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Enhanced disinfection of wastewater by combining wetland treatment with bioelectrochemical H_2O_2 production

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Abstract

A highly-loaded constructed wetland (up to 44 ± 21 gCOD m^{-2} d^{-1}) was connected to a bioelectrochemical system (BES) to produce hydrogen peroxide for disinfection purposes. The anode delivered a current from the wetland effluent up to 3.5 A m^{-2} (maximum 62% anodic efficiency) but was limited in the supply of organic carbon. Hydrogen peroxide could be produced *in situ* in wetland effluent. Production rates were tested at various current densities with a maximum rate of 2.7 g $m^{-2}_{electrode}$ h^{-1} (4 h at 10 A m^{-2}, 41% cathodic efficiency). Little difference was observed between production rate in wetland effluent or a 0.3% NaCl solution. The resulting hydrogen peroxide (0.1%) was used to disinfect wetland effluent successfully (<75 CFU ml^{-1} after 1 h contact time). The combination of wetland water treatment with peroxide production in a BES thus enables generating higher water qualities, including disinfected water, without external input of chemicals.

1. Introduction

With a growing world population, the pressure on safe (drinking) water supplies increases (Un-Habitat, 2010; Unep, 2008). Numerous technologies exist to provide clean and safe water flows. However, most technologies are only viable at larger scales and/or require maintenance and quality control by trained operators. Therefore it is not possible to supply people in remote areas or in developing countries with adequate wastewater treatment and supply of fresh water. Constructed wetlands (CWs) are a basic form of wastewater treatment where (domestic) wastewater is treated in case no access to advanced wastewater treatment facilities is available. Although, at first glance, this seems a basic form of treatment, this technique is widely applied, in low as well as highly populated areas (Karathanasis et al., 2003; Puigagut et al., 2007; Rousseau et al., 2004). Moreover, wetland water treatment systems are also employed in intensive horticulture systems (Gruyer et al., 2013). Removal of contaminants occurs by the combined action of among others; 1) sorption on bed material and plant roots, 2) microbial transformations and 3) plant uptake (Vymazal, 2005). The main drawback of such a treatment system with little to no operational controls is the (seasonal) variability of organic carbon, nutrient and pathogen removal resulting in variable performance. This leads to effluent qualities (in terms of organics and/or nutrients) that are not always in compliance with regulations (Karathanasis et al., 2003; Kern & Idler, 1999; Puigagut et al., 2007), although various studies also state that wetland treatment confers good removal on some parameters (Gruyer et al., 2013; Rousseau et al., 2008). In General, microbial indicators in wetland effluent are not in agreement with the most stringent water regulations i.e. for drinking water (Council Directive 98/83/EC). However removal efficiencies between 93 - 99.9% can be obtained for microbial contaminants (Gruyer et al., 2013; Karathanasis et al., 2003). Removal of microbial indicators does not seem to be correlated to the plant species present in the wetland (Karathanasis et al., 2003).

Besides treatment of wastewaters, (constructed) wetlands have been suggested as a source of electrical power generation by the use of a plant-microbial fuel cell (plant-MFC) (De Schamphelaire et al., 2008; Fang et al., 2013; Strik et al., 2008). Electrical power is generated based on the subsequent action of plant photosynthesis, root exudation processes and oxidation of organic matter by microorganisms with electron transfer to an anode. Plant derived organic carbon is enters the soil by means via rhizodeposition processes. Various

microorganisms can, under anaerobic conditions, oxidize this organic carbon and generate electrons that can be transferred to a conducting material, the anode electrode. In this paradigm, the electrons are transferred over an external load to the cathode where oxygen reduction to water takes place (De Schamphelaire et al., 2008; Strik et al., 2008). A plant-MFC can be integrated in a green roof, agricultural settings or in constructed wetlands (De Schamphelaire et al., 2008; Fang et al., 2013; Helder et al., 2013; Strik et al., 2008). However, plant-MFCs suffer from low performance due to large internal resistances, inconsistent substrate supply and competing reactions (Timmers et al., 2011; 2012). This is in contrast with reactor-based MFCs (or bioelectrochemical systems, BES) where a more optimized configuration and feed flow can lead to relatively high power densities (Aelterman et al., 2006; Logan et al., 2006; Rabaey et al., 2005). Interestingly, the cathode reaction can be tuned towards the production of hydrogen peroxide from air-derived oxygen, enabling disinfectant production from wastewater (Fu et al., 2010; Modin & Fukushi, 2013; Rozendal et al., 2009).

Here, a combined system (Figure S1 in electronic supporting information) is introduced which aims to maximize the benefits of both wetland and BES. In this new concept, part of the wetland will act as a rapid filter, retaining and transforming suspended solids into soluble organics. The soluble organics combined with rhizodeposits are fed into the anode compartment of a BES. Using this procedure more engineering control (mixing, flow rates) can be achieved to supply organic carbon to the microorganisms on the anode electrode, compared to anodes in sediment systems. The bacteria on the anode will oxidize organic matter into electrical current and CO_2. At the cathode O_2 is reduced to H_2O_2. The BES is controlled by a potentiostat so that favourable potentials for both reactions are maintained. The produced hydrogen peroxide is subsequently used for downstream disinfection of (at least part of) the effluent of the wetland-BES system. In case nutrient removal is not sufficient, the effluent of the anode can be sent through another section of wetland with this effluent being sent through the cathode for final disinfection. Now a cleaner water flow is achieved that can be used for irrigation or possibly for direct human use instead of discharge to surface waters. Furthermore, year-round operation, also during wintertime can be achieved as the anode biocatalysts can be adapted to operation at low temperatures

whereas plant activity of the wetland can be at a lower level (Bergdolt et al., 2013; Helder et al., 2013; Jadhav & Ghangrekar, 2009; Patil et al., 2010).

As described above, all four components of this new concept have separately been described in literature namely; 1) the use of constructed wetlands for wastewater treatment, 2) the use of a bioelectrochemical system to directly produce an electrical current from wastewater, 3) the use of a bioelectrochemical system to produce H_2O_2 and 4) the use of H_2O_2 for disinfection of wastewater (Labas et al., 2008; O'sullivan & Tyree, 2007; Vargas et al., 2013). However, it is not known whether wetland effluent can be used for H_2O_2 production and what the disinfection requirement for the resulting wetland effluent is.

Therefore, the goal of this work was to conduct an integrated study on the feasibility of the combined wetland-BES concept, focusing on chemical oxygen demand (COD) removal, H_2O_2 production in wetland effluent and disinfection efficiencies of wetland effluent. In other words, 1) can wetland effluent drive current generation and subsequent H_2O_2 production in a BES and 2) which fraction of the wetland effluent can be disinfected with this rate of H_2O_2 production.

2. Materials and Methods

2.1 Wetland construction and operation

Two labscale constructed wetlands (58*47*43 cm) were operated in a horizontal subsurface mode in a greenhouse. At the bottom of the container 3 drain tubes (diameter: 6.5 cm) wrapped in geotextile were installed (Deschacht plastics, Belgium). The bed consisted of 10 cm course sand (diameter: 0.2-1.6 cm) on top of a layer of 15 cm gravel (diameter: 0.8-2.5 cm). The top layer was planted with sods with an equal amount of freshly developing shoots and rhizomes of common reed (*Phragmatis* sp.) originating from an operational CW (De Pinte, Belgium). Influent wastewater from the domestic wastewater treatment plant of Dendermonde, Belgium and from the hospital Maria Middelares (Gent, Belgium) was collected after screen filtration and stored at 4 °C until it was sent through the wetland. Operation of the wetland started with domestic wastewater but switched to hospital wastewater as the latter contained more COD. Removal rates are expressed per m^2 wetland surface.

2.2 Bioelectrochemical system construction and operation

The bioelectrochemical system used for producing current from wetland effluent consisted of two Perspex frames with an inner diameter of 5 * 20 * 2 cm and a wall thickness of 2 cm sandwiched between two Perspex endplates (13 * 28 * 2 cm). The two compartments were separated by a cation exchange membrane (Ultrex CMI-7000, Membranes international Inc, USA). Rubber gaskets (3 mm thick) were used to create a watertight seal between all Perspex parts. The cathode was a custom made gas diffusion electrode (GDE) (Pant et al., 2011) with an integrated current collector and a total projected area of 100 cm^2. The anode consisted of carbon felt (3.28 mm thick, Alfa Aeasar, Germany) and was used as received, projected area of 100 cm^2. The anode current collector was a steel mesh (inox AISI 304, mesh width: 5.45 mm, wire thickness: 0.8 mm, Omnimesh, Belgium) with two leads protruding through the rubber gaskets for external connections. Both anode and cathode were placed against the membrane in order to limit diffusion resistances. Liquid connections were provided via the endplates. A Ag/AgCl reference electrode (RE-1B, Biologic, France) was inserted through the anode endplate and placed close to the anode electrode. The cathode compartment was open to the air and did not contain any liquid. Before inserting the cathode into the reactor, the membrane side was wetted to ensure adequate liquid contact. Cloth filtered (Liplisse 3 Cloth, Libeltex, Belgium) wastewater (for start-up purposes) or wetland effluent were added to the anode directly without any other treatment. Anode inoculum was the effluent of a MFC that was continuously operated in the lab for the specific purpose of providing inoculum. Cell potential over a 500 Ω resistor and anode or cathode potential were measured continuously (Data acquisition unit 34970A, Agilent, The Netherlands) during start-up of the BES. During experimental periods, the anode was controlled at a potential of 0 vs. Standard Hydrogen Electrode (SHE) with a potentiostat (VSP, Biologic, France). Polarization curves were recorded at a scanrate of 1 mV s^{-1} following a 20 min stabilization period in open circuit. Electrochemical calculations were performed according to Logan et al. (2006).

2.2.1 BES for hydrogen peroxide production.

This reactor was of the same design as the one used for current production. The anode electrode was a dimensionally stable (DSA) Ir coated Ti mesh (Ta/Ir; dimensions: 5*20*0.1 cm; specific surface area: 1 m^2 m^{-2}, Magneto Special Anodes, The Netherlands) with an

integrated 5 mm diameter rod of similar material as a current collector. The cathode electrode consisted of a carbon felt (dimensions: 5*20*0.3 cm, Alfa Aesar, Germany) interwoven with 2 carbon rods (dimensions: 0.5*30 cm; P48677-CMG, Morgan, Belgium) from the short side of the carbon felt with 1.5 cm spacing. To ensure adequate electrical contact between the rods and the felt, conductive carbon cement (Leit C, Laborimpex, Belgium) was used. An anion exchange membrane (AMI-7001, Membranes international Inc, USA) was used to separate the two compartments to prevent any diffusion of metal ions towards the cathode. The Ag/AgCl reference electrode was inserted through the cathode endplate and placed close to the cathode electrode. The cathode potential was maintained at ~ -0.23 vs. SHE throughout all experiments by sparging O_2 via two inlets at the bottom of the cathode compartment, therefore the catholyte contained a dissolved oxygen concentration of 8 mg L^{-1} . Anode and cathode were operated in batch with a total liquid volume of 0.5 L each and a recirculation rate of 1.08 L h^{-1}. The anolyte consisted of wetland effluent and two different catholytes (0.3 % NaCl and wetland effluent) were used. The electrochemical cell was operated in a galvanostatic mode (VSP, Biologic, France) for 24 h per current density and electrolyte combination. Per electrolyte combination, four current densities were tested, starting from a biological relevant current density of 2.5 A m^{-2} in incremental steps of 2.5 A m^{-2} up to 10 A m^{-2}. Between each current density/electrolyte combination the anode and cathode compartment were rinsed for at least 24 h with 0.3% NaCl.

The reference electrodes were regularly monitored versus a calomel electrode (+244 mV vs. SHE; QIS, the Netherlands). Removal rates, production rates, current and power densities are expressed per m^2 membrane projected surface area.

2.4 Chemical analysis

Chemical oxygen demand was determined by means of a standard kit according to the manufacturer's procedure (Nanocolor ® COD, Macherey-Nagel, Germany). Soluble COD was determined after filtration over a 0.45 µm filter. pH was determined using a handheld probe (SP10B, Consort, Belgium). Dissolved oxygen was determined with a handheld O_2 probe (HQ30D, Hach Lange, Germany). Hydrogen peroxide concentrations were determined by means of a spectrophotometric method adapted from O'Sullivan and Tyree (2007). Briefly, 1 ml of appropriate diluted sample (in 0.3 % NaCl) was added to 1 ml 1.8 M H_2SO_4 and 24 mM

TiO_5O_4*xH_2O (5% Ti basis, Sigma Aldrich, Germany). Absorbance was read after 10 min. incubation at room temperature at 405 nm. A linear standard curve from 0-70 mg L^{-1} was used to quantify H_2O_2.

2.5 Disinfection tests

H_2O_2 was added to stirred real wetland effluent (Aquafin, Belgium) at room temperature to final concentrations of 0.1% and 0.01%. Disinfection effectiveness was determined by (selective) plate counting and by means of flow cytometry. Samples were taken before addition of H_2O_2 and at intervals of 5 or 10 minutes, up to an hour after addition of H_2O_2. Peroxidase (~ 3U ml^{-1} final concentration) was added to the sample to stop the action of H_2O_2. Appropriate dilutions were made in 8.5 g NaCl L^{-1} sterile physiological solution. Total plate counts were determined on R2A agar after 24 h of incubation due to the presence of spreader colonies after 48h. Enterococci were determined after incubation for 48 h at 37°C on *Enterococcus* agar (Difco, BD, Belgium). Total coliforms were determined after overnight incubation at 37°C on MacConkey agar (Oxoid, UK). Flow cytometry analysis of bacterial presence was included to account for viable but nonculturable cells (VBNC) after disinfection (Hoefel et al., 2003; Wang et al., 2010). Total bacteria viability analysis based on membrane integrity was performed by means of flow cytometry according to Van Nevel (Van Nevel et al., 2013). The procedure was adjusted to 0.4 µM Propidium Iodide (PI) and 13 minutes incubation at 37 °C. Presence of peroxidase did not affect flow cytometry determinations (not shown).

3. Results & Discussion

This chapter describes a concept with an initial filtering of wastewater via a high rate wetland, followed by the bioelectrochemical production of peroxide and subsequent disinfection of wetland effluent. To demonstrate the concept (Figure S1), 2 lab-scale wetlands were operated to study organics removal, secondly a BES was coupled to a wetland to study current production from wetland effluent, thirdly electrochemical H_2O_2 production in real wetland effluent was investigated and finally disinfection experiments with H_2O_2 were performed with real wetland effluent to determine the needed concentration.

3.1 Wetland COD removal and anode performance

The wetlands in this study produced an effluent flowrate of 21.4 ± 7.2 L $m^{-2}_{wetland}$ d^{-1} at a concentration of 161 ± 53 mg $COD_{soluble}$ L^{-1} (Table 5.1, stage 2). The wetland effectively operated as a filter, since suspended COD (i.e. total COD – soluble COD) removal efficiency was 95 ± 7.4 % whereas soluble COD was less efficiently removed, 72. ±6.6 %, before coupling of the BES to the effluent. In this particular case, remaining soluble COD serves as a source of reducing equivalents to drive current generation in the anode of a BES.

Comparing the loading rate of the labscale wetlands with full scale wetlands shows that the wetlands in this study received a 1-10 times higher COD loading rate (Karathanasis et al., 2003; Kern & Idler, 1999; Puigagut et al., 2007). This indicates that loading rates can be increased on existing wetlands when aiming only at rapid filtration. However, full scale wetlands are usually operated with a pretreatment step (Karathanasis et al., 2003; Kern & Idler, 1999; Puigagut et al., 2007). COD effluent concentrations of the labscale wetland were in compliance with effluent concentrations according to Belgium regulations (< 125 $mgCOD_{total}$ L^{-1}) however relative removal needs to be improved (total COD removal efficiency of 70% is required) (Rousseau et al., 2004; Vlarem_II, 2012). Additional COD removal was achieved by the anode of the BES (Table 1). This resulted in an average biologically an average biologically generated current of 58 mA $m^{-2}_{electrode}$ from the effluent of the wetland and an extra 36% decrease of effluent COD_{total} concentrations (Table 1, stage 2 vs. stage 3).

The coulombic efficiency during this period amounted to 4.0 % indicating that also other processes played a role, such as settling of solids or conversion with other electron acceptors. The bioanode performance was limited by the amount of $COD_{soluble}$ available, as spiking (starting day 25) of the wetland influent yielded higher current densities, up to max. 3.5 A m^{-2}, equal to 25 gCOD m^{-2} d^{-1} with coulombic efficiency of 62% (Figure 1a, Table 1; Stage 4). This indicates that the BES/wetland combination was underloaded in terms of anode performance. This finding is corroborated by polarization curves (Figure 1b) where the anode potential changed more at higher currents compared to the cathode potential (2.7 times more change in anode potential vs. cathode potential at currents > 0.95 A m^{-2}). The higher loading(~ 50%, Table 1 stage 3 and 4) resulted in a net power output during polarization of maximum 150 mW m^{-2} (Figure 1c)

The higher $COD_{soluble}$ content led to an increase of the $COD_{soluble}$ concentration in the effluent of the BES, surpassing the discharge limit (Rousseau et al., 2004; Vlarem_II, 2012). In the proposed concept a second wetland will provide a polishing step to remove residual organics and nutrients so discharge limits can be met (Figure S1).

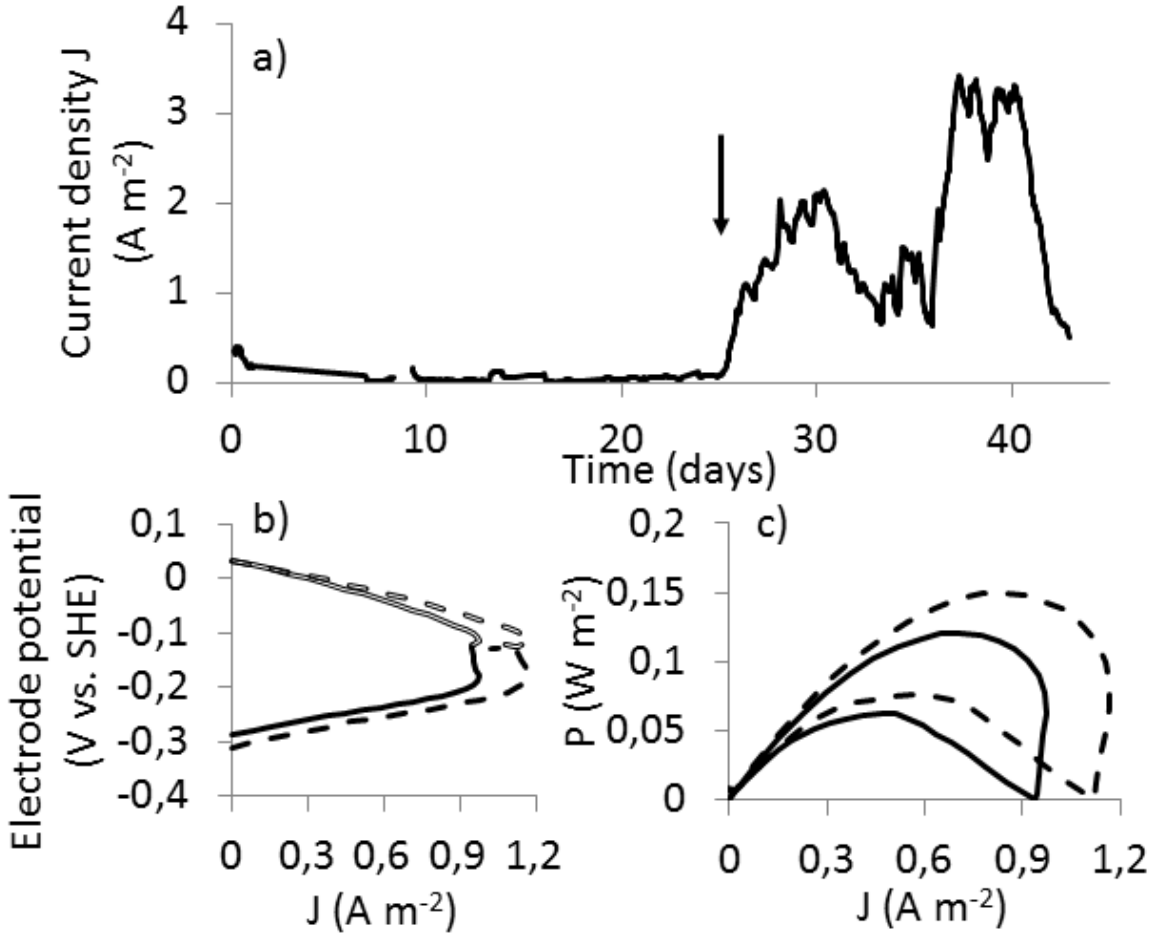

Figure 1: a) Current density in function of time for a BES operated on wetland effluent. Arrow indicates start of spiking wetland influent, see § 3.1. b) anode (solid lines) and cathode (open lines) potentials and c) power density curves during polarization measurements on day 36 (_ _) and 42(____).

Table 1: Overview of COD and flow rates on both wetlands and the coupled system.

Stage	1) start-up			2) increase solids			3) coupling of BES to effluent					4) increase soluble organics				
Wastewater source	Domestic wastewater			Hospital wastewater			Hospital wastewater					Hospital wastewater				
Operating days	36			47			25					18				
	WL1	**WL2**	**n**	**WL1**	**WL2**	**n**	**WL + BES**	**n**	**WL**	**n**	**BES**	**n**	**WL + BES**	**n**	**WL**	**n**	**BES**	**n**
Loading rate (gCOD $m^{-2} d^{-1}$) †																		
total	20.3 ± 2.6	20.2 ± 2.3	4	26.6 ± 15.5	29.9 ± 17.7	9	19.1 ± 9.1	5					44.2 ± 23.5	5				
solids	2.3 ± 1.1	1.7 ± 1.6	4	12.6 ± 8.0	15.9 ± 10.6	9	9.2 ± 9.4	4					5.5 ± 4.5	4				
soluble	18.0 ± 2.9	19.0 ± 3.6	4	14.0 ± 9.0	13.9 ± 8.5	9	12.6 ± 5.3	6					46.5 ± 21.3	4				
Removal rate (gCOD $m^{-2} d^{-1}$) †											*(gCOD $m^{-2} d^{-1}$)**						*(gCOD $m^{-2} d^{-1}$)**	
total	15.6 ± 1.7	15.7 ± 1.8	3	23.5 ± 14.0	26.7 ± 18.2	7			18.1 ± 15.4	2	10.6 ± 6.3	2			30.8 ± 21.9	5	40.5 ± 44.5	5
solids	2.1 ± 1.3	2.1 ± 0.2	3	12.4 ± 7.9	16.7 ± 11.9	7			12.6 ± 14.3	2	5.1 ± 5.8	2			6.1 ± 4.0	2	20.3 ± 39.2	4
soluble	13.5 ± 0.4	13.6 ± 1.7	3	11.1 ± 7.6	12.9 ± 8.2	7			5.6 ± 1.2	2	5.7 ± 0.5	2			32.5 ± 26.2	4	44.7 ± 34	5
Flow rate (L d^{-1})																		
in	10.6 ± 1.3	10.6 ± 1.4	6	11.2 ± 5.6	11.1 ± 5.4	16			14.3 ± 3.4	10	4.3 ± 1.0	11			10.8 ± 5.5	8	4.8 ± 2.9	8
out	5.5 ± 2.4	6.3 ± 3.1	9	5.8 ± 2.0	5.5 ± 2.9	20			10.6 ± 2.0	8	#				9.3 ± 2.4	8	#	
Effluent concentration (mgCOD L^{-1})																		
total	81.0 ± 58.4	130.0 ± 45.5	12	187.2 ± 89.8	164.7 ± 152.9	12					98.0 ± 32.2	5					267.4 ± 75.3	5
solids	6.1 ± 6.5	21.4 ± 26.5	12	28.2 ± 51.4	16.9 ± 34.7	8					11.8 ± 5.7	4					9.6 ± 8.1	5
soluble	81.5 ± 47.8	109.2 ± 50.2	12	160.5 ± 53.3	160.4 ± 186.4	13					93.2 ± 31.2	6					257.8 ± 71.7	5

#: in = out in the BES.

*: rates for the BES are calculated per projected membrane surface area.

†: loading rate considered for a bed height of 30 cm.

WL: Wetland

BES: Bioelectrochemical system

n: number of samples

3.2 Peroxide production in wetland effluent.

From an applied perspective, producing peroxide directly in wetland effluent is the most attractive option as no separate cathodic liquid supply is needed and the peroxide is immediately produced in the flow to be disinfected. To determine the effectiveness of peroxide production at various current densities in wetland effluent, 0.3 % NaCl was used as a control. 0.3 % NaCl was chosen to compare with other studies on bioelectrochemical peroxide production (Modin & Fukushi, 2013; Rozendal et al., 2009). A maximum rate of peroxide production of 2.7 g m^{-2} h^{-1} was achieved at 10 A m^{-2} after 4 hours in the batch cycle. No clear difference was observed between a catholyte of 0.3% NaCl or wetland effluent (Figure 2a). This indicates that there is little need for an additional cathodic water supply, thus the disinfectant can be produced *in situ*. The maximum hydrogen peroxide production rate was achieved at a cathodic coulombic efficiency of 40%. However, higher cathodic coulombic efficiencies could be achieved with a maximum efficiency of 51% achieved at 1.7 g m^{-2} h^{-1} at a current density of 5 A m^{-2} and 7 hours contact time (Figure 2b). In all cases a contact time of 24 h was too long in terms of overall rate and efficiency (Figure 2b), as the peroxide is gradually decomposing. Other works have shown that peroxide production rates can be increased by ~ 33% (Modin & Fukushi, 2013) and efficiencies by ~ 40% (Figure 2). These improvements can be mainly attributed to reactor design (5 mL cathode vs. 500 mL, this work) and the use of cathode material (gas diffusion electrodes (Modin & Fukushi, 2013; Rozendal et al., 2009) vs. standard carbon felt (this work)). The combination of the anion exchange membrane with the high cathodic pH caused a minor transfer of hydroperoxyl to the anode compartment with a maximum anodic peroxide concentration of 0.0015% after 24 h at 10 A m^{-2}, some 2.6% of the total peroxide quantity produced.

Instead of using a biologically generated current, one can opt for a pure electrochemical system. In the case of the highest achieved rate at 10 A m^{-2} an energy investment of 6 W m^{-2} was needed. This amounts to 2.5 kWh $kg_{peroxide}^{-1}$ and with an assumed energy price of € 0.1 kWh^{-1}, a minimum production cost of € 0.22 $kg_{peroxide}^{-1}$ can be obtained. Other benefits of electrochemical peroxide production include the possibility of generating active chlorine compounds at the anode electrode and the ability of altering the pH of the anode and cathode solutions. Active chlorine compounds can aid in disinfection at the anode, however care should be taken to limit the occurrence of disinfection by-products (Wang et al., 2010). When using wetland effluent as the water source for the anode and cathode, which has a

low buffer capacity, an increase in cathodic pH and decrease in anodic pH was readily observed (Figure 2c). This phenomenon is usually regarded as a drawback for use of BES in other applications, due to associated energy losses (Timmers et al., 2012), but here it can become a positive attribute as it will also aid in disinfection.

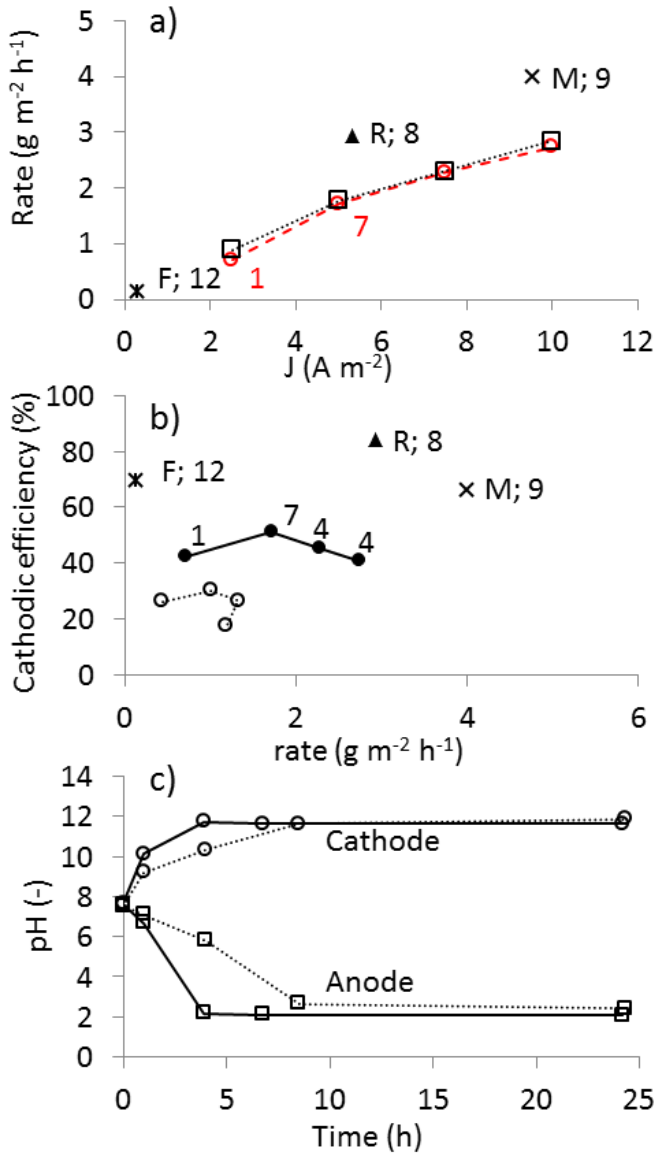

Figure 2: a) hydrogen peroxide production rates at increasing current densities with 0.3% NaCl (··□··) and wetland effluent as catholyte (—○—). All data points are maximum rates obtained at 4 hours contact time except where numbers are added. b) Maximum (—●—) and 24h (—○—) rate of hydrogen peroxide production in function of cathodic coulombic efficiency. Time of maximum rate is indicated. c) pH profile for the anode (□) and cathode(○) compartment during H_2O_2 production in wetland effluent at 2.5 Am^{-2} (.....) and 10 Am^{-2} (____). Outcomes of comparable studies: F: Fu et al. (2010), R: Rozendal et al. (2009), M: Modin et al. (2013).

3.3 Peroxide requirement for disinfection of wetland effluent

The concentration of hydrogen peroxide needed for effective disinfection determines the required quantity and thus the cathodic production rate and further BES dimensions. 0.01% hydrogen peroxide lead to a 50% removal of total bacterial counts within wetland effluent on R2A agar after 1 h contact time. Increasing the concentration 10 times to 0.1% lead to an almost complete removal of culturable bacteria (3 log reduction to <75 CFU ml^{-1}) after 1 h contact time (Figure 4a.). These results are in line with pure culture kinetic and modelling studies (Labas et al., 2008; Vargas et al., 2013). A similar trend is observed for total coliforms, as determined by selective plating on MacConkey agar (Figure 3a), where the higher concentration of peroxide resulted in a higher disinfection efficiency. No culturable *Enterococci* were detected by means of selective plating on enterococcus agar (<75 CFU ml^{-1}). These results indicate that peroxide is a non-selective disinfectant.

Flow cytometry analysis with viability staining, based on membrane integrity, indicated a far less efficient disinfection as compared to selective plate counts, which is consistent with previous work (Hoefel et al., 2003; Wang et al., 2010). Both concentrations of disinfectant showed a similar increase in damaged cell counts (Figure 3b). Examining the increase in percentage of dead cells over time reveals a similar pattern for both concentrations (Figure 4c) with a maximum increase in dead cells of 35% after 40 min contact time for 0.1% H_2O_2. The difference between plate counts and flow cytometry results indicates that although cell membrane integrity seems to be intact, the microorganisms were in a VBNC state (Hoefel et al., 2003; Wang et al., 2010).

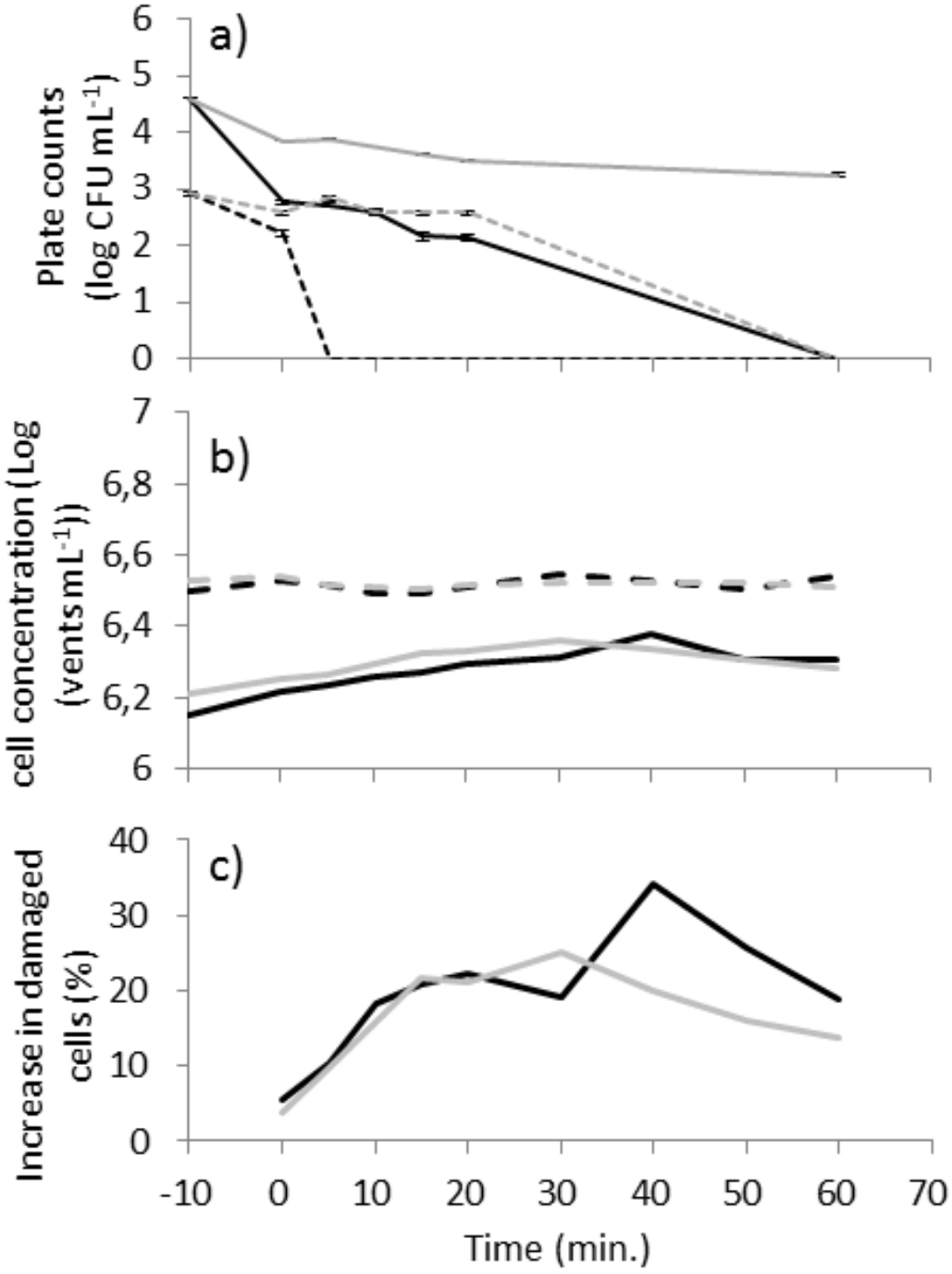

Figure 3: Disinfection performance of 0.1% and 0.01% H_2O_2 on wetland effluent. a) plate counts 0.1% total bacteria: ——, 0.1% coliforms: – – , 0.01% total bacteria: ——, 0.01% coliforms: – – . Error bars indicate 95% confidence interval as determined with the Poisson distribution, not all error bars are visible. Values at 0 are below detection limit of 75 CFU ml^{-1} for total bacteria and 150 CFU ml^{-1} for coliforms. b) Flow cytometry based viability staining for 0.1% and 0.01% H_2O_2. Intact cells 0.1%: – – 0.01%: – – , damaged cells 0.1%: —— 0.01%: ——, c) increase in dead cells for 0.1%: —— and 0.01%: —— H_2O_2.

3.4 Wetland & BES dimensions and configuration

With the results presented here a case study can be made on the design of a constructed wetland for water treatment. Considering a municipality of 750 person equivalents (PE), producing 100 L wastewater containing 0.5 gCOD L^{-1} per inhabitant (Kern & Idler, 1999; Vymazal, 2005) a flowrate of 75 m^3 d^{-1} or a mass flowrate of 37.5 kgCOD d^{-1} can be expected. At the highest loading rate as determined in this work (45 gCOD m^{-2} d^{-1}, Table 1), 850 m^2 of wetland is needed to filter the solids from the wastewater flow. This resulted in a wetland effluent of 170 mgCOD L^{-1}. The COD in the effluent can be further treated with an anode. Taking a BES and operating it with a bioanode producing 2.5 Am^{-2} at 40 % coulombic efficiency (suboptimal conditions from the maxima reported earlier) leads to the notion that 44 gCOD $m_{electrode}^{-2}$ d^{-1} can be processed. When using a membrane electrode assembly (MEA) of 1*0.05*1 m^3 (5 cm width for one assembly is a reasonable estimate (Dekker et al., 2009; Rozendal et al., 2008)), 880 gCOD can be treated per $m_{reactor}^3$ per day. To treat the complete COD load of the wetland effluent, a 15 m^3 reactor would be needed, which is evidently beyond the scope or need. The cathode was able to produce 17 gH_2O_2 $m_{electrode}^{-2}$ d^{-1} at 2.5 A m^{-2} (Figure 2a), one m^3 of BES will thus produce 340 gH_2O_2 d^{-1}. To achieve a good disinfection (i.e. viable heterotrophic count < 100 CFU mL^{-1} (Council Directive 98/83/EC)), 0.1% H_2O_2 is needed (Figure 3a). Therefore 340 L d^{-1} of disinfected water can be produced per m^3 of reactor if the purpose is to produce water for consumption. Considering that an average person consumes about 3 L of water per day, a 7 m^3 reactor would suffice in this example. If the purpose is to lower the infectious pressure, depending on the needed concentration of H_2O_2, a higher flow of water can be produced. Moreover setting the current density, irrespective of anode performance, can lead to higher rates of H_2O_2 production (Figure 3a). Performance can even be enhanced by incorporating the additional benefits of active chlorine and pH as stated before. With the data provided by Rozendal et al. (2008), the total installed reactor costs can be estimated at € 5800 m^{-3} for a system with 20 MEA m^{-3}.

4. Conclusions

In this work an integrated process for wetland wastewater treatment with subsequent disinfection via (bio)electrochemical H_2O_2 production was studied. The lab scale wetland was able to operate with loading rates up to 44 gCOD $m_{wetland}^{-2}$ d^{-1} and provide an almost solid-

free effluent to the anode of a bioelectrochemical system (BES). H_2O_2 production at the cathode for disinfection was directly feasible in wetland effluent, up to rates of 2.7 g $m_{electrode}^{-2}$ h^{-1}. Finally, a system configuration is proposed that can be applied in conjunction with wetland water treatment facilities e.g. greenhouse horticulture water recycling or clean water production in off-grid locations.

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Enhanced disinfection of wastewater by combining wetland treatment with bioelectrochemical H_2O_2 production

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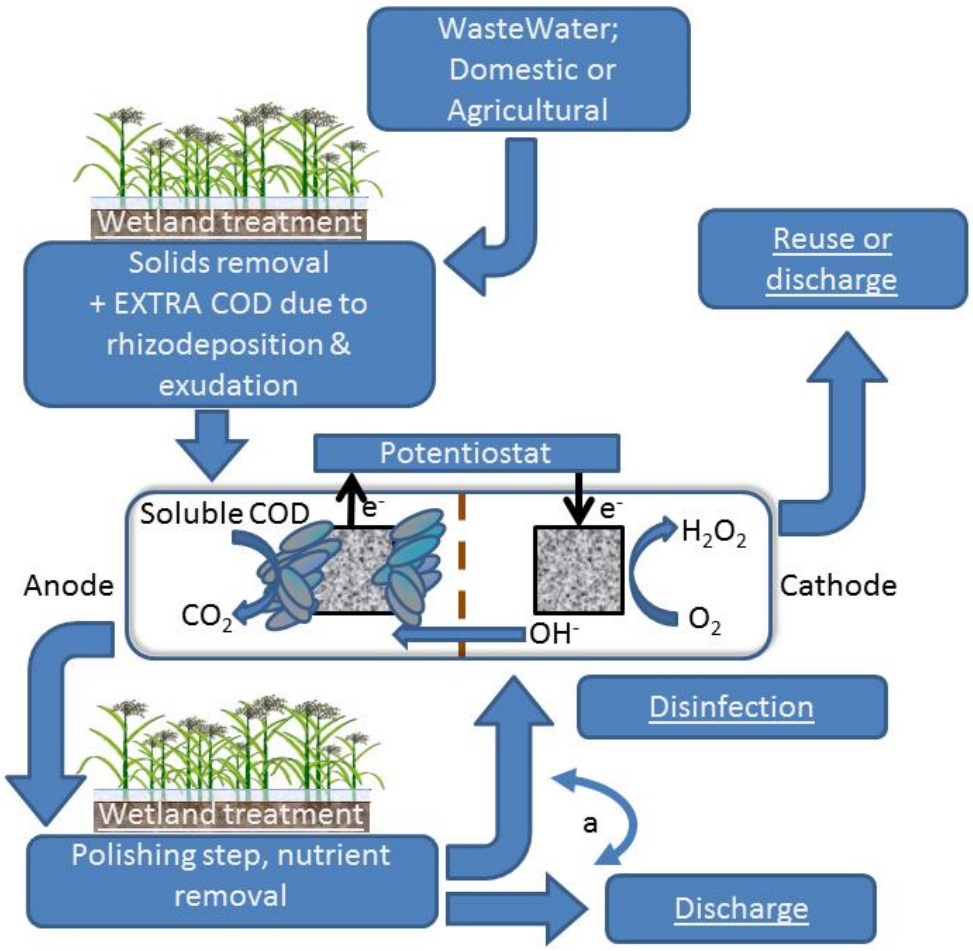

Figure S1: Conceptual overview of wetland wastewater treatment with enhanced disinfection by means of a bioelectrochemical system (BES). At the anode soluble organic matter is biologically oxidized to an electrical current. At the cathode oxygen is abiotically reduced to hydrogen peroxide. a: flow rate between these two options can be adjusted to meet demand.