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Abstract

This study examined for the first time the use of bioelectrochemical systems (BES) to entrap, decompose and oxidise fresh algal biomass from an algae-laden effluent. The experimental process consisted of a photobioreactor for a continuous production of the algal-laden effluent, and a two-chamber BES equipped with a graphite granules and carbon-felt anodic filter facilitating the physical removal and oxidation of the algal biomass from the effluent. Results showed that the BES filter could retain *ca*. 90% of the suspended solids (SS) loaded. A coulombic efficiency (CE) of 36.6% (based on particulate chemical oxygen demand (PCOD) removed) was achieved, which was consistent with the highest CEs of BES studies (operated in microbial fuel cell mode (MFC)) that included additional pre-treatment steps for algae hydrolysis. Overall, this study suggests that a filter type BES anode can effectively entrap, decompose and *in situ* oxidise algae without the need for a separate pre-treatment step.

Key words: Bioelectrochemical systems, waste stabilisation pond, algal biomass, suspended solids, anodic filter

1. Introduction

Waste stabilisation ponds (WSPs) are well suited for tropical climate areas to treat municipal wastewater especially from remote communities (Mara et al., 1998). Typically, a WSP system consists of serially-connected anaerobic, facultative and maturation ponds (Mara et al., 1992). Facultative ponds (FPs) facilitate much of the oxidation of organic matter through a mutualistic relationship between algae and bacteria, and the process relies heavily on natural factors (wind, sunlight, temperature) to fulfil the requirements of such mutualistic relationship (Pedahzur et al., 1993; Tharavathi & Hosetti, 2003). However, due to the reliance on these uncontrollable factors the removal of dissolved organic carbon (DOC) and

suspended solids (SS, concentration of up to 200 mg L^{-1}) are often inadequate (Ellis & Mara, 1983; Mara et al., 1998; Mara et al., 1992). With recent enforcement of stringent discharge limits (e.g. <30 mg L^{-1} of SS), most utilities have considered using additional processes to polish up the WSP effluent (Mara & Johnson, 2007). Rock filters have been identified as a simple and an economical technology for this purpose (Mara & Johnson, 2006; Short et al., 2007). As WSP effluent passes through a rock filter, SS are physically retained and subsequently oxidised biologically over time. However, excessive accumulation of SS can obstruct passive aeration and creates anaerobic conditions inside the rock filter, compromising COD removal (Saidam et al., 1995). Hence, alternative treatment technologies that could overcome these challenges are desirable for the wastewater industry.

Bioelectrochemical systems (BES) are an emerging wastewater treatment technology that can facilitate anaerobic oxidation of organic matter. These systems typically consist of a separated anode and a cathode chamber. Electrochemically active bacteria oxidise organic matter in the anode chamber using an inert electrode such as graphite as a final electron acceptor (Logan et al. 2006). The electrons donated by the bacteria subsequently flow through an external circuit to the cathode, where the electrons are accepted by electron acceptors such as dissolved oxygen. The anodic compartment of a BES could be engineered to functionally resemble a rock filter, i.e. to entrap and oxidise SS and DOC in wastewater. In this study, graphite granules and felts in the anode chamber simulated filtration aspects of a rock filter and the graphite media similar to rock media facilitated the formation of biofilms. A forced passage of WSP effluent through the anodic filter bed enabled entrapment of SS such as algal biomass in the anodic chamber. The hydrolytic, fermentative and electrochemically active bacteria resident on biofilm then facilitated hydrolysis, fermentation

and oxidation of soluble organic matter and SS entrapped in the BES anode chamber under complete anaerobic conditions. Since COD removal is facilitated under complete anaerobic conditions, this BES configuration (here on referred to as a "BES filter") addresses limitations of rock filters and could become a good alternative technology to replace rock filters.

The oxidation of DOC in a BES anode has been extensively reported in past literature (Hou et al., 2016; Kim et al., 2016; Venkidusamy et al., 2016). However, entrapment and oxidation of SS (specifically algal biomass) on a BES anode is yet to be fully investigated. There are a few studies in literature that have examined the potential use of a BES anode to oxidise algal biomass (Inglesby et al., 2012; Nishio et al., 2013; Strik et al., 2008). The algal feedstocks used in these studies, however, were subjected to a pre-treatment (thermal, physical, chemical and biological) step (Cui et al., 2014; Hur et al., 2014; Kondaveeti et al., 2014; Velasquez-Orta et al., 2009). This pre-treatment step was carried out with an assumption of difficulties to facilitate a hydrolytic, fermentation and/or a direct oxidation of algal biomass in a BES anode. For instance, a pre-treatment of algal biomass using ultrasound (3.5 W mL^{-1} for 20 min) enabled Wang et al. (2012) to achieve a coulombic efficiency (CE) of 25% using a dual chamber BES. Velasquez-Orta et al. (2009) also were able to achieve a similar CE (28%) in a single chamber BES by having a pre-treatment step that involved mechanical breakage of algal cells (drying and grinding). Walter et al. (2015) for the first time reported an attempt to continuously treat algal biomass using a BES and this required a continuous operation of a pre-digester that had a hydraulic retention time (HRT) of 10 days. The pre-digested algal biomass was continuously fed into the BES anode and current densities as high as 9.03 A m^{-3} were achieved. In their studies, the pre-digestion step was found to be critical to achieve higher current densities.

The aim of this study was to evaluate the use of the BES filter to entrap and oxidise untreated algal biomass without reliance on a separate pre-treatment step. This feature may be advantageous in practice as no additional pretreatment unit is required.To mimic algal discharge from a failed WSP, a reactor was operated in a continuous mode to enable an unrestricted supply of algal biomass to BES filters. The performance of the BES filter was assessed under two different settings: (i) close-circuit operation with current generation; (ii) open-circuit operation without current generation. Over the period of reactor operation, parameters such as current, total COD (TCOD), soluble COD (SCOD), particulate COD (PCOD), SS, volatile fatty acids (VFAs) were determined and coulombic efficiency (CE) of this study was compared with others that used algae as a feedstock in BES.

2. Materials and Methods

Figure 1 illustrates the experimental setup of this study, which was composed of two reactors: (1) an algal reactor – that facilitated a continuous supply of algae and (2) a BES reactor – which entrapped and oxidised algae discharged from the algal reactor.

2.1 Algal reactor design and operation

The algal biomass was continuously grown in a photobioreactor having a working volume of 14 L. An algal growth medium (BG-11) was continuously fed into the photobioreactor at a constant flow rate. The total flowrate from photobioreactor into the two BES filters (closed circuit reactor (R1) and open circuit (control) reactor (R2)) matched the influent (BG-11) flowrate into photobioreactor. The BG-11 medium adapted from Rippka et al. (1979) contained (per litre of de-ionized water) 1500 mg $NaNO_3$, 40 mg $K_2HPO4.3H_2O$, 75 mg

$MgSO_4.7H_2O$, 36 mg $CaCl_2.2H_2O$, 6 mg Citric acid, 6 mg Ferric ammonium citrate, 1 mg disodium magnesium ethylenediaminetetraacetic acid (EDTA), 20 mg Na_2CO_3 and 1 mL of trace element solution prepared as detailed in Cheng et al. (2008). Algal culture was obtained by exposing 5 L of primary effluent (Subiaco wastewater treatment plant, WA, Australia) to natural sunlight for a period of 14 days and 150 mL of the resulting culture was used as the inoculum for the algal bioreactor. The photobioreactor was initially operated in batch mode (with no continuous inflow of BG-11 medium) for a period of 17 days and was subsequently switched over to a continuous mode of operation. Two fluorescent lamps (24W; cool white, Philips), mounted at an approximate angle of 45° were placed 10 cm away from reactor to provide continuous light to the photobioreactor. Mixing was achieved using an overhead stirrer (250 rpm) and continuous aeration was carried out using an aquarium pump (4.2 L h^{-1}). Influent flow rate was set based on the growth rate of algae and the growth rate was determined by quantifying increase of biomass over time. A range of measurements (e.g. SS, TCOD, SCOD and optical density (OD_{600})) were carried out to quantify increase of algal biomass.

2.2 The configuration of the BES filters

Two identical Perspex BES reactors were used in this study (Figure 1). The anodic chambers of the BES reactors (dual chamber) were further subdivided into two compartments (with each 14 cm × 12 cm × 2 cm in dimension) using two layers of graphite felt (a single layer thickness of *ca.* 5 mm and surface area of 168 cm^2, MGM-Carbon Industrial, Ltd Co., China). Close to the top of reactor, a T shaped stainless steel sheet (15 cm^2) was firmly sandwiched between the two layers of graphite felt and was used as a current collector.

Algal biomass was fed continuously into the first compartment of the anodic chamber which was packed with graphite granules (3-5 mm in diameter, porosity 48%, KAIYU Industrial (HK) Ltd.). Two graphite rods (5 mm diameter, length 12 cm) were embedded in the granular bed and were also used as current collectors. The other compartment, which was adjacent to the cation exchange membrane (that separated the anodic and the cathodic chamber) was not packed with any media and the effluent port was located in this compartment. The location of the effluent port forced passage of algal media through both graphite granules and the felt that separated the two compartments enabling entrapment of algal biomass in the first compartment of the anodic chamber. The anolyte (algal medium, 480 mL) was continuously re-circulated (150 mL min^{-1}) from the second to the first compartment of the anodic chamber through a recirculation bottle (150 mL) using a peristaltic pump (Cole-Parmer, Victoria, Australia). The re-circulation bottle facilitated pH adjustment (feedback dosing of 1 M NaOH to control pH at 7) and maintenance of anaerobic conditions in anolyte (by sparging head space of the bottle with N_2 gas for 3 min at every 20 min interval). Online monitoring of pH was carried out using pH electrodes (Ionode, QLD, Australia) and pH control and recording of data was carried out with the assistance of a programmable logic controller (Compact-Rio, National Instrument, Austin, TX, USA) and a LabView software (National Instrument, Austin, TX, USA).

A silver-silver chloride (Ag/AgCl) reference electrode (MF-2079, RE-5B, BASi Bioanalytical Systems, Inc., IN, USA) was mounted in the second compartment of the anodic chamber and all electrode potentials (mV) were monitored in reference to the reference electrode (ca. +197 mV vs. standard hydrogen electrode). Of the two BES filters that were operated, one was operated in close circuit mode allowing current to be produced (R1) and the other was operated in open circuit mode with no current production (control, R2). To

enable precise control of anodic potentials, minimise cathodic limitations and overcome ohmic limitations of the system, a poised potential of +200 mV was applied to the anode of R1 using a potentiostat (VMP3, BioLogic, Claix, France). The current produced in R1 and the working electrode potential (WE) of R2 was measured using the potentiostat and the programmable logic controller was used to control and record measurements of the potentiostat.

The cathodic chamber of the BES was also packed with graphite granules and two graphite rods (5 mm diameter, length 12 cm) were embedded in the granular bed as current collectors for the cathode. A phosphate buffer of pH 7.05 (5.356 g L^{-1} K_2HPO_4 , and 2.62 g L^{-1} KH_2PO_4) was used as catholyte and was continuously recirculated (150 mL min^{-1}) through the cathodic chamber using a peristaltic pump (Cole-Parmer, Victoria, Australia). All reactors were operated at ambient temperature (22-25℃).

2. 3 Start up and operation of BES filters

The anodic chambers of R1 and R2 were inoculated with 1% (v/v of anodic chamber) of returned activated sludge (RAS, Subiaco wastewater treatment plant, Western Australia). The BES filter operation can be divided into five stages. Initially, the effluent of the photobioreactor was continuously fed into the anodic chambers of R1 and R2. At a flow rate of 0.34 mL min^{-1} similar to influent reaching compartment 1 of anodic chamber, effluent was discharged from compartment 2 of anodic chamber using peristaltic pumps (Cole-Parmer, Victoria, Australia). This mode of operation continued for a period of 34 days (Stage 1). Both anodic and cathodic chambers of R1 and R2 were covered with black rubber to prevent light-dependent phototrophic activities.

While maintaining continuous mode of operation, on day 35, 1% (v/v of anodic chamber) anaerobic digester sludge (Beenyup wastewater treatment plant, Western Australia) was added into anodic chambers of R1 and R2 to introduce hydrolytic and fermentative bacteria into the system. Subsequently the reactors were closely monitored for a period of 39 days (Stage 2). After 74 days of operation at a poised potential of +200 mv, different anodic poised potentials were applied over a period of 8 days to R1 to examine electrochemical properties of the established biofilm (Stage 3). Stage 2 conditions were thereafter continued for another 22 days. During Stage 4 (starting on day 104), the BES filters were switched to batch mode operation for a period of 40 days (until current of R1 decreased approximately to 0 mA). On day 144 a concentrated volume of algal biomass was injected and reactor performance was monitored in batch mode until the current returned back to the same baseline level (Stage 5).

2.4 Assessment of electrochemical properties of R1 (stage 3)

To evaluate anodic activity of the established biofilm in R1, the poised potential was varied. In addition to OCP, potentials of -600, -500, -400, -200, 0 and +200 mV were examined over a period of 8 days (days 74-81). At each applied potential, current was allowed to reach steady state over a period of 24 h (1 HRT) and two anolyte samples were collected prior to change over to the next setting. To minimise possible shifts of anodic microbial community structure and examine the effect of applied potential on the biofilm activity, the duration of exposure to each of the applied potentials was restricted to 1 HRT. TCOD and SCOD were immediately measured on collection of the samples and PCOD was calculated by subtracting SCOD from TCOD. Volatile fatty acids (VFAs) measurements were carried out after filtering

the samples through 0.22 μm syringe filters (Merck Millipore, USA). The samples were stored at -20ºC until analysed for VFAs. All measured parameters were plotted against the electrode potentials.

2.5 Concentration of algal biomass for stage 5 injection

Algal biomass from photobioreactor was collected into Imhoff cones and was allowed to settle for 3 hours. The sediment layers at the bottom of cones were collected and centrifuged (4000 × *g* for 30 minutes) and supernatants were discarded. Pelleted algae were resuspended in deionised water and centrifuged once again and the supernatant was discarded. The algal pellet was then re-suspended in 25 mL of algal growth medium. TCOD and SCOD of the concentrated algal biomass were 13,125 ± 121 mg L^{-1} and 42 ± 6.1 mg L^{-1}, respectively.

2.6 Analysis and calculations

SCOD and TCOD analyses were performed using HACH reagents (cat no. TNT 821; method 8000, LR) and a spectrophotometer (GENESYS 20, Thermo Scientific). Algal biomass densities were monitored measuring absorbance (A) at 600 nm using the spectrophotometer and normalising the absorbance values to optical density (OD) using optical pass length (L) of 1 cm and equation OD = A/L. Coulombic efficiency (CE) (i.e., the percentage of electrons recovered as anodic current from the PCOD removed) of the anodic reaction was calculated as detailed in literature (Logan et al., 2006). Particulate matter was quantified by measuring SS. SS was measured as described in standard methods for examination of water and wastewater (APHA, 1992). VFAs were measured by an external laboratory (Department of Agriculture Western Australia, Australia) using a method detailed by Christophersen et al. (2008). In brief a gas chromatograph (GC) fitted with a flame ionization detector (Agilent 6890 series) and capillary column (HP-FFAP, 30 m × 0.53 mm × 1.0 m, Agilent) was used.

The operational temperatures of the oven, injection port and the detector were 100°C, 260°C and 265°C, respectively. The data was processed with Chemstation software (Agilent) and the following VFAs were determined: acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, valeric acid and caproic acid. The concentrations of individual VFAs was converted to SCOD. All analytical measurements were carried out in duplicate.

3. Results and Discussion

3.1 The photobioreactor for a continuous supply of algal biomass

In this study, a photobioreactor was used to provide a continuous supply of algal biomass to the BES filters. As shown in Figure 2, a maximum and stable optical density of approximately 0.2 nm cm^{-1} was achieved after 14 days of process initiation. The OD_{600} changes of the photobioreactor were analogous with PCOD and SS changes. Considering OD_{600} is indicative of algal biomass abundance, the increase of SS and PCOD in this instance can be attributed to an increase of algal biomass in the reactor. The SCOD concentration remained relatively stable throughout the experimental period and hence the increase of TCOD was largely a result of an increase of particulate organic matter, which in this instance was algal biomass. Considering 14 days was required to achieve maximum biomass concentration, the specific growth rate of the algal biomass was estimated to be 0.07 d^{-1}. Based on this specific growth rate, the photobioreactor was operated in a continuous mode with inflow and outflow rates maintained at 0.69 mL min^{-1} to achieve a HRT of 14 days. This enabled an algal biomass loading of 250 ± 11.3 mg $L^{-1}d^{-1}$ to each of the BES filters. The SS discharged from a failed WSP is largely in the form of algal biomass and according to Ellis

and Mara (1983) the SS concentrations of such a pond could be up to 200 mg L^{-1}. Accordingly, the photobioreactor of this study resembled a failed WSP.

3.2 Process start up and initial examination of the BES performance (stage 1)

After inoculating both R1 and R2 BES filters with RAS, the anodic chambers of both reactors were continuously fed with algal biomass from the photobioreactor for a period of 34 days. Each reactor received algal biomass containing influent at a flow rate of 0.34 mL min^{-1} and this enabled maintenance of a hydraulic retention time (HRT) of 1 day. According to past literature, a duration of 34 days is more than sufficient to enable establishment of an active anodophilic biofilm (Cheng et al., 2012). During this period there was also an unlimited supply of organic matter for anodic oxidation due to entrapment of SS (> 50% of SS fed were entrapped) in the BES filters (Figure 3E). However, R1 only showed a marginal increase of current (<1 mA) and the anodic potentials of R2 also did not show a significant decrease and remained at approximately -70 mV (Figure 3B). Walter et al. (2015) also reported a similarly low current (0.03 ± 0.006 mA) with direct input of algal biomass into BES anode. Although algal biomass (approximately 4.2 g loaded over 34 days) is a source of organic matter, it does not appear to be readily oxidisable by the anodic bacteria. There was no measurable change of SCOD and VFAs in effluent of both R1 and R2 during this period of operation indicating a limited hydrolysis of algal biomass. This suggested a limited abundance of hydrolytic and fermentative bacteria in the inoculum (RAS) used.

When the efficiency of the BES filters to entrap and oxidise algal biomass was quantified, an effective removal of SS was noted in both reactors. The SS removal gradually improved over

time from approximately 50% to a maximum of 80% over the period of 34 days. The entrapment of algal biomass in the BES filters may have improved the further retention of algal biomass. However, when CE was calculated based on total amount of coulombs loaded (derived from PCOD removed) and coulombs recovered (as measurable current) only a 4.1% efficiency was noted during the initial 34 days of operation. This further confirmed the limited ability of the anodic biofilm to retrieve electrons from oxidation of retained algal biomass.

3.3 Addition of anaerobic sludge enhanced current generation (stage 2)

With previous data suggesting inability of the resident bacteria in the anodic chamber to facilitate hydrolysis and fermentation of algal biomass, it was decided to re-inoculate both R1 and R2 with some anaerobic digester sludge. The inoculation with digester sludge was to increase the abundance of hydrolytic and fermentative bacteria in the anodic chambers of both R1 and R2 to facilitate the hydrolysis and fermentation steps, which appeared to be limiting the current generation as indicated by the low VFAs concentration in the BES filter effluents (Figure 3).

On inoculating with anaerobic biomass on day 35, an increase of anodic current was noted within a very short period of time (<5 days) (Figure 3A). The working electrode potential of the open circuit control (R2) also gradually declined from -70 to a steady -420 mV after 35 days of inoculation (Figure 3B). These results suggested an increased availability of electron donor for the anodic bacteria and the digester biomass appeared to have contributed towards this increase by releasing the reducing power entrapped in algal biomass. Algal biomass is largely composed of complex proteins, carbohydrates and lipids and past literature

demonstrates the importance to hydrolyse these compounds to simple monomers before they could be oxidised by bacteria (Middelboe et al., 1995; Nishio et al., 2013; Vergara-Fernandez et al., 2008). Over a period of 70 days the current increased up to 6.2 ± 0.15 mA with a stable SS loading rate of approximately 250 ± 13.3 mg L^{-1} d^{-1}.

The increase of current in R1 was expected to coincide with an increase of SCOD in R2 effluent. While no significant increase of SCOD was observed in R1 effluent, a gradual increase of SCOD (from 75.5 ± 6.1 mg L^{-1} to 144 ± 9.3 mg L^{-1}) was noted in the effluent of R2 during stage 2 of reactor operation (Figure 3C). When SCOD of R2 effluent was further analysed for VFAs, VFAs increase from 0.7 ± 0.2 mg- SCOD L^{-1} to 28.3 ± 3.8 mg - SCOD L^{-1} was observed during the same period of operation. There were no measurable concentrations of VFAs in R1 BES filter effluent. Compared to R2 control, of the low SCOD in R1 effluent was likely a result of SCOD oxidation in anodic chamber of R1 via current generation pathways.

To infer the prevalence of SCOD removal pathways in R2, a coulombic balance was calculated between R2 and R1. Since the loading of TCOD (algal biomass) in both R1 and R2 were identical, the total coulombs available for both R1 and R2 were also identical. If one assumed that the lower amount of SCOD in R1 (compared with that in R2) was exclusively caused by current production, then one would expect the coulombic balance between this amount of SCOD (16,960 coulombs) and the current (19,890 coulombs) to be close. However, a noticeable difference of 2,930 coulombs was noted due to a shortfall of coulombs in SCOD of R2. Such a shortfall may be due to unaccountable electron sinks such as methanogenesis in R2. Further studies are required to verify this.

3.4 The influence of anodic potential on the performance of BES filter (stage 3)

On inoculation of BES filters with anaerobic biomass the current generation of R1 gradually increased at stage 2. To investigate to what extent anodic current production could influence effluent quality (quantified as TCOD, SCOD, PCOD and VFA), R1 was exposed to different anodic potential set points (OCP, -600, -500, -400, -200,0 and +200 mV). Figure 4A and B detail how effluent quality and current production changed in response to different set points. The increase of anodic potential increased current production (from 0 mA to 5.78 ± 0.14 mA) demonstrating the prevalence of an active anodic biofilm in R1. The current negatively correlated with both SCOD and PCOD concentrations in the R1 effluent. Specifically, an increase of current from 0 to 5.78 ± 0.14 mA resulted in a SCOD and PCOD reduction from 137 ± 3.8 to 78 ± 0.5 mg L^{-1} and from 10.3 ± 0.3 to 5.2 ± 0.1 mg L^{-1}, respectively (Figure 4A and B). While the influence of current on R1 effluent SCOD concentrations was apparent, the influence of current on R1 effluent PCOD concentrations remained unclear. One explanation could be given on the lines of a feedback inhibition of hydrolytic bacteria (Wang et al., 2012). The exposure of anode to unfavourable anodic potentials could potentially have caused feedback inhibition of hydrolytic bacteria due to a build up of SCOD with low current generation, resulting in a higher discharge of PCOD from R1.

An accumulation of SCOD in R1 with an application of unfavourable potentials confirms co-existence of hydrolytic and fermentative pathways with current generation pathways. According to Kondaveeti et al. (2014), the hydrolysis of algal biomass produces a range of different compounds and acetate is one of the major end products. In this study under open circuit conditions, acetic and propionic acids were observed accumulating in R1 at a rate of

28.1 ± 1.3 mg-SCOD L^{-1} d^{-1} and 5.2 ± 0.88 mg-SCOD L^{-1} d^{-1}, respectively. No other VFAs were observed in noticeable concentrations. When exposed to favourable anodic potentials, VFAs accumulation was almost non-existent in R1 suggesting that these two VFAs (acetate and propionate) in particular may be oxidised anodically via current generation pathways. Being a non-fermentable substrate, the decrease of acetate in particular has to occur via current generation pathway. Further, under open circuit, the SCOD concentration (137 ± 3.8 mg L^{-1}) far exceeded the total VFAs concentration (28.1 ± 1.3 mg-SCOD L^{-1}) in R1. This confirms the prevalence of other forms of organic carbon as SCOD. When exposed to favourable potentials, the SCOD in R1 decreased from 137 ± 3.8 mg L^{-1} to 78 ± 0.5 mg L^{-1} (close to the SCOD levels observed in effluent of the photobioreactor).

3.5 The BES filter is a self-cleansing filter(stage 4)

The BES-filter successfully removed up to 92 % of algal biomass (SS removal) that was continuously loaded over a period of 104 days (Figure 3E). If the entrapped algae was not continuously removed through some mechanisms, inevitably the filter should have clogged. Even with continuous loading of algae (250 ± 13.3 mg $L^{-1}d^{-1}$) for 104 days, the BES-filter showed no signs of clogging suggesting that a self-cleansing mechanism existed in both R1 and R2. A self-cleansing mechanism in this instance refers to mechanisms that involves decomposition of algae into gases (i.e. CO_2 or CH_4) or soluble compounds. To demonstrate the self-cleansing ability of the BES-filters, the feeding of algal biomass into R1 and R2 was discontinued (stage 4 – from day 104 to 143). Thereafter, R1 continued to maintain the current (6.16 ± 0.23 mA) for a period of 4 days. Subsequently over a period of 40 days the current gradually declined to <1 mA (Figure 3A). In R2 the anodic potential gradually declined and became more negative (from -430 to -503 mV) (Figure 3B).

R2 BES-filter also showed accumulation of SCOD and at the end of 134 days a stable concentration of approximately 1198 ± 64.2 mg L^{-1} was observed (Figure 3D). This corresponded to an average SCOD accumulation rate of 35.1 mg $L^{-1}d^{-1}$. A similar trend was recorded for VFAs accumulation (10.76 mg-SCOD L^{-1} d^{-1}). In contrast only a marginal increase of SCOD (from 75 ± 5.8 to 193 ± 12.8 mg L^{-1}; accumulation rate 3.93 mg L^{-1} d^{-1}) was observed in R1 BES filter. This confirms an efficient removal of SCOD in R1 and it also implies a marginal dilution impact of SCOD with a continuous feed of algal biomass. The dilution impact in R2, however, could have been more profound as a significant increase of SCOD was observed during stage 4 operation. If continuous mode of operation can be assumed to minimise occurrence of a feedback inhibition of hydrolytic pathways (by preventing excessive build up of SCOD), feedback inhibition may be one explanation to the non-linear accumulation of SCOD during stage 4 (no continuous feeding) operation of R2 (Wang et al., 2012). Other contributing factors may be a possible increase of methanogenesis (due to increase of SCOD concentrations) and a gradual exhaustion of substrate (i.e. algal biomass).

The build up of SCOD in R2 was likely due to hydrolysis and/or fermentation of the entrapped algal biomass. VFAs, also quantified, is a subset of SCOD, and this fraction only accounted for 30% of the total SCOD. The remaining 70% was likely constituted by chlorophyll, amino acids, sugars, alcohols and other fermentation products (He et al., 2009; Lee et al., 2008; Velasquez-Orta et al., 2009). The low SCOD concentration in R1 suggested that the anodic bacteria were capable of oxidising some of these compounds. Although SCOD concentrations of R1 were low during stage 4 operation, a gradual increase of SCOD was noted possibly due to an accumulation of the non-anodically oxidisable SCOD fraction.

In continuous mode of operation the SCOD released from algae was diluted with new influent, and hence the SCOD concentrations in both the influent and effluent of R1 were only marginally different.

3.6 The coulombic efficiency of R1 – a calculation suited for a BES filter

This study for the first time reports of a BES filter designed to trap and anodically oxidise algal biomass without any separate pre-treatment. Unlike in most BES studies reported in past literature the influent COD of this study was primarily in the form of PCOD. Hence, calculation of CEs was carried out based on PCOD (TCOD – SCOD) entrapped ($PCOD_{IN}$ – $PCOD_{OUT}$) in the BES filter. To date there are no reports of any BES that has been directly fed with algal biomass as previous studies have used hydrolysed algal biomass. Since no SS was introduced, CEs in these BES were calculated based on SCOD only. SCOD removed ($SCOD_{IN}$ – $SCOD_{OUT}$) was considered as the coulombs removed and the current produced was used to calculate coulombs recovered. Accordingly, a comparison of CE of this study with those of others should be done with caution and with an understanding of this difference in the calculation of CEs.

From stage 1 to the beginning of stage 4 (a total of 104 days), the BES filters were operated in continuous mode and the algal biomass loading rate into the BES filters was approximately 250 ± 13.3 mg-SS $L^{-1}d^{-1}$. Accordingly, each BES filter would have received 12.6 g-SS of algal biomass throughout this period. Of this 12.6 g-SS, the BES filters were able to retain 9.9 g-SS of algal biomass. This 9.9 g-SS algal biomass was equivalent to 8.2 g of PCOD. This approach was taken also to derive algal biomass retention (quantified as PCOD) at regular

time intervals and was used to report CEs (calculated based on an average current measured between time intervals) over time (Figure 3A). Accordingly, a gradual increase of CE was observed and during steady state of operation, the system achieved a CE of 36.6 %. During stage 4, continuous loading of algal biomass was terminated by switching the system from continuous to batch mode. Interestingly, even without the supply of carbon, the system continued to produce current for a period of 40 d. This suggests an *in situ* supply of electrons and confirms the prevalence of hydrolytic and fermentative pathways in the anodic chamber of R1.

To further consolidate response of anodic biofilm to algal biomass a concentrated mass (TCOD and SCOD 13,125 ± 121 mg L^{-1} and 42 ± 6.1 mg L^{-1} respectively) of algae was injected to R1 in stage 5. As can be seen on Figure 3A, the current gradually increased from 0 mA to 2.2 mA over a period of 8 days and subsequently declined to previous background levels within the next 7 days (i.e. by day 158). The CE for stage 5 operation was 28.5 %. This further confirms the anodic biofilm's ability to decompose and *in situ* oxidise algal biomass.

To examine whether CEs calculated using PCOD is a reasonable measure of efficiency of the BES filter, CEs were also derived over time using TCOD measurements. Since SCOD in influent and effluent were quite similar, CEs calculated based on TCOD removal were only marginally different to CEs calculated based on PCOD (data not shown). For example, during steady operation of BES filter a CE of 36.6 % and 35.5 % was observed when PCOD and TCOD were used respectively for CE calculations.

4. Implication of the findings

This study proposed a new BES configuration that could potentially polish WSP effluent contaminated with high levels (up to 200 mg L^{-1}) of algal biomass. The aim was to propose a technology that would enable overcome limitations of rock filters. Based on the filtration concept of a rock filter, for the first time, Mohammadi Khalfbadam (2016) demonstrated a BES filter configuration that could remove and oxidise particulate and soluble organic matter from municipal wastewater. This study extended the previous findings by demonstrating that algal biomass also could be entrapped (up to 92 %) and decomposed anaerobically via electrochemical pathways in these BES filters. This is the first report of a BES anode that enabled algal biomass decomposition and oxidation. Past researches to date stressed the importance of having a pre-treatment step prior to anodic oxidation of algae. The CE (36 %) of this study was consistent with CEs of BES operated in MFC mode (e.g. a CE of 28 % and 25% by Velasquez-Orta et al. (2009) and Wang et al. (2012) respectively) that used a pre-treatment step to enable solubilisation of algae. However, the BES of this study was operated in microbial electrolysis cell (MEC) mode. If the BES of the above studies were also operated in MEC mode to overcome ohmic resistance and cathodic limitations, it is likely that these studies would likely have achieved higher CEs to that of what is reported in the present study (Cusick et al., 2010). The findings of this study could have economic (due to the elimination of the pre-treatment step), environmental (elimination of the pre-treatment may reduce footprint) and process implications (a finely tuned symbiotic relationship of hydrolytic, fermentative and anodic bacteria could enhance oxidation of algae) by eliminating the need for a separate pre-treatment process.

5. Conclusions:

For the first time a BES filter was demonstrated to replace the need of a pre-treatment step to facilitate anodic oxidation of algae. Showing no signs of clogging, the BES filter was able to entrap 92 % of SS from influent. The results indicate *in situ* hydrolysis, fermentation and anodic oxidation of entrapped algal biomass and a CE of 36.6 % was noted during stable operation of reactor. In addition to VFAs, the anodic biofilm was able to successfully oxidise other organic compounds.

6. Acknowledgments

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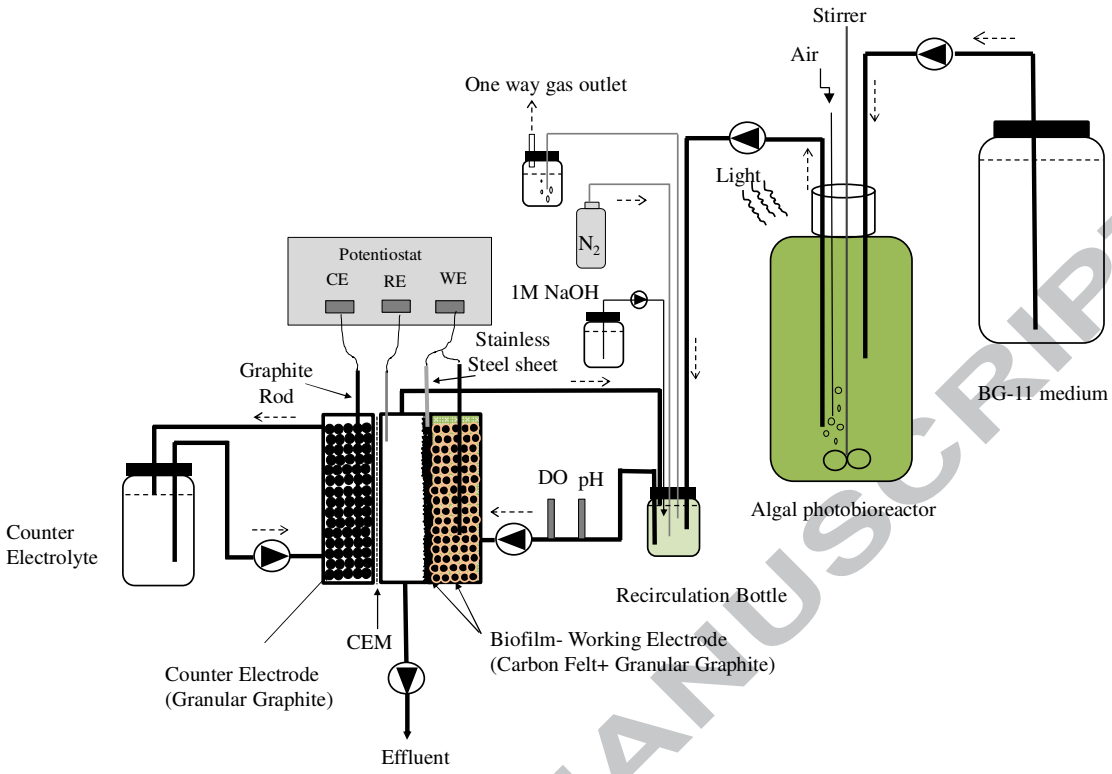

Figure1. Schematic diagram of the BES filter operated in continuous mode. CE= counter electrode; RE= reference electrode; WE= working electrode; CEM= cation exchange membrane; BG-11 medium= algal growth medium.

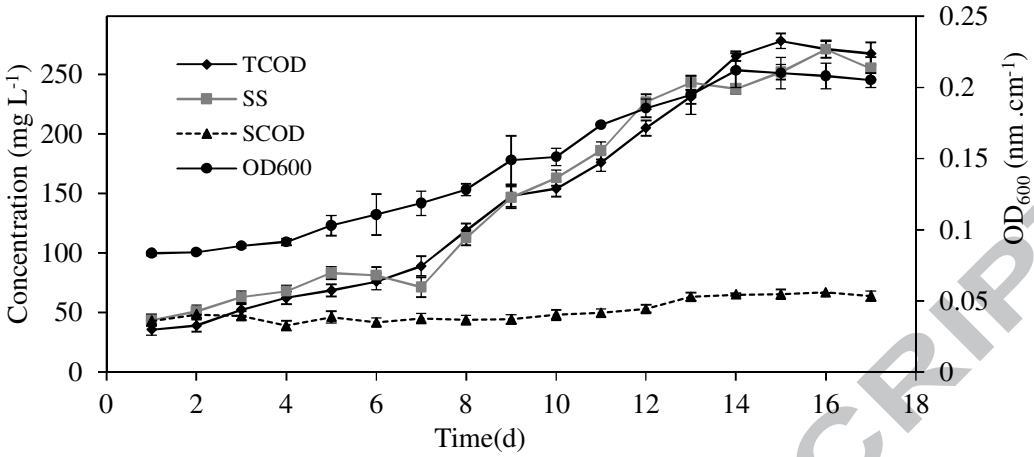

Figure 2. Changes in total chemical oxygen demand (TCOD), suspended solids (SS), soluble chemical oxygen demand (SCOD) and optical density measured at 600 nm (OD_{600}) of algal biomass in the photobioreactor. (Algal growth rate= 0.07 d^{-1}).

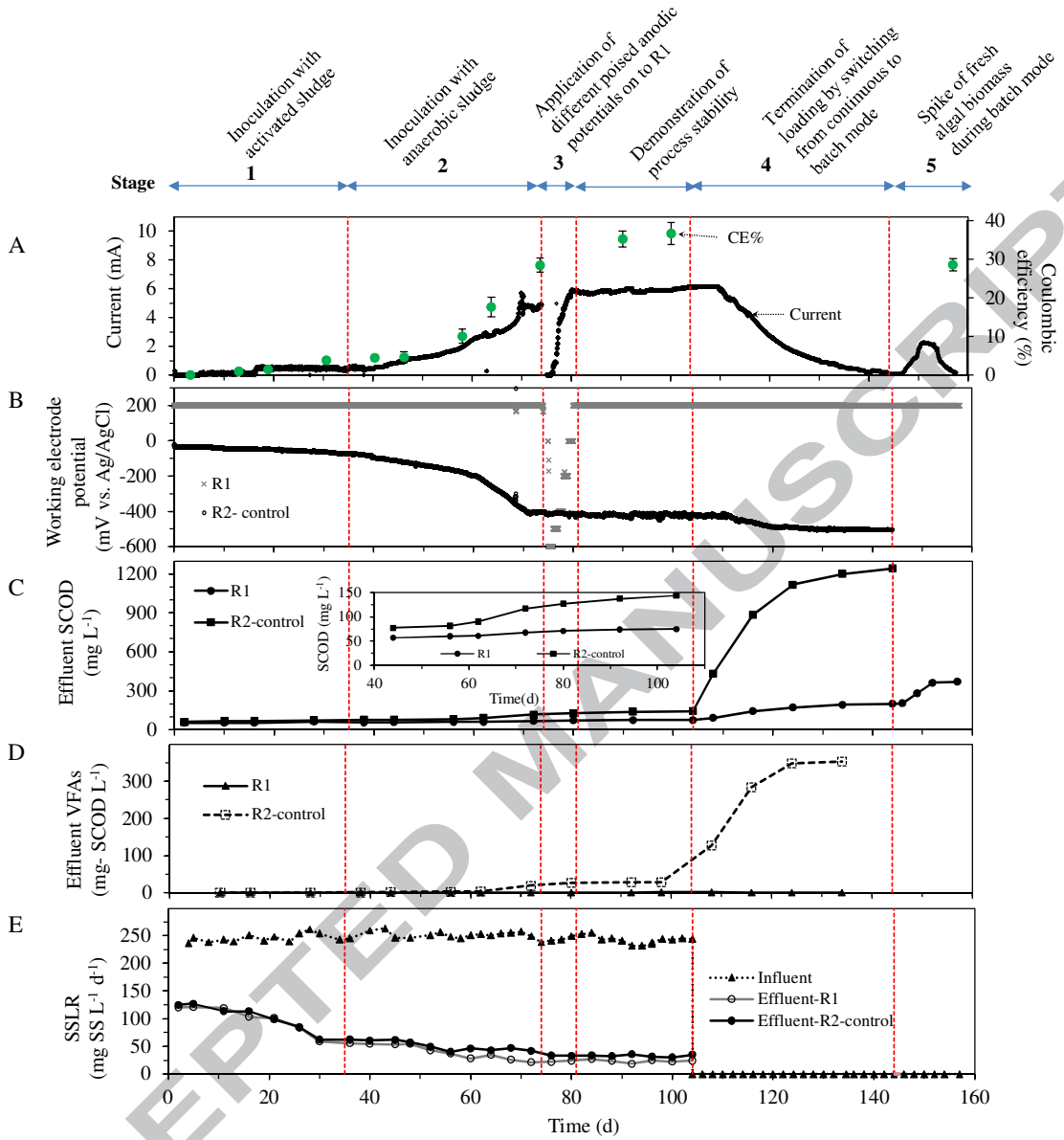

Figure 3. (A) Current generation and coulombic efficiency , (B) working electrode potential of R1 and R2, (C) soluble chemical oxygen demand (SCOD) in R1 and R2 effluents, (D) volatile fatty acids (VFAs) in R1 and R2 effluents calculated as SCOD, and (E) suspended solids loading rate (SSLR) and suspended solids removal of R1 and R2 over time. Numbers (1-5) represent different stages of operation used for the BES filters. (R1: close circuit reactor, R2: open circuit reactor- control, CE: coulombic efficiency).

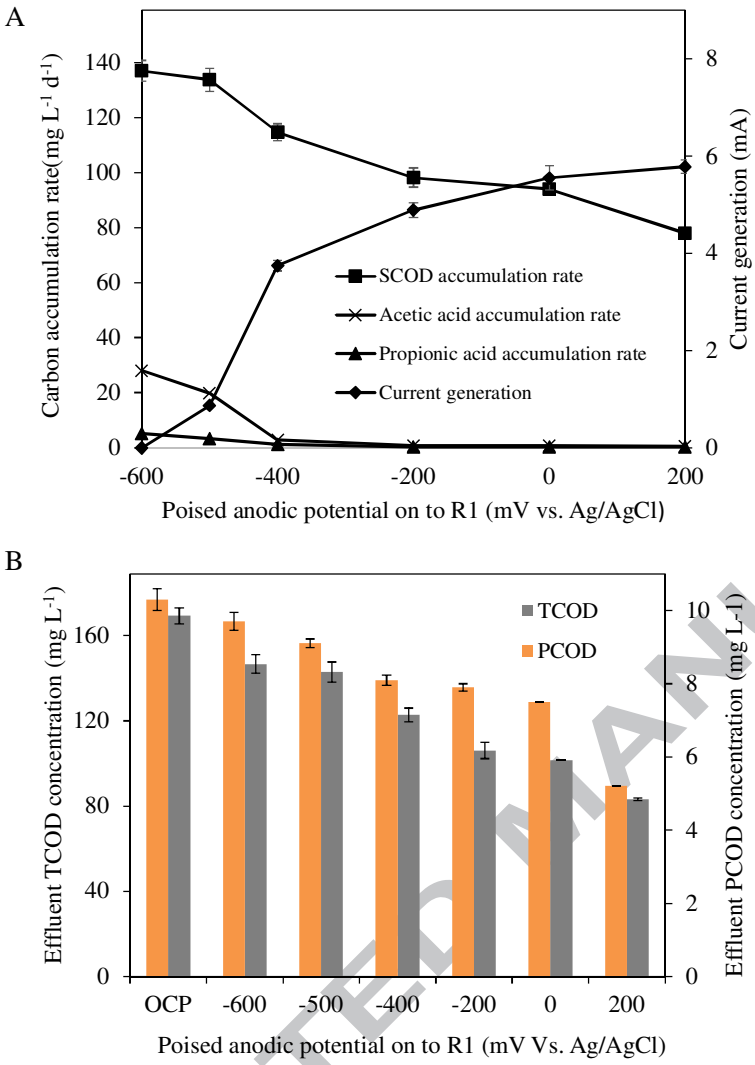

Figure 4. (A) Current generation and carbon (soluble chemical oxygen demand (SCOD), acetic acid and propionic acid) accumulation rate at anodic potentials ranging from -600 to +200 mV. The error bars of current profile are indicative of the stable current measured over the latter 5 h of a 24 h exposure to a set potential. (B) Total (TCOD) and particulate (PCOD) chemical oxygen demand (COD) concentrations at open circuit potential (OCP) and anodic potentials ranging from -600 to +200 mV.

Highlights

- A BES filter that facilitates entrapment and anodic oxidation of untreated algae.
- With no signs of clogging, the filter removed 90 % of SS from influent.
- A CE of 36.6 % was achieved with anodic oxidation of untreated algae.