the biofilm could

readily use the graphite granules (growth surface) as an electron acceptor without any lag period. The current production also coincided with a slight decrease in the anolyte pH (Fig. 2a), as the anodic oxidation of oxalate would liberate protons (reaction 1). Resuming the anolyte pH to ~9.2 by adding NaOH (i.e. the optimal pH for the aerobic biofilm) resulted in a sharp increase in current, asserting that the biofilm was alkalophilc (Fig. 2a).

$$C_2O_4^{2-} + 2H_2O \rightarrow 2HCO_3^- + 2H^+ + 2e^- \quad (1)$$

Oxalate measurement further confirmed that the current production was associated with oxalate removal (Fig. 2b). Clearly, the biofilm could degrade oxalate immediately even when the electron acceptor was drastically switched (from oxygen to graphite anode). Noteworthy, such a rapid onset of biofilm activity is opposing our previous finding, in which under the same conditions (i.e. anodic potential, pH, salinity and anolyte composition) activated sludge inoculum failed to adapt as an anodic oxalotrophic biofilm for efficient oxalate removal (Weerasinghe Mohottige et al., 2017b). The fact that in this study, a pre-acclimatised aerobic oxalate-degrading biofilm could rapidly (instantly) start up a BES for oxalate removal under saline and alkaline conditions is promising.

3.2. Increasing hydraulic loading increased current and oxalate removal when the anolyte had a pH of ~9.

The biofilm was further characterised for its ability to convert (remove) oxalate into current at various HRTs (Fig. 3). Decreasing HRT (from 24 to 3 h) increased the current (Fig. 3a). This suggested that the anodic biofilm activity was limited by the oxalate availability. In fact, increasing the oxalate loading rate (decreasing HRT)

resulted in linear increases in both the current ($R^2 > 0.99$) and the oxalate removal rate ($R^2 > 0.99$) (Fig. 3d). The maximum current production was ~120 mA (363 A/m^3) as recorded at a HRT of 3 h. The results were reproducible as the biofilm was able to reproduce similar current (e.g. at HRTs of 12 h and 6 h).

Notably, at a very low HRT of 1 h the current was drastically reduced (Fig. 3c). Fig. 3b shows that after the HRT was reduced from 12 h to 1 h, the current initially increased as expected due to increased oxalate loading (from 27 mA to 111 mA). However, the current only lasted for a short period (~80 min) and then a collapse was recorded (Fig. 3b). Such a collapse was likely due to an increasing anolyte pH initiated by the higher flux of the alkaline influent (pH 12.5). As such, the acidity generated from the oxalate oxidation (reaction 1) was not sufficient to sustain the anolyte pH at ~9. Clearly, the gradual increase (within 20 mins) in the anolyte pH to 10 coincided with a current drop (from 106 mA to 42 mA), and with further increase in the anolyte pH to 11.3 (maximal), the current drastically decreased to 5 mA (Fig. 3b). Overall, this suggested that at anolyte pH higher than ~9.7, the biofilm became less proficient to convert oxalate into current. Hence, the optimal pH of the anodic biofilm was lower than 9.7, aligned with the optimum pH (pH 9) recorded for the aerobic biofilm prior to inoculation and acclimatisation in the BES (Weerasinghe Mohottige et al., 2017a).

To rectify the suppression caused by the pH rise (i.e. due to the overloading of alkalinity at elevated influent load), and to test if the now impacted anodic biofilm activity could be readily revived, the HRT was reverted to 12 h (Fig. 3b, at ~day 6.3). As expected, with a higher HRT the anolyte pH gradually returned to ~9 again, and the current was also gradually increased to a similar level (~26 mA) as noted earlier with the same HRT (Fig. 3a).

In terms of coulombic conversion, a reasonably high and stable CE of ~ 70% was recorded at most tested HRTs (Fig. 3c). This suggested a good ability of the biofilm to electrochemically oxidise the oxalate. As near complete oxalate removal was recorded at most HRTs tested (Fig. 3d), a CE of 70% means that 30% of the oxalate was removed via non-bioelectrochemical pathways such as fermentation or methanogenesis. Since prior to this study methanogenic activities were detected with the aerobic biofilm (Weerasinghe Mohottige et al., 2017a), this unaccountable loss of oxalate in the BES may also be due to methanogenesis. However, further studies are required to elucidate these losses.

3.3. The oxalotrophic biofilm remained proficient in generating current even at low anode potentials

Anode potential is a critical factor determining the performance of a BES process. Typically, a more positive anode potential is favourable for microbial energy gain (Torres et al., 2009; Wagner et al., 2010). However, some studies showed that lower anode potentials enabled higher anodic current (Aelterman et al., 2008; Torres et al., 2009). From a BES operational standpoint, it is desirable if an anodic biofilm could produce maximal current at a low anode potential as this would minimise the energy loss in the anodic process (Aelterman et al., 2008). Hence, it would be meaningful to determine the effect of anode potential on the performance of our oxalate-degrading BES (Fig. 4).

When the anode potential was varied between -300 mV and +200 mV, no noticeable changes in current (~70 mA) and cathode potentials (~-1250 mV) were recorded (Fig. 4a), suggesting that the anodic activity of the biofilm remained stable.

However, current began to decrease when the potential reduced to below -300 mV (Fig. 4a, ~4.5 d), suggesting that the bioelectrochemical oxalate oxidation was limited by the anode potential (Aelterman et al., 2008; Cheng et al., 2008). Even at a very low potential of -600 mV, the biofilm could still generate a notable current (10 mA). This was not surprising because under the tested condition (pH 9, oxalate 25 mM), oxalate oxidation is thermodynamically feasible as long as the anode potential was higher than -644 mV (see supplementary information). The ability of the biofilm to anodically oxidise oxalate was also evident from the relationship between steady-state currents and anode potentials (Fig. 4b). Such relationship again confirmed that the biofilm was proficient in generating current even at a very low anode potential (e.g. at -500 mV), and both the oxalate removal and CE could be sustained at a relatively stable range (near 100% and 60%, respectively) in all the tested conditions (Fig. 4b). In terms of oxalate removal rate, when the BES was operated in open circuit mode a notable rate was recorded (3 $kg/m^3.d$), plausibly due to alternative degradation pathways as previously mentioned. However, compared with close-circuit operation a much lower oxalate removal was recorded in open-circuit mode operation (34% vs. 97%), confirming that most of the oxalate was electrochemically oxidised by the anodophilic biofilm. When the anode potentials became more favourable (i.e. from -600 mV to -300 mV), oxalate removal rate was remarkably increased from 4.2 $kg/m^3.d$ to 8.75 $kg/m^3.d$ (Fig. 4c).

3.4. Simultaneous acetate degradation by the established oxalotrophic anodic biofilm

Apart from oxalate, other simple organics such as acetate are also commonly present in alumina refinery process water (McKinnon & Baker, 2012; Tilbury, 2003).

Unlike oxalate that directly affects the alumina product quality and yield (Power et al., 2012), these compounds may affect oxalate degradation in a microbial treatment process. In the presence of acetate and oxalate, oxalotrophic microorganisms may prefer to metabolise acetate over oxalate for cell growth (a metabolic phenomenon known as diauxic growth) (Dijkhuizen et al., 1978; Krulwich & Ensign, 1969; Whiting et al., 1976). For example, Dijkhuizen et al. (1978) found that *Pseudomonas oxalaticus*, an oxalate metabolizing microorganism, preferred to metabolise acetate over oxalate when both substrates were present in anaerobic condition. Hence, in this study the effect of acetate on oxalate degradation in the BES was investigated by gradually increasing acetate concentration in the influent (from 0 to 30 mM), while maintaining the oxalate concentration constant (25 mM) (Fig. 5a).

The results showed that increasing acetate concentration increased the current almost instantly and linearly (Figs 5b and d), suggesting that in the presence of oxalate the established oxalotrophic anodic biofilm could also readily convert acetate into anodic current. Apparently, there was no sign of diauxic metabolism recorded. The addition of acetate did not affect the oxalate removal rate, and the biofilm concurrently removed both oxalate and acetate at efficiencies of nearly 100% (Fig. 5c).

To test if the addition of acetate would affect the overall coulombic conversion efficiency of the anodic process, the CE% was calculated (based on the total amount of electrons retrieved as current versus the amount of electrons dissipated as substrate removal (both acetate and oxalate) in the BES anode) (Fig. 5d). Compared with the previous experiment where oxalate was used as the sole substrate, the CE% increased from 71% to 80% with the increasing molar ratio of acetate in the feed (Fig. 5d). However, although with nearly complete removals of both acetate and oxalate (Fig. 5c),

it remained uncertain whether the improved CE% was due to a more proficient anodic conversion of oxalate or acetate. Nonetheless, the result confirmed that the anodic oxalate removal was highly efficient and was not impacted by the presence of acetate. Indeed, the anodic biofilm could efficiently oxidise both substrates.

3.5. Changes in microbial community compositions at different stages of the BES operation

The effectiveness of the strategy of using the so-called "aerobic biofilm-electrode assemblage" to start up a BES for oxalate removal was demonstrated. Understanding the temporal changes in the microbial communities involved in this process would be valuable to develop this effective strategy. Hence, the microbial communities of the biofilm at different stages (1^{st}: prior to inoculation; 2^{nd}: during active BES operation with oxalate as the sole substrate; and 3^{rd}: during active BES operation with both oxalate and acetate as the substrate) were characterised and compared (Fig. 6).

The microbial communities of the six biofilm samples were compared using principal coordinate analysis (PCoA), which measured the similarity amongst the samples based on phylogenetic diversity (Fig. 6a). Each point on the PCoA plot represents a sample and a closer distance between two points indicates smaller differences between the two microbial communities. In general, the sequences of all tested samples were distinctively clustered based on the aforesaid three operational stages (Fig. 6a). A notable shift of the clusters occurred after the aerobic biofilm (BR-1 and BR-2) was inoculated to the BES (BES-21D and BES-75D), suggesting that the microbial composition was remarkably changed when the biofilm was forced to use an

anode instead of oxygen as an electron acceptor to oxidise the oxalate. When both acetate and oxalate were made available as substrates, the microbial composition of the biofilm shifted again to form a new distinct cluster (BES-99D and BES-103D) (Fig. 6a). This affirmed that the substrate characteristic was influential on microbial composition of the anodic biofilm. Similar conclusion could be derived from the unweighted pair group method with arithmetic mean (UPGMA) clustering (Fig. 6b). Again, the microbial communities in all samples were clustered according to the three distinct stages, signifying that the biofilms at these unique operational stages were phylogenetically different.

The relative abundances of microbial families of the aerobic biofilm (inoculum) and those acclimatised in the BES are depicted in Fig. 6c. Before inoculated into the BES, the dominant microbial families in the aerobic biofilm inoculum were *Rhodobacteraceae* (~50%) and *Rhodocyclaceae* (~ 18%). Among the members of the *Rhodobacteraceae* family, *Paracoccus* was the dominant genus (47%) in the aerobic reactor biofilm. Microorganisms belonging to this genus have been shown as being able to oxidise oxalate. For instance, Anbazhagan et al. (2007) isolated a *Paracoccus* strain capable of oxidising oxalate under aerobic and alkaline (pH 8) condition (Anbazhagan et al., 2007). Another pure culture of *Paracoccus* (*P. homiensis DRR-3*) was also found to be electrochemically active and was able to produce anodic current in a microbial fuel cell (Jothinathan & Wilson, 2017).

After inoculation and acclimatisation in the BES, the abundances of families *Rhodobacteraceae* (20%) and *Rhodocyclaceae* (12%) in the biofilms decreased remarkably (Fig. 6c). During the time when the BES was operated with oxalate as the sole electron donating substrate, the abundance of *Oxalobacteraceae, Idiomarinaceae,*

Clostridiaceae and *Balneolaceae* increased considerably. As expected, after the BES influent was supplemented with acetate, the microbial community became more diverse with several families becoming more abundant (from <3% to >10%) (e.g. *Rhodobacteraceae*, *Oxalobacteraceae* and *Marinicellaceae*). Of special interest among these bacterial families is *Oxalobacteraceae*, which is a well-known oxalotrophic bacterial family containing both aerobic and anaerobic strains characterised in past studies (Baldani et al., 2014; Sahin, 2003). Further, under the family *Oxalobacteraceae,* genera *Oxalicibacterium* and *Oxalobacter* are known oxalate degraders, of which, *Oxalobacter* sp. are known to be obligatory anaerobic (Baldani et al., 2014). Since the abundance of *Oxalobacteraceae* remarkably increased after the aerobic biofilm was inoculated to the BES (under anaerobic condition), it is plausible that the increased abundance of *Oxalobacteraceae* was attributed to an increased growth of *Oxalobacter* sp. Indeed, *Oxalobacter formigenes* is a widely studied oxalotrophic strain that requires acetate as a growth supplement (Allison et al., 1985; Baldani et al., 2014). The increased abundance of *Oxalobacteraceae* (from 7.3% to 11.4%) recorded after the BES influent was supplemented with acetate was a result of the enrichment of this species. Although, no report had so far confirmed that *Oxalobacteraceae* strains were electrochemically active, the fact that our biofilm could efficiently convert both oxalate and acetate into current (CE 80%) suggests that *Oxalobacteraceae* strains might play a role in producing current in the described BES process. However, further studies are required to confirm this.

3.6. Implication of the findings

Overall, this study offered a number of new findings. The most notable one is that an aerobic oxalotrophic biofilm could rapidly switch their final electron acceptor from oxygen to an electrode for oxalate oxidation, enabling a rapid and successful start-up of a BES process. Second, the BES could efficiently remove oxalate (>97%) from an alkaline and saline influent, and allowed a coulombic conversion of oxalate into current at the highest efficiency reported in the literature (CE >70%). Third, the well-known oxalate degrading bacterial family *Oxalobacteraceae* became more abundant over time in the oxalate-degrading BES. Fourth, the established oxalotrophic biofilm could simultaneously convert both acetate and oxalate into anodic current at a high CE of 80%. These are new knowledge that have not been previously reported.

The practical implication of these findings can be appreciated by comparing the performance of the described BES process with other industrial scale oxalate-removing bioprocesses (Table 1). Here, the selected aerobic processes in the comparison were operated with alkaline alumina refinery spent liquor and residue lake water, and are considered as benchmark for this comparison. In terms of oxalate removal, it is clear that all aerobic bioprocesses were better than the BES processes, with the highest rate (41.2 $kg/m^3.d$) recorded from a pilot scale bioreactor. However, the recovery of resources (such as energy and caustic) are not allowed in aerobic reactors. Although the laboratory-scale bioreactor (from which the aerobic oxalotrophic biofilm-graphite granules were harvested for this work) removed oxalate at a lower rate (23.5 $kg/m^3.d$), it could be operated at a considerably higher hydraulic loading rate (HRT 3.5 h vs. ≥14.7 h) (Table 1). This is attractive because a lower operational HRT enables a smaller reactor foot-print, and hence a lower capital investment.

In the literature, only limited studies have explored the use of BES for oxalate removal. Bonmati et al. (2013) reported for the first time that oxalate could be completely removed in a BES. However, only a very poor anodic conversion of oxalate was recorded (CE 21%), and it was unclear if the anodic oxalate removal could be carried out under alkaline and saline conditions. Under industrial relevant conditions (high alkalinity and salinity), the BES established in the current study enabled oxalate removal at a rate comparable to the laboratory-scale aerobic process (19.6 vs. 23.5 $kg/m^3.d$) (Table 1). To this end, using BES for treating alumina refineries liquor can be considered attractive.

The start-up strategy adopted in this study also deemed practically attractive. In our previous study, a similar BES loaded with plain graphite granules was seeded with activated sludge. However, start-up was not successful even after a prolonged period (>50 days), with only negligible oxalate removal recorded (0.4 $kg/m^3.day$) (Weerasinghe Mohottige et al., 2017b). Having an effective and reliable source of microbial inocula is highly desirable for any industrial microbial processes. A process demanding a long start-up period requires high operational cost, which is obviously undesirable. The fact that an aerobic oxalotrophic biofilm could readily start up a BES process is encouraging, as this can reduce down time operation of the process (e.g. during circumstances such as reactor failure, process inhibition (see supplementary information figure S3)).

Last but not the least, the described BES processes could also facilitate caustic production, which can be considered apart from oxalate removal, as an additional benefit for the alumina industry. Since the industry is well known for its high demand for caustic, this aspect should be further researched. Finally, as with many other novel

processes, further optimisation and development of the described BES process are desired.

4. Conclusions

For the first time, an aerobic oxalotrophic biofilm pre-grown on a graphite carrier was demonstrated as an effective agent to readily start-up a BES under alkaline and saline conditions. The biofilm could rapidly switch from using oxygen to graphite as electron acceptor for efficient anodic oxalate oxidation (CE 70%). The established biofilm could simultaneously degrade both oxalate (25 mM) and acetate (30 mM) with removal of 97 – 99 % removal efficacy (HRT 12 h). The microbial community of the established anodic biofilm deviated notably from the initial aerobic biofilm, with known oxalotrophic families (e.g. *Oxalobacteraceae*) became increasingly abundant over time.

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Supplementary data

E-supplementary data of this work can be found in e-version of this paper online.

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Figure Captions

Fig. 1. A schematic diagram of the BES process consisting of anodic cell and cathodic cell filled with graphite granules, a recirculation line and a computer for process monitoring and control.

Fig. 2. (a) BES current generation and pH profile during start up with biofilm granules from aerobic bioreactor, using influent with 25 mM sodium oxalate. The anode potential was +200 mV and HRT of 1 day for first 4 days. b) Oxalate concentration in anolyte inflow and outflow streams for the first 4 days.

Fig. 3. BES performance at various HRTs at an anode potential of -300 mV vs. Ag/AgCl, in-reactor pH of ~ 9 and with sodium oxalate as only electron donor. a) Variation of HRT and current production over time. An enlarged view of area covered by red dotted box is given in (b). c) Average current and CE (%) at various HRTs. d) Oxalate removal percentage and average current produced at various COD loading rates.

Fig. 4. BES performance at various poised anode potentials from -600 mV to +200 mV vs. Ag/AgCl at in-reactor pH of 9 – 9.5 with 25 mM sodium oxalate as only electron donor and anodic chamber HRT of 6 h. a) Current production and electrode potentials over time. b) Average current produced, oxalate removal percentage and CE (%) at various poised anode potentials. c) Positive linear relationship between current and oxalate removal rate.

Fig. 5. Increase in current production with increasing acetate concentrations in the feed with sodium oxalate (25 mM) at anode potential of -300 mV vs. Ag/AgCl, HRT of 12 h and in-reactor pH of 9.5. a) Increase in oxalate to acetate ratio over time. b) Current production, and influent oxalate and acetate concentrations over time. c) Oxalate and

acetate removal efficiencies over time. d) Relationship of average current production and CE with oxalate to acetate ratio.

Fig. 6. a) Principle component analysis (PcoA) profile (unweighted Unifrac) for samples collected from the aerobic bioreactor (BR-1 and BR-2) and BES (BES-21D, BES-75D, BES-99D to BES-103D) indicating clustering of samples. b) Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of unweighted unifrac distances sample set. The values at the branches show the similarity of the samples. The two plots highlight the clustering of samples according to reactor type and carbon source available. c) Stacked bar plot of the relative abundance bacterial families in the aerobic bioreactor and BES fed with oxalate and oxalate + acetate. Family that represent less than 3% of the total microbial community composition were classified as “others”.

Table 1. Comparison of oxalate removal performance of various aerobic and bioelectrochemical reactor processes.

Process	Electron acceptor	Influent	Reactor size (m^3 or L)	HRT h	In-reactor pH	Oxalate removal rate* $kg/m^3.d$	Oxalate removal %	Current density* A/m^3	CE %	Reference
Aerobic reactor										
Pilot scale aerobic biofilm reactor	Oxygen	Refinery lake water	3.8 m^3	20	9.6	41.2	100	n.a	n.a	(McSweeney, 2011)
Full scale aerobic biofilm reactor	Oxygen	Refinery lake water	450 m^3	14.7	9.7	30.6	100	n.a	n.a	(McSweeney, 2011)
Full scale aerobic reactor	Oxygen	Refinery lake water + oxalate thickener discharge	270 m^3	20	10	40.3	100	n.a	n.a	(McKinnon & Baker, 2012)
Lab scale aerobic biofilm reactor	Oxygen	Synthetic liquor	1.5 L	3.5	9.2±3	23.5	100	n.a	n.a	(Weerasinghe Mohottige et al., 2017a)
BES reactor										
Dual chamber BES reactor	Graphite granules anode	Synthetic liquor	0.34 L	6.96	-^	10.3	100	29.8	21±2	(Bonmati et al., 2013)
Dual chamber BES reactor	Graphite granules anode	Synthetic liquor	0.5 L	24	9	0.4	2	3.0	39	(Weerasinghe Mohottige et al., 2017b)
Dual chamber BES reactor	Graphite granules anode	Synthetic liquor	0.5 L	3	9.1±4	19.6	97	363.1	73.5	This study

* = Normalised to active void volume of the bioreactor (i.e. anodic chamber of BES).

^ = In-reactor anolyte pH value is not available in this reference.

CE = Coulombic efficiency

n.a = not applicable.

Fig. 1.

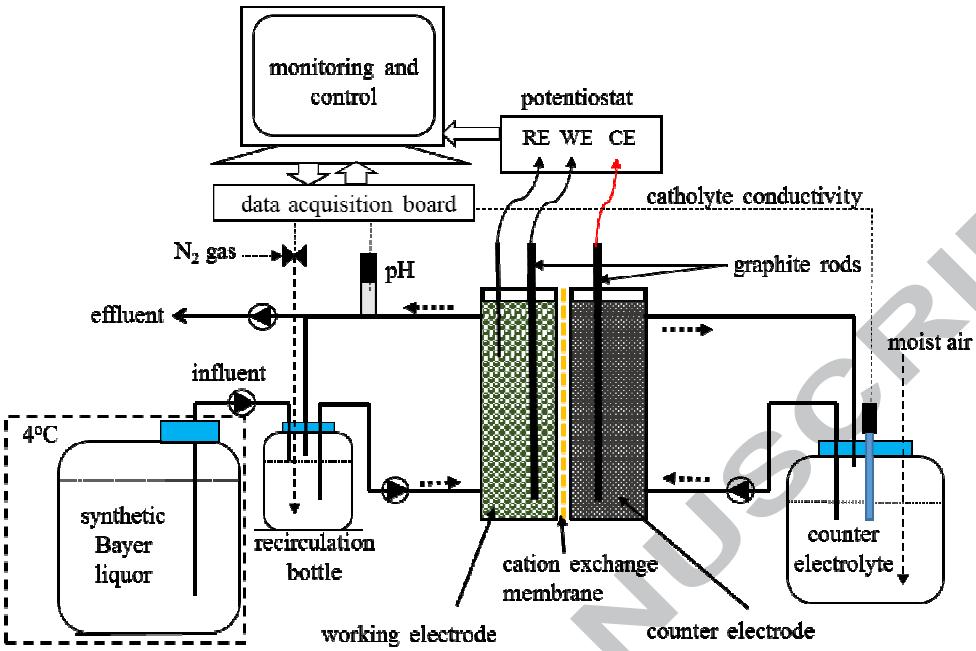


Fig. 2.

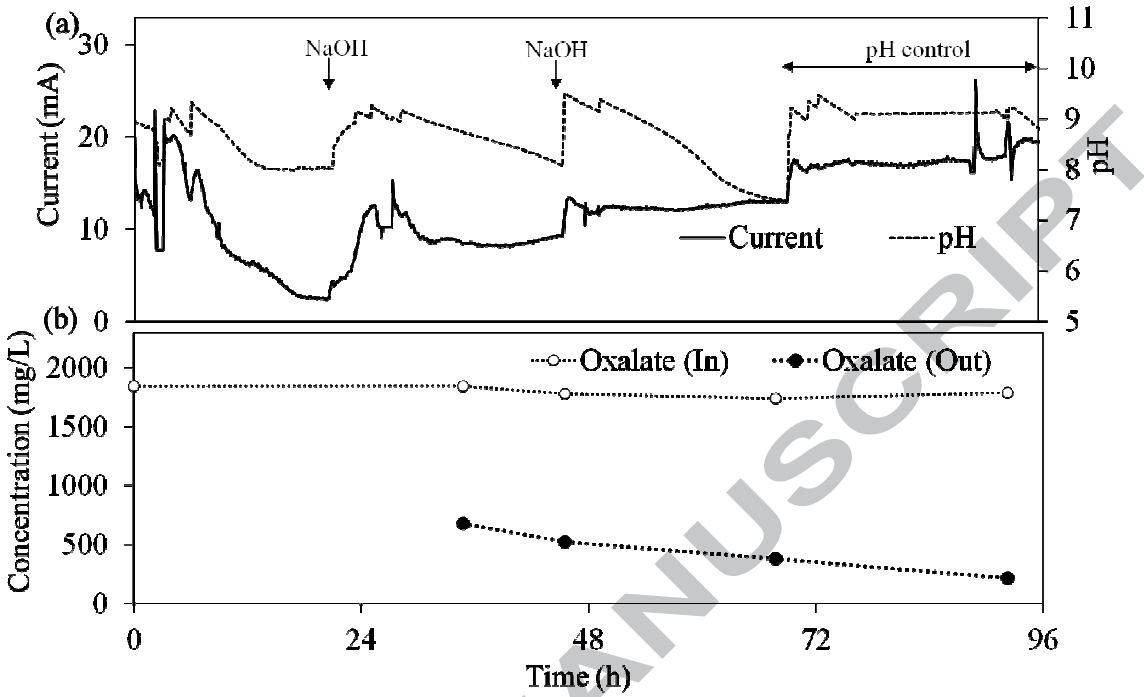
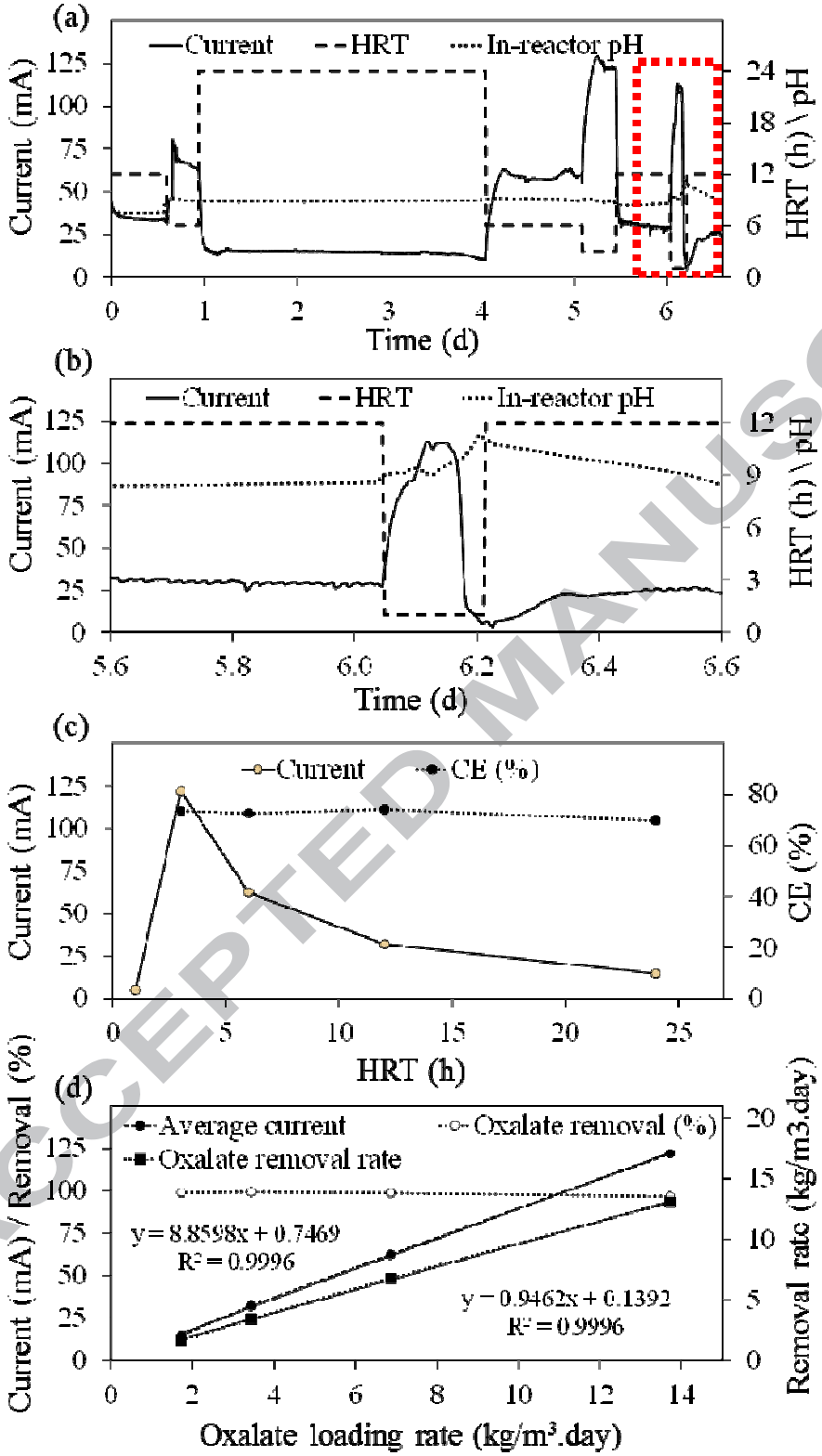


Fig. 3.



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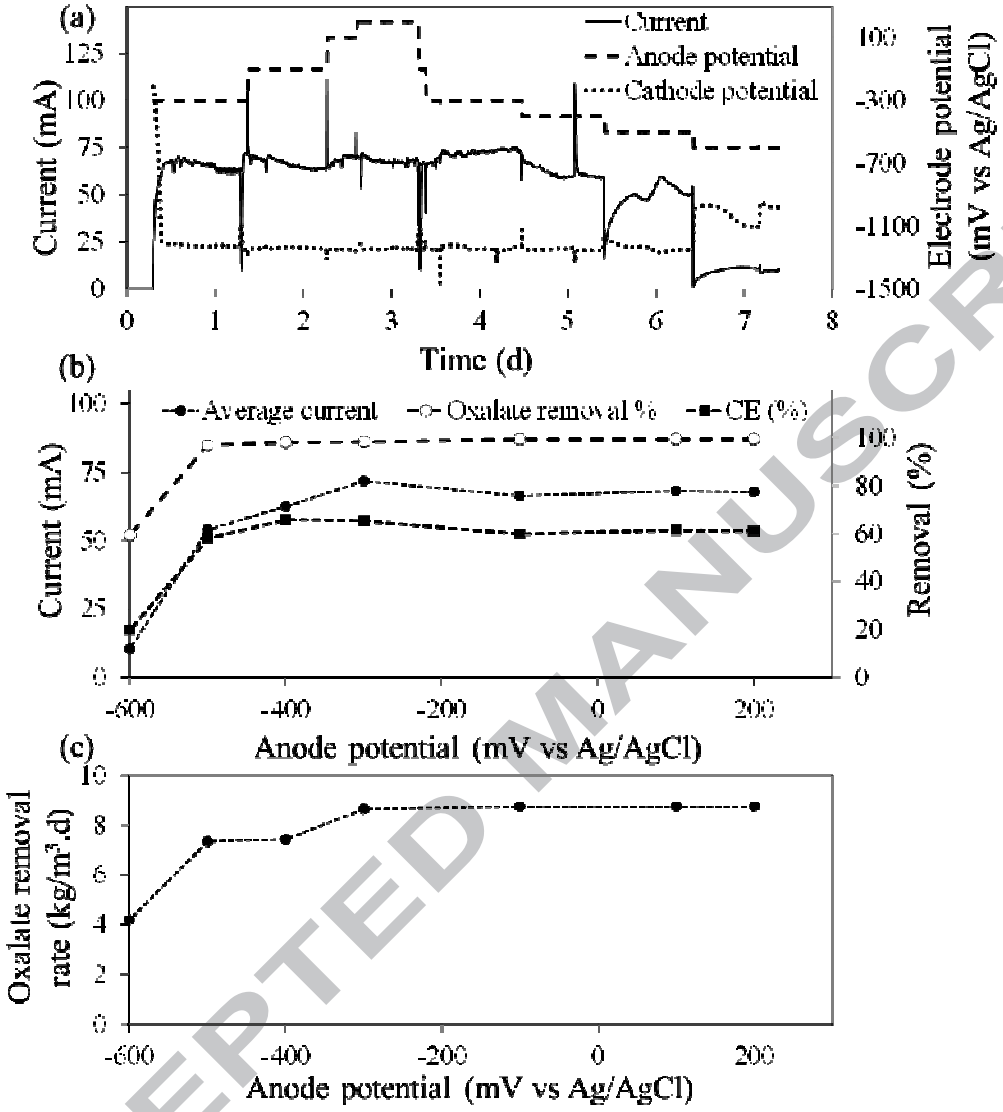

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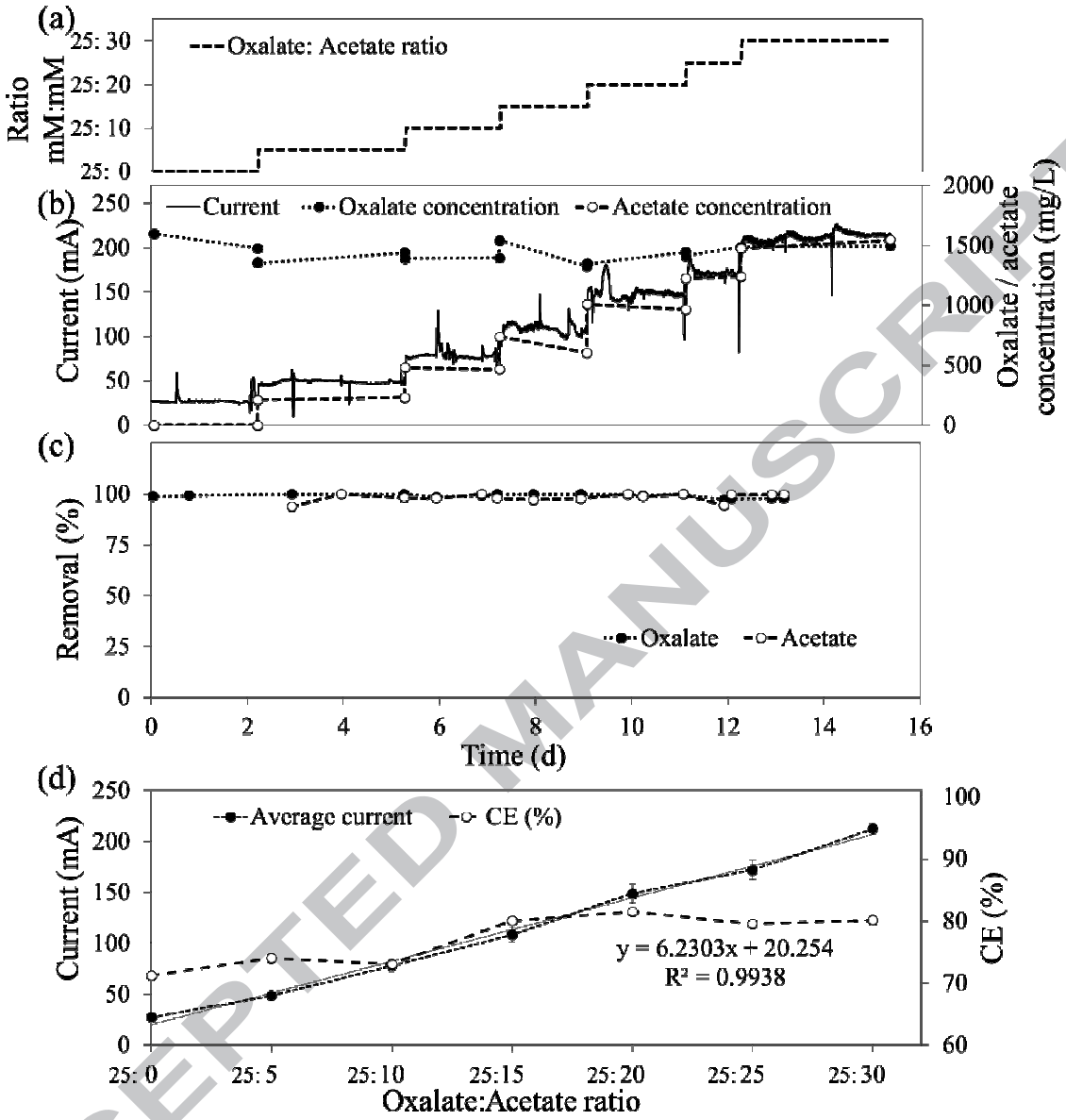
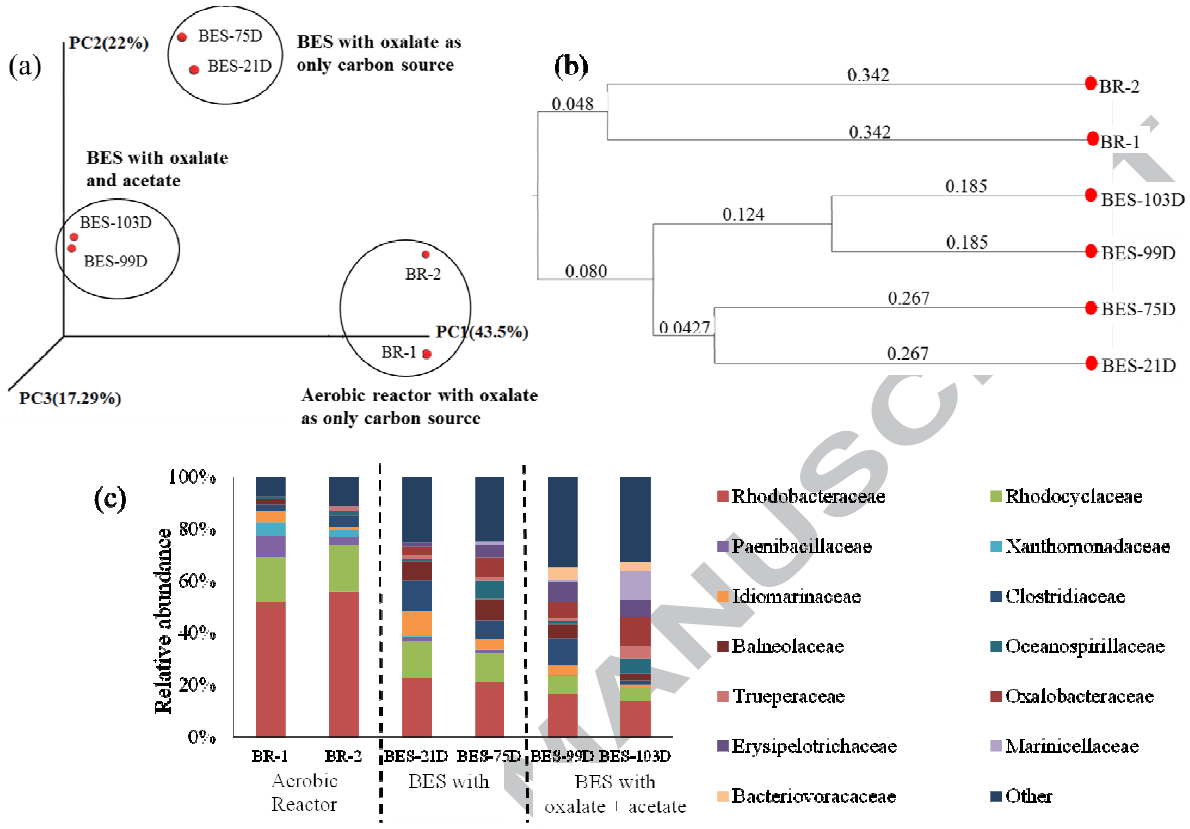


Fig. 6.



Highlights

- A bioelectrochemical system (BES) was successfully started up for oxalate removal
- Aerobic oxalotrophic biofilm pre-grown on graphite granules was used as inoculum
- The biofilm could rapidly switch from using oxygen to graphite as electron acceptor
- Highest coulombic efficiency (>70%) for anodic oxalate oxidation in the literature
- *Oxalobacteraceae* strains became abundant signifying their role in the BES process