UHASSELT
KNOWLEDGE IN ACTION

Maastricht University

Faculteit Geneeskunde en Levenswetenschappen
School voor Levenswetenschappen

master in de biomedische wetenschappen

Masterthesis

BIOCHAR AS ELECTRON SHUTTLE AND SUBSTRATE MATERIAL FOR ELECTROACTIVE BACTERIA IN A BIOELECTROCHEMICAL SYSTEM TO ENHANCE REMOVAL OF PESTICIDES FROM WASTEWATER

Inez Roegiers

Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen, afstudeerrichting milieu en gezondheid

PROMOTOR :

dr. Sofie THIJS

BEGELEIDER :

De heer Robin BONNE

2017
2018

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7th of June, 2018

DANKWOORD

Deze thesis is een ware ontdekkingsreis geworden. Als biomedische masterstudent lijkt dit onderwerp een misschien niet zo voor de hand liggende keuze, toch was ik meteen verkocht door het idee. Het opstarten van het project aan de universiteit Hasselt was niet een van de meest voor de hand liggende dingen. Vele obstakels werden overwonnen, maar dat alles resulteerde wel in een mooie basis voor toekomstige studenten die zich verder op dit onderwerp willen storten aan de Universiteit Hasselt.

Eerst en vooral zou ik mijn dagelijkse supervisor Robin Bonné en promotor Sofie Thijs willen bedanken voor de goede begeleiding, steun, feedback en opgedane kennis. Natuurlijk ook bedankt aan mijn medestudent die voor een gelijkaardig project koos en waarmee ik zoveel uren heb doorgebracht in het labo, Thessa Van Limbergen. Bedankt ook aan de mensen van X-lab, die een groot deel van mijn project mogelijk hebben gemaakt en vaak opbouwende feedback hebben gegeven: Prof. dr. Jean Manca, Rob, Ragha, Michel, Rik, Mieke en Dieter. Bedankt ook aan Tom Haeldermans en Feredica Mare, voor het maken en karakterizeren van de biochars. Karolien, heel erg bedankt om me op het laatste nog te helpen met het maken van SEM beelden!

Bedankt ook aan Jesse, mama, papa, Esther, Laura en Dries voor alle steun!

Natuurlijk ook een oprechte dankuwel aan de mensen van CMET, die mij hebben geholpen om in de laatste weken voor mijn thesis nog een project op te starten! Dr. Kun Guo, thank you to make time to set the experiment up and to supervise me so well, I know time was very limited, so I really appreciate your help. Dr. Jan Arends, bedankt voor de vele feedback en de mogelijkheid om dit experiment uit te voeren aan de Universiteit Gent!

En -niet te vergeten – danku aan de mensen van het IMDEA Agua instituut van de universiteit van Alcalá, Spanje die waarschijnlijk de meest onvergetelijke ervaring van deze stage mogelijk gemaakt hebben! Ten zeerste bedankt voor de goede opvang en vele feedback die we daar gedurende tien dagen hebben gekregen. Dr. Sara Tejedor Sanz, thank you for the daily supervision, Prof. dr. Abraham Esteve-Núñez, thank you for the invitation, we certainly learned a lot!

Bedankt aan iedereen die van deze al fantastische stage een nog betere ervaring hebben gemaakt!

Inez

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LIST OF ABBREVIATIONS

BES: bioelectrochemical systems

MFC: microbial fuel cell

MEC: microbial electrolysis cell

EAB: electroactive bacteria

GAC: granular activated carbon

WE: working electrode

RE: reference electrode

CE: counter electrode

CA: chronoamperometry

CV: cyclic voltammetry

SEM: scanning electron microscopy

FISH: fluorescence in situ hybridization

HPLC: high-performance liquid chromatography

OD: optical density

I: current (Ampère)

U: potential (Volts)

R: resistance (Ohms)

PCP: pentachlorophenol

Gs: *Geobacter sulfurreducens*

MC: mixed culture

SAMENVATTING

Inleiding: De pesticide concentraties in oppervlakte waters overschrijden regelmatig de toegelaten limieten in Vlaanderen. De oorzaak ligt vaak bij spoelingsresten van pesticiden in de landbouwsector, die niet voldoende gereinigd worden door bestaande waterzuiveringsinstallaties en zo in het milieu terecht komen. Een mogelijke oplossing voor een nieuw reinigingssysteem is de implementatie van bioelectrochemische systemen (BES) in bestaande waterzuiveringssystemen. In deze toepassingen oxideren electroactieve bacteriën substraten en geven ze die electronen door aan een anode, die gebruikt wordt als elektron donor. Biochar, een op koolstof-gebaseerd materiaal geproduceerd door de pyrolyse van biomassa onder anaerobe condities, wordt gesuggereerd als nieuwe toevoeging aan BES. Door zijn vermogen om elektronen door te geven van bacteria naar de elektrode zou de degradatie van bepaalde stoffen versneld kunnen worden. Biochars worden algemeen aanzien als een meer milieu-vriendelijke, economischere vervanging voor granulaire geactiveerde kool (GAC), die nu al gebruikt worden in BES als anode materiaal. In deze scriptie werden de volgende hypotheses gesteld: elektro-actieve bacteriën kunnen pirimicarb and thiacloprid afbreken of biotransformeren in een niet-electrochemisch systeem, en dit proces wordt versneld wanneer er GAC of biochar wordt toegevoegd. De tweede hypothese stelt dat de toevoeging van biochar aan het anodische deel van een BES de verwijdering van pesticides bevorderd.

Materiaal en methoden: De experimenten voor de degradatie van pesticiden door EABs te testen werden uitgevoerd in anaerobe flesjes of tubes. Een mixed culture en *G. sulfurreducens* bacteriën werden blootgesteld aan 10 ppm thiacloprid of pirimicarb, al dan niet met de toevoeging van biochars of GACs. Na een incubatie periode van twee weken werd de pesticide concentratie gemeten met high-performance liquid chromatography en bacteriële groei met een Bradford assay. Het tweede deel bestond uit het vergelijken van de stroomdichtheden, electrochemische processen en biofilm ontwikkeling van drie GACs in een acht-electrode BES reactor. Chronoamperometry, cyclic voltammetry en scanning electron microscopy werden hiervoor gebruikt.

Resultaten: Over het algemeen toonden de resultaten toonden geen significante daling van pesticide concentraties wanneer we de condities met bacteriën vergeleken met de abiotische condities. Wel werd er vastgesteld dat de toevoeging van een GAC of biochar de pesticide concentratie significant deed dalen. De GAC-partikels uit de anodes van de acht-electrode BES toonden een dichte kolonisatie met *G. sulfurreducens* na twee weken. De stroomdichtheden geproduceerd door de anodes lagen significant veel hoger dan de controles.

Discussie en conclusie: De hypothese dat electroactieve bacteriën thiacloprid en pirimicarb kunnen afbreken werd niet bevestigd op basis van de verworven resultaten. De daling in totale pesticide concentratie na toevoeging van GACs of biochars zou kunnen verklaard worden door hun mogelijke katalytische eigenschappen. De GACs lijken alle geschikt als anode materiaal in een BES set-up. De volgende stap zou het vergelijken van biochars zijn in dezelfde BES set-up. Het gebruiken van BES voor de remediatie van pesticiden staat nog in zijn kinderschoenen, maar door dit onderzoek is nu een eerste stap gezet richting een milieuvriendelijkere en goedkopere manier om pesticiden uit afvalwater te verwijderen.

ABSTRACT

Introduction: Pesticides in surface waters regularly exceed regulatory limits in Flanders. Pesticide rinsing facilities are major sources of contamination of water nearby agricultural sites, and existing wastewater treatment facilities do not completely remove these micro-pollutants. A possible solution is the implementation of bioelectrochemical systems (BES) in existing remediation applications. In these applications, electroactive bacteria oxidize substrates and use the anode as electron acceptor. Biochar, a carbon-based material made through pyrolysis of waste biomass, is suggested as a new addition to BES that shuttles electrons from bacteria to the anode and thereby enhances the breakdown of pollutants. Granular activated carbons (GACs) are already used as addition to the anodic compartment, however, there are some downsides (production costs, regular replacements), and biochar seems to be an interesting, environmental-friendly, alternative. We hypothesized that electroactive bacteria can degrade the pesticides pirimicarb and thiacloprid in a non-electrochemical system, and that this degradation is increased when a granular activated carbon or biochar is added. Second, we hypothesized that the addition of biochar made from waste biomass to a bioelectrochemical system improves the removal of pesticides from wastewater, and this via diverse mechanisms, acting as a bacteria-colonization substrate, an electron shuttle, and mediating redox reactions

Materials & methods*:* The experiments to test degradation or biotransformation of thiacloprid and pirimicarb were conducted in anaerobic vials or tubes. A mixed culture and *G. sulfurreducens* bacteria were spiked with 10 ppm pesticides. After an incubation period of two weeks, pesticide concentrations in medium were measured with high performance-liquid chromatography. Protein concentrations in medium were measured with the Bradford assay to determine bacterial growth. Afterwards, similar experiments were conducted, but with the addition of a granular activated carbon (GAC) or biochar. For the second part, the performances of three GACs as anodic material were compared in a BES reactor by performing chronoamperometry, cyclic voltammetry and scanning electron microscopy.

Results: The results showed almost significant decrease in pesticide concentrations between conditions with bacteria and abiotic control groups. However, the total pesticide concentrations significantly decreased when a GAC of biochar was added. The GAC particles in the eight-electrode BES set-up showed colonization by *G. sulfurreducens*.

Discussion & conclusion: The hypothesis that electroactive bacteria can break down to biotransform pesticides cannot be confirmed based on the results that were obtained in these experiments. However, the total pesticide concentrations significantly decreased when a GAC of biochar was added, which could be because of catalytic properties of biochars and GACs. Based on the results of the test in the eight-electrode BES, GAC seems to be a suitable substrate for EAB in a BES. More research is necessary to say more about the possible effects of biochar and GAC on pesticide breakdown, and the mechanisms are responsible for these effects. These tests are a first step into the bioremediation of pesticides in a biochar-enhanced BES.

INTRODUCTION

I. The environment, emerging contaminants and purification systems

In the year 2050, the world's population will be reaching an estimation of 9.7 billion people. As a result, the demands for food, clean drinking water and energy will increase, but their availability might not [1,2]. Fresh, accessible water, which now makes up about 1% of the total amount of water on this earth, will get increasingly scarce. A growing number of emerging contaminants such as pesticides, pharmaceuticals and hormones enter the water supplies in developing countries as well as industrialized nations. To decontaminate polluted water in an effective, non-costly way that does not stress the environment even more is a challenge [3].

Pesticides are widely used, especially in the agricultural sector but also in disease control management. They include groups such as insecticides, fungicides, and herbicides, each targeting different groups of organisms [4]. In 2014, the agricultural sector in Flanders used about three million kg of pesticides [5]. In the same year, the amount of pesticides present in surface water exceeded regulatory limits in Flanders in more than 50 % of the measurement locations [6]. One of the main benefits of the use of pesticides in agriculture is increased productivity by reducing losses of crops by targeting insects, weeds and diseases. However, the extensive use of pesticides also raises questions about the possible adverse effects it has on the ecosystem, and organisms living in it. Pesticides enter the biosphere via many different routes and are, in high concentrations, toxic to a wide variety of organisms [4]. The problem of contaminated ground- and surface water near agricultural sites often starts at the pesticide mixing, loading and rinsing facilities. One percent of the pesticides that are used in agriculture, which is about 100 tons a year, end up as rest products in surface- and ground-water nearby. One way of limiting this effect is by treating the wastewater sufficiently, so almost no pesticides are released into the environment [7,8].

Methods currently used for the treatment of micropollutants such as pesticides in wastewater comprise physicochemical methods and biological methods such as membrane filtration, activated carbon and advanced oxidation processes. These methods are commonly high in costs and oxygen use [9,10] and only remove a fractions of the micropollutants [11,12]. An example of a physicochemical installation in agriculture is the Sentinel® unit manufactured by Allman & Co. Ltd (Birdham, UK). This unit is currently used by the fruit research center PC Fruit, which delivered the pesticide wastewater for this research project. The method for purification in this unit is based on sand- and activated carbon filtration and is very effective. Unfortunately, the installation and operational costs are high and moreover, the activated carbon regeneration is expensive. Therefore, creating a more cost-efficient version of this unit is an interesting option to explore. Biological methods include, amongst others, constructed wetlands. These wetlands are designed to use the natural processes that occur in wetlands in a controlled environment for the treatment of wastewater. An emerging method to treat wastewater is using bioelectrochemical systems (BES) and electroactive bacteria (EAB). BES can be implemented in CWs and have great potential for the removal of emerging contaminants in wastewater [9,13–15] (*Supplemental Figure 18*).

II. Bioelectrochemical systems (BES)

An emerging technology that can be used as an energy efficient way to treat polluted wastewater is a bioelectrochemical system (BES). In these systems, electroactive bacteria (EAB) are used to drive reactions that take place at the anode and/or cathode. Anode respiring bacteria produce CO_2, protons (H^+) and electrons when oxidizing substrates. The electrodes then flow through an external circuit and combine with protons that migrated to the cathodic chamber, to produce then either water (if oxygen is present) of H_2 (when no oxygen is present) at the cathode[16].

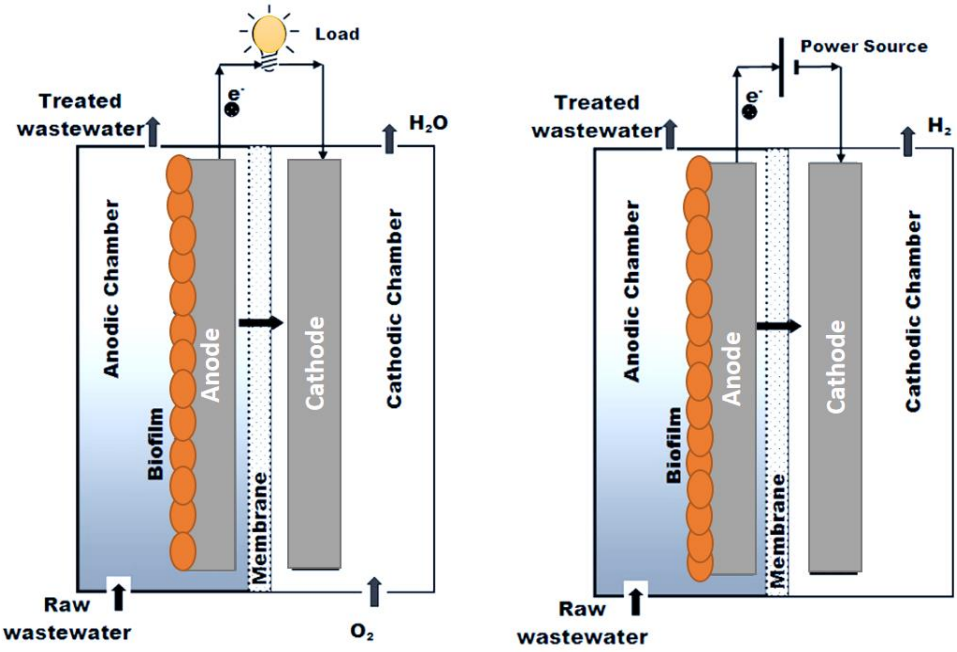

Figure 1 *Schematic representation of a BES in microbial fuel cell (MFC)-configuration, where H_2O is produced at the cathode (**left**) or microbial electrolysis cell (MEC)-configuration, where H_2 is created at the anode (**right**). Both configurations oxidize substrates at the anode and can be used for wastewater treatment applications. (Figure obtained from Escapa et al. 2014)*[16]

Two well-known BES-configurations include microbial fuel cells (MFC) and microbial electrolysis cells (MECs) (*Figure 1*). A microbial fuel cell (MFC) can be seen as a battery powered by bacteria instead of chemicals. In this set-up, the oxidation reaction at the anode happens spontaneously. Because of the presence of oxygen at the cathode, the cathodic potential is raised and thereby makes the oxidation of compounds at the anode a thermodynamically favorable reaction. Here, H_2O is created at the anode. By oxidizing organic compounds, electrons are received by the EABs and donated to an electrode, hereby conversing extracted electrons to current [17]. A milestone in this field was the discovery of the complete oxidation of organic compounds by bacteria of which the electrons are then donated to electrodes in an efficient electron transfer process via direct contact between the bacteria and the anode[18]. Microbial electrolysis cells (MECs) are another configuration of BES. In MECs, no oxygen is present at the cathode and thus, in order for oxidation to occur at the anode, a certain amount of current input is required [19–21]. H_2 is then created as a product at the cathode (instead of H_2O in an MFC). MECs and MFCs used in wastewater remediation can also be referred to as microbial remediation cells (MRCs). The idea of microbial BES cleaning wastewater from contaminants, while at the same time generating power, has gotten a lot of attention the past decade. However, producing

large amounts of power whilst purifying water can be a challenge. Instead, focusing on bioremediation without energy production seems to be a better approach [22].

III. Electroactive microorganisms in BES

In BES, electroactive microorganisms can transfer electrons outside their cell surface to other substances or electrodes, linking extra- and intracellular electron transfer mechanisms with each other. There are many electroactive microorganisms in nature and most are used in applications such as MFCs and MECs. Examples include the gram-negative proteobacteria *Geobacter sulfurreducens* and *Shewanella oneidensis.*

Geobacter sulfurreducens and mixed cultures in BES

G. sulfurreducens (*Geobacteraceae,* Proteobacteria*)* is one of the most promising EAB to be used in MFCs and MECs because of their relatively high-power output and high coulombic efficiency. *G. sulfurreducens* bacteria are known for their ability to produce biofilms with a relatively great depth (up to a few 100 µm). The growth of a biofilm on electrodes depends on the growth conditions, type of electron donor, material, surface area and applied potential to the electrode. The thicker the biofilm, the more current is produced [19]. They are Gram-negative rods that are known to oxidize acetate with the reduction of Fe(III). They can oxidize many compounds such as Mn(IV), U(VI) and humic substances and are able to transfer these electrons with a high efficiency to ethanol, fumarate, hydrogen or other small molecular weight organic acids. When no terminal electron donor is present in the environment of the bacteria apart from an electrode, the bacteria will use the anode as electron acceptor via diverse electron transfer mechanisms [19]. It is known that *G. sulfurreducens* exhibits multiple types of c-type cytochromes and multicopper proteins that have essential roles in the extracellular electron [23–25] transfer on their outer surface[26]. An example is OmcB, an essential Fe(III)-reducing cytochrome, located in the outer membrane, that is highly expressed when *G. sulfurreducens* grown on electrodes [23,27]. Mixed cultures are often used at the anode or cathode in BES. These cultures are more robust and can also generate a high-power output, similar to pure *G. sulfurreducens* cultures [25]. Synergistic interactions are seen in mixed cultures on or near electrodes, which makes them able to convert more complex substrates compared to pure *G. sulfurreducens* cultures [28]. In a BES, an artificial potential can be poised on bacteria at an electrode. Since the metabolic energy of the bacteria is dependent in the standard potential of the electron donor or acceptor, changing the potential causes selective pressure on a mixed culture, leading to more productive communities [29,30].

Mechanisms for extracellular electron transfer (EET)

Three major mechanisms are used by EABs to transfer electrons to extracellular electron acceptors. The first one, direct electron transfer (DET), involves redox-active proteins present on the outer cell is membrane of the microorganism that are in direct contact with the electron acceptor, called cytochromes [24]. Another form of DET is the presence of filamentous structures on the microorganisms such as pili or nanowires that can conduct electrons. In both *Geobacter* and *Shewanella* species [31,32], filaments have been found to have conductive properties. These nanowires are part of the biofilm matrix and conducts electrons from the EAB to the solid electrode surface [33] (*Figure 2, Left*). The second mechanism, mediated electron transfer (MET) occurs via soluble molecules present nearby the microorganisms. Previous studies have

shown that, when not directly in contact with Fe(III), some microorganisms are still able to reduce Fe(III) due to soluble substances (such as flavins and humic substance) accumulating between Fe(III) and the microorganisms that can promote electron shuttling [34–36] (*Figure 2, Middle*). The third one is indirect electron transfer (IET), which is based on the electrochemical synthesis of a wide range of microbial electron donors and acceptors. Here, the compounds used as electron donor or acceptor undergo irreversible redox processes, thereby creating new compounds such as hydrogen or formic acid. Additionally, electroactive (metabolic) substances can be secreted by microorganisms and transfer electrons between the microbes and electrodes [19] (*Figure 2, Right*).

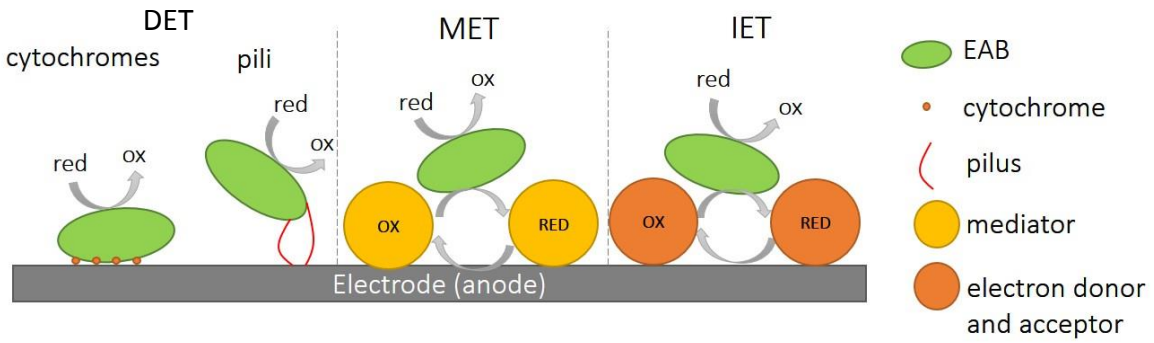

Figure 2 Illustration of three extracellular electron transfer (EET) mechanisms of EAB (Figure based on Sydow et al., 2014)[19]

IV. BES systems in remediation applications

BES can remove contaminants through different mechanisms

The overall principle of remediation in a BES is the difference in the redox potential between the contaminants and the electrodes. Different mechanisms are known so far for remediation of various contaminants and are thoroughly described in a review paper by Wang *et al.*., 2015. For example, oxidized contaminants such as azo dyes or perchlorinated contaminants can be used as electron acceptors and can thus be anaerobically reduced at the cathode. Other examples include the sorption of a trace pollutant or metal by an electrode, helping these pollutants to be conversed. On site remediation, where multiple contaminants are remediated at once, requires a combination of all the techniques described above [13].

The removal of micropollutants with BES

Micropollutants are compounds that are present in wastewater at very low concentrations (µg/L or ng/L). Examples include pesticides, pharmaceuticals, and personal care products, which are products that are not easily biodegradable. Because of these low concentrations, removing them through available wastewater treatment systems is not perceived as an easy task. The removal of these micropollutants through BES might be an innovative way to remove these compounds from wastewater. Because of the wide variety of microbial communities that can be used in a BES, combined with the possibility of various oxidation- and reduction reactions, BESs have the potential to be used for the removal of these micropollutants. Wang *et al.* tested the removal of 26 micropollutants in single- or two chamber MFCs, using carbon cloth as the anode. Their results show that the removal of positively charged micropollutants was higher, and that the properties of the compounds determined how well they were removed[29]. The bacterial community in a

BES for the removal of micropollutants can be quite specific, but more research need to be done as to which bacteria can be used for the degradation of certain specific compounds. Many studies have shown that electrodes, able to accept or donate electrons to micro-organisms, can significantly accelerate contaminant removal processes. An accelerated electron transfer process was seen when a species could metabolize the contaminant, as well as the electrode. A mixed culture of bacteria can synergistic interactions between contaminant-metabolizing species and bacteria that are electrochemically active on the electrode [(13)].

V. Enhancing the anodic part of a BES with biochar

There are many attractive aspects about the potential use of BES in applications like CWs or the Sentinel unit for the removal of contaminants from wastewater [(37)]. The anode material used in MECs is similar to the materials used in MFCs, and literature has shown that a good anodic material should have (1) a low resistance and high conductivity; (2) chemical stability; (3) large surface area for the bacteria to attach on; (4) strong biocompatibility [(31)]. A popular material for the anode in MECs and MFCs are carbon-based materials such carbon cloth, graphite felt, carbon mesh and so on, because of their biocompatibility, low costs and high conductivity. Moreover, these materials are chemically stable in anaerobic conditions in MECs[(33)]. Expanding the surface area in the anodic compartment even more with carbon-based materials, to obtain a higher surface area and potentially a higher current output, would be a next step in enhancing BES for the treatment of wastewater.

Granular activated carbon (GAC) as addition to the anodic compartment in a BES

By adding granular activated carbon (GAC) to the anodic compartment, the electrode surface is enlarged, which increases the attachment surface for the bacteria [(38)]. A large surface is preferred so the bacteria can form a biofilm, and, consequently, produce more current. GACs also have great adsorptive capacities and can thus already remove contaminants by adsorption[(38)]. In previous research was shown that the biodegradation rate of a biofilm growing on GAC in a MFC is much higher compared to biofilms that grow on non-adsorbing medias[(39)]. The downside of using GACs, however, is the fact that after a prolonged use, the adsorbing capacities decrease, and the GAC needs to be replaced because of lower efficiency rates, which is expensive in the long run [(41)]. A solution could be the use of biochar.

Biochar, a more environmentally alternative for GACs

Biochar is a carbon-rich solid material produced by thermal decomposition of diverse waste biomass species under oxygen-limited conditions (pyrolysis), with a large pore structure (>100 nm). When pyrolyzing, the biomass maintains the same 3D-structure that is very similar morphology, however at a temperature higher than 800°C, the cellular structure that is comprised by lignin, is converted to conductive graphite. The main properties that make biochar an interesting possible addition to a MEC are: (1) the porous structure, which can immobilize the contaminants and as a consequence decreases their bioavailability and ecotoxicity (2) the ability to act as an electron shuttle and donate and receive electrons from electroactive bacteria, (3) the ability to act as a substrate for electroactive bacteria and plants [(40)]. Biochars show electroconductive features, which makes them an interesting potential addition to MFCs and MECs. In 2012, Liu *et al.* showed that biochar can facilitate interspecies electron transfer. Also, when compared to GACs, the anaerobic

metabolism of the biofilm was as higher. Biochar thus has many environmental benefits and comes at a very low production cost. It can become an improvement in MFCs and MECs for large-scale wastewater treatment operations [41]. Biochar has a longer lifetime compared to GACs, and wood-derived biochar has already been studied as a possible replacement for GACs in contaminant removal applications. Biochar is also a low-cost material compared to GACs, and shows additional benefits because of additional carbon sequestration and soil amendment features [42].

Biochar as substrate material for EABs in BES for the removal of pesticides

Research has shown that biochar and GACs not just adsorb contaminants, but are also able to catalyze their chemical transformation [43–45]. Oh *et al.* found that biochar stimulated the chemical reduction of nitro herbicides by shuttling electrons between reductants and organic contaminants [46]. Surface redox-active moieties or RAMs on biochars seem to play an important role in these processes. Biochar has also been observed as electron shuttle between Shewanella oneidensis MR-1 and Fe(III) minerals in a study by Kappler *et al*., thereby promoting microbial reduction of ferrihydrite [47]. Tong *et al*. showed that the addition of biochars to paddy soil enhanced the microbial transformation rate of pentachlorophenol, due to increased EET of micro-organisms because of their growth on biochar [44] . In 2015, Yu *et al.* examined the reductive dechlorination of the pesticide pentachlorophenol (PCP) by *G. sulfurreducens* in the presence of different biochars (pyrolyzed at different temperatures), to understand how biochar affects the bioreductions of contaminants. Their findings showed that biochar significantly enhances reductive dechlorination of a pesticide called pentachlorophenol. However, this study also gave rise to many new questions that need answering regarding the potential beneficial use of biochar in BES, and the possible breakdown or biotransformation of pesticides by electroactive bacteria in the presence of biochars [40].

In this study, the possible degradation of two neonicotinoid insecticides by a mixed culture or pure *G. sulfurreducens* culture is examined. Two pesticides present in the wastewater of PC Fruit (Sint-Truiden, Belgium), thiacloprid and pirimicarb, were chosen because of their low biodegradability rates, high prevalence in the wastewater of PC Fruit and possible hazardous effect to the aquatic environment. Both are neonicotinoid insecticides and disrupt the nervous system of insects [48,49]. As an addition, pentachlorophenol (PCP), an insecticide and herbicide was used in this research as a well-studied pesticide, used in wood preservation [50]. Additionally, the addition of biochar to the anodic BES compartment and how this affects pesticide removal will be studied.

VI. Hypothesis, research questions and objectives

The focus of this thesis will lie on the potential use of electroactive bacteria and biochar in BES to remove pesticides from wastewater. We hypothesized that electroactive bacteria can degrade or biotransform the pesticides pirimicarb and thiacloprid in a non-electrochemical system, and that this degradation is increased when a granular activated carbon or biochar is added to serve as electron shuttle between the contaminant and the EABs. Second, we hypothesized that the addition of biochar made from waste biomass to a bioelectrochemical system improves the removal of pesticides from wastewater, and this via diverse mechanisms, acting as a bacteria-colonization substrate, an electron shuttle, and mediating redox reactions. To evaluate this, the research is divided in two main parts:

Part One: Are pesticides degraded by a pure of mixed culture and is the degradation improved when adding a GAC or biochar in a simple, non-electrochemical system? Following objectives were made:

I. Assess the tolerance of a pure or mixed culture of bacteria to different concentrations of pesticides.
II. Determine whether the bacteria can degrade/bio-transform pesticides by using the pesticides as either an electron donor- or acceptor.
III. Assess the effect of adding a conductive material in the form of a commercial GAC on pesticide concentrations.
IV. Determine whether adding biochar enhances pesticide removal, and how it compares to GAC.

Part Two: How do the performances of GACs and biochars as anode material in a BES compare regarding bacterial attachment on the anodes and produced current densities? Following objectives were made:

I. Understand in an MEC how anodes enhanced with biochar perform compared to GAC as anode material in terms of bacterial attachment and current densities.
II. Compare performances of GACs and biochars as addition to the anodic compartment in a larger BES set-up, looking at current densities and biofilm characterization.

I. General procedures

1. Bacteria, media and cultivation conditions

Geobacter sulfurreducens (DSM 12127, DSMZ, Leibniz, Germany) and a mixed culture (grown from silt collected from Demer, Diepenbeek, Belgium) were used as bacterial inoculum in the experiments. Bacteria were grown in fresh water medium (FWM)[51], composed of 2.5g L^{-1} $NaHCO_3$, 0.25g L^{-1} NH_4Cl, 0.6g L^{-1} $NaH_2PO_4*H_20$, 0.1g L^{-1} KCl, 10ml L^{-1} vitamin mix and 10ml L^{-1} mineral mix (*Supplemental Table 2*). This medium was used in all experiments and is referred to as FWM. For culturing, 20mM Na-acetate ($NaC_2H_3O_2$, 1.64 g L^{-1}) and 40mM Na-fumarate ($C_4H_2Na_2O_4$, 6.40 g L^{-1}) was added as electron donor and acceptor respectively. Medium was prepared by combining all components mentioned above and was then transferred to 12 mL tubes for anaerobe culturing (Chemglass Life Sciences, Vineland, USA). Medium was then flushed for 15 minutes with N_2, the headspace was flushed for 5 minutes with N_2 (image of purging system can be found in *Supplemental Figure 21*) and the tube closed with a tight butyl seal (Rubber B.V., Hilversum, The Netherlands). Then with a sterile needle, 2 mL of CO_2 was injected in the headspace and N_2 was added to reach 1,4 bars over pressure. The final headspace gas composition was approximately 80:20 $N_2/C0_2$. Tubes were autoclaved for 15 minutes at 121°C (Liquid A program, Tuttnauer, Breda, The Netherlands). Afterwards, 1 mL of pure or mixed bacterial culture was injected and the tubes were placed in a dark room at 30°C. Routinely, bacteria were also cultured in 60 mL glass vials (548-0609, VWR, Radnor, USA), in the same medium as described above. Media was flushed for 15 minutes with N_2, followed by 5 minutes of headspace flushing with N_2 and injecting 12 mL of CO_2. To maintain active cultures, a transfer was done every 2 weeks.

Pesticides were spiked in the media from concentrated stock solutions, to reach final concentrations of 10 mg L^{-1}. A stock of 10.000 mg L^{-1} thiacloprid (PESTANAL®, 37905-100mg-r, analytical standard, Sigma-Aldrich, St. Louis, USA) was made dissolved in DMSO. A pirimicarb (PESTANAL®, 45627, analytical standard, Sigma-Aldrich, St. Louis, USA) solution of 1.000 mg L^{-1} was made in Milli-Q water, filter sterilized (45 µm, VWR, Radnor, USA) and flushed with N_2 gas for 10 minutes. A 10.000 mg L^{-1} pentachlorophenol (PCP) (97%, P2604-5G, Sigma-Aldrich, St. Louis, USA) solution was prepared in methanol (99,8%). Pesticides were injected under sterile conditions using a Hamilton syringe (VWR, Radnor, USA). Physicochemical properties of the pesticides can be found in *Supplemental Table 1*.

2. Biochars and granular activated carbons (GACs)

The biochars were made by the chemistry department at Hasselt University. Two of the used biochars were made by of the following waste biomasses: coffee beans and AB-wood (industrial wood mix). The particles were grinded until they reached a diameter of less than 1 cm using a Retsch SM100 cutting mill (Haan, Germany). The particles were then pyrolyzed at 450°C in a modified reactor [52]. Another biochar used in the experiments was made from *Thypha latifolia* (Typhaceae, Wijers, Limburg). The whole plant was used and

the pyrolyzation process was performed in a closed oven at 500°C. Three commercial granular activated carbons (GACs) were provided by Chemviron Carbon (Écaussinnes, Belgium): HPC MAXX 830, Filtrasorb® 400 and Cyclecarb® 301. The properties of the biochars and GACs were predetermined and can be found in *Supplemental Table 3*.

3. Bradford Protein Assay

Protein concentrations were determined by the Bradford method with bovine serum albumin (BSA, ThermoFisher Scientific, Massachusetts, USA) as a standard, using the Quick Start Bradford Dye Reagent (Bio-Rad, Hercules, CA, USA) for soluble protein quantification. First, a standard curve was made, diluting BSA in Milli-Q water (linear range from 125–2000 µg/mL). 50 µL of each solution was then mixed with 50 µL of a 1M NaOH solution and vortexed. Afterwards, the dilutions were mixed with the Bradford dye, incubated for 5 minutes and the absorbance was measured at 595 nm using UV detector (Shimadzu UV-1602, Shimadzu, Kyoto, Japan). Medium was sampled from the vials or tubes and then vortexed for 30 seconds. 500 µL NaOH was then added to 500 µL sample, then vortexed for 5 seconds. 100 µL of this mixture was then added to 1 mL Bradford dye and after 5 minutes of incubation, the absorbance was measured at 595 nm.

4. Pesticide extractions

To extract pesticides from the culture media, a modified Quechers protocol was used[53]. 4 mL medium sample was transferred into 15 mL disposable polypropylene centrifuge tubes and centrifuged at 4000 rpm (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) for 15 minutes to pellet the bacterial cells. 3 mL acetonitrile (ACN) hyper solvent for HPLC analysis (VWR, Radnor, USA) was added to 3mL sample and the tubes were immediately shaken for one minute. 1,2g $MgSO_4$, 0.3g NaCl, 0.3 g $C_6H_5Na_3O_7$ * 2 H_2O (trisodium citrate dihydrate) and 0.15g $C_6H_8Na_2O_8$ (disodium hydrogen citrate sesquihydrate) were added to introduce phase partitioning. The tubes were immediately shaken for 5 minutes and centrifuged for 5 minutes at 4000 rpm. 2 mL of the upper ACN layer of the extract was filtered through a 0.22µm PTFE filter (VWR, Radnor, USA), transferred into HPLC autosampler vials (VWR, Radnor, USA) and analyzed via high-performance liquid chromatography (HPLC) as described in the section below. The pesticide concentration adsorbed by the granular activated carbons and biochars were extracted in 3 mL acetonitrile by the process of sonication for two hours. Samples were then filtered with a 0.22 µm PTFE filter and the pesticide concentration was determined by HPLC.

5. High-performance liquid chromatography (HPLC)

Pesticides were separated on a C18 column (ACE Equivalence 5 C18, 5µm particle size, EQV-5C18-2546, Advanced Chromatography Technologies Ltd, Aberdeen, UK) on a HPLC system Chromaster (Hitachi Chromaster, VWR, Radnor, USA) with a 5160 pump, 5280 autosampler, 5310 column oven and 5430 diode array detector (Hitachi, Tokyo, Japan). For pirimicarb, a 30:70 Milli-Q water : ACN phase was used for 5 minutes. Thiacloprid was run in Milli-Q water : ACN on the following gradients: 0-3 minutes: 90:10; 3-10 minutes: 80:20; 10-15 minutes: 50:50; 15-17 minutes: 90:10. Pentachlorophenol ran on a 20:80 Milli-Q

water : ACN phase for 14 minutes. The retention time of Pirimicarb was 4,2 minutes, for thiacloprid 14,7 minutes and pentachlorophenol 11,9 minutes. Pirimicarb was monitored at a wavelength of 244 nm, thiacloprid was detected at 242 nm and pentachlorophenol at 213 nm.

6. Measurements of acetate consumption

The 76785 FluoroSELECT™ Acetate Assay Kit (Sigma-Aldrich, USA) was used to determine the amount of acetate still left in medium. Standard protocol by Sigma-Aldrich was used with the following adaptations: Five acetate standards were made instead of just one (1mM, 0,75 mM, 0,5 mM, 0,25mM and 0mM) and fluorescence intensity was measured at an excitation wavelength of 495 nm and an emission wavelength of 590 nm with FLUOStar Omega (BMG LABTECH, Ortenberg, Germany).

7. Scanning electron microscopy (SEM)

To evaluate cell attachment to the activated carbon and the biochar, the GAC or biochar-attached fraction was studied by scanning electron microscopy. Samples were first fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer for up to 12 hours at 4°C, then washed 3 times in 0.1 M phosphate buffer at 4°C for 10 minutes each, dehydrated further in an ethanol/water mixture of 50%, 70%, 80%, 90%, 95% and 100% for 10 minutes each (dehydration in 100% ethanol was done 3 times), and at last immersed twice for 30 seconds in pure hexamethyldisilazane (Sigma Aldrich, St Louis, MO, USA) followed by 10 minutes of air-drying. Before SEM analysis, the samples were sputter coated by an Auto Fine Coater (JFC-1300, JEOL, Peabody, USA) with a coater containing a gold target (81001 Gold target (57x0.1), JEOL, Peabody, USA) and the analysis was done with a benchtop SEM (TM3000, Hitachi, Tokyo, Japan). Images were processed and analyzed using the software ImageJ 2.0.0 (developed by National Institutes of Health, Bethesda, USA).

8. Fluorescence in-situ hybridization (FISH)

Paraformaldehyde (PFA)-fixation was performed on GAC and biochar samples according to Amann, 1995 [(54)]. The samples were fixated and hybridized in Eppendorf tubes. A 1,4% paraformaldehyde (PFA) solution was added to the samples for 24h at 4°C. Then, the tubes were shaken and the supernatant was removed. A washing step with 1x PBS (8,0 g L^{-1} NaCl; 0,2 g L^{-1} KCl; 1,44 g L^{-1} NaH_2PO_4; 0,2 g L^{-1} NaH_2PO_4, pH = 7) was performed three times: 1 mL 1x PBS was added to each sample, then resuspended with a pipette tip and the supernatant was removed. 300µL 1xPBS was then added to the samples, with the same amount of molecular biology grade 100% ethanol (not denatured), mixed well and stored at -20°C. The samples were then dehydrated by adding 50%, 80% and 100% ethanol successively (3 minutes each) and air dried. Next, hybridization was performed on the samples. A 30% formamide (FA) solution was made by combining 270 µL of NaCl, 30 µL of Tris/HCl, 750 µL of ddH2O, 450 µL of FA and 1,5 µL of 10%SDS. Afterwards, the solution was filter sterilized. 24 µl hybridization buffer was added to each dried sample, as well as 3 µl of each probe (six probes were added in total, concentration 50 ng/µl). The samples were then hybridized in a moisture chamber. The Eppendorf tubes were placed in a rack and silver foil, and consequently in a water bath at 46°C for 3 hours. Afterwards, the formamide was washed off by pouring approximately 2 mL of washing buffer (0,02 M Tris-HCl; 0,01 % SDS; 0,005 M EDTA; 0,1 M NaCl and ddH2O) in the Eppendorf tubes. Samples

were then places in a water bath for 20 minutes at 48°C. Afterwards, the washing buffer was removed, the samples were washed with ddH_2O and air dried at room temperature. The samples were then placed in folded aluminum foil and stored at -20°C until microscopic examination. The probes that were used were the following: Fluo-GEO3-A, Fluo-GEO3-B and Fluo-GEO3-A to target Geobacter genera, with the helpers HGEO3-4 and HGEO3-4, and then also Cy3-EUB338 or Cy5-EUB338 to target most bacteria (probe specifications in *Supplemental Table 4*). Probes were synthesized by IDT (Coralville, USA). The samples were stained with DAPI (4′,6-diamidino-2-phenylindole) to stain all nucleic acids. Images were taken in a Leica TCS SP5 AOBS Spectral Confocal Microscope, equipped with four lasers (405, Ar, Kr/Ar, and He/Ne) (405, 561, and 633) and 4 prism spectrophotometer detectors. The objective used was a HCX APO L U-V-I 40.0x0.80. The software was the Leica Confocal Software (LCS) for multi-dimensional image series acquisition. The Images were processed and analyzed using ImageJ 2.0.0.

11. Statistical analysis

Statistical analysis was performed in SPSS (IBM Analytics, New York, USA). Independent sample t-tests and ANOVA analysis were used for comparing group averages (using a significance level of $\alpha = 0,05$). If the ANOVA result was significant, Tukey HSD was used as the post hoc test for all pairwise comparisons. Outliers were removed using a ± 25 percent interquartile distance cut-off. All variables were checked for normality using the Shapiro-Wilk test and homogeneity of variances (Levene's test or Brown-forsythe).

If there were two independent variables (e.g. medium and bacteria type) possibly having an effect on the dependent variable (e.g. pesticide concentration) the second one was controlled by grouping values of the dependent variable based on the second variable. For example, when the effect of medium type on pesticide concentration was tested, observed pesticide concentrations were grouped by bacteria type. Within each of these groups there are concentration values for every medium and the average of these values was compared using ANOVA analysis.

II. Experimental procedures

1. Pesticide tolerance

Pre-grown bacterial cells (log-phase) were anaerobically and aseptically transferred to 12 mL tubes containing 9 mL FWM with 24 mM Na-fumarate as an electron acceptor and 2g L^{-1} Na- carbonate as buffer. In the treatment condition, thiacloprid was added as electron donor in the following concentrations: 0 – 0,625 – 1,25 – 2,5 – 5 – 10 mg L^{-1}. The experiment was repeated with Na-acetate as electron donor in the same concentrations. Each condition was replicated in threefold. Thiacloprid was spiked from the stock solution in DMSO. Tubes were incubated at 30°C in a dark environment for two weeks and optical density (OD) was measured every 2 days with a spectrophotometer (Novaspec, Pharmacia LKB, Stockholm, Sweden) at a wavelength of 600 nm. The Gompertz model was used for statistical analysis. Details can be found in *Supplemental Section 1: Gompertz Model*. A more detailed figure of the set-up can be found in *Supplemental Figure 19*.

2. Anaerobic pesticide degradation in growing cultures

Bacteria were pre-grown in FWM containing 20 mM Na-acetate and 40 mM Na-fumarate in 60 mL vials. From log-grown cells, a 5 % solution of mixed culture or *G. sulfurreducens* was transferred to FWM containing 10 mg L^{-1} of pesticide (thiacloprid, pirimicarb or pentachlorophenol) or no pesticides (control group). Experiments were conducted in 60 mL glass vials, filled with 39 mL FWM and with or without 20mM Na-acetate and 40 mM Na-fumarate as electron donor or acceptor. 1 mL of the pre-grown bacterial suspension was spiked in each vial under anaerobic sterile conditions. Vials without bacteria were taken along as controls. The vials were incubated in a dark chamber at 30°C for two weeks. Each condition was replicated in threefold. At the end of the experiment, samples were collected for pesticide concentration determination with HPLC. Bacterial growth was measured with the Bradford assay. All methods used are described in the sections above. A more detailed figure of the set-up can be found in *Supplemental Figure 20, A*.

3. Anaerobic pesticide degradation in growing cultures with granular activated carbons

The degradation experiments were conducted in 12 mL tubes with 9 mL FWM and 20 mM Na-acetate as electron donor. For Cyclecarb®301, FWM with Na-fumarate was set up as a control. 0,25 g of a commercial GAC (2,5g L^{-1}) (HPC MAXX 830, Filtrasorb® 400 and Cyclecarb® 301) was added to each tube (amount of GAC was based on a paper by Chen *et al.*., 2014) [45]. Bacteria (1 mL, mixed culture and *G. sulfurreducens*) and pesticides (thiacloprid and pirimicarb) (10 mg L^{-1}) were inoculated under sterile and anaerobic conditions. As a control, no bacterial cells were added to tubes. At the end of the experiment, samples were collected for pesticide concentration determination with HPLC, and bacterial growth was measured with the Bradford assay. Tubes were placed in a temperature-controlled room at 30°C in the dark for two weeks. A more detailed image of the set-up can be found in *Supplemental Figure 20, B*.

4. Anaerobic pesticides degradation in growing cultures with biochars

Degradation experiments were conducted in 12 mL tubes filled with 9 mL FWM medium with 20 mM Na-Fumarate as electron acceptor and 0,25 g of biochar. The biochar types tested were based on *Thypha*, AB-wood and coffee bean biochars. Bacteria (mixed culture) and pesticides (thiacloprid and pirimicarb) (10 mg L^{-1}) were inoculated under sterile and anaerobic conditions. As controls, no bacterial cells were added to the tubes. A condition with a GAC (Cyclecarb®301) was added to control for adsorption by the biochars. Experiments were performed in triplicates. At the end of the experiment, samples were collected for pesticide concentration determination with HPLC, and bacterial growth was measured with the Bradford assay. Samples of the biochar and GAC were collected for FISH analysis after the experiment. Tubes were placed in a temperature-controlled room at 30°C in the dark for two weeks. A more detailed figure of the set-up can be found in *Supplemental Figure 20, C*.

5. Comparing GACs in an eight-electrode microbial electrolytical cell (MEC)

A fine stainless steel (SS) mesh (RVS 554/64: material AISI 316L, Solana, Schoten, Belgium) was cut in pieces of 4 x 2 cm, then folded into small pockets (projected surface area of $2cm^2$). The bottom of each mesh was sewn with a SS wire (AISI 304 Wire; 0,5mm diameter, Advent Research Materials, UK). Afterwards, the mesh was filled with GACs (HPC MAXX 830, Filtrasorb® 400 and Cyclecarb® 301) until completely full. The average weight of the SS steel mesh of the electrodes was 1,25 ± 0,03 g. The average weight of the GACs inside the pockets was 0,30 ± 0,00 g. Each condition was made in duplicate. Two empty meshes were used as control electrodes. The ends of the wire used to sew the mesh were twisted and heat-shrink tubing was applied around these twisted wires to maintain a tight connection. The mesh and wires served as current collectors for the anodes. An overview of the anodes can be found in *Supplemental Figure 23.*

The experiment was conducted at the Center for Microbial Ecology and Technology (CMET, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium), and the eight-electrode MEC reactor was designed by Dr. Kun Guo [56]. On the first day, the reactor was installed by connecting the electrodes to the reactor and additionally adding 750mL of FWM with 20 Mm Na-acetate as electron donor. The cathode was a folded piece of stainless steel mesh and was separated from the reactor space by an anion exchange membrane (AMI-7001, Membranes International, Ringwood, USA). 60 mL of FWM (20mM Na-acetate) was added to the cathodic space. In total there were eight anodes, functioning as working electrodes (WEs), one cathode functioning as the counter electrode (CE) and one reference electrode (RE) (Ag/AgCl 3M KCl) (BASi®, Lafayette, USA) (*Figure 3 and Supplemental Figure 22*). The medium was flushed with a N_2/CO_2 (80:20) gas mixture for 20 minutes. The anodes were polarized for 24 hours on -0,2V vs. Ag/AgCl. The next day, 40 mL of a mixed culture inoculum, which was taken from the effluent of an operating BES from Dr. Kun Guo (CMET, unpublished work), containing ± 90% *G. sulfurreducens* bacteria, was injected in the reactor. The electrodes were again polarized at -0,2V vs. Ag/AgCl until the end of the experiment. A magnetic stirrer (Snijders Scientific, Tilburg, The Netherlands) with a stirring bar (length: 4 cm) was used to mix the solution continuously at a speed of 350 rpm. The experiment was conducted in a temperature-controlled room at 28°C. Samples (GAC granules) were collected from the SS mesh after the experiment for SEM analysis. Pre-inoculation and at the end of the experiment, cyclic voltammograms (CVs) were

conducted in the same medium to investigate the electrochemical activity on the electrodes under acetate turnover conditions, meaning that CVs were performed when there was an excess of Na-acetate in the medium and the bacteria were metabolizing Na-acetate. A cyclic voltammogram is a technique where a potential is varied over time and the corresponding produced current is recorded. Because of the varying potential, redox reactions will happen that give more information about the electrochemical reactions taking place on the electrode. CVs were performed within a potential window between −0.6 and 0 V (vs Ag/AgCl) at a scan rate of 1 mV/s. In the beginning, a CHI 1000C Multi-Potentiostat (CHI Instruments, Austin, TX, U.S.A.) was used to conduct chronoamperometry and cyclic voltammetry experiments for all eight electrodes at the same time. When the current of the electrodes containing GACs exceeded 10 mA (e.g. the current limit of the CHI Potentiostat), all eight electrodes were connected together and controlled by a Biologic VSP potentiostat (BioLogic Science instruments, Seyssinet-Pariset, France), recording the sum of all currents. In the end of the experiment, the eight electrodes were disconnected from each other and measured one by one by chronoamperometry to record the current produced by each electrode, and current densities were calculated. Each time a drop in current (to ± 0 mA) was observed, the medium was either replaced (FWM with 20mM Na-acetate) or a Na-acetate solution in ddH_2O (reaching a final concentration of 2,67 g L^{-1} in the reactor) was injected.

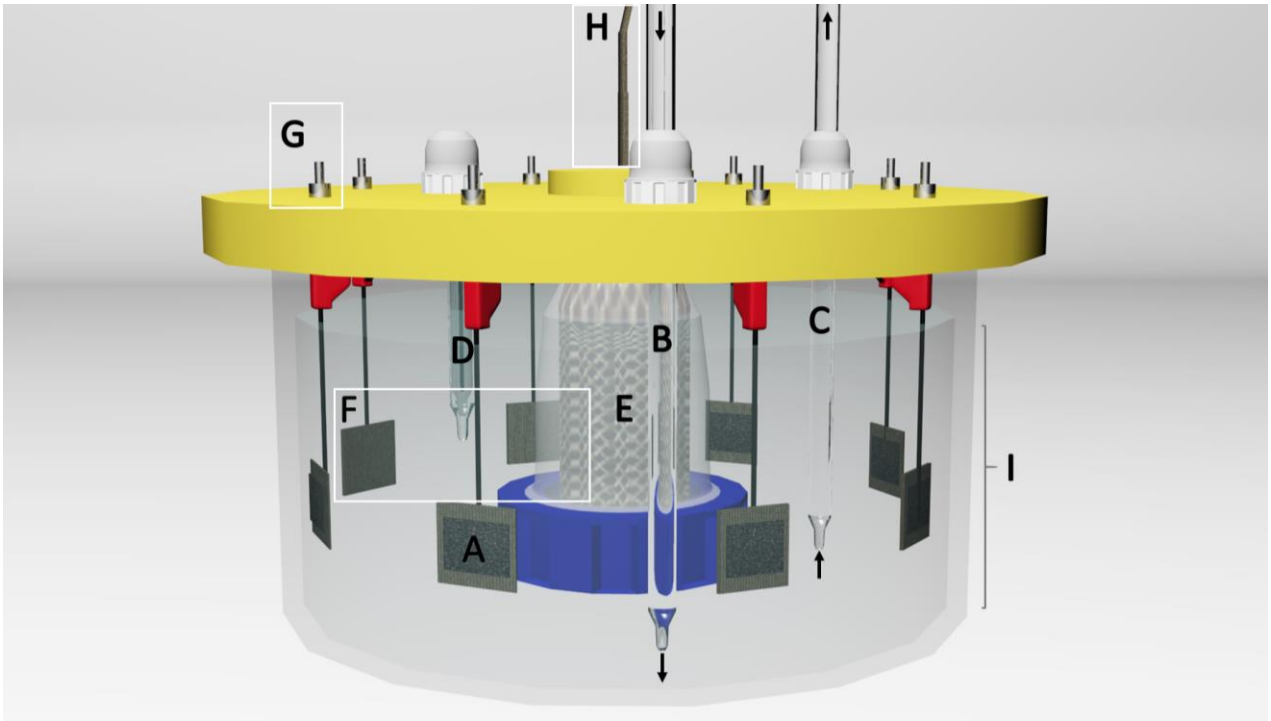

Figure 3 *Eight-electrode MEC reactor used in this experiment (design by Dr. Kun Guo, CMET). A: Anode (stainless steel mesh filled with GAC) B: Pipette for influx; C: Pipette for efflux; D: Reference electrode (Ag/AgCl in 3M KCl); E: Cathode compartment with stainless steel mesh as cathode; F: Control electrodes (empty stainless steel mesh); G: Connection points to potentiostat; H: Cathodic mesh to connect to potentiostat; I: The reactor was filled with FWM (20 mM Na-acetate). Artwork by Dries Peeters.*

6. Comparing GACs as addition to the anodic compartment in a larger BES set-up

Three GACs (HPC MAXX 830, Filtrasorb® 400 and Cyclecarb® 301) were studied in a larger BES set-up as addition to the anodic compartment. Each GAC had granules with a diameter of 1-2mm and was mixed with fine sand with a diameter less than 1mm (Cobo Garden, Niel, Belgium) in a 50:50 (v/v) ratio, reaching a total volume of 100 mL. The control condition consisted of 100 mL of fine sand only. The glassware was made by Adams and Chittenden Scientific Glass (Berkeley, USA) (5 cm diameter, 20cm in height) with aerated cathodic compartment, four sampling points and a porous glass filter at the bottom (pore size: 16-40 µm, ROBU®, Hattert, Germany). The system was operated in batch mode. FWM was prepared as described above with the addition of 20mM Na-acetate as electron donor, and 200 mL was added to each glass ware. The cathodic electrode was made of carbon felt (diameter: 4 cm; thickness: 2,5 cm) (Mersen, Paris, France), with a titanium wire attached to connect the electrode to either a potentiostat or Arduino system. A layer of fine sand (2 cm) was added between the anodic and cathodic compartment. The anodic electrode was a graphite rod (length: 5cm; diameter: 1cm) (Mersen, Paris, France) with an attached titanium wire. The bottom of the glass ware was flushed with N_2 gas two hours. Two glass wares of each condition were injected with 1 mL of mixed culture bacteria three days after the set up. The potential of each anode (WE) was set at 0,5 V vs the cathode (CE) and current generation was recorded by chronoamperometry using a home-made Arduino based measuring system the first 20 days of the experiment (*Figure 4* and *Supplemental Figure 24*). Samples of the medium were taken on day 0 and day 23 to measure Na-acetate. On the final day, cyclic voltammograms were performed in the same medium to investigate the electrochemical activity of the biofilms at the electrodes under acetate turnover conditions in a window between −0.8 and 0,8V (vs Ag/AgCl) at a scan rate of 10 mV/s with a VERSAStat 3F Potentiostat (Princeton Applied Research, Oak Ridge, USA). Set-up was placed in a dark room at room temperature.

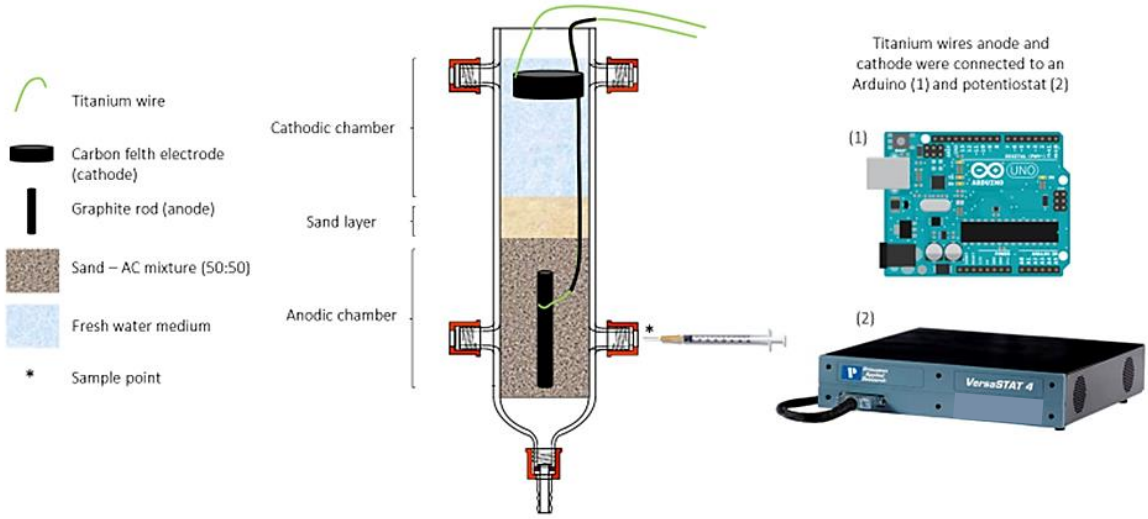

Figure 4 Larger BES set-up. The anodic compartment contained 100 mL of a GAC-fine sand mixture or fine sand (control); a 2 cm layer of fine sand was added on top of the anodic compartment, separating the anodic and cathodic compartment. Titanium wires were used to connect the electrodes to electronical devices. Samples were taken on day 0 and day 23 to check Na-acetate consumption by the bacteria from a specific sampling point (*). CA was recorded during the first 20 days of the experiment by a home-made Arduino based system (1). CVs were performed on each set-up at the end of the experiment (on day 23) using a potentiostat (2)

RESULTS

We hypothesized that electroactive bacteria can degrade or biotransform the pesticides pirimicarb and thiacloprid in a non-electrochemical system, and that this degradation is increased when a granular activated carbon or biochar is added to serve as electron shuttle between the contaminant and the EABs. To answer the first hypothesis, we started with assessing the tolerance of a pure *G. sulfurreducens* culture to different concentrations of thiacloprid. The second part was setting up experiments with bacteria (mixed culture and *G. sulfurreducens*) in vials or tubes. This existed of three main parts: (1) bacteria with pesticides; (2) bacteria with pesticides and three GACs; (3) bacteria with pesticides plus three biochars and one GAC. At the end of each experiment, concentrations of pesticides were quantified by HPLC, and the concentration in the medium and/or GACs/biochar with bacteria was compared to a control group without bacteria. FISH analysis was performed on biochar and GAC particles. To gain more insight in bacterial growth, a Bradford assay was performed on media samples from the vials or tubes.

Second, we hypothesized that the addition of biochar made from waste biomass to a bioelectrochemical system improves the removal of pesticides from wastewater, and this via diverse mechanisms, acting as a bacteria-colonization substrate, an electron shuttle, and mediating redox reactions. The second hypothesis was answered by running tests in two different BES set-ups: (1) in an eight-electrode BES, in which the performance of eight electrodes, six of which containing three GACs, was compared by looking at maximum current densities and bacterial attachment and (2) in a larger BES set-up, comparing three GAC-sand mixtures and one sand condition. Chronoamperometry (CA) was performed on these BES set-ups, as well as cyclic voltammetry (CV).

Part One: Are pesticides degraded by a pure of mixed culture with or without the addition of biochar of GAC?

I. Growth rates of a pure culture are not significantly affected by different concentrations of thiacloprid

To test the tolerance of thiacloprid for the bacteria, bacterial growth was measured every two days by OD_{600} measurements. A graph was made to check the optical density (OD) in function of the time for each thiacloprid or acetate concentration. The Gompertz model was used to estimate growth parameters for each concentration (*Figure 5, C*).

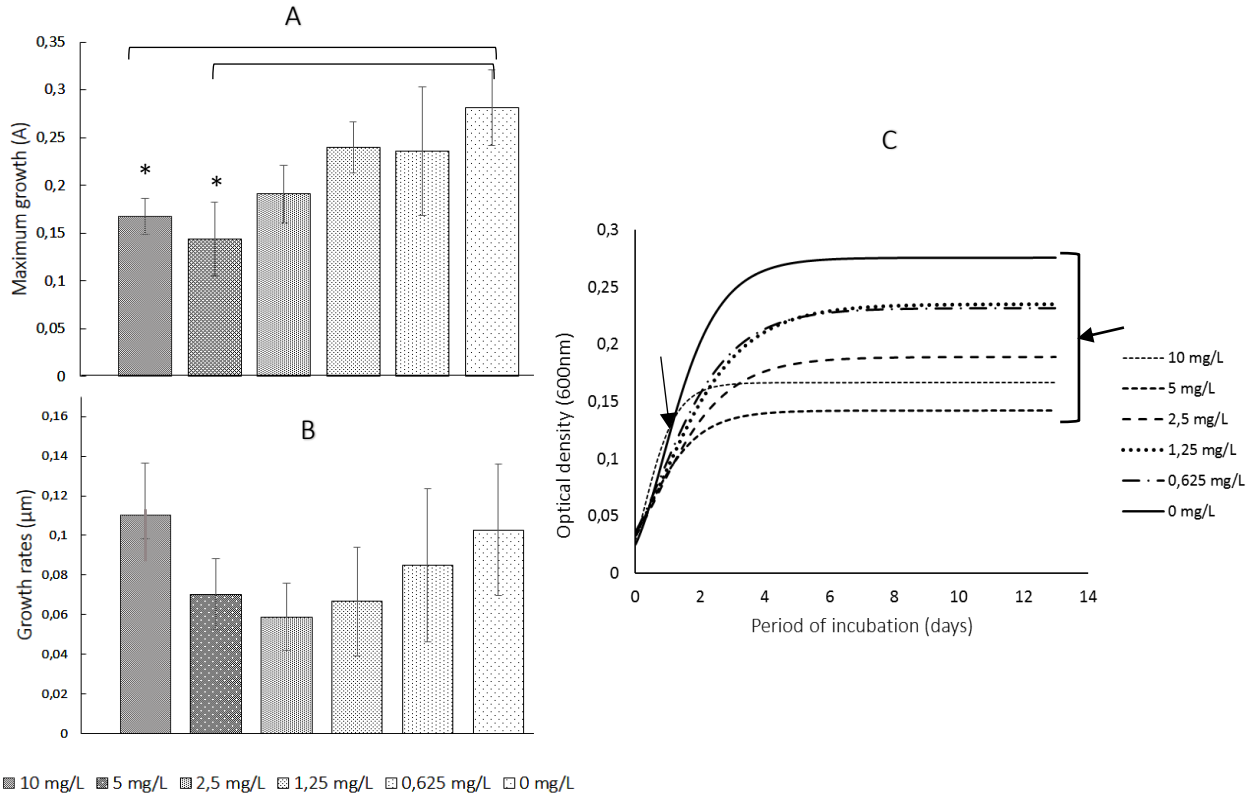

***Figure 5 A**: Maximum growth absorbance values (parameter A) are pictured for different concentrations of thiacloprid. * indicates a statistically significant difference ($p \leq 0,05$); **B**: Growth rates (parameter μ_m) are shown for different concentrations of thiacloprid; **C**: Bacterial growth modelled with Gompertz model for each concentration of thiacloprid. The left arrow indicates growth rates (μ_m), the right arrow indicates maximum growth (A)*

The log phase is observed from day 0 until day 2. Maximum turbidity is observed after two days, which means the bacteria have reached the stationary phase. For thiacloprid, no significant difference in growth rate was found between different concentrations with ANOVA (*Figure 5, B*). However, the maximum turbidity value for 0 mg L^{-1} was significantly higher compared to the 5 and 10 mg L^{-1} conditions ($p \leq 0,05$) (*Figure 5, A*). The same test was performed for different concentrations of Na-acetate (*Supplemental Figure 25 B, C and D*). Here, 1,25 mg L^{-1} shows a significant difference ($p \leq 0,05$) in maximum turbidity compared to all other conditions These results indicate that a higher concentration of thiacloprid influences the maximum growth of the bacteria, however the growth rate is not influenced. In further experiments, a concentration of 10 mg L^{-1} was used.

II. *No significant decrease is found in pesticide concentration without GAC or biochar between bacteria and control groups is found, except for pirimicarb by G. sulfurreducens in Na-acetate medium.*

Mixed culture and *G. sulfurreducens* were exposed to 10 mg L^{-1} thiacloprid, pirimicarb and pentachlorophenol. For pirimicarb and thiacloprid, three different mediums were used, containing both electron donor and acceptor (respectively Na-acetate and Na-fumarate) or only an electron donor or acceptor. HPLC measurements of the pesticide concentrations in the vials after two weeks of incubation gave an indication if there was any decrease in conditions with bacteria. Bradford assay results show bacterial growth by measuring the protein concentration in medium after two weeks of incubation.

- *The effect of bacteria on the pesticide concentration*

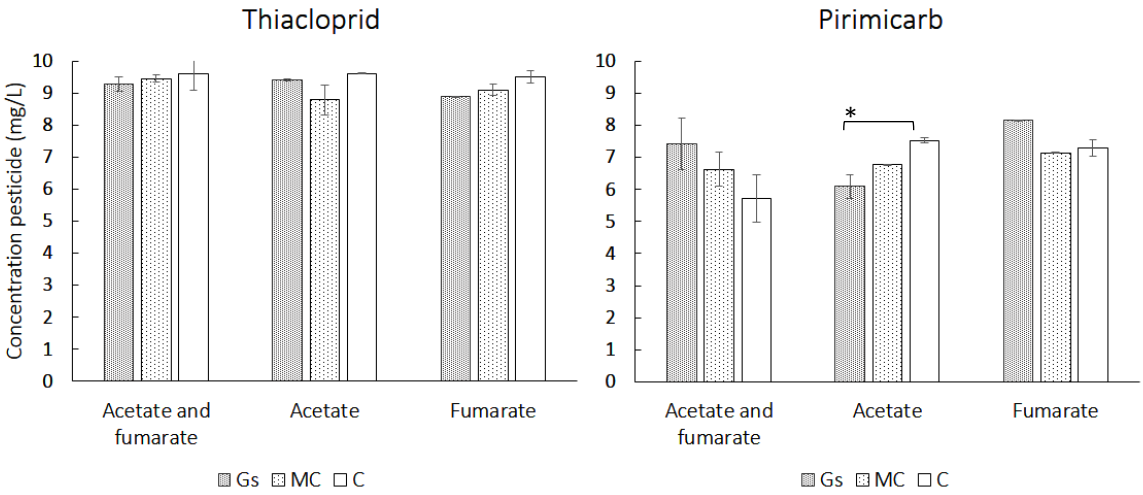

Figure 6 *Pesticide concentrations measured with HPLC for thiacloprid and pirimicarb. Values represent means of either triplicates or duplicates ± 1 SD. Gs = G. sulfurreducens; MC = Mixed culture; C = Control. * indicates a significant difference with p ≤ 0,05*

For thiacloprid (*Figure 6*) and pentachlorophenol (*data not shown*), HPLC results indicate that the mixed and pure culture both do not show a significant decrease of the pesticide concentration after two weeks in any of the conditions compared to the no bacteria control group. For pirimicarb, a significant decrease ($p \leq 0,05$) was found in vials with *G. sulfurreducens* compared to the control condition in Na-acetate medium. ANOVA analysis was performed to compare group means.

- *Bacterial growth*

The protein concentration was measured using the Bradford method and is directly correlated with bacterial growth. ANOVA analysis was used to compare group means. For thiacloprid and pirimicarb (*Figure 7, left*), *G. sulfurreducens* grew significantly better in medium with Na-acetate and Na-fumarate compared to the other two media types ($p \leq 0,01$). Mixed culture grew significantly better for both pesticides in medium with Na-acetate and Na-fumarate or Na-fumarate only compared to medium with Na-acetate only ($p \leq 0,01$).

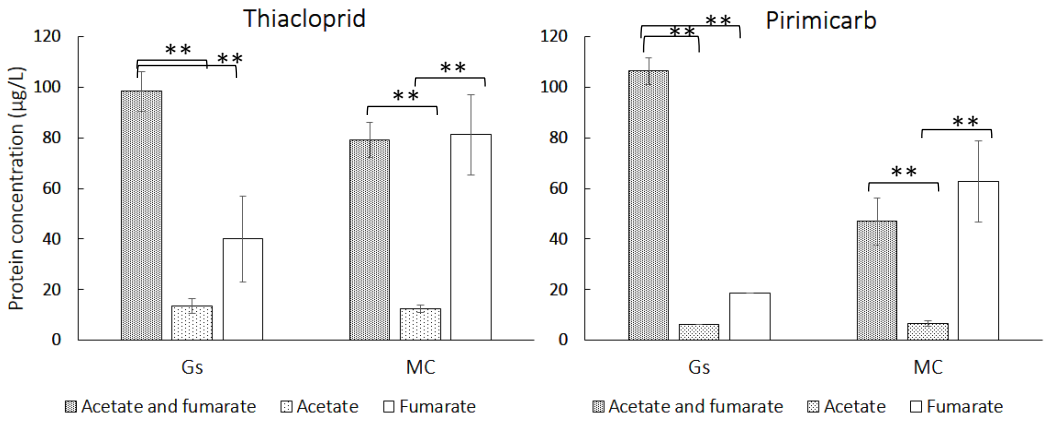

Figure 7 *Bacterial growth measured in medium in vials after a two-week incubation period. ** indicates a statistical difference (p ≤ 0,01). Gs =* G. sulfurreducens*; MC = Mixed culture. Values are means of triplicates or duplicates ± 1SD.*

- *Conclusion*

No significant decrease in pesticide concentration in conditions with bacteria compared to no bacteria controls, except for pirimicarb by *G. sulfurreducens* in Na-acetate medium. For pirimicarb and thiacloprid, *G. sulfurreducens* shows the best growth in medium with both Na-acetate and Na-fumarate, the mixed culture grows best in medium containing Na-acetate and Na-fumarate or Na-fumarate only.

III. The bacteria do not show significant degradation of the pesticides in the presence of GACs

The concentration of two pesticides (thiacloprid and pirimicarb) was examined after two weeks. A pure and a mixed culture was exposed to pesticides in tubes, the only difference being the addition of three GACs: HPC MAXX 830, Filtrasorb®400 and Cyclecarb®301. The concentration of the pesticides was checked in the medium and for Cyclecarb®301, the fraction of pesticides adsorbed by the GACs was determined as well. Protein concentrations were measured with the Bradford assay. The medium that was used was FWM containing Na-acetate for all three GACs and for Cyclecarb®301, one condition was made with FWM containing Na-fumarate as a control. Here, the effect of the addition of GAC on pesticide concentrations and bacterial growth is examined.

- *The effect of bacteria on the pesticide concentration in medium*

The pesticide concentrations in medium were grouped per GAC type (Filtrasorb®400, HPC MAXX 830 and Cyclecarb®301) to control for the effect of the GACs on pesticide concentration. The bacteria type was used as the independent variable resulting in three groups (*G. sulfurreducens*, mixed culture and control) that were compared. No significant difference was found between pesticide concentrations in medium in the presence of *G. sulfurreducens* or mixed culture bacteria compared with the control group. Similar results were found for pirimicarb. For the pesticide concentrations in the presence of Cyclecarb®301, no analysis was done because there were less than three observations for each group.

- *The effect of GACs on the pesticide concentration in medium*

The pesticide concentrations measured in medium were grouped by bacteria type (*G. sulfurreducens*, mixed culture and control) to control for the effect of bacteria on pesticide concentration (giving us three

dependent variables for ANOVA analysis). GAC type was used as the independent variable resulting in three groups (Filtrasorb®400, HPC MAXX 830 and Cyclecarb®301) that were compared for each dependent variable with ANOVA. No significant difference was found between pesticide concentrations (in medium) comparing different GAC's. Similar results were found for pirimicarb.

- *The effect of medium type on the pesticide concentration in medium*

The pesticide concentrations in medium containing Na-fumarate and Na-acetate were compared for Cyclecarb®301. GACs Filtrasorb®400 and HPC MAXX 830 were only observed in medium with Na-acetate, so they were not included in this analysis. The pesticide concentrations in total (medium + adsorbed by Cyclecarb®301) were first grouped by bacteria type (*G. sulfurreducens*, mixed culture and control) to control for the effect of bacterial presence, resulting in three dependent variables. This time the medium type (Na-acetate or Na-fumarate) was used as the independent variable. This means only two groups were compared (for each of the dependent variables) with an independent sample t-test was used. The total thiacloprid concentration (in the presence of *G. sulfurreducens* ($p \leq 0,05$) and a mixed culture ($p \leq 0,01$)) is significantly higher in Na-fumarate medium compared to Na-acetate medium. A similar analysis was performed for pirimicarb. The total pirimicarb concentration (in the presence of *G. sulfurreducens*) is significantly higher ($p \leq 0,05$) in Na-acetate medium compared to Na-fumarate medium. No significant difference was found between mediums in the absence of bacteria (control group) using the non-parametric Mann-Whitney U test.

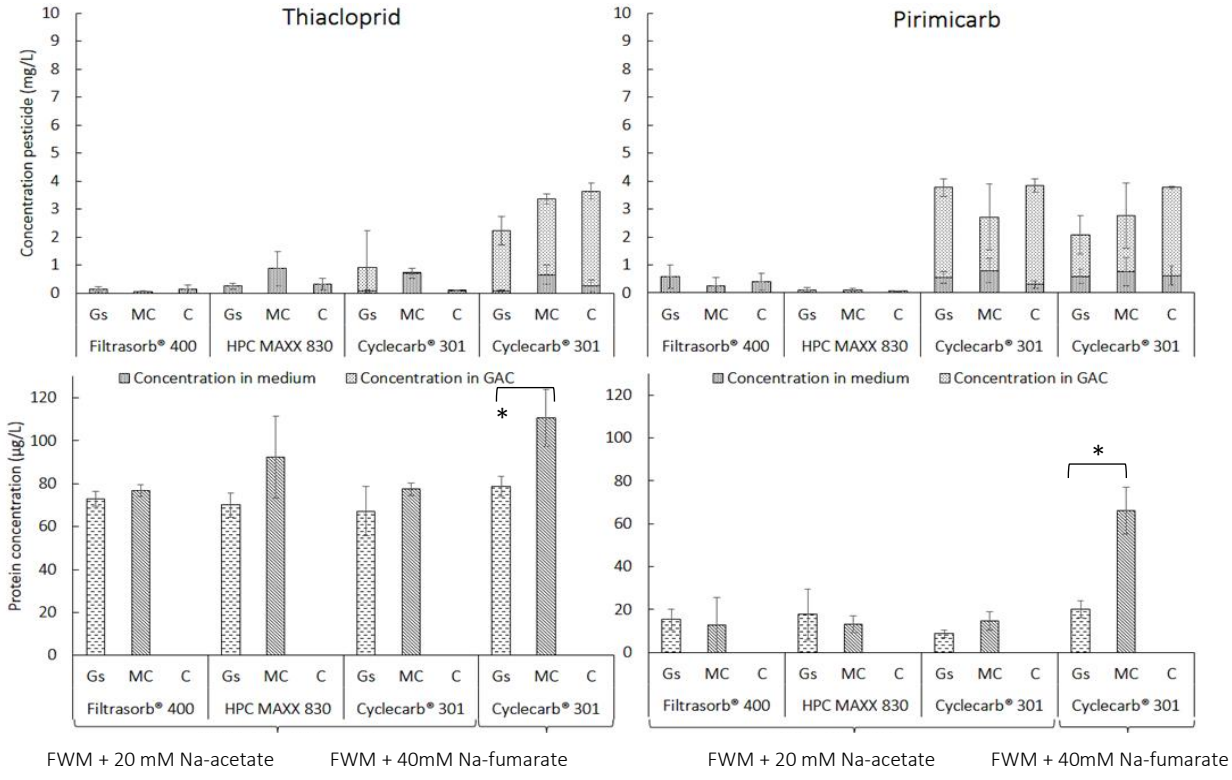

Figure 8 *Above, HPLC results are pictured for thiacloprid and pirimicarb. Below, Bradford protein concentrations are pictured. The concentration adsorbed by the GACs was only measured in Cyclecarb®301 in both medium types. Gs = G. sulfurreducens; MC = mixed culture; C = no bacteria control. * indicates a statistical difference ($p \leq 0,05$); Values are means of triplicates ± 1 SD.*

- *Bacterial growth*

For the Bradford assay results, the same statistical methodology was used as described above, using protein concentrations as our dependent variable values. For both pesticides, no significant difference was found in bacterial growth using different GACs and no significant difference was found when comparing protein concentrations of the *G. sulfurreducens* and mixed culture with GACs in medium with Na-acetate. However, a significant result was found in Na-fumarate medium using Cyclecarb®301, namely a higher protein concentration for the mixed culture compared to *G. sulfurreducens* ($p \leq 0,05$) for thiacloprid and pirimicarb. The protein concentrations in mediums with either Na-fumarate and Na-acetate were compared for Cyclecarb®301 (Filtrasorb®400 and HPC MAXX 830 were only observed in medium with Na- acetate, so they were not included in this analysis). Protein concentrations for *G. sulfurreducens* were not significantly different using different mediums. Protein concentrations for mixed culture were significantly higher using medium with Na-fumarate compared to mixed culture grown in Na-acetate for thiacloprid ($p \leq 0,05$) and pirimicarb ($p \leq 0,01$).

- *Conclusions*

No evidence was found for the degradation or biotransformation of thiacloprid and pirimicarb by electroactive bacteria in the presence of a GAC. The type of GAC did not affect pesticide concentrations. Medium choice only influenced pesticide concentrations when bacteria were present. This could indicate an interaction between the electroactive bacteria and the used medium Na-acetate (an electron donor) or Na-fumarate (an electron acceptor). The type of GAC did not influence bacterial growth. The medium type did not influence growth of *G. sulfurreducens*, but mixed cultures were observed to grow better in medium with Na-fumarate.

IV. The addition of a biochar does not affect pesticide concentrations

Pesticide concentrations of thiacloprid and pirimicarb were measured after two weeks in tubes containing mixed culture and no bacteria control tubes. There was an addition of three biochar (made from AB-wood, *Typha* and coffee beans) and one GAC (Cyclecarb®301). Pesticide concentration was measured in medium and in the biochar and GAC. All tubes contained FWM with Na-fumarate.

- *The effect of bacteria and biochars/GAC on pesticide concentrations*

No significant decrease in total pesticide concentration between mixed culture and control groups was observed for each of the pesticides. The adsorbed concentration of thiacloprid is significantly higher for the coffee-based biochar compared to the AB-wood biochar in the presence of bacteria ($p \leq 0,05$). The total concentration of thiacloprid (in medium and the absorbed fraction) is significantly lower in the presence of the GAC Cyclecarb®301 compared to the coffee- ($p \leq 0,01$) and AB-wood ($p \leq 0,05$) biochars. No statistically significant results were found for pirimicarb.

- *Bradford results*

No significant results were found for thiacloprid. For pirimicarb, protein concentrations were significantly higher when in the presence of Cyclecarb®301, compared to the AB-wood ($p \leq 0,01$) and *Typha* ($p \leq 0,01$) biochars. After controlling for the influence of the biochar on the protein concentration by grouping protein concentrations per biochar, an independent samples t-test was used (for each of the biochars/GAC) to compare bacterial growth for both pesticides.

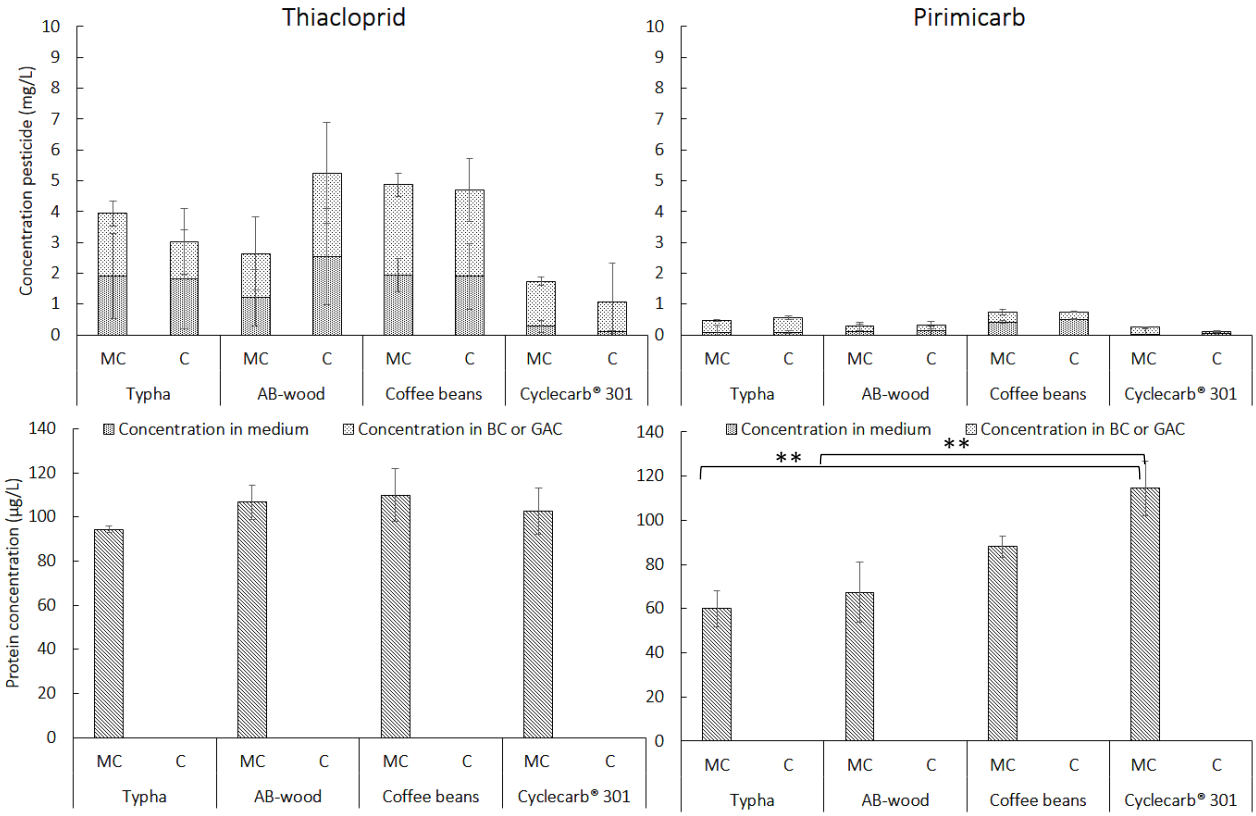

Figure 9 *Upper part: Pesticide concentrations found in medium and biochar or GAC after two weeks, measured with HPLC. Left = Thiacloprid; Right = Pirimicarb. Lower part: Protein concentrations measured in medium. Gs = G. sulfurreducens; MC = Mixed culture; C = no bacteria control. Shown values are means of triplicates ± 1SD. ** Indicates a statistical difference with p ≤ 0,01*

- *Fluorescence in situ hybridization show colonization of mixed culture and G. sulfurreducens bacteria on GAC and biochar particle*

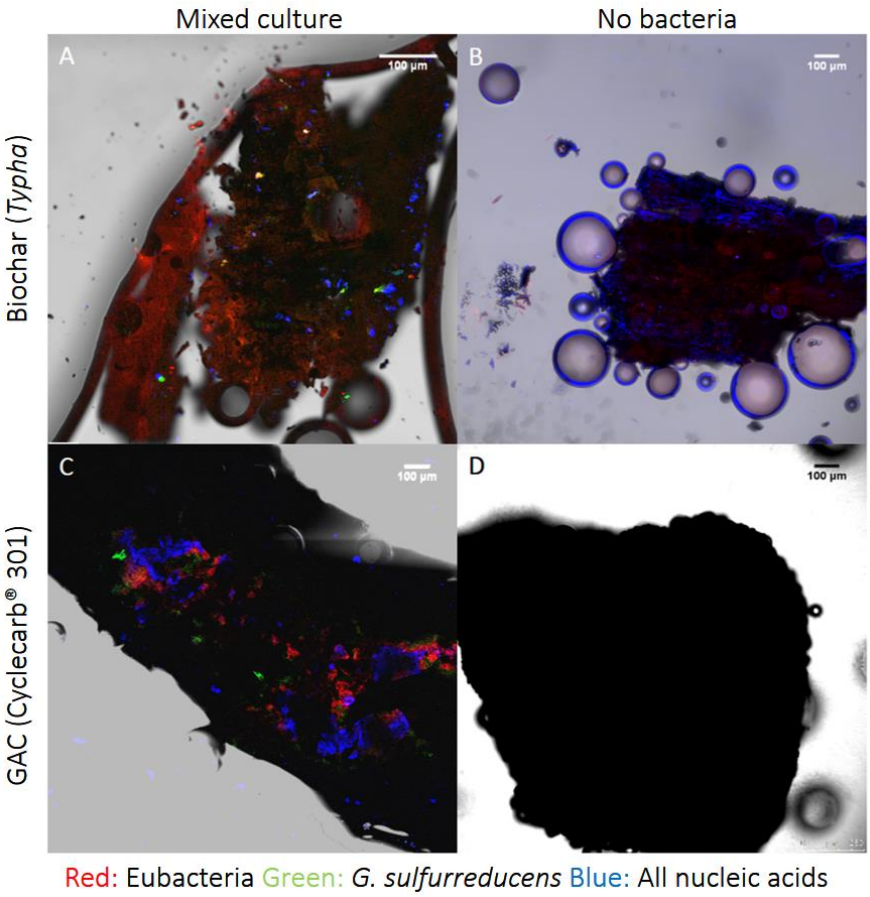

Figure 10 *FISH images. All particles in these images were exposed to 10 ppm pirimicarb and are part of the experiment Part IV.* ***A****: biochar (Coffee beans) with mixed culture;* ***B****: Control biochar (Coffee beans), no bacteria;* ***C****: GAC (Cyclecarb® 301) with mixed culture;* ***D****: Control GAC (Cyclecarb® 301), no bacteria. DAPI (blue signal) shows all nucleic acids; G. sulfurreducens (green signal); Eubacteria (red signal)*

The electroactive biofilm of biochar (mixed culture and control) and GAC (mixed culture and control) particles exposed to 10 mg L^{-1} pirimicarb were characterized by FISH. By performing this analysis, the colonization and species present on the surface of the particles was investigated. The biochar particle with mixed culture shows the presence of *G. sulfurreducens*, as well as eubacteria (*Figure 10,A*; *Supplemental Figure 26*). When comparing to the control biochar particle (without mixed culture), less eubacteria were found, as well as no *G. sulfurreducens* bacteria (*Figure 10 B; Supplemental Figure 27*). On the GAC with mixed culture, eubacteria and *G. sulfurreducens* were found (*Figure 10 C; Supplemental Figure 28*). The control GAC particle showed no presence of any bacteria or nucleic acids (*Figure 10 D*; *Supplemental Figure 29*).

- *Conclusions*

The presence of a mixed culture did not affect pesticide concentrations compared to the control group, meaning no evidence is found for degradation of pesticides. The GAC did not adsorb more pesticides compared to the biochars. For pirimicarb, evidence was found that the mixed culture grew better in the

condition with Cyclecarb®301 compared to the biochars. FISH analysis show colonization by *G. sulfurreducens* and eubacteria on the biochar and GAC particles.

V. Significant decreases in pesticide concentrations are observed when adding GAC or biochar to medium

When comparing the total pesticide concentrations of the second experiment with concentrations found in similar conditions when a biochar or GAC was added (the fourth experiment), concentrations were significantly lower for thiacloprid ($p \leq 0,05$) and pirimicarb (only mixed culture in Na-fumarate could be compared). Pirimicarb shows the largest decreases ($p \leq 0,01$) compared to the conditions with no GAC or biochar. As stated before, no significant decrease in concentrations between the mixed culture and control groups without bacteria was observed. These results show that the bacteria likely do not influence pesticide concentrations, but the biochars and GACs do. The total concentration of thiacloprid (in medium and the absorbed fraction) is significantly lower in the presence of the GAC Cyclecarb®301 compared with the control without GAC or biochar (*Figure 11*, statistical differences are not indicated in order to avoid overcomplication of the figure).

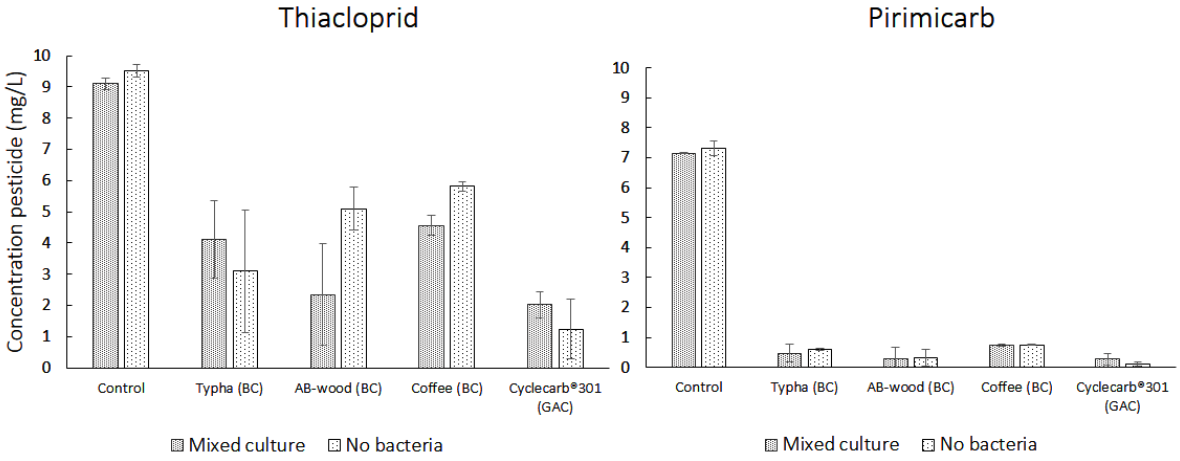

Figure 11 *Total Pesticide concentrations of control (no GAC or biochar, experiment 2) compared with total pesticide concentrations (medium + adsorbed) when a GAC or biochar was added (experiment 4) in medium with Na-fumarate. Values are averages of pesticide concentrations for either duplicates (experiment 2) or triplicates (experiment 4) with ± 1SD.*

Part two: Comparison of the performances of GACs as anodic material a bioelectrochemical system

I. In an eight-electrode BES, the current densities produced by the biofilm are significantly higher for GAC-anodes compared to the control, and cyclic voltammetry analysis shows that GAC acts as a capacitator.

Three GACs (Cyclecarb®301, HPC MAXX 830 and Filtrasorb®400) were compared in a microbial electrolysis cell inoculated with a mixed culture and filled with FWM containing 20mM Na-acetate as electron donor and the anodes functioning as electron acceptor. CVs were performed two times (pre-inoculation and after two weeks) for electrochemical activity characterization. Chronoamperometry was performed during the whole experiment, with the working electrodes polarized at -0,2V vs Ag/AgCl.

Chronoamperometry results are shown in *Supplemental Figures 30* and *31*. Initially, the currents of the electrodes were all measured separately. The day before inoculation, the currents produces by all electrodes remained ± 0mA (*Data not shown*). Half a day after inoculation, a steep increase in current was seen for all electrodes with GACs (*Supplemental Figure 30*). At the end, when the biofilm on the electrodes was fully developed, the maximum current was recorded per electrode and the average current densities (mA g^{-1} GAC and mA cm^{-2}) were calculated for each electrode. Statistical analysis (ANOVA) showed no significant difference in current densities calculated per gram of GAC between the GACs. When comparing current densities per projected surface area (mA cm^{-2}) using an ANOVA, the current density of the control was significantly lower than current densities produced by the GAC conditions ($p \leq 0,05$) (*Figure 12*).

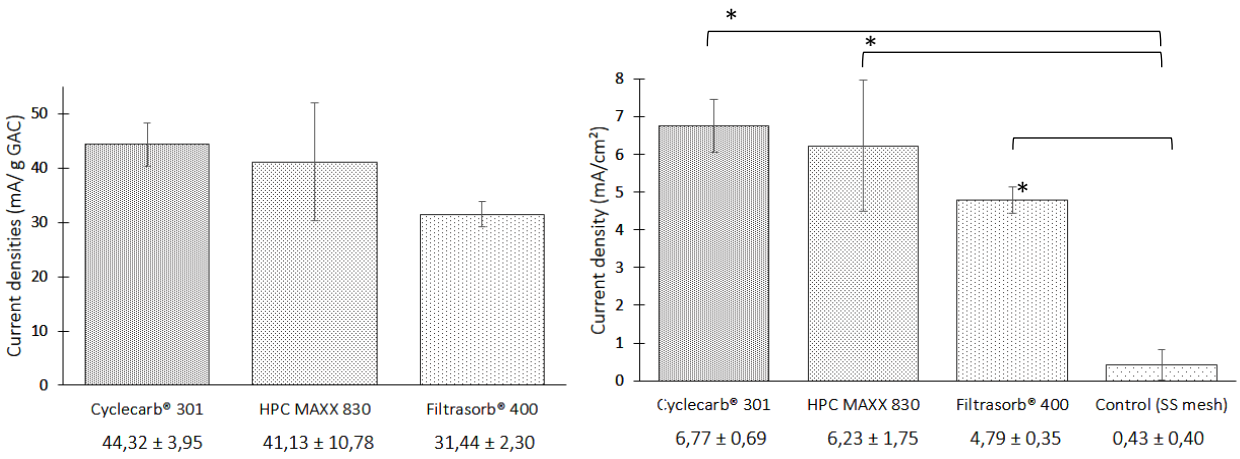

Figure 12 Left: *Maximum current densities (mA g GAC^{-1}) at -0,2V vs Ag/AgCl, averages calculated for each condition (Cyclecarb® 301, HPC MAXX 830, Filtrasorb® 400).* ***Right:*** *Maximum current densities (mA cm^{-2}) at -0,2V vs. Ag/AgCl for each condition. The maximum current recorded for each electrode at the end of the experiment was recorded, an average was calculated per condition and consequently. Values are averages of current densities (mA cm^{-2} or mA g^{-1}) of two replicates ± 1 SD. * Indicates a statistical difference of $p \leq 0,05$*

Before inoculation (*Figure 10, A*) and after two weeks (*Figure 10, B*), turn-over cyclic voltammograms were performed on each electrode. A difference in maximum current is observed between the CV curves of the control electrodes and the other electrodes containing GACs. The CV curve for each GAC-condition is unlike the classic sigmoidal shape that is normally seen for anodic biofilms under Na-acetate turnover conditions (56). The control CVs pre-inoculation show reduction peaks around -0,2 and -0,3V, that are likely related reduction of metals in the stainless steel (*Figure 13, C*). Electrochemical catalysis likely to be Na-acetate oxidation can, however, be seen on the CV curve of both control electrodes in *Figure 11, D*. Here, the oxidation of acetate starts around -0,45V and reaches a plateau around -0,2V. With the visible eye, a small amount of pink biofilm could be seen on the control electrodes after 14 days that could be responsible for the possible oxidation of acetate that is seen here. The shape of the CV curves for Cyclecarb® 301, HPC MAXX 830 and Filtrasorb® 400 indicate that the GACs all act as capacitators, meaning they are storing electrons.

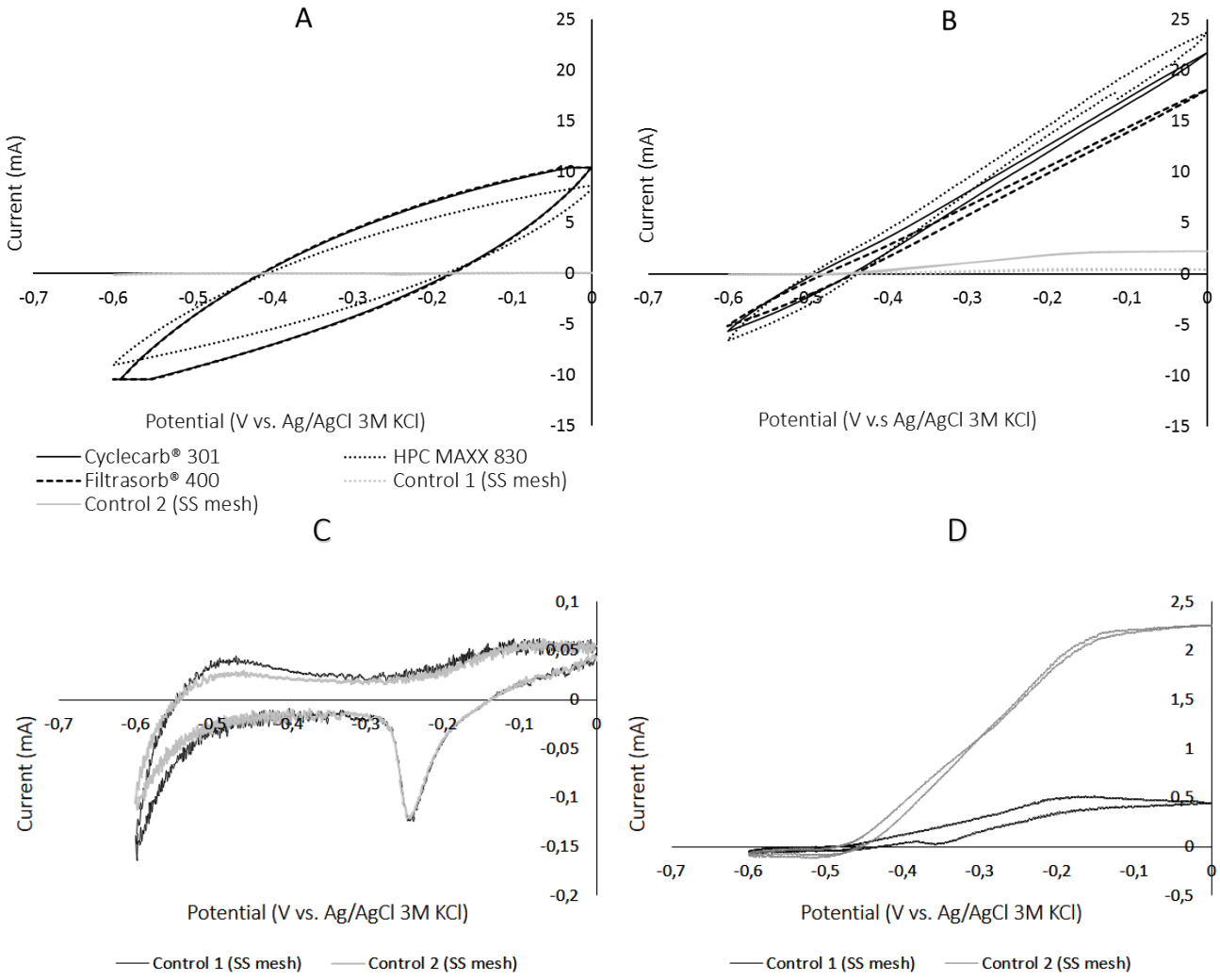

*Figure 13 A and B show cyclic voltammograms (every time, the second scanning cycle of a total of two scanning cycles is displayed) of five of eight electrodes (one electrode from each GAC condition, and two reference electrodes). **A**: Before inoculation (one electrode of each condition is shown, for the control group two electrodes is shown; **B**: Two weeks after inoculation (one electrode of each condition is pictured here, for the control group two electrodes are pictured). In C and D, CVs of the control electrodes are pictured. **C**: Pre-inoculation. **D**: Two weeks after inoculation.*

Pink biofilms on the electrodes were visible after 14 days of incubation, suggesting that the main bacteria present in the biofilm were *G. sulfurreducens* bacteria (*Figure 15*). Scanning electron microscopy (SEM) images were taken from GAC granules inside the SS mesh pockets for each condition, to look at attachment of the bacteria. All GACs show a nearly full coverage with bacteria (*Figure 14, Supplemental Figures 32* and *33)*. These images suggest that the GAC granules can serve as a suitable surface for bacterial attachment. The biofilms on the GAC show typical characteristics of a *G. sulfurreducens*-dominant biofilm *G. sulfurreducens* cell bodies have a typical length around 1,7 µm and a width of 0,4µm, as is seen in these images. Measures of bacteria are based on 10 observations in SEM images of *G. sulfurreducens* on electrode surfaces by Bond and Lovley [57]. The wires seen in *Figure 13, C* have a thickness of around 130 nm (measured with Image J), suggesting that these are not conductive nanowires (who usually have a diameter of 3 to 5 nm [58]), but rather other biofilm-related structures.

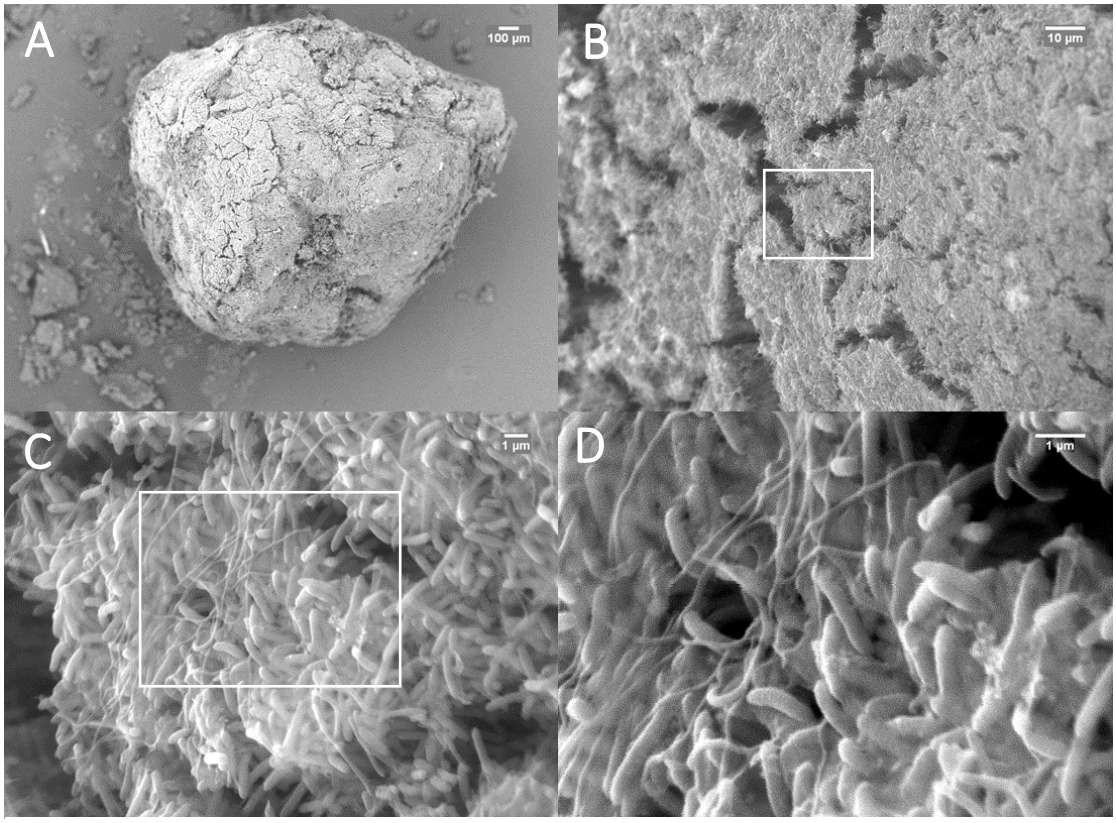

Figure 14 *Scanning electron microscopy images of a granule obtained from electrode 1, containing Cyclecarb® 301.* ***A****: magnification x60;* ***B****: magnification x1200;* ***C****: magnification x7000;* ***D****: magnification x15000.*

Figure 15 Left*: A pink biofilm has grown on the SS mesh of the electrode containing GAC after two weeks (here: E2 containing Cyclecarb® 301);* ***Right****: Little to no biofilm has grown on a control electrode (empty SS mesh) (here: E7)*

II. Measurements obtained from larger BES set-up show inconsistencies

The performance of three GAC-sand mixtures and one sand control was compared using chronoamperometry (CA) and cyclic voltammetry (CV). Medium samples were taken from the anodic compartment on the first day (before inoculation) and the last day (day 23) to assess Na-acetate consumption of the bacteria. CA and CV results showed great inconsistency and were inconclusive (data not shown). The measurements of Na-acetate in medium samples also showed many inconsistencies. The medium that was added had a concentration of 20 mM Na-acetate, however, measurements on the first day (pre-inoculation) to determine the concentration of Na-acetate, did not show consistency and in most cases, values of Na-acetate were found that were significantly higher or lower than the 20mM starting concentration (*Figure 11*), making the other measurements for Na-acetate consumption unreliable and inconclusive. Chronoamperometry results in show inconsistencies (*Supplementary Figure 34*). The highest current outputs were found up for the biotic conditions of Cyclecarb®301 and HPC MAXX 830, (0,17 and 0,22 mA) respectively. However, for the abiotic HPC MAXX 830 condition, similar maximal current was found, suggesting that the bacteria were likely not responsible for the current that was produced.

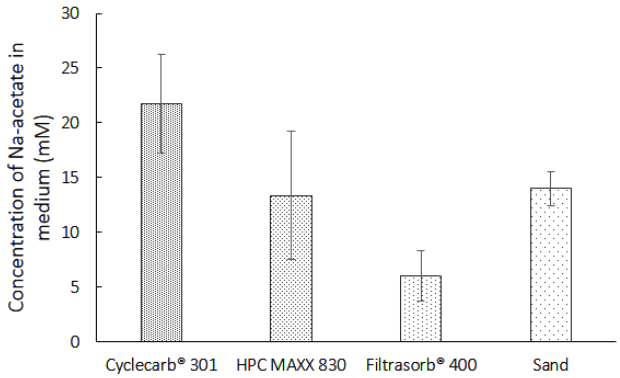

Figure 16 *Concentrations of Na-Acetate (mM) taken on day 0 (before inoculation). Medium was added containing 20mM of Na-acetate, however concentrations measured in samples taken from the set-ups shows inconsistencies, making further results unreliable. Means of triplicates are pictured with ± 1 SD.*

I. *Part One: Are pesticides degraded or biotransformed by a pure or mixed culture and is this process improved with the addition of biochars or granular activated carbons?*

We hypothesized that electroactive bacteria can degrade or biotransform thiacloprid and pirimicarb, and that these processes are accelerated when a granular activated carbon (GAC) or biochar is added. In literature, the anaerobic degradation of these pesticides has not been studied yet. Our research shows that the addition of biochar and GACs significantly decreases total pesticide concentration, compared to conditions that only contained electroactive bacteria. However, statistical analysis shows that these decreases were likely not caused by the bacteria themselves, since a significant decrease in pesticide concentration was only found between *G. sulfurreducens* and the control group in medium with Na-acetate. In the following sections, the results are discussed.

- *Pesticide tolerance*

The growth rates of the bacteria were not influenced by various concentrations (1 until 10 mg L^{-1}) of thiacloprid, meaning that the concentration that was used for exposure in later experiments (10 mg L^{-1}) did not inhibit bacterial growth. This conclusion can only be made for thiacloprid, but it does provide a first insight as to how the growth of the *G. sulfurreducens* is influenced by pesticides. The experiments were set-up in such a way that only thiacloprid was added as electron donor. However, in the condition where no electron donor was added to the medium, the highest maximum turbidity of the bacteria was observed. This result indicates that traces of Na-acetate were still present in the vial of the *G. sulfurreducens* culture that was used for the inoculation. In the future, this experiment should be repeated with an inoculum that does not contain Na-acetate traces. The method used to measure bacterial growth, optical density (OD_{600}), is not the most accurate method. Measuring pesticide or Na-acetate concentrations with HPLC would have been more precise. Another more accurate option would be measuring Na-fumarate consumption by the bacteria. As a last remark, OD measurements were performed every two days. Since the log growth phase of bacteria is mostly seen on day one, essential measuring points were missed, so the exponential growth phase was not properly shown.

- *Degradation of pesticides by G. sulfurreducens or a mixed culture*

The hypothesis that electroactive bacteria can break down to biotransform pesticides cannot be confirmed based on the results that were obtained in these experiments. However, the total pesticide concentrations significantly decreased when a GAC or biochar was added. In a similar set up, Yu *et al.* showed that *G. sulfurreducens* was a weak dechlorinating-respiring bacterium, and that without the presence of biochar, 11.1% (± 1.2%) of the pentachlorophenol was degraded by *G. sulfurreducens* after 21 days [40]. These findings could not be confirmed in our experiments for pentachlorophenol (PCP). However, for pirimicarb, a similar result was found for *G. sulfurreducens* in Na-acetate medium.

- *The effect of the addition of a BC or GAC on pesticide concentrations*

The decrease in pesticide concentration after the addition of GACs or biochars could possibly be explained by the catalytic properties of biochars and GACs. As stated before, research has shown that biochar and GACs are not just able to adsorb contaminants, but can also catalyze their chemical transformation [43–45]. A more detailed image of possible biotransformation processes because of the addition of GACs and BCs could be provided by gas chromatography mass spectrometry (GC-MS), since HPLC chromatograms showed unidentified peaks of potential breakdown products for all pesticide conditions after two weeks. The total concentration of thiacloprid is significantly lower in the presence of the GAC Cyclecarb®301 compared to two of the biochars. However, the GAC did not adsorb more pesticides. This difference might be explained by certain properties of the GAC but could also be explained by the fact that only thiacloprid concentrations, and not possible degradation products, were measured. Results also indicated that the type of GAC did not influence total pesticide concentrations. Although the electrical conductivities of Cyclecarb®301 and HPC MAXX were 40-fold higher compared to Filtrasorb®400, no difference was observed in pesticide concentration. As for the biochar, Yu *et al.* suggests that the pyrolyzation temperature of the biochars made a significant difference in contaminant removal rates by the bacteria. Here, the pyrolyzation temperatures of the biochars was between 450 (AB-wood and coffee) and 500°C (*Typha*), with corresponding conductivities of 0,23; 3,12 and 30,93 mS cm^{-1}. The similar pyrolyzation temperatures might explain for the fact that no difference was seen in pesticide concentrations between the biochars. However, the electrical conductivities between the GAC and biochars show large differences. Yu *et al*. (2015) stated that the higher removal rates were associated with higher conductivities, this cannot be confirmed based on these results. In the future, comparing biochars made at different temperatures in the same set-up would be a logical next step, since Yu *et al.* (2015) found that the higher the production temperatures, the better the conductivity and the faster the component (PCP) was dechlorinated [40].

- *Growth of bacteria*

The growth of the mixed culture was significantly higher in the presence of a GAC compared to two of the biochars. This could potentially explain how the use of GAC's resulted in lower total pesticide concentrations compared to biochars without a significant difference in medium or adsorbance concentrations. *G. sulfurreducens* grew best in medium with both Na-acetate as electron donor, and Na-fumarate as electron acceptor without biochar or GAC. When providing both an electron donor- and acceptor to the bacteria, it is expected for the bacteria to grow better, since they can receive electrons and transfer them to an electron acceptor. No direct evidence was found that the bacteria can use the pesticides as electron donor- or acceptor. Mixed culture grew best in medium containing Na-fumarate, also when biochars and GACs were added. Mixed cultures are known for their synergistic effects, and therefore might be able to grow better in medium with only an electron acceptor [13].

- Conclusion and future perspectives

So far, the evidence for degradation or biotransformation of thiacloprid or pirimicarb by either a mixed culture or *G. sulfurreducens* bacteria is not conclusive enough to confirm the hypothesis. The addition of biochars and GACs did decrease pesticide concentrations and additional research needs to be done to gain

more insights as to why this is the case. GC-MS analysis could provide more insight about which degradation products are formed in different conditions. Wang *et al.* tested the removal of 26 micropollutants in single- or two chamber MFCs, using carbon cloth as the anode. Their results show that the removal of positively charged micropollutants was higher, and that the properties of the compounds determined how well they were removed [29]. A similar experiment could be performed in the future, studying the removal of a mixture of pesticides in an electrochemical system. Many studies have shown that electrodes, able to accept or donate electrons to micro-organisms, can significantly accelerate contaminant removal processes. The addition of a working electrode as an anode, set at a certain potential, might enhance degradation of pesticides by electroactive bacteria. Also, the bacterial community in a BES for the removal of micropollutants can be quite specific, and more research need to be done as to which bacteria can be used for the degradation of certain specific compounds. Exploring the degradation of pesticides in a BES might also provide more answers as to how the pesticides are metabolized by the bacteria. For the degradation of azo dyes was in an MFC was found that a co-substrate was required for the degradation of these dyes. Here, the co-substrate acted as electron donor for the EABs on the anode, and the EABs were then able to use part of these electrons to reduce azo dyes [11,13]. Similar mechanisms could be at play here, but more research needs to be done to make conclusions. An interesting remark is that electron transfer between a component and the bacteria is typically energetically driven by the redox potential difference. The reduction of a component by *G. sulfurreducens* (which has a standard potential below -150mV) occurs when it is thermodynamically favorable, meaning that the electrons flow from a high to a low potential. Thus, determining the redox potentials of thiacloprid and pirimicarb could also provide insights as to if *G. sulfurreducens* or mixed culture bacteria can degrade these pesticides. The experimental set-up could also be improved for future experiments. The culturing conditions might not have been ideal, since the preferred 80:20 N_2/CO_2 ratio was not always accurately achieved. Adding more sampling points in time of the medium in the tubes or vials, might provide more accurate information about how pesticide concentrations vary through time. HPLC measurements showed large standard deviations, meaning that this technique can still use optimization for the accurate determination of pesticides. These tests provided valuable information and insights about the possible degradation of pesticides by bacteria and biochar. However, additional research still needs to be done to be able to say more about the mechanisms at work in these experiments.

II. *Part Two: Compare performances of GACs and biochars inoculated with a mixed culture in a bio electrochemical system*

The hypothesis for this part was that the addition of biochar made from waste biomass to a bioelectrochemical system (BES) improves the removal of pesticides from wastewater. However, results about pesticide degradation indicated that the bacteria are not capable of degrading thiacloprid and pirimicarb. Therefore, the hypothesis was changed to: "Biochars or granular activated carbons serve as a suitable substrate for the bacteria in the anodic compartment of a bioelectrochemical system". Due to time restrictions, the hypothesis was only tested for three GACs in two different bioelectrochemical set-ups.

- *Comparing GACs in an eight-electrode BES set-up*

The maximum current densities of the GAC-containing electrodes were all in the range of 4,8 – 6,8 mA cm^{-2}, compared to a maximum current density of 0,14-0,71 mA cm^{-2} for the control stainless-steel electrodes. Stainless steel has a low biocompatability and produces low current densities, and thereby is not seen as a suitable anode material in a BES. To illustrate, a study of Kun *et al*. (2014), measured current densities of 0.06 ± 0.01 mA cm^{-2} for stainless-steel electrodes, using the same set-up as and similar conditions[56]. The values for the control electrodes here are somewhat higher, but this could be since the control electrodes were not properly rinsed before applying them in the reactor. They could have been contaminated with GAC particles, making it easier for the biofilm to grow on these electrodes. As described in Baudler *et al.* (2015), an electrochemically active biofilm grown in Na-acetate medium on a graphite electrode at a fixed potential typically delivers current densities in a range of 0,5-1,5 mA cm^{-2}(59). The current densities produced by the GAC-anodes are significantly higher compared to when graphite is used at the anode. A current of 0,6mA for a single activated carbon granule (particle polarized at-0,3V vs. Ag/AgCl) was measured by Borsje *et al.* in 2016 (60). This illustrates that high currents can be achieved with GAC granules in a bioelectrochemical system. The results of this experiment thus show that a faradic current is produced by the biofilm, probably through oxidation of Na-acetate by the biofilm since the currents dropped significantly when all the Na-acetate was depleted. On each type of GAC granule sampled from the electrodes, a well-developed biofilm with multiple layers of bacteria was found with SEM analysis. The biofilms on the GAC show typical characteristics of a *G. sulfurreducens*-dominant biofilm G. sulfurreducens, which was expected for these specific conditions (electrodes polarized at -0,2V vs. Ag/AgCl and Na-acetate as electron donor)[58].

Filtrasorb® 400 has a low conductivity (1,3 mS cm^{-1}) compared to Cyclecarb ®301 (55,8 mS cm^{-1}) and HPC MAXX 830 (55,8 mS cm^{-1}). However, this difference is not seen in maximum current densities generated by the electrodes. Capacitive effects of the GACs were observed when performing cyclic voltammetry pre-inoculation and on fully-grown biofilm after two weeks of incubation. Under the experimental conditions in this work, the CVs for each GAC electrode shows no redox reactions taking place when the potential is varied between 0,6 and 0V. A mechanism that could explain these results is when Na-acetate is oxidized at the anode, a faradaic current is generated by this oxidation. The electrons are then transferred to the GAC particle, and together with the transport of cations, an electric double layer is formed inside of the pores, causing the charge to be stored. This process is described in more detail in a paper by Borsje *et al.* (2016) [60]. A capacitive effect is often seen when the electrode exists out of porous materials similar to these GACs.

The high the surface area and porosity, the larger the capacitive effect becomes [61]. GACs are known for their large internal effective surfaces (typically between 500 and 1500m² g^{-1}), and here Filtrasorb®400 and Cyclecarb®301 both have total surface areas of respectively 1050 and 900 m² g^{-1} (the total surface area of HPC MAXX 830 not known). This could be an explanation for the capacitive effects that were seen, however other factors such as pore size, surface groups and so on could also play a role [60].The difference in thickness of the CVs of the GAC electrodes pre-inoculation and after biofilm formation, is likely because the GAC granules were not as closely packed together and O_2 was still trapped between the granules in small air pockets in the beginning, whereas the granules were more tightly packed at the end of the experiment.

To conclude, a biofilm of electroactive bacteria was present on the GAC granules and high current densities were produced by each GAC compared to the control electrodes, suggesting that the biofilm on the granules was electroactive and was converting Na-acetate and transferring these electrons to the electrodes. In cyclic voltammograms, the oxidation of Na-acetate is not visible due to the capacitive effects of the GAC. Based on these results, GAC seems to be a suitable substrate for EAB in a MEC. The next step would be to compare other materials in this set-up, starting with different biochars with known characteristics, in the same way as was performed here.

- *Comparing GACs in a larger BES set-up*

Comparing the performance of GACs in a fluidized-bed MFC reactor did not show conclusive results due to errors in the set-up. In the future, a different method should be used for sampling, since the concentrations of Na-acetate found in medium samples taken before inoculation were significantly different than the concentration present in the medium. The set-up we used seems to be unreliable because of bad connections between the current collector and the Arduino-based system that was used to record current. Future experiments should include a reference electrode. Setting a known potential on the working electrodes to the reference electrode, might help to make the set-up more reproducible. Also, instead of immediately testing three GAC-sand mixtures, a simpler set-up should be tested first.

- *Conclusion and future perspectives*

Based on the results of the test in the eight-electrode BES set-up, GAC seems to be a suitable substrate for EAB because of the formation of an electroactive *G. sulfurreducens* biofilm and high current densities that were produced. Biochars could also be examined in the eight-electrode BES set-up as anode material, and the performance and bacterial attachments could be compared to the GACs that were already tested. The larger BES set-up still needs improvements.

CONCLUSION & SYNTHESIS

The hypothesis that electroactive bacteria can break down to biotransform pesticides cannot be confirmed based on the results that were obtained in these experiments. However, the total pesticide concentrations significantly decreased when a GAC of biochar was added. The decrease in concentration could be because of the catalytic properties of biochars and GACs. Based on the results of the test in the eight-electrode BES set-up, GAC seems to be a suitable substrate for EAB in a BAS because of the formation of the high current densities that were produced by the electrochemically active biofilm on the GAC granules. Additional research is necessary to say more about the possible effects of biochar and GAC on pesticide breakdown, and the mechanisms are responsible for these effects. Biochars could also be examined in the eight-electrode BES set-up as anode material, and the performance and bacterial attachments could be compared to the GACs that were already tested. The use of BES in bioremediation of micropollutants such as pesticides from wastewater is a promising and emerging method and these tests are a first step into the bioremediation of pesticides in a biochar-enhanced BES.

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I. Gompertz model

Gompertz growth curves were used for modelling bacterial growth. In a study by Zwietering *et al*. (1990)(62) several sigmoidal functions (logistic, Gompertz, Richards, Schnute, and Stannard) were compared to describe a bacterial growth curve. In the cases tested, the modified Gompertz equation was statistically sufficient to describe the growth data of Lactobacillus plantarum and was easy to use. The same comparison of models was carried out with growth data from other microorganisms. With these data, the Gompertz model was accepted in 70% of the cases.

The Gompertz model uses 3 parameters (A, μ_m and λ) corresponding to 3 bacterial growth phases and its equation is written as:

Equation 1 Gompertz model (equation obtained from Zwietering et al.*, 1990)*

$$y = A\exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\}$$

The first parameter A refers to the maximum value the population size will reach before stabilizing (assuming sufficient substrate). Graphically A is the upper asymptote of the curve when time reaches infinity. μ_m is an estimator for the growth rate of the population assuming a constant growth rate. It is defined as the tangent in the inflection point. Finally λ is the time it takes for the bacterial growth rate to accelerate from zero to a constant growth rate μ_m. The lag time λ could also be interpreted as the recovery time after transferring a microbial population to a new habitat. Graphically it is represented by the x-axis intercept with the tangent in the inflection point.

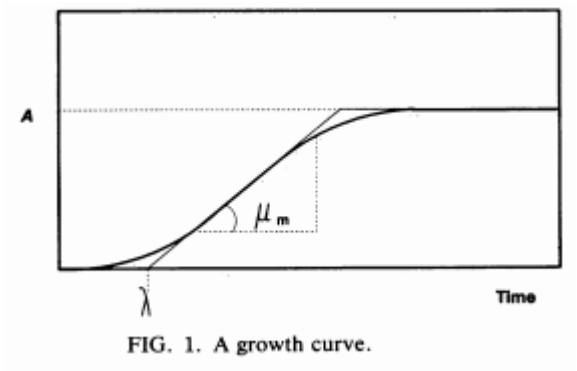


FIG. 1. A growth curve.

Figure 17 Illustration of the parameters of the Gompertz model (Figure obtained from Zwietering et al.*, 1990)*

Parameters for this thesis were estimated using the non-linear regression function in SPSS. Starting values for parameters A, μ_m and λ were chosen by plotting the actual observations and looking at respectively the maximum, tangent and the x-axis intercept. Because for this thesis population size was measured every 2 days and the bacteria recovery time was very short (< 1 day) it was not possible to make an accurate estimation of the lag time (parameter λ) because there were not enough observations.

II. Tables

Table 1 *Physicochemical properties of the pesticides used in the experiments.*

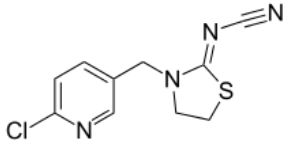
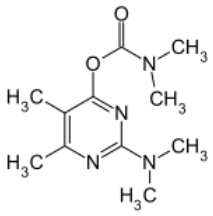
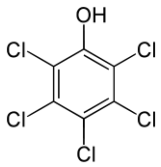
	Thiacloprid	Pirimicarb	Pentachlorophenol
IUPAC name	{(2Z)-3-[(6-Chloropyridin-3-yl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide	(2-Dimethylamino-5,6-dimethylpyrimidin-4-yl) N,N-dimethylcarbamate	2,3,4,5,6-Pentachlorophenol
Provider	Sigma-Aldrich	Sigma-Aldrich	Sigma-Aldrich
Molecular formula	$C_{10}H_9ClN_4S$	$C_{11}H_{18}N_4O_2$	C_6HCl_5O
2D chemical structure			
Molecular weight (g/mol)	252,72	238,29	266,34
Kind of pesticide	Insecticide	Insecticide	Insecticide and herbicide
Concentration in pesticide wastewater from PC Fruit (µg L^{-1})	993	634	/
Water solubility (mg L^{-1})	185	2,7	20

Table 2 *Ingredients of vitamin solution and mineral mix added to fresh water medium (FWM).*

1 Liter

Ingredient	1 L
Milli-Q H_2O	800 ml
In Fridge	
Biotin	0.002 g
Pantothenic Acid	0.005 g
B-12	0.0001 g
p-aminobenzoic acid	0.005 g
Vitamin Box	
Thioctic Acid (alpha lipoic)	0.005 g
Nicotinic Acid	0.005 g
Thiamine	0.005 g
Riboflavin	0.005 g
Pyridoxine HCl	0.01 g
Folic Acid	0.002 g
Complete volume with Milli-Q H_2O to	1000 ml

1 Liter

Ingredient	1 L
Milli-Q H_2O	800 ml
NTA Trisodium Salt (Free acid)	1.5 g
$MgSO_4$	3.0 g
$MnSO_4 * H_2O$	0.5 g
NaCl	1 g
$FeSO_4 * 7 H_2O$	0.1 g
$CaCl_2 * 2 H_2O$	0.1 g
$CoCl_2 * 6 H_2O$	0.1 g
$ZnCl_2$	0.13 g
$CuSO_4 * 5 H_2O$	0.01 g
$AlK(SO_4)_2 * 12 H_2O$	0.01 g
H_3BO_3	0.01 g
$Na_2MoO_4 * 2 H_2O$	0.025 g
$NiCl_2 * 6 H_2O$	0.024 g
$Na_2WO_4 * 2 H_2O$	0.025 g
Complete volume with Milli-Q H_2O to	1000 ml

Table 3 *Properties of commercial granular activated carbons (GACs) and biochars used in the experiments.*

	FILTRASORB® 400	Cyclecarb® 301	HPC MAXX 830	Thypha biochar	Coffee biochar	AB-wood biochar
Supplier	Chemviron Carbon	Chemviron Carbon	Chemviron Carbon	Home-made	Home-made	Home-made
Form	Granules	Granules	Granules	Powder	Granular	Granular
Precursor	Buituminous coal	All kinds of activated coal	Virgin coal	*Thypha latifolia*	Coffee beans	AB-wood mix
Size (used in experiments) (mm)	1-2	1-2	1-2	1-2	1 - 2	1 - 2
Mean particle diameter (mm)	1	1,1	/	/	/	/
Bed Density (for adsorber sizing, backwashed and drained, kg/m^3)	425	450	330-400	/	/	/
Total surface area (N_2 BET surface area (m^2g^{-1})	1050	900	/	/	/	/
Methylene blue number	300	/	/	43,30 ± 0,37	37,13 ± 0,71	29,79 ± 0,98
Moisture content (as packed, max., %w/w)	3	2	5	/	2,86 ± 0,39	4,01 ± 0,68
Dry conductivity (mS/cm)	1,3	55,8	55,8	30,93 ± 0,15	3,12 ± 0,08	0,23 ± 0,02
Wet conductuvity (mS/cm)	0,7	38,5	38,5	/	/	/
pH (measured after 24h)	/	/	/	10,62 ± 0,022	10,04 ± 0,02	8,03 ± 0,11
Remark	- Produced by steam activation of selected grades of bituminous coal that have first been pulverized then agglomerated. - Has both high adsorption capacity and a high number of transport pores. This gives the carbon a greater selectivity for the removal of micropollutants such as pesticides in the presence of high concentrations of natural organic matter	- Granular reactivated carbon - Exhausted carbon from costumers is recycled and pooled together. Recycling by thermal reactivation involves processing the exhausted carbon in a special furnace at over 800°C.	The HPC Series of virgin coal (not obtained from mining)-based granular activated carbons are specifically designed to provide a rapid rate of adsorption and low resistance to flow with liquids of low to medium viscosities.	- Made from the whole *Typha latifolia* plant - Pyrolyzed at 500°C	- Made from coffee beans - Pyrolyzed at 450°C	- Made from an industrial waste-wood mix - Pyrolyzed at 450°C

Table 4 Probe specifications used in FISH analysis on GAC and biochar particles. All probes were purchased from IDT (Coralville, USA).

Target	probe	Sequence	Fluorochrome	FormamidE (%)
Geobacter cluster A Geobacter cluster B Geobacter cluster C Helper 1 Helper 2	GEO3-A GEO3-B GEO3-C **HGEO3-3** **HGEO3-4**	CCGCAACACCTAGTACTCATC CCGCAACACCTAGTTCTCATC CCGCAACACCTGGTTCTCATC **GTTTACGGCGGGTACTACC** **CACTGCAGGGGTCAATAC**	Fluoresceine	30
Eubacteria	EUB338	TGAGGATGCCCTCCGTCG	ATTO550	30
Non specific	NONE	ACT CCT ACG GGA GGC AGC	Fluoresceine	30

III. Figures

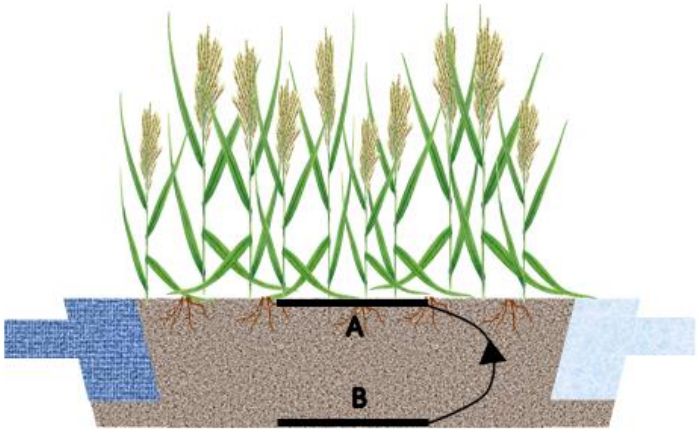


Figure 18 *BES implemented in a constructed wetland (CW). A: Cathode; B: Anode; Arrow indicates flow of electrons*

Concentration (mg/L)	Thiacloprid	Sodium acetate
10	1	19
	2	20
	3	21
5	4	22
	5	23
	6	24
2.5	7	25
	8	26
	9	27
1.25	10	28
	11	29
	12	30
0.625	13	31
	14	32
	15	33
0	16	34
	17	35
	18	36

Figure 19 *Experimental set-up tolerance experiment. Concentrations were varied from 10 – 0 mg L^{-1} for thiacloprid as well as Na-acetate. Each condition was set up in triplicates.*

A

49	43	37	31	22	16	10	1	*Geobacter sulfurreducens*
50	44	38	32	23	17	11	2	
51	45			24			3	
52	46	39	33	25	18	12	4	Mixed culture
53	47	40	34	26	19	13	5	
54	48			27			6	
55		41	35	28	20	14	7	No bacteria
56		42	36	29	21	15	8	
57				30			9	
Ac + Fum	*Ac + Fum*	*Fum*	*Ac*	*Ac + Fum*	*Fum*	*Ac*	*Ac + Fum*	
PCP	Control	Pirimicarb			Thiacloprid			

B

28	19	10	1	28	19	10	1	*Geobacter sulfurreducens*
29	20	11	2	29	20	11	2	
30	21	12	3	30	21	12	3	
31	22	13	4	31	22	13	4	Mixed culture
32	23	14	5	32	23	14	5	
33	24	15	6	33	24	15	6	
34	25	16	7	34	25	16	7	No bacteria
35	26	17	8	35	26	17	8	
36	27	18	9	36	27	18	9	
Fum	*Ac*			*Fum*	*Ac*			
Pirimicarb				Thiacloprid				
Cyclecarb® 301	Cyclecarb® 301	HPC MAXX 830	Filtrasorb® 400	Cyclecarb® 301	Cyclecarb® 301	HPC MAXX 830	Filtrasorb® 400	

C

31	13	7	1	31	13	7	1	Mixed culture
32	14	8	2	32	14	8	2	
19	15	9	3	19	15	9	3	
20	16	10	4	20	16	10	4	No bacteria
21	17	11	5	21	17	11	5	
22	18	12	6	22	18	12	6	
Fum				*Fum*				
Pirimicarb				Thiacloprid				
Cyclecarb® 301	COF BC	AB BC	Typha BC	Cyclecarb® 301	COF BC	AB BC	Typha BC	

Figure 20 *Set-up of experiments 2, 3 and 4. Ac = Na-acetate; Fum = Na-fumarate; PCP = Pentachlorophenol; In part A, control is condition without pesticide; Each time, a control condition was made without bacteria; In part C, BC =biochar; COF = Coffee bean biochar; AB = AB-wood mix biochar.*

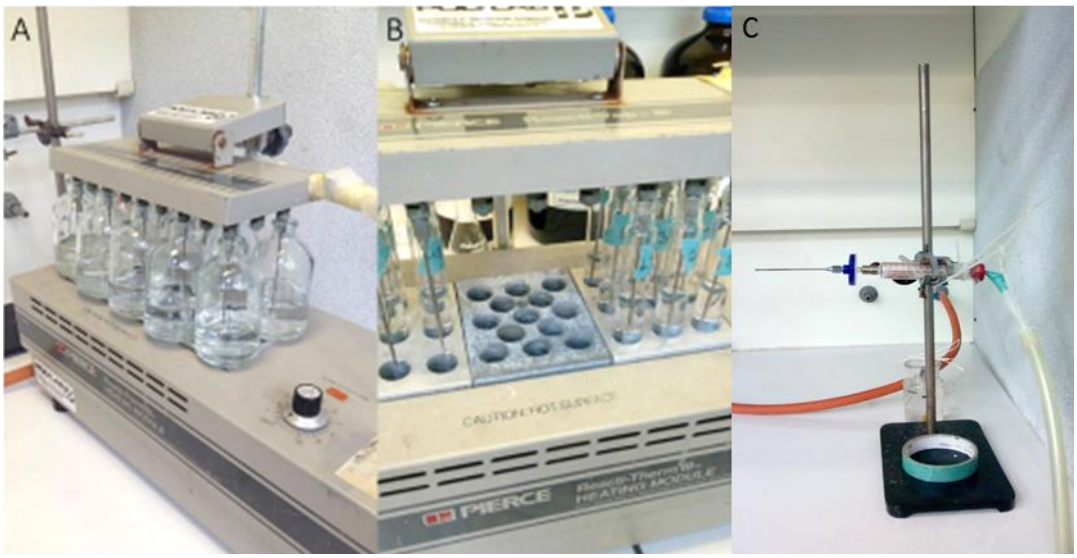


Figure 21 *Purging system.* ***A****: Vials are being flushed with* N_2 *;* ***B****: Tubes are being flushed with* N_2*;* ***C****: Sterile set-up for purging with* N_2

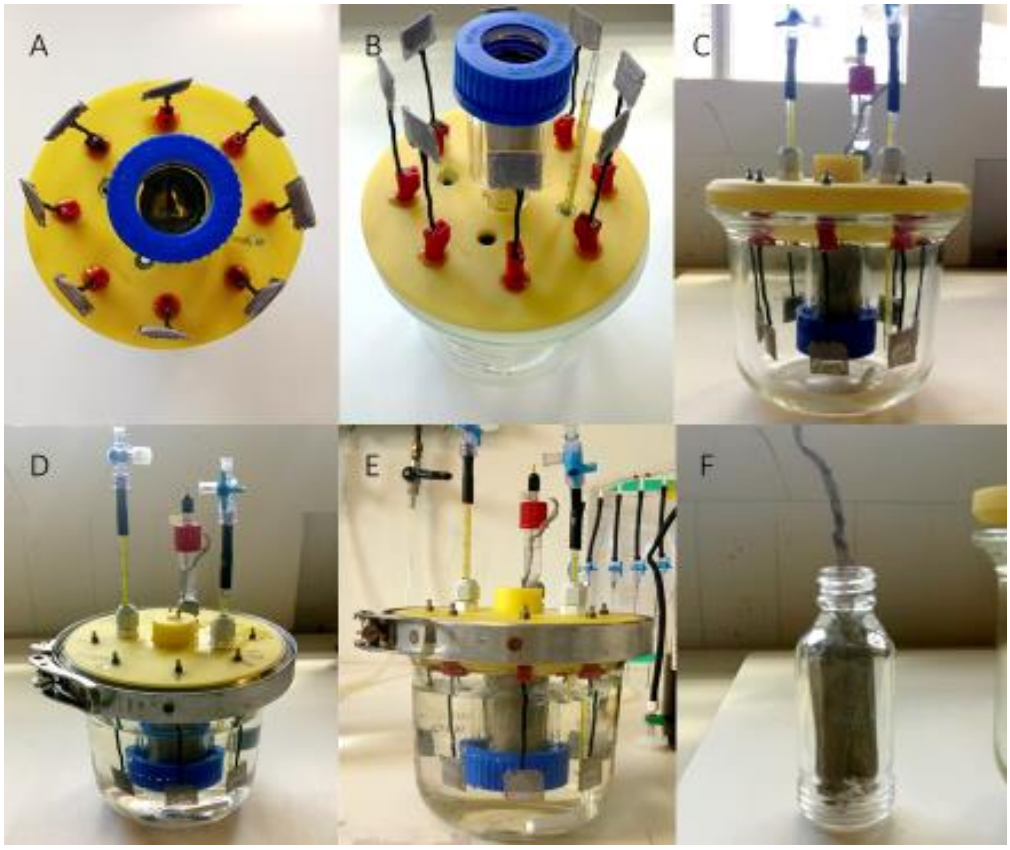


*Figure 22 Set-up 8-electrode BES reactor (CMET, Ghent University, design by Dr. Kun Guo) **A + B**: Reversed top part of reactor, with connected WEs and CE. **C:** Reactor with one counter electrode (CE), eight working electrodes (WEs) and one reference electrode (RE). **D**: Sealed reactor, filled with 20mM acetate FWM. **E:** Medium is flushed with N2/CO2 mixture. **F**: Counter electrode (CE): glass bottle filled with SS mesh as a cathode.*

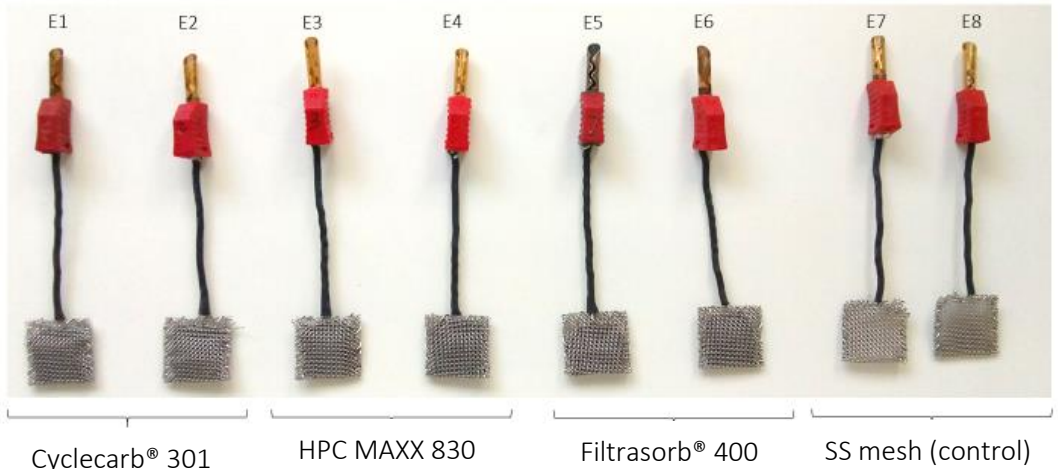


Figure 23 Design of the working electrodes. SS mesh was folded (projected surface area of 2 cm²) and filled with three kinds of GAC (Cyclecarb® 301, HPC MAXX 830 and Filtrasorb® 400) or left empty as a control.

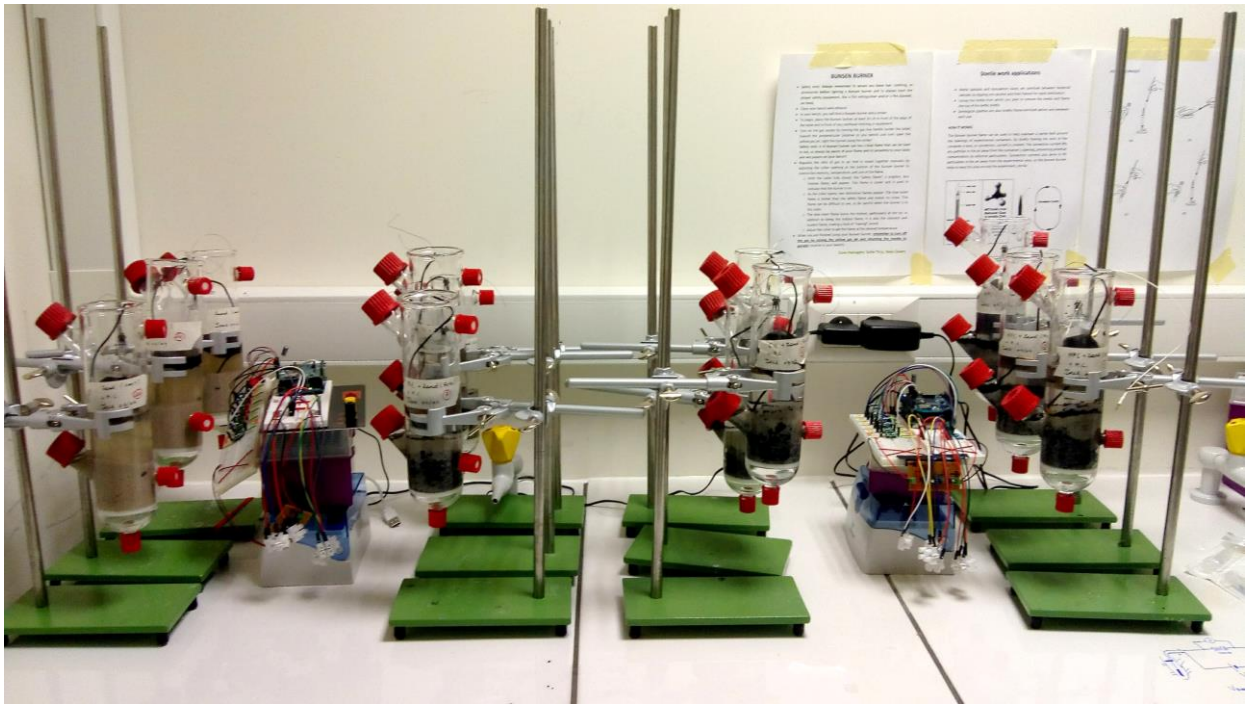


Figure 24 *Picture of the larger MFC reactor set-up (experiment 6)*

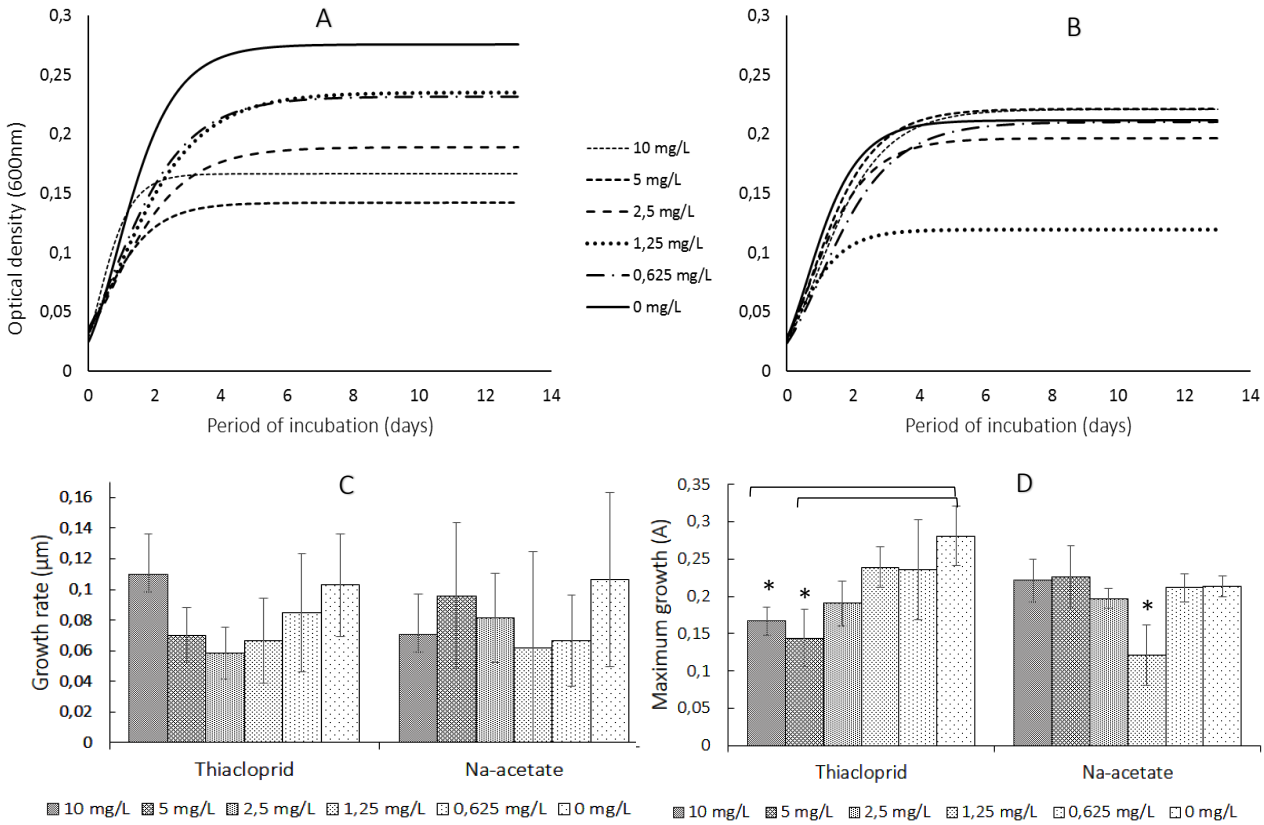


Figure 25 *Results tolerance experiment. **A**: Bacterial growth for different concentrations of thiacloprid modelled with Gompertz; **B**: Bacterial growth for different concentrations of Na-acetate modelled with Gompertz. **C**: Growth rates for different concentrations of thiacloprid and Na-acetate. **D**: Maximum growth for different concentrations of thiacloprid and Na-acetate. * indicates a significant difference with p ≤ 0,05. Averages were calculated of triplicates (± 1 SD).*

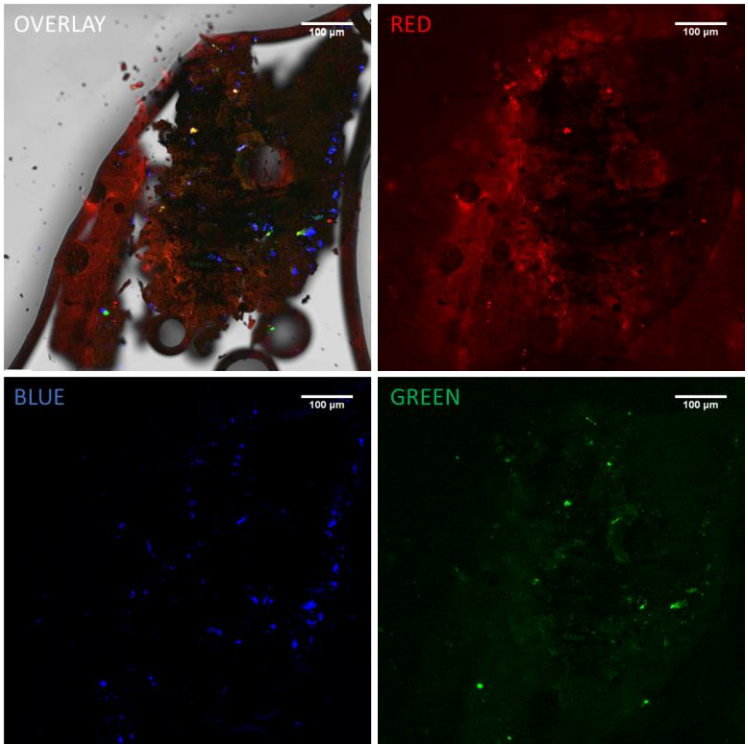


Figure 26 FISH on biochar particle, inoculated with mixed culture and exposed to 10 mg L^{-1} pirimicarb (Blue = DAPI and colors all nucleic acids; Green = G. sulfurreducens*; Red = Eubacteria).*

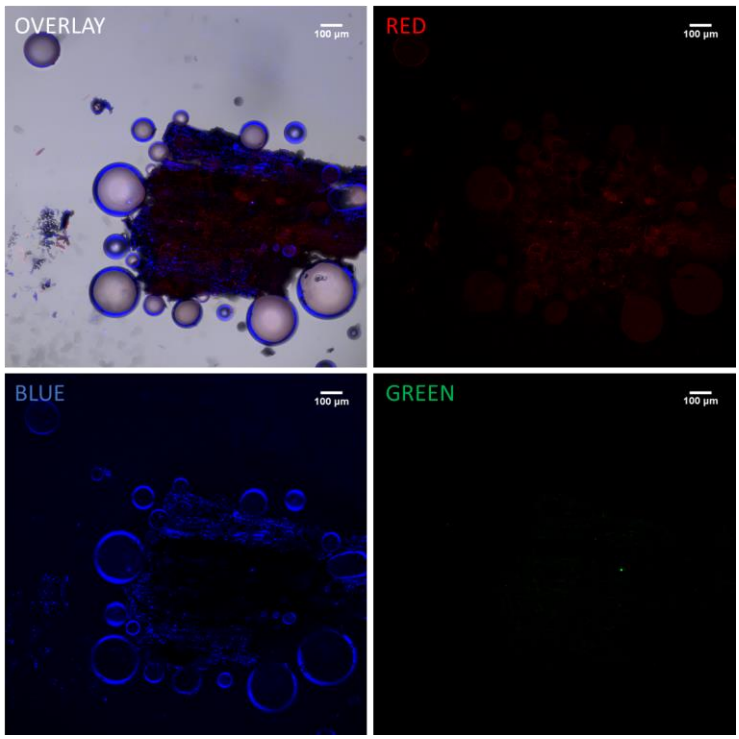


Figure 27 FISH on control biochar particle (no bacteria), exposed to 10 mg L^{-1} pirimicarb (Blue = DAPI and colors all nucleic acids; Green = G. sulfurreducens*; Red = Eubacteria). Some eubacteria were observed, but no* G. sulfurreducens *was found.*

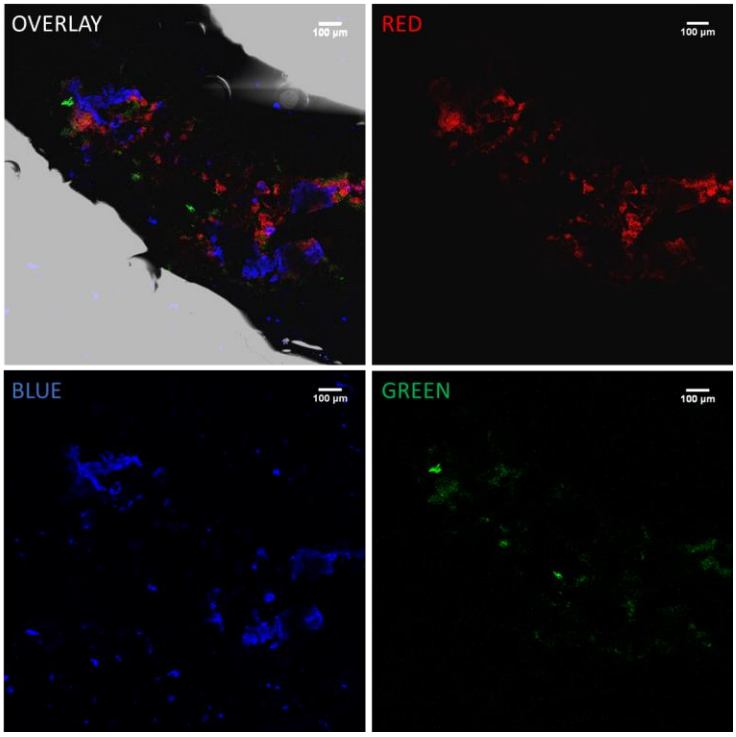


Figure 28 *FISH GAC particle (Filtrasorb® 400) inoculated with a mixed culture, exposed to 10 mg L^{-1} pirimicarb (Blue = DAPI and colors all nucleic acids; Green =* G. sulfurreducens*; Red = Eubacteria). Some* G. sulfurreducens *was found on this particle, although not as much compared to the biochar particle shown in Figure 24.*

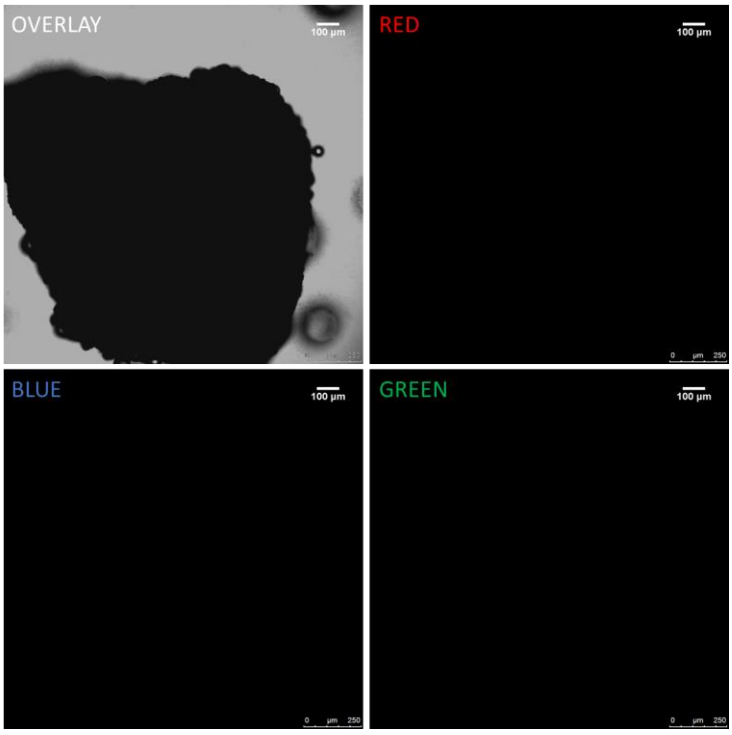


Figure 29 *FISH control GAC particle (Filtrasorb®400) (no bacteria), exposed to 10 mg L^{-1} pirimicarb (Blue = DAPI and colors all nucleic acids; Green =* G. sulfurreducens*; Red = Eubacteria). No bacteria were observed.*

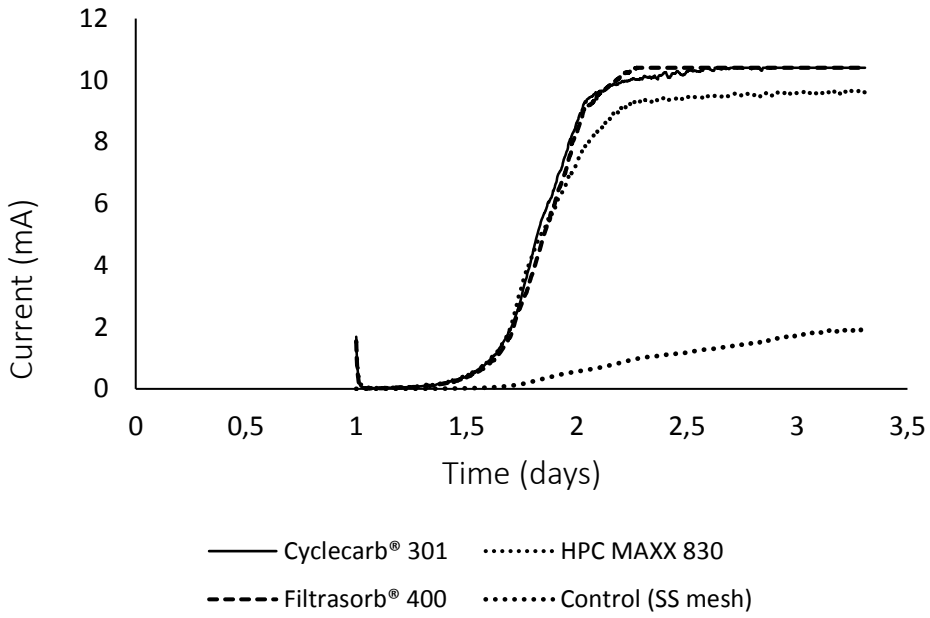


Figure 30 *Chronoamperometry results of the eight-electrode reactor set-up. E = electrode. E1 and E2 contain Cyclecarb® 301. E3 and E4 contain HPC MAXX 830; E5 and E6 contain Filtrasorb® 400. E7 and E8: control (empty mesh).*

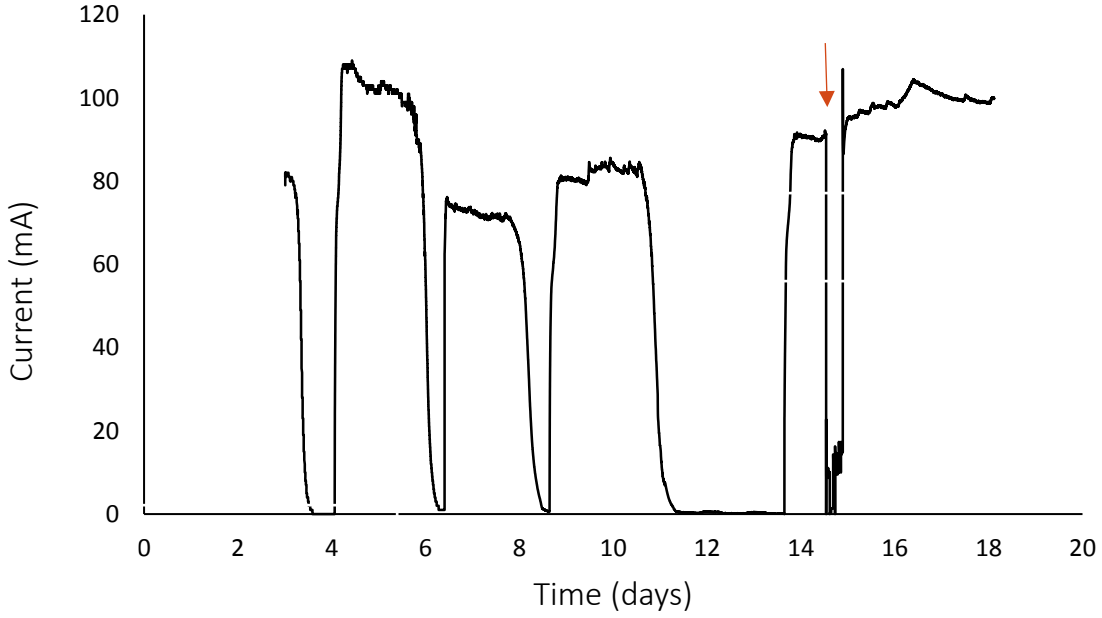


Figure 31 *Chronoamperometry was recorded for eight working electrodes at the same time (after the electrodes containing GACs all exceeded 10 mA after inoculation and a configuration change was made). Each time a drop in current was recorded, medium was refreshed or Na-acetate was spiked in medium. The drop on day 15 indicated by the red arrow indicates recorded currents for each electrode separately, to register maximum current densities for each electrode*

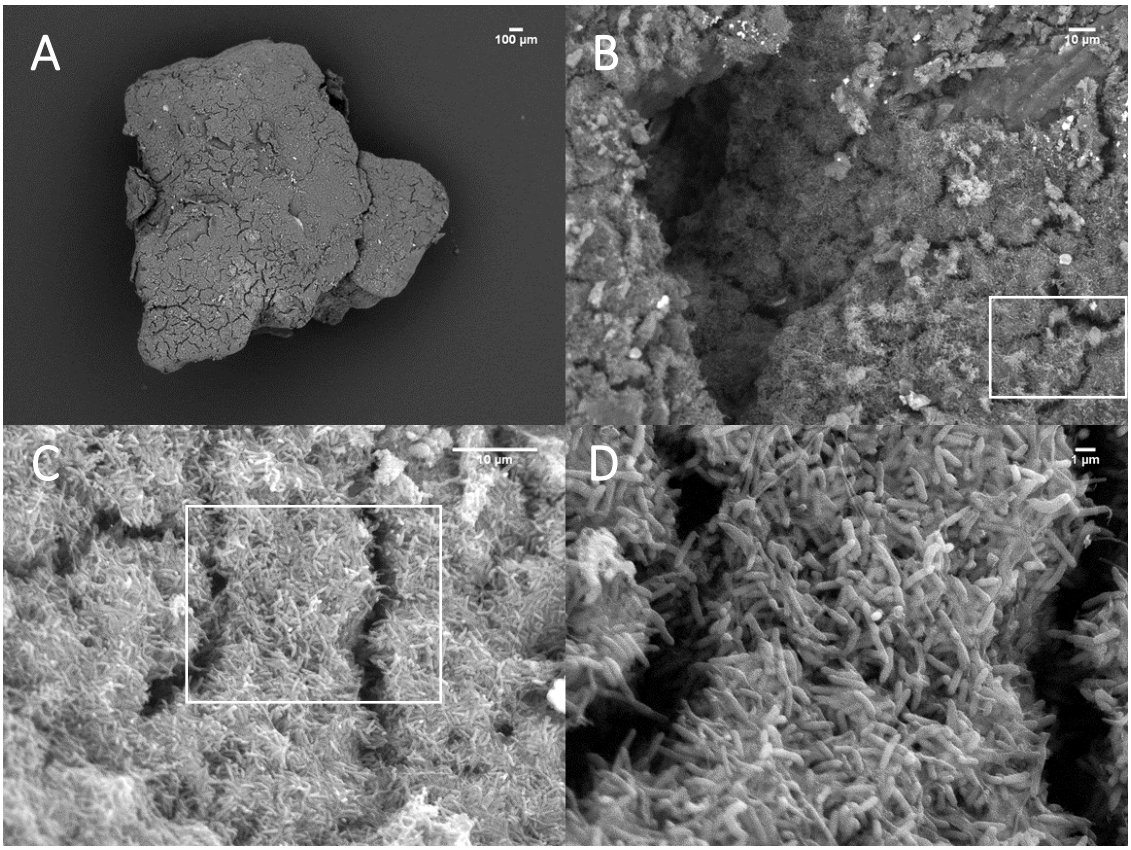


*Figure 32 Scanning electron microscopy images of a granule obtained from electrode 4, containing HPC MAXX 830. **A**: magnification x40; **B:** magnification x800; **C**: magnification x2500; **D**: magnification x6000. In B, a full coverage of the granule surface is observed.*

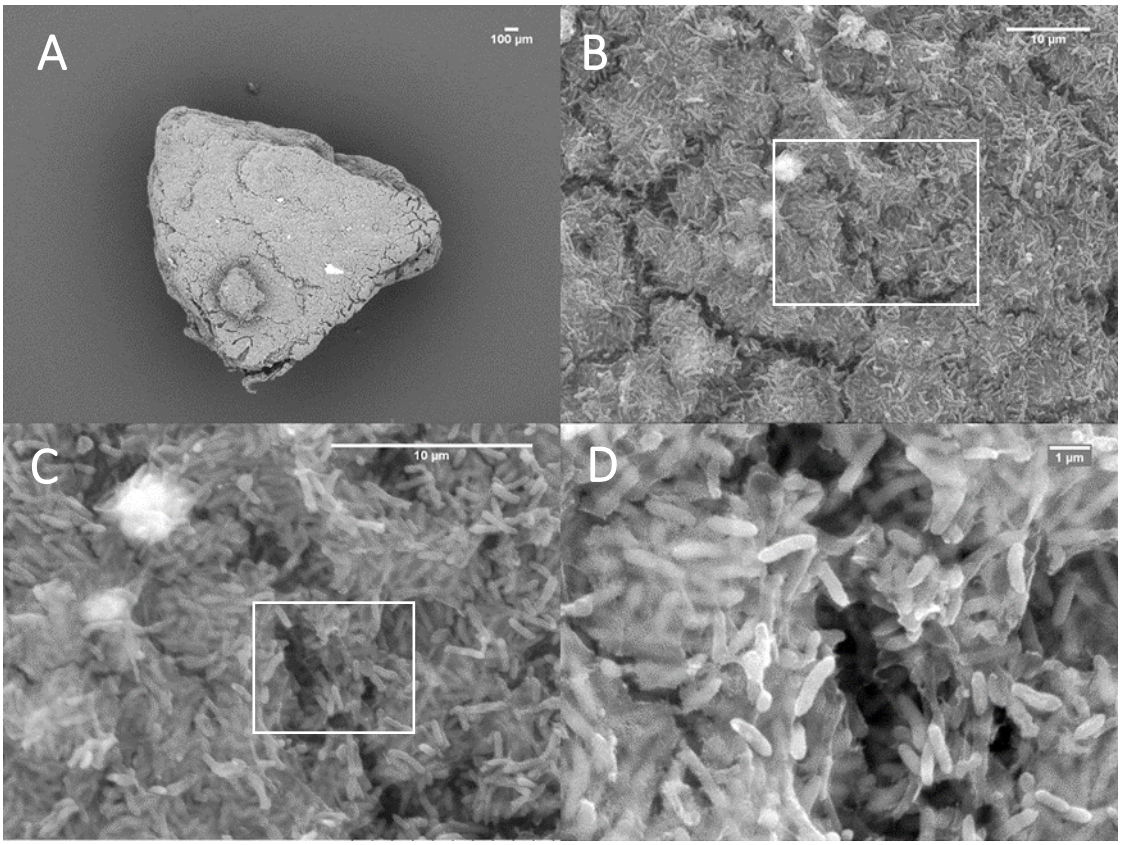


Figure 33 *Scanning electron microscopy images of a granule obtained from electrode 6, containing Filtrasorb® 400.* ***A****: magnification x40;* ***B****: magnification x 2500;* ***C****: magnification x6000;* ***D****: magnification x12000. In B, full coverage of the granule surface is observed, similar to the other GACs.*

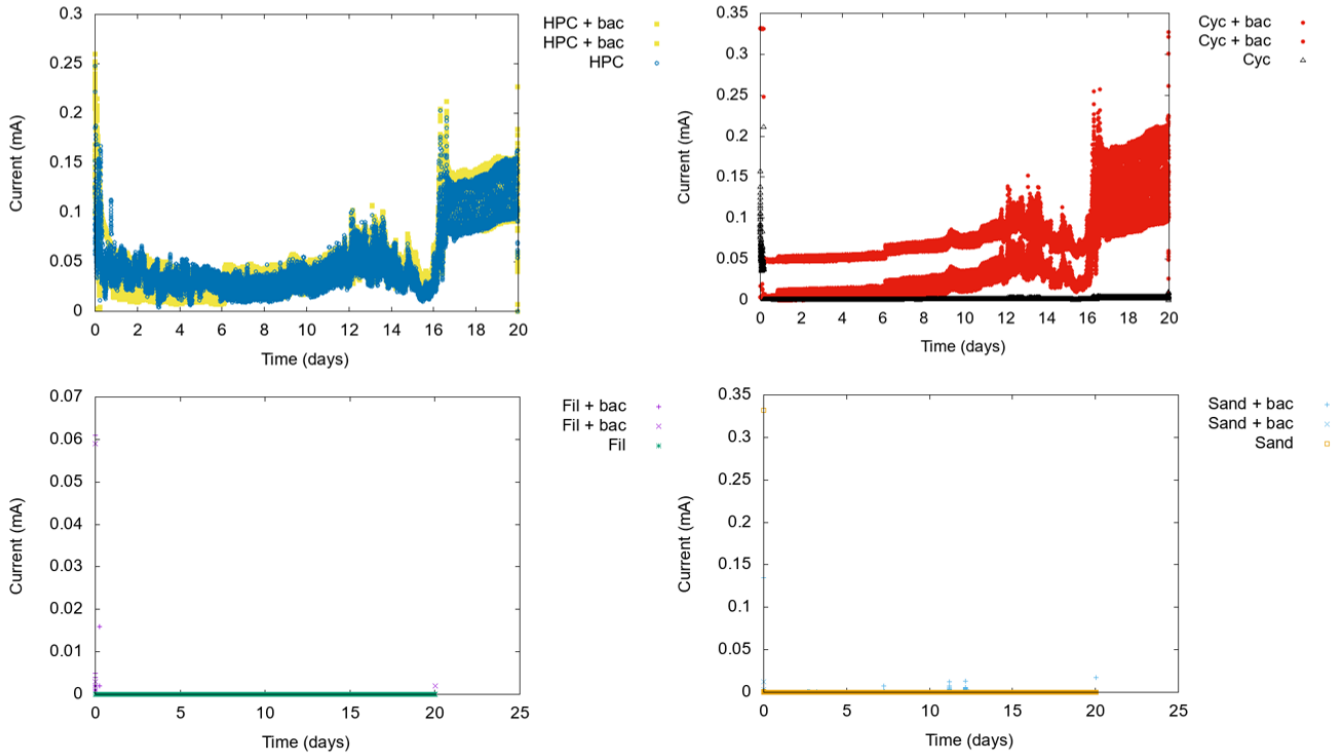


Figure 34 Chronoamperometry (CA) results for the experiment in a larger MFC set-up. HPC = HPC MAXX 830; Cyc = Cyclecarb®301; Fil = Filtrasorb ® 400; For each condition, two set-ups were inoculated (+ bac, addition of a mixed culture) and one abiotic control was made.

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BIOCHAR AS ELECTRON SHUTTLE AND SUBSTRATE MATERIAL FOR ELECTROACTIVE BACTERIA IN A BIOELECTROCHEMICAL SYSTEM TO ENHANCE REMOVAL OF PESTICIDES FROM WASTEWATER

Richting: **master in de biomedische wetenschappen-milieu en gezondheid**
Jaar: **2018**

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