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Maastricht University

Faculteit Geneeskunde en Levenswetenschappen
School voor Levenswetenschappen
master in de biomedische wetenschappen

Masterthesis

The removal of Ibuprofen and Diclofenac in Biochar included Microbial Electrochemical Remediation (BiMER)

Thessa Van Limbergen

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

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De transnationale Universiteit Limburg is een uniek samenwerkingsverband van twee universiteiten in twee landen: de Universiteit Hasselt en Maastricht University.

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Een woordje vooraf

Als ik nu, op het einde van het jaar, terugkijk naar waar we gestart zijn, naar hoeveel er veranderd is. Dan denk ik dat ik gerust mag zeggen dat ik trots ben op wat we bereikt hebben. Ik zeg we, want ik zou nergens zijn zonder de hulp van een aardig hoopje mensen.

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Thessa

Samenvatting

Inleiding: De aanwezigheid van medicijnresten in water is een bron van bezorgdheid. De huidige technologieën zijn onvoldoende en er is nood aan een nieuwe efficiënte en goedkope manier om water te zuiveren. Een veelbelovende techniek is de integratie van elektrisch geleidend materiaal zoals biochar met microbiële elektrochemische technieken. Hierdoor kunnen elektro-actieve bacteriën zoals *Geobacter sulfurreducens* afvalwater saneren door respiratie via de elektroden of elektronshuttles. In deze paper wordt de hypothese gesteld dat ibuprofen en Diclofenac door anaerobe vertering (AV) uit het afvalwater worden verwijderd in Biochar included Microbial Electrochemical Remediation (BiMER; biochar bevattende microbiële elektrochemische sanering).

Materialen & methoden: AV van geneesmiddelen werd onderzocht door elektro-actieve bacteriën (*Geobacter sulfurreducens* of een gemengde cultuur) bloot te stellen aan diclofenac of ibuprofen. Na incubatie werd de concentratie bepaald met HPLC en bacteriegroei met Bradford-assay. In aangrenzende experimenten werden vergelijkbare opstellingen gebruikt met aanvullend elektrisch geleidend materiaal (biochar). Verder werd de AV van acetaat onderzocht in BiMERs met verschillende biochar/zand-mengverhoudingen.

Resultaten: Noch de zuivere cultuur, noch de gemengde cultuur kan de geneesmiddelen efficiënt verwijderen zonder enig elektrisch geleidend materiaal. Biochar zou de afbraak kunnen verbeteren, maar dit was afhankelijk van het farmaceutisch product. Hoge biochar/zand-mengverhoudingen resulteerden in een lagere elektrochemische weerstand en maakten een goede kolonisatie en groei mogelijk.

Discussie en conclusie: De analyse van verdere experimenten en meer onderzoek is nodig om een beter begrip van het AD-proces te krijgen.

Abstract

Introduction: Pharmaceutical traces in wastewater are a growing concern in wastewater treatment. Current technologies are inadequate and there is need for a new low-cost, efficient treatment technology. A novel approach could be the integration of electroconductive materials such as biochar to microbial electrochemical technologies. Here electroactive bacteria such as *Geobacter sulfurreducens* could remediate wastewater by respiring through electrodes or electron shuttles. In this paper it is hypothesized that ibuprofen and diclofenac are removed from wastewater by anaerobic digestion (AD) in a biochar included microbial electrochemical remediation cell (BiMER).

Materials & methods: AD of pharmaceuticals was investigated by exposing electroactive bacteria (*Geobacter sulfurreducens* or a mixed culture) to diclofenac or ibuprofen. After incubation, contaminant concentration was determined with HPLC and bacterial growth with Bradford assay. In adjoining experiments, similar set-ups were used with additional electroconductive material (biochar). Additionally, the AD of acetate was investigated in BiMERs with different biochar/sand mixing ratios.

Results: Neither the pure culture, nor the mixed culture could degrade the pharmaceuticals efficient in the absence of any electro-conductive material. Biochar could improve degradation, but it was dependent on the pharmaceutical. High biochar/sand mixing ratios resulted in a lower electrochemical resistance and allowed good colonisation and growth.

Discussion & conclusion: The analysis of further experiments together with more research is needed to acquire a better understanding of the AD process.

Inhoud

List of abbreviations

AC: activated carbon

ACN: acetonitrile

AOP: advanced oxidation process

BiMER: Biochar included Microbial electrochemical remediation

CAM: chronoamperometry

CV: cyclic voltammetry

CW: constructed wetland

EET: extracellular electron transfer

FWM: fresh water medium

FWM_A: fresh water medium with acetate

FWM_AF: fresh water medium with acetate and fumarate

FWM_F: fresh water medium with fumarate

GC-MS: gas chromatography – mass spectroscopy

GS: *Geobacter sulfurreducens*

HPLC: high pressure liquid chromatography

MC: mixed culture

MEC: microbial electrolysis cell

MET: microbial electrochemical technology

MFC: microbial fuel cell

MER: microbial electrochemical remediation

NB: no bacteria

SEM: scanning electron microscopy

TGA: thermogravimetric analysis

WWTP: wastewater treatment plant

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1 Introduction

1.1 Wastewater and the problem of micro-pollutants

The growing world population and the development of life quality cause an increase in water use and therefor urban and industrial wastewater production. Only a minor 20% of globally produced wastewater is discharged in the environment with proper treatment. This is less than 8% in low-income countries due to a lack of infrastructure, technical or institutional capacity and financing (1). The remaining 80% causes significant water pollution.

One type of emerging contaminants in wastewater are pharmaceuticals. Pharmaceuticals have been present in drinking water for quite some time. Only up until recently, people are able to quantify their presence. This means that their regulation is inexistent or still in process and that their removal still contains some knowledge gaps (2,3). Little is known about the long-term effects of exposure to pharmaceuticals on the environment or their chronic health effects (4).

Pharmaceuticals can reach the surface and ground-water by disposal of the medicines in the sewage system, which are not broken down in current wastewater treatment processes (4–6). Alternatively, pharmaceuticals on landfill sites can seepage to the groundwater and contaminate the environment (3,7). Third, agricultural over-use of antibiotics and excretion by the animals is another major route of dispersal of pharmaceuticals in the environment (8).

Although the levels of these pharmaceuticals are a thousand times lower (micro to nanograms) than the minimal therapeutic dose, the contaminants are still considered as “micro-pollutants” (3,9). Meaning it could still be useful to monitor and resolve them. The levels found in finished drinking water vary substantially per component, the detected concentration of ibuprofen or diclofenac for instance were 3 ng/L and 6 ng/L respectively (10), while the concentration of phenazone or propyphenazone reached up to 250 400 ng/L and 80 120 ng/L (11). The levels also vary between countries: clofibric acid for instance was found with a maximum concentration of 5.3 ng/L in Italy (12) while in Germany the maximum detected concentration went up to 270 ng/L (13). The most commonly detected pharmaceutical products are anti-inflammatories and analgesics, antidepressants, antiepileptics, lipid-lowering drugs, β-blockers, antiulcer drugs and antihistamines, antibiotics and other substances (2).

1.2 Current wastewater treatment technologies

Sewage water is treated in a wastewater treatment plant (WWTP), but the removal of pharmaceutical residues with conventional techniques is inadequate due the low concentrations but also due to their molecular structures (4–6). The fact that there is no superior way of removing them, means there is also currently no regulation on the permitted concentrations of pharmaceuticals in the environment (2).

Conventional wastewater treatment includes the preliminary removal of large materials followed by metabolization by aerobic bacteria. The secondary effluents can be used after primary, secondary of advanced treatment, depending on its purpose. Additionally, most effluents are disinfected with chlorine or a more unconventional method like ozonation or irradiation. The remaining sludge from the treatment processes are also further processed and disposed (14). Due to the frequently synthetic origin of pharmaceuticals, microorganisms have trouble metabolizing them. Research indicated that the primary treatment can remove some pharmaceuticals, but not all substances such as ibuprofen (15). While another study stated that WWTP can eliminate ibuprofen, paracetamol and acetylsalicylic acid but not diclofenac (16). Studies have shown that chlorine and chlorine dioxide is effective for some pharmaceuticals such as diclofenac or sulfamethoxazole. It reacts selectively to compounds with electron dense functional groups. But in general, most micropollutants are

not completely removed in the treatment process and conventional treatment systems are insufficient for pharmaceutical removal.

Some more unconventional treatment technologies are activated carbon adsorption, advanced oxidation processes (AOP) based on ozone, UV or gamma radiation or electro-oxidation with/without active chlorine generation (2). Activated carbon is efficient for some compounds but not all, depending on the characteristics of the carbon. In addition, the underlying mechanisms and interactions are still being investigated. One advantage of activated carbon is that it does not generate any toxic of active compounds. A disadvantage is that their capacity is limited. Advanced oxidation processes are based on the oxidation of compound with generation of free radicals with HO* being the most potent. AOPs based on ozone and H_2O_2 have been proven to be successful in the removal of ibuprofen and diclofenac with a mineralization rate of 98% (17). AOP based on UV radiation is an excellent technology since most pharmaceuticals are photoactive. For instance, diclofenac is rapidly decomposed by photooxidation (18–20), while ibuprofen does not absorb solar light (21,22). AOP with gamma radiation also causes the formation of free radicals which can react with different compounds, but its implementation is limited due to the little knowledge about its safety and performance. Electrochemical oxidation is a popular electrochemical technique for the remediation of wastewater based on the application of high cell voltage to oxidise pollutants (23). Mainly, there are a lot of different techniques all with their own conveniences, but also their limitations. A more specific method is needed to reduce pharmaceutical pollution in the environment.

There are some other problems with the current wastewater treatment methods besides the inadequacy such as the price tag of treatment and its energy demand. Even though the low capital cost is an advantage it is negligibly when compared to other high costs that are accompanied. The expenses are rather high for chemical consumption, maintenance, sludge handling and labour (24). The activated sludge process, or aerobic digestion, is accountable for a yearly 21 billion kWh electrical energy consumption in the US alone. With most of this energy going into the aeration and pumping of the system. In the UK, wastewater treatment is accountable for 3-5% of the national electricity consumption. This together with the growing ecological footprint and the already rising cost of energy due to depletion of the resources will lead to an acceleration in the price of wastewater treatment (25). The high costs, high energy demand, the growing ecological footprint and inadequate removal all emphasizes the need for new treatment technologies that are more cost-friendly, environment-friendly and more sensitive and specific.

1.3 A new flow in the purification technologies

Nature has its own way of cleaning water. Clay minerals, humic matter, and microorganisms all have the capacity to decontaminate water. Humans have turned these natural processes into a technology: constructed wetlands (CWs), using soil, vegetation and organisms to treat water. The process of water purification occurs via filtration, sedimentation, precipitation, sorption-desorption, biodegradation, oxidation-reduction and ion-exchange (26). CWs are very sustainable treatment technologies due to their non-energy and -chemical intensive processes for the return of nutrients to the environment (27).

CWs could however be made more efficient by the addition of some electrochemistry, namely microbial electrochemistry. Microbial electrochemistry combines the activities of electroactive microorganisms (or electricigens) with electro-conductors to enhance chemical processes. When implemented into CWs, the term METLands is used for microbial electrochemical technologies (MET) in constructed wetlands (28). Microbial electrochemistry has previously been successfully employed in microbial fuel cells (MFCs) for power production, in microbial electrolysis cells (MECs) for the synthesis of chemical compounds, and microbial electro remediating cells (MERs) for the degradation of pollutants.

The practical implementation of a METLand, consists of two compartments, a cathode and anode of which at least one is connected to a microbial process (29). In the anaerobic side, electro-active bacteria will take electrons from an electron donor (*e.g.* acetate, or organic compounds in wastewater or brewery water) and give them to an electrode (the anode), a process called extracellular electron transfer (EET). Because of the EET capacity the electroactive bacteria don't use oxygen as terminal electron acceptor which causes them to be viable in anoxic environments. The electrons are subsequently shuttled to the aerobic side (cathode) where finally oxygen will serve as electron acceptor and forms water by joining with hydrogen atoms that can move freely between the two cells via a semi-permeable membrane. The electrons that are transferred from anode to cathode produce a measurable current. The energy yield from this process is however not substantial and is rather used as an indicator for the degradation rate. The processes in the anaerobic (Eq. 1) and aerobic (Eq. 2) side can be put into equations:

$$\text{Organic matter} \rightarrow CO_2 + e^- + H^+ \quad (1)$$

$$e^- + O_2 + H^+ \rightarrow H_2O \quad (2)$$

Electroactive bacteria, also called electricigens (30), have very ingenious features to transfer electrons over their cell membrane, unique to this class as not all organisms can do this. Some example species are *Geobacter sulfurreducens* and *Shewanella oneidensis*. Electrons are conducted by electricigens through transmembrane proteins (31–33) that use different methods to transfer electrons to acceptors: direct contact, iron chelating agents, conductive pili, shuttling through mediators that are excreted, through a conductive biofilm etc. Electron shuttles and chelators can be produced by the electricigens themselves and may help the electron transfer when the microbial population is dense. If the density is low, the concentrations of these chelators and shuttles could be rather low as well and direct transfer is favourable. The conductive biofilm is among others the case for *Geobacter sulfurreducens*. Here, riboflavin is an electron carrier that is not completely freely soluble but loosely bound to the biofilm, making it highly conductive (34).

1.4 Towards improving MET technologies using biochar

As mentioned before, the process of cleaning wastewater in METs relies on the bacteria and their vicinity to the anode: the closer the electricigens are, the more efficient the process is. This culminates in an active area that is approximately equal to the surface of the anode. One way of improving electron transfer and thus speeding up the remediation process is by adding electroconductive material to the anodic compartment to enhance the electrically conductive surface area.

Previous studies have already proven the use of coke granules for increasing the active surface area (35). They have shown that the effluent with residual values was 3-4.5 times lower when comparing cokes to gravel (28). Other studies used activated carbon as substrate (36,37). Arends et al. also investigated the effect of carbon granule size and mixing ratios on substrate oxidation rate at the anode side and power output. Though with cokes having a rather negative environmental impact (38), a more ecological alternative is preferred. A candidate is the use of conductive organic carbon material, in the form of biochar.

Biochar is a carbon rich material that is very similar to cokes and charcoal. It is a solid material made through the thermochemical conversion of different input sources in an oxygen-limited environment (39). Biochar has electroconductive characteristics dependent on the pyrolysis temperature. There are some differences between cokes, charcoal and biochar. The former two are produced in an unsustainable way, charcoal being a big driver of deforestation and cokes of resource depletion (40). The pyrolysis process of biochar is nowadays a lot less polluting when compared to the traditional technologies and the main purpose is to find a new fate for waste biomass. The by-products formed are considered to be bioenergy as well (bio-oil and bio-gas) a more environmental friendly form of energy (41). Another difference is the use of the material itself. While biochar is primarily used in soil improvement, the opposed are mainly used as fuel. Biochar is also very porous, it has

minimal tars, a fixed high carbon content and a high surface area, making it an excellent host for bacteria and fungi in soil and maybe as well in water.

Many studies have focused on using biochar as sorbent (42–44) or electron shuttle (45) in closed set-ups not coupled to anode and cathode. Another study investigated the use of biochar for the removal of azo dyes in an bio-electrochemical set-up (46) However, what is lacking is a rigorous investigation of the use of biochar in METs to remove micropollutants, acting both as sorbent, but also as substrate for electroactive bacteria, and interface where redox reactions can take place to completely transform or detoxify the pollutants besides sorption.

1.5 Research objectives

Given the potential of increasing anode surface area using conductive material thereby speeding up degradation processes and the sorption capacity of biochar, the aim of this study is to increase the knowledge concerning anaerobic digestion of electricigens in the presence of biochar. It is therefore hypothesised that adding biochar to microbial electrochemical remediation will lead to a more efficient removal of pharmaceuticals; and this will be dependent on the type and mixing ratio of biochar. To evaluate this, the performance of different types of biochar was investigated through the clean-up of ibuprofen and diclofenac, mixing ratios were investigated in terms of clean-up efficiency while also permitting plant growth.

To this end, we (1) assessed whether the electricigens (a pure *Geobacter sulfurreducens* culture or a mixed culture from the river Demer) could digest two pharmaceuticals (diclofenac or ibuprofen), (2) evaluated if the previous assessment was improved with the addition of biochar (Typha, wood or coffee bean based) or activated carbon from Chemviron, (3) designed a BIMER (Biochar included Microbial Electrochemical Remediation cell) and assessed the mixing ratio of Typha biochar for increasing the degradation of the pharmaceuticals in an electrochemical cell. Many advanced technologies were used to elaborate said objectives. Initial experiments were performed in small glass vials, to assess degradation and growth HPLC and protein determination were used (respectively), biochar colonisation was investigated through scanning electron microscopy (SEM). In the second part, selected parameters were transferred to a larger system, a microbial electrochemical remediation cell, with the addition of an electrochemical circuit which allowed to perform chronoamperometric measurement and cyclic voltammetry. To keep the set-up simple, no pharmaceuticals were added. Instead the concentration of acetate was measured. The formation of biofilm was investigated through thermogravimetric analysis. Gas chromatography-mass spectroscopy was used to investigate breakdown products.

This thesis will describe the degradation of diclofenac and ibuprofen by electroactive bacteria, in the presence of biochar or activated carbon. Going from a simple set-up to more complex systems in MECs.

2 Materials and methods

2.1 Biochar and Activated carbon material, pharmaceuticals and solvents

The biochars used in this research were a bulrush char (*Typha latifolia*), wood mix, and coffee beans. The granular activated carbons used were HPC MAXX 830, Filtrasorb 400, or Cyclecarb 301, kindly provided by Chemviron, Teluy, Belgium. Sodium-diclofenac (≥98%) was purchased from Acros Organics, Geel, Belgium. Sodium-Ibuprofen (≥98%) was purchased from VWR, Leuven, Belgium. Methanol Chromasolv™ ≥99.9% pure, and Acetonitrile HiPerSolv ChromaNorm® ≥99.9% pure for extractions and HPLC were purchased from VWR, Belgium.

2.2 Biochar pyrolysis

Bulrush was collected (Zonhoven and Westerlo, Belgium), dried, chopped fine to cm pieces, shredded and sieved to 2 mm pieces before going into the pyrolysis process. For the pyrolysis, a custom-made reactor was used (Fig. 1). 121 gram of the 2 mm sieved material was used per production batch. The reactor heated up with steps of 10°C/min until an internal temperature of 450-500°C was reached where the biomass was pyrolyzed for 45 minutes. An Archimedes' screw rotated the biomass around with a speed of 400 rpm and nitrogen gas (N_2) was supplied with a speed of 2x 70 ml/min.

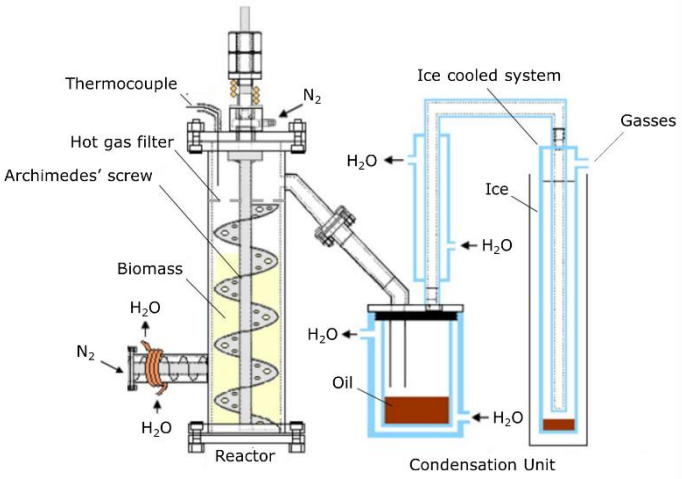


Figure 1: Scheme of the pyrolysis reactor set up. H_2O: water, N_2: dinitrogen gas

2.3 Biochar characterisation

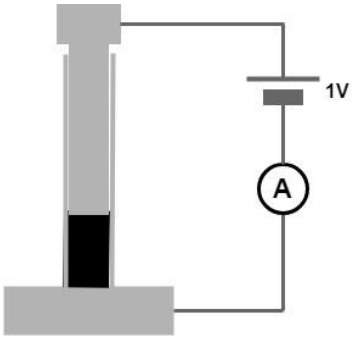
2.3.1 pH and wet conductivity

Samples were weighed and dried in an oven. After drying, MiliQ water was added, diluting the biochar with a factor of 1:10. Samples were incubated in a shaking water bad and after 24 hours, the samples were filtered with a Rotilabo® Rundfilter 14A (Carl Roth, Karlsruhe, Germany) and the remaining liquid was collected. Wet conductivity was measured with WTW LF 340 conductivity meter with a WTW TetraCon 325 conductivity probe (Weilheim, Germany). The pH of the liquid was measured with Knick pH meter 764 Multi Calimatric (Berlin, Germany). Samples were compared to MiliQ as control.

2.3.2 Sorption capacity

For the two-point sorption test samples of either 15 mg or 120 mg of biochar were dried and added to 30 ml mineral salts solution supplemented with 40 ppm ibuprofen (mineral salts solution, per l: 65.7 mg sodium bicarbonate ($NaHCO_3$), 3.75 mg potassium chloride (KCl), 294 mg calcium chloride dihydrate ($CaCl_2.2H_2O$), and 123 mg magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), pH 8 with Tris). Samples were incubated for 24 h at room temperature in a shaking incubator. After the 24-hour incubation the samples were filtered and the ibuprofen concentration in the supernatant was subsequently measured with a Ultraspec 7000 spectrometer at 210 and 230 nm absorption maxima and compared against a standard curve of Ibuprofen (0-40 ppm). Samples were weighed in triplicates.

2.3.3 Dry conductivity



Dry conductivity was measured with a custom set-up (designed by Robin Bonné, built by Johan Soogen) (Fig. 2). It consists of a metal and weights 100 g with an additional 150 g on top. A volume of 5 cm^2 of dry biochar or water-saturated biochar was added to the system and subsequently a voltage of 1V was applied to the system and current was measured. Conductivity determined out of the systems resistivity (Eq. 3). The resistivity was calculated from the measured current with the help Pouillet's law (Eq. 4). Samples were tested in triplicate.

$$C = 1 / R \quad (3)$$

$$R = \rho * L / A \quad (4)$$

Figure 2: Scheme of electrical circuit used for dry conductivity measurements. Char (black) is applied to measurement system (grey) and a voltage (1 Volt) is applied. A: ampere meter, V: volt

2.4 Bacteria and culture conditions

Geobacter sulfurreducens strain 12127 was obtained from DSMZ (Braunschweig, Germany). A mixed bacterial culture was established by adding some scoops of sediment from the Stiemer in Diepenbeek to a sterile anaerobic flask with fresh water medium (47). The mixed culture was incubated at 30°C in the dark for five days and then 1:10 transfer was performed to a new flask, incubated for 1 week. This was the stock solution to use at the start of experiments.

The bacteria were routinely cultured in fresh water medium (FWM; Appendix 1), in 27 ml Hungate culture tubes (VWR, Belgium). Briefly, to prepare the medium tubes, all compounds were weighted separately, brought to pH 8, and then 10 ml was transferred per tube. The medium was flushed for 15 min with N_2 gas, followed by 5 min of N_2 flushing in the headspace, and sealing with butyl rubber stopper and screw cap. After sealing, 3.4 ml of CO_2 gas was added and additional 3.4 ml of N_2 gas to increase the pressure to 20.3 psi. The vials were sterilized in a Tuttnauer 3870 EL autoclave (Liquid A program, Tuttnauer, Breda, Netherlands). After sterilization bacteria and heat sensitive products were added to the vials.

2.5 Anaerobic degradation of pharmaceuticals by growing cultures

2.5.1 Without biochar or activated carbon

One ml of growing cells of *G. sulfurreducens* and mixed culture cells (log-growth phase), were transferred to 10 ml of sterile FWM medium in Hungate tubes, supplemented with 10 ppm Ibuprofen sodium salt or 10 ppm Diclofenac sodium salt (added from 10 000 ppm stock solutions in DMSO). The conditions of pesticide degradation tested were in medium containing of 20 mM acetate and 40 mM fumarate (FWM_AF), 20 mM acetate only (FWM_A), or 40 mM fumarate only (FWM_F). All conditions were tested in threefold and compared to no bacteria controls. Samples were incubated at 30°C for 14 days before HPLC analyses and Bradford.

2.5.2 With activated carbon (AC)

The experimental set-up was similar as above, except that 25 mg of AC (Filtrasorb 400, HPC MAXX 830 or Cyclecarb 301; sieved over 2 mm) was added to the Hungate tubes prior to sterilisation. After autoclaving and cooling down, 1ml of pre-grown bacteria culture (*G. sulfurreducens* or mixed cultures) and 10 ppm of the pharmaceutical was added to the vials. Conditions tested were in the presence of 20 mM acetate as donor, except for Cyclecarb 301, here both medium with acetate and medium with fumarate was tested, in triplicates. Samples were incubated for 14 days prior to sampling for HPLC analyses and Bradford.

2.5.3 With biochar

Similar to the AC experiment, three types of biochars were tested; two biochars were kindly provided by the chemistry department of Hasselt University (wood mix and coffee beans), the third one was self-made with help the chemistry department and was made from bulrush plants. The biochars were added in 25 mg to 10 ml medium in the Hungate tubes, prior to sterilisation by autoclaving. After autoclaving and cooling down, 1ml of mixed bacterial culture was added to the vials, and 10 ppm of the pharmaceutical. Conditions tested were in the presence of 40 mM fumarate, in triplicate. Samples were additionally compared to another condition with Cyclecarb 301. Samples were incubated for 14 days prior to sampling for HPLC analyses and Bradford.

2.5.4 With one biochar, one AC or no material with iron spike

The experiments as described above were repeated for the bulrush biochar, and Cyclecarb 301, and a control without conductive material, with an extra iron citrate ($FeC_6H_5O_7$) as electron donor. Similarly, 25 mg of the biochar, AC was added to the vials with FWM prior to autoclaving. Then 1 ml of mixed culture or *G. sulfurreducens* and 10 ppm of pharmaceutical. All conditions were tested with 40mM fumarate, in triplicate. Samples were incubated for 14 days prior to sampling for HPLC analyses and Bradford.

2.6 Anaerobic degradation of Acetate in a Biochar included Microbial Electrochemical Remediation cell

A Biochar included Microbial Electrochemical Remediation cell (BiMER) was used to investigate the anaerobic diclofenac/ibuprofen degradation capacity of a mixed culture. The set up consisted of a piece of custom made glassware from Adams & Chittenden (Berkeley, USA) filled with a different (volume) percentage of biochar mixed with sand (Fig. 3). The biochar used in this experiment was bulrush. The percentages used were 100%, 50%, 25% and a 0% control with only sand (1 mm diameter; Cobco Garden; Niel, Belgium). To create an electrochemical cell, electrodes were added to the system: the current collector consisted of a 5 cm x 1 cm graphite rod (Mersen Benelux, Wemmel, Belgium) while the cathode was made of a carbon felt circle with a diameter of 4 cm and a thickness of 2.5 cm (Mersen Benelux, Wemmel, Belgium). Both electrodes were connected to an Arduino system using 0.7 mm thick titanium wires covered with heath shrinking tubing.

The glassware was filled as followed: first the biochar mixtures were made by adding the appropriate volume (0, 25, 50 or 100 ml) of biochar to a beaker and adding sand until a total volume of 100 ml was acquired. The mixture was added to the glassware together with the anode. Next, the mixture was topped with a 2-3 cm sand layer to separate the biochar mixtures from the rest of the system, creating an anaerobic and aerobic zone. The whole system was filled with fresh water medium with an acetate concentration of 20 mM and the cathode was added at last. To remove the oxygen from the anodic compartment of cell, the whole system was flushed with N_2 by puncturing the bottom septum of the cell with a needle connected to the gas supply. Every cell was flushed for two hours.

After 2 days of stabilisation, two BiMERs of each condition were inoculated with a mixed culture sample (1 ml), while the third set-up remained bacteria free. Liquid samples were taken at the start-up day and after 14 days (for acetate assay and Bradford assay), a biochar sample was taken after 14 days (for thermogravimetric analysis).

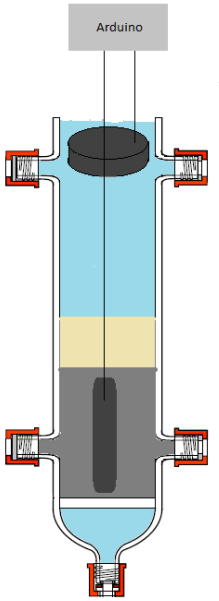


Figure 3: Design of the biochar included remediation cell (BiMER). The BiMER set-up consists of anaerobic compartment with biochar/sand mixture (dark grey) and graphite rod (black rod), sand layer (beige), and aerobic compartment with carbon felt electrode (black circle). System is saturated with FWM (blue). Both electrodes are connected to an Arduino system (light grey).

2.7 Pharmaceutical extraction procedure

For the extraction of Ibuprofen and Diclofenac, three ml of water sample was collected and centrifuged in an Eppendorf 5810 R centrifuge (Hamburg, Germany) for 15 min at 4000 rpm. Next, to three ml of supernatants, an equal volume of acetonitrile (HiPerSolv, VWR, Leuven, Belgium) was added. After shaking briefly, salts were added (1.2 g anhydrous magnesium sulphate (Alfa Aesar, Haverhill, USA), 0.3 g sodium chloride (VWR), 0.3 g trisodium citrate dihydrate (Sigma-Aldrich) and 0.15 g disodium hydrogen citrate sesquihydrate (Sigma-Aldrich)). The mixture was shaken vigorously for five minutes and centrifuged (5 min, 4000 rpm). The top layer was filtered with a 0.2 µm PTFE syringe filter (VWR) and transferred to a High-Performance Liquid Chromatography (HPLC) vial.

The sorption capacity of the ACs and biochar was investigated as well. For this, culture medium was discarded, and three ml of acetonitrile as added to the biochar. Samples were sonicated for two hours and subsequently filtered over 0,2 µm PTFE membranes prior to HPLC analysis.

2.8 HPLC analyses

HPLC was performed using an ACE Equivalence C18 column (EQV-5C18-2546, VWR, Leuven, Belgium; 4.6 x 250 mm, octadecyl as functional group, pore size of 11 nm, particle size of 5 µm, surface area of 280 m^2/g, a carbon load of 15%) and using the Hitachi Chromaster instrument consisting of the 5160 pump, 5280 autosampler, 5310 column oven and 5430 diode array detector (VWR, Oud-Heverlee, Belgium). The mobile phase used for Ibuprofen and Diclofenac consisted of 3:1 ACN:MetOH which flowed with a speed of 1 ml/min. For ibuprofen the retention time was 3.3 minutes, peaks measured at 264 nm. Diclofenac was measured at 276 nm after 3.127 minutes.

2.9 Gas Chromatography (GC)

For GC analyses, one ml of an HPLC extract was completely dried under N_2 gas and the precipitate was resuspended in two ml of chromatography-grade ethyl acetate (VWR, Leuven, Belgium). A small amount of anhydrous sodium sulphate (Alfa Caesar, Leuven, Belgium) was added for dewatering, and next the supernatant was transferred to a new vial and dried under N_2. 100 µl of BSTFA derivatising agent (Sigma, Overijse, Belgium) was added, and incubated overnight at 80°C, subsequently 1 µl of the reaction was injected in a Trace 1310 Gas chromatograph (Thermo Fisher) coupled to an ISQ-LT mass spectrometer (Thermo Fisher). The samples were injected (splitless, 0.5 min) into a 30 m DB-5MS column (5% phenyl 95% polydimethylsiloxane, 0.25 mm diameter; Agilent Technologies, Heverlee, Belgium) with an injection temperature of 280°C and a helium flow rate of 1.2 ml/min. The GC had a temperature program with start at 35°C, increased to 350°C with a rate of 15°C/min and stabilised at 350°C for 10 minutes. The mass spectrometer had a transfer temperature of 280°C and ion source temperature of 250°C. The samples were ionised by electron ionization. The MS scanned for 2.5 minutes with a scan time of 0.3 seconds.

2.10 Thermogravimetry

Samples were dried and sieved to separate the biochar particles from the sand particles. Samples were then send to the Research support centre for geological techniques of the Complutense University of Madrid, Spain for thermogravimetric analysis.

2.11 Bradford assay

The growth of the bacteria in different conditions was monitored with a Bradford assay. An adapted version of the protocol was used. In brief, a standard series was made of bovine serum albumin (BSA) (0 to 1500 mg/L) and an equal volume of 1M NaOH was added to achieve a higher protein yield. Of this, 20 µL was added to 1 mL of Bradford dye reagent 1X (BIO-RAD, Hercules, USA) and incubated for five minutes. After incubation the absorbance was measured with a Shimadzu 1602 spectrometer (Kyoto, Japan) at 595 nm. For the samples, a similar sample preparation was performed: an equal volume of NaOH was added to a 1 ml sample, and 100 µL solution was added to 1 ml of Bradford dye. Protein concentration of the samples was determined based on the standard curve analyses with BSA, and the concentration factor of 5 was taken into account. The mean protein concentration was calculated and normalized to the equivalent control condition.

2.12 Acetate assay

The FluoroSELECT™ Acetate Assay Kit (Sigma-Aldrich, Saint Louis USA) was used to measure concentrations of acetate, according to manufacturer's instructions with slight modifications. In brief, more standard concentrations were added (0mM; 0.25mM; 0.5mM; 0.75mM; 1mM) instead of only the 1mM standard and blanc. The samples were diluted with MiliQ (1:20) to make them fall below the upper detection limit. The fluorescence was measured in a black 96 well microplate (Greiner Bio-One, Kermsmünster, Austria) with a Fluostar Omega plate reader from BMG Labtech (Ortenberg, Germany) with an excitation wavelength of 495 nm and an emission wavelength of 590 nm.

2.13 Chronoamperometry

A self-made Arduino system allowed to do some chronoamperometric measurements. The system consisted of an Arduino® Uno, including a self-made amplification system and voltage source and was connected to the anode and cathode of the microbial electrochemical remediation cell. The obtained data was saved to a micro-SD card and analysed after the experiment.

2.14 Cyclic voltammetry

Some cyclic voltammetry measurements were performed with a VersaSTAT 3F from Ametek (Devon-Berwyn, USA). The applied voltage was set using VersaStudio software and went from -500 mV to -1 V, back to 1 V en eventually back to -500 mV for two cycles with a scan rate of 0.1 V/s. A line was fitted through the voltammogram and the slope was used the determine the resistance of the cell.

2.15 Scanning electron microscopy (SEM)

Activated carbon samples were fixed in 2.5% glutaraldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer (Appendix 4) overnight at 4°C. After overnight incubation, the samples were washed three times for ten minutes in phosphate buffer. Next, the samples were gradually dehydrated in ethanol (one time ten minutes of 50%, 70%, 80%, 90% and 95% ethanol in water and three times ten minutes of 100% ethanol). The final step consisted of immersing the samples twice for 30 seconds in hexamethyldisilazane (Sigma-Aldrich) and drying them. The samples were coated with a gold layer by a Jeol JFC-1300 sputter coater (Jeol Europe, Zaventem, Belgium) and analysed with a JCM6000 plus SEM (Jeol Europe) with the following specifications: acceleration voltage of 7.57 nA, pulse height analyser mode T3 (high resolution), counting rate of 11502 cps and high vacuum.

2.16 Statistics

Results were evaluated in a statistical package program (SPSS Inc, Chicago, USA). Normality was assessed with the Shapiro-Wild test. If data was normally distributed and homoscedastic, a one-sample t-test and a one-way ANOVA were used. If the data was not normally distributed, a log transformation was performed, and normality was checked again. If this was still not the case, a Kruskal-Wallis test was performed. Significance was determined on a level of $\alpha = 0.05$, unless mentioned otherwise.

3 Results

3.1 Bulrush char characteristics

One biochar was made from bulrush (*Typha latifolia*) using a closed reactor pyrolysis procedure at 500°C, and 1,55 kg input material was converted into 40 gram of biochar material, after washing and drying. The other biochars used in this study (wood mix and coffee) were characterised previously (F. Mare; Table 1). The ACs were characterised by Chemviron (Appendix 5).

The pH of the Typha char was 10.6 (± 0.02) compared to 7.0 for MiliQ. The mean wet conductivity of the biochar is 30.9 (± 0.15) mS/cm compared to 11.1 mS/cm for MiliQ. Dry conductivity was additionally measured and resulted in a conductivity of 79.4 mS/cm when dry and 2 294.9 mS/cm when saturated with water.

Table 1: pH, wet conductivity and sorption of used biochars.

	Typha	Coffee beans	Wood mix
pH-H_2O (± SE)	10.6 (± 0.01)	10.0 (± 0.02)	8.0 (± 0.1)
Wet conductivity (mS/cm (± SE)	30.9 (± 0.1)	3.1 (±0.08)	0.2 (± 0.02)
Sorption (mg/g ± SE)	0	37.1 (± 0.7)	29.8 (± 1.0)

Mean values ± standard error (SE). Wet conductivity is measured in milli-Siemens per centimetre (mS/cm), sorption in milligram per gram biochar (mg/g). For the sorption test methylene blue was used for the coffee and wood char, for the Typha char ibuprofen was used, method remained the same.

For the two-point sorption test, 40 ppm of ibuprofen in a mineral salt solution was added to a 15 mg or 120 mg of biochar sample. After 24h of incubation, the mean ibuprofen concentration in the water of the 15 mg samples was 39.2 (± 0.2) ppm and 41.3 (± 0.4) ppm for 230 nm and 210 nm respectively. For the 120 mg samples the remaining ibuprofen concentration was 43.0 (± 0.1) ppm and 51.0 (± 0.6) ppm for 230 and 210 nm. The 40-ppm control without biochar yielded a concentration of 38.1 ppm (230 nm) and 39.3 ppm (210 nm). This indicates that the Typha char shows poor sorption capacity for the polar sodium ibuprofen compound.

3.2 Anaerobic degradation of Ibuprofen and Diclofenac

3.2.1 Without biochar or activated carbon

Without external electron shuttles as biochar or activated carbon, no significant degradation of the pharmaceuticals was observed by either mixed culture or *Geobacter sulfurreducens* (Appendix 6.1; Fig. 9A, Table 2). Though growth analysis shows that the cells are not inhibited by 10 ppm of either diclofenac or ibuprofen in the medium (Appendix 6.1; Fig. 9B), and most growth, as expected, was observed for the FWM_AF conditions, containing the donor/acceptor pair.

In detail, for Diclofenac, mixed culture showed more growth than *Geobacter* for all tested media conditions (Kruskal-Wallis, $p < 0,05$). For example, mixed culture reached a protein concentration of 82.2 (± 12.6) mg/L in FWM_FA, 70.2 (± 5.3) mg/L in FWM_F and 21.8 (± 0.2) mg/L in FWM_A, while *Geobacter* reached concentrations of about 54.7 (± 8.0) mg/L, 30.2 (± 6.3) mg/L and 69.1 (± 3.8) mg/L for FWM_AF, FWM_F and FWM_A respectively (Appendix 6.1, Table 3).

For Ibuprofen, the concentration of mixed culture and pure culture are more alike: 75.6 (±4.5) mg/L and 71.8 (±14.9) mg/L for *Geobacter* and mixed culture in FWM_AF, 60.2 (±20.9) mg/L and 68.0 (±1.9) mg/L for *Geobacter* and mixed culture in FWM_F, and 23.1 (± 2.7) mg/L and 21.1 µg/ml (±3.7) for *Geobacter* and mixed culture in

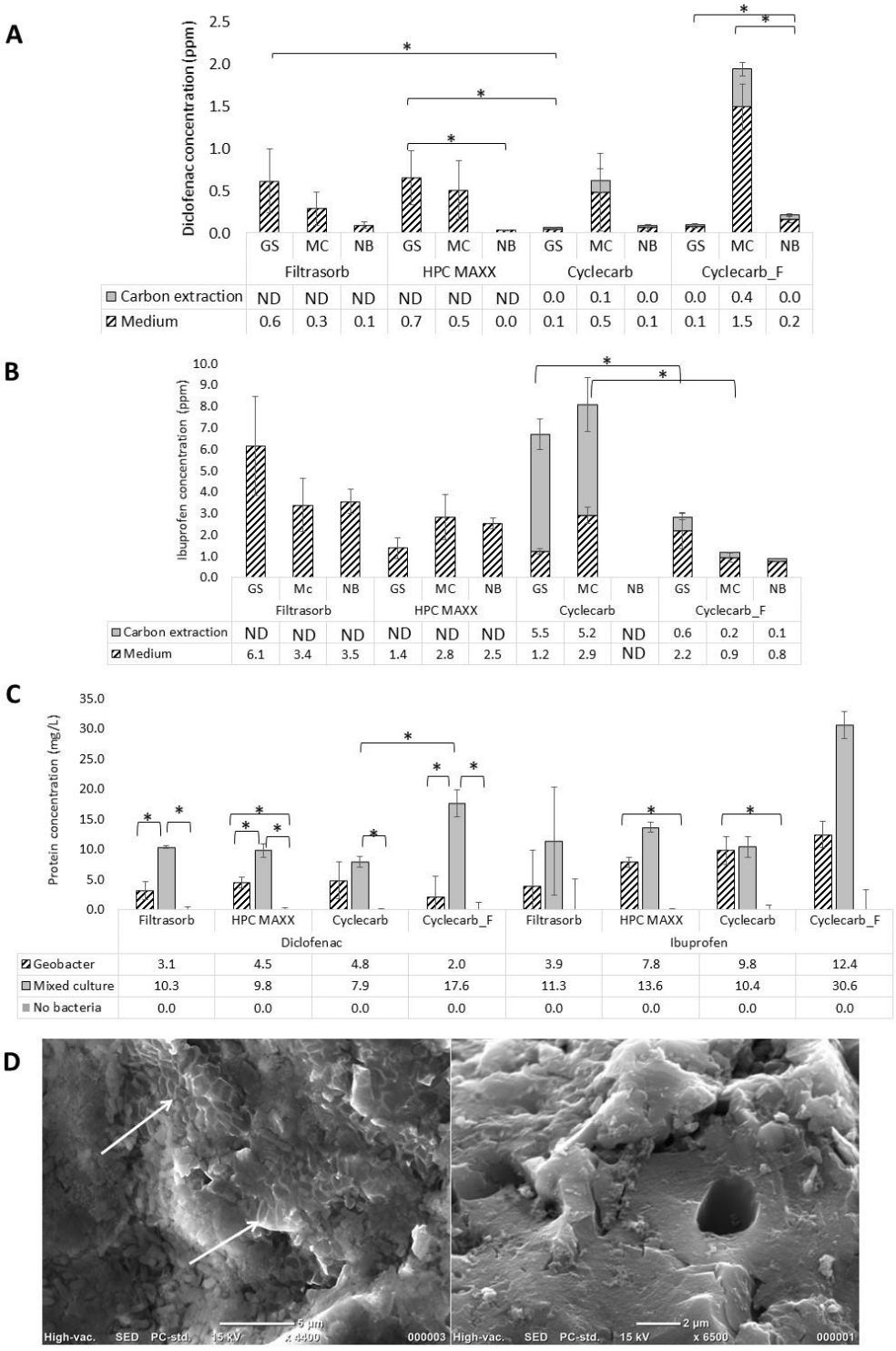


Figure 4: Results of the anaerobic digestion of pharmaceuticals in the presence of activated carbon. **(A)** Concentration of diclofenac (in ppm) after a 14-day incubation of bacteria (mixed culture of *Geobacter sulfurreducens*) to 10 ppm of diclofenac and different activated carbons. Compared to abiotic control. Grey bars represent concentration extracted from carbon, striped bars indicate concentration in medium Y-axis represents concentration in ppm. Error bars represent standard error. Significant differences indicated with * (significant if $P < 0.05$) **(B)** Concentration of Ibuprofen (in ppm) after a 14-day incubation of bacteria (mixed culture of *Geobacter sulfurreducens*) to 10 ppm of ibuprofen and different activated carbons. Compared to abiotic control. Grey bars represent concentration extracted from carbon, striped bars indicate concentration in medium. Y-axis represents concentration in ppm. Error bars represent standard error. Significant differences indicated with * (significant if $P < 0.05$) **(C)** Protein concentration (in mg/L) of bacteria exposed to diclofenac or ibuprofen. Bacteria (*Geobacter sulfurreducens* or mixed culture) were exposed to 10 ppm of diclofenac (left) or ibuprofen (right) and cultured with different activated carbons. Protein concentration in mg/L on Y-axis. All compared and normalized to abiotic control. Error bars represent standard error. **(D)** Scanning electron microscopy image of a Filtrasorb 400 activated carbon particles. Left panel: particle exposed to a mixed culture and

diclofenac. Magnification factor: x4400, scale in image (5 µm). Right panel: particle exposed to diclofenac but incubated without bacteria. Magnification factor: x6500, scale in image (2 µm). F: fumarate, GS: *Geobacter sulfurreducens*, MC: mixed culture, mg/L: millligrams per litre, NB: no bacteria, ND: no data, Ppm: parts per million.

FWM_A respectively. For ibuprofen, all growth was significant compared to control, no significant difference was observed when comparing *Geobacter* with mixed culture (Appendix 6.1; Table 3).

3.2.2 With activated carbon

No clear breakdown was observed with the addition of activated carbon as electron shuttle. Concentrations are lower in comparison to the samples from the experiment without activated carbon. This is due to the sorption effect of activated carbons. The addition of Filtrasorb to the breakdown of diclofenac for instance, resulted in concentrations of 0.6 (±0.4) ppm, 0.3 (±0.2) ppm and 0.1 (±0.04) ppm for *Geobacter*, mixed culture and no bacteria respectively (Fig. 4A). Extraction of pharmaceuticals from the carbon granules resulted in highly variable data. Consequently, no appropriate conclusions could be drawn. By comparing the results of the cyclecarb (in FWM_A) with ibuprofen, it is observed that for by mixed culture its total remaining concentration (medium + extracted from granules) is 8.2 (± 1.7) ppm (Fig. 4B). The *Geobacter* samples on the other hand only have a total of 6.7 (± 0.8) ppm left. But no significance could be obtained due to the loss of data from the control condition (Appendix 6; Table 4).

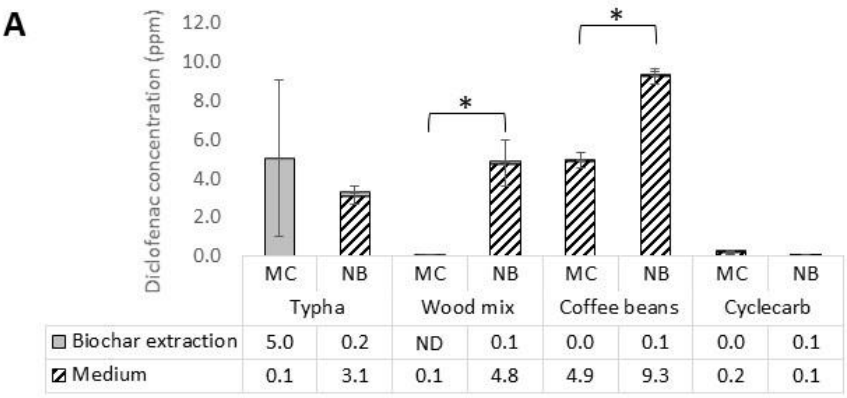
Another strong effect of the carbon is the influence on bacterial growth. Protein concentrations are much lower than without activated carbon. In detail, mixed culture samples grew better for all diclofenac samples: 10.3 (±0.2) mg/L, 9.8 (±1.1) mg/L, 7.9 (±0.9) mg/l and 17.6 (±2.2) mg/L compared to 3.1 (±1.5) mg/L, 4.5 (±0.9) mg/L, 4.8 (±3.1) mg/L and 2.0 (±3.5) mg/L for *Geobacter* samples. The same is observed for ibuprofen but less distinct (Fig. 4C). However, almost all conditions with ibuprofen had a significant difference from their analogues without conductive material (Appendix 6.2; Table 5)

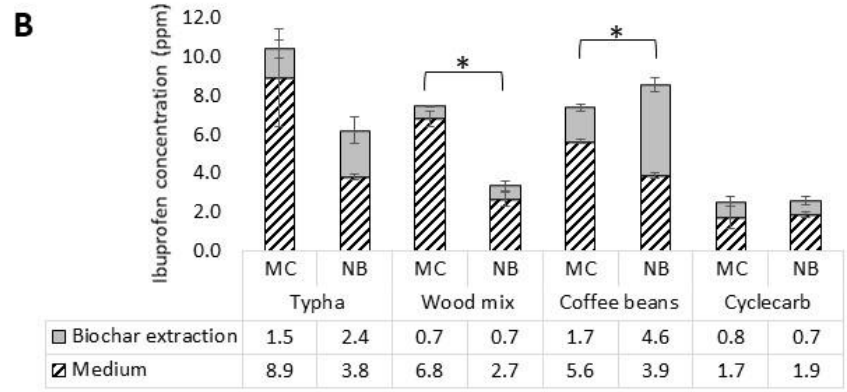
A low protein concentration in the medium may be explained by the microbial colonization of the carbon granules; this was evaluated through SEM. Two samples were investigated: a Filtrasorb carbon granule with mixed culture and diclofenac and its abiotic control (Fig. 4D, Appendix 6.2; Fig. 10). In the biotic samples there are clear imprints of the bacteria on the activated carbon granules, which aren't visible in the abiotic control.

3.2.3 With biochar

Looking at the remaining concentration Diclofenac in the medium and extracted from the biochar, sorption is also an important factor for the biochars (Fig. 5A). Though the effect is not as pronounced as with the cyclecarb activated carbon where there is almost no diclofenac left (0.2 (± 0.2) ppm and 0.1 (± 0.006) ppm for mixed culture and no bacteria) and even after sonication the concentration of diclofenac has not risen much (0.3 (± 0.1) ppm for mixed culture and 0.1 (± 0.01) ppm for no bacteria). The biochar from coffee beans does seem to show some promising results: while the total remaining diclofenac concentration with no bacteria is 9.4 (± 0.5) ppm (very close to the spiked 10 ppm), the mixed culture seems only to have a remaining concentration of 4.9 (± 1.2) ppm (Appendix 6.3; Table 6). For ibuprofen, no clear results could be drawn for the remaining concentration in vials with bacteria is higher than the concentration in the vial without bacteria (Fig. 5B).

The results for the coffee bean char can be confirmed by the Bradford results (Fig. 5C) where it is observed that the bacteria exposed to coffee bean char have the highest protein concentration (72.9 (± 9.9) ppm) (Appendix 6.3; Table 7). Due to the irregular results of the other two biochars (Typha and Wood mix), the results of the Coffee bean char should be handled with some scepticism.





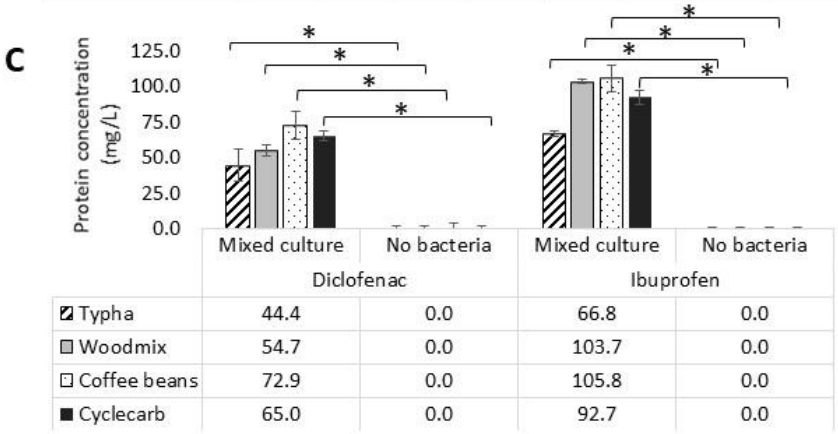


Figure 5: Results of the anaerobic digestion of pharmaceuticals in the presence of biochar. (A) Concentration of diclofenac (in ppm) after a 14-day incubation of bacteria (mixed culture of *Geobacter sulfurreducens*) to 10 ppm of diclofenac and different biochars. Compared to abiotic control. Grey bars represent concentration extracted from biochar, striped bars indicate concentration in medium. Y-axis represents concentration in ppm. Error bars represent standard error. Significant differences indicated with * (significant if $P < 0.05$) **(B)** Concentration of Ibuprofen (in ppm) after a 14-day incubation of bacteria (mixed culture of *Geobacter sulfurreducens*) to 10 ppm of diclofenac and different biochars. Compared to abiotic control. Grey bars represent concentration extracted from biochar, striped bars indicate concentration in medium. Y-axis represents concentration in ppm. Error bars represent standard error. Significant differences indicated with * (significant if $P < 0.05$) **(C)** Protein concentration (in mg/L) of bacteria exposed to diclofenac or ibuprofen. Bacteria (*Geobacter sulfurreducens* or mixed culture) were exposed to 10 ppm of diclofenac (left) or ibuprofen (right) and cultured with different biochars. Protein concentration in mg/L on Y-axis. All compared and normalized to abiotic control. Error bars represent standard error.MC: mixed culture, mg/L: millligrams per litre, NB: no bacteria, ND: no data, Ppm: parts per million.

3.2.4 With one biochar, one activated carbon or no material

The HPLC results for the vials containing diclofenac clearly recapitulate what was observed in the previous experiments (Fig 6A). Without any conductive material, there is no breakdown (9.6 (± 0.1) ppm for *Geobacter*, 10.9 (± 0.2) ppm for mixed culture and 10.2 (± 0.5) ppm for control). The addition of activated carbon causes a significant decrease in contaminant concentration due to sorption (0.2 (± 0.02) ppm for *Geobacter*, 2.3 (± 0.1) ppm for mixed culture and 0.5 (± 0.2) ppm for no bacteria), while the Typha biochar has a similar but smaller sorption effect (8.2 (± 0.2) ppm for *Geobacter*, 7.6 (± 0.3) ppm for Mixed culture and 8.4 (± 0.1) ppm for no bacteria) (Appendix 6.4; Table 8). Yet, no clear breakdown is found in this experiment. Ibuprofen shows very irregular results: the sorption effect of the activated carbon is less prone, but more visible with the biochar. Out of these results no precise conclusions could be drawn.

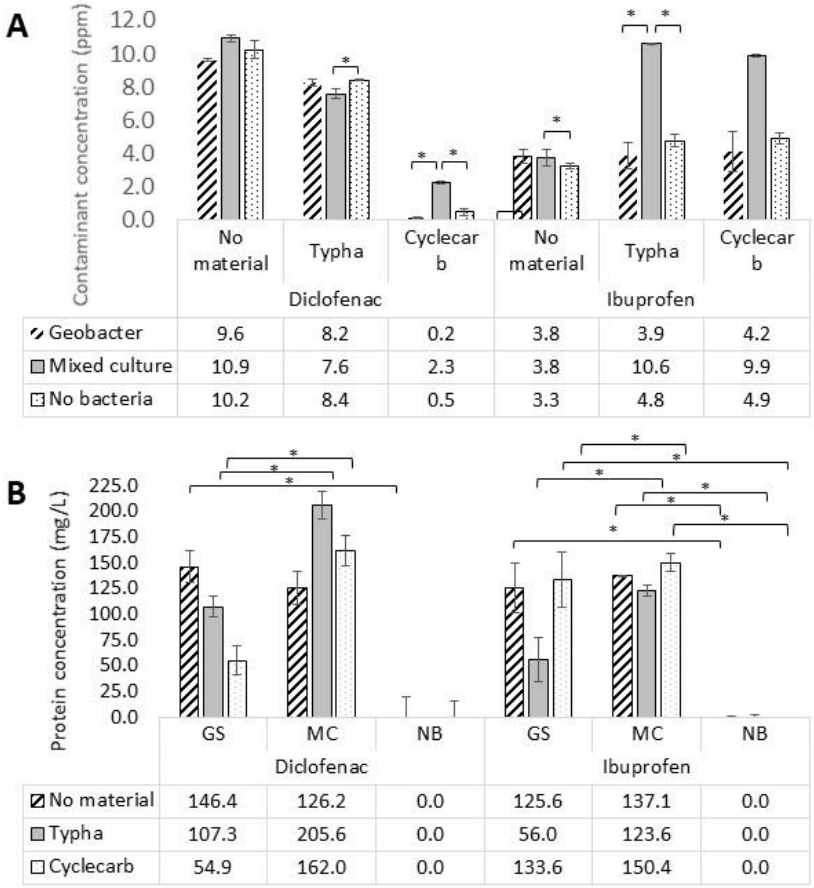


Figure 6: Results of the anaerobic digestion of pharmaceuticals in the presence of one biochar, one activated carbon or no conductive material (A) Concentration of Diclofenac (left) or Ibuprofen (right) in ppm after a 14-day incubation of bacteria (mixed culture of *Geobacter sulfurreducens*) to 10 ppm of pharmaceutical and Typha biochar, Cyclecarb activated carbon or no material. Compared to abiotic control. Y-axis represents concentration in ppm. Error bars represent standard error. **(B)** Protein concentration (in mg/L) of bacteria exposed to diclofenac or ibuprofen. Bacteria (*Geobacter sulfurreducens* or mixed culture) were exposed to 10 ppm of diclofenac (left) or ibuprofen (right) and cultured with either Typha char, cyclecarb carbon or no material. Protein concentration in mg/L on Y-axis. All compared and normalized to abiotic control. Error bars represent standard error. Significant differences indicated with * (significant if $P < 0.05$) GS: *Geobacter sulfurreducens,* MC: mixed culture, mg/L: millligrams per litre, NB: no bacteria, Ppm: parts per million.

The Bradford assays (Fig. 6B) shows that *Geobacter sulfurreducens* in the presence of diclofenac grows best without conductive material (146.4 (± 16) mg/L), although this might be explained by the colonization of the carbon and biochars. Colonization would cause the bacteria to grow on the carbons and biochars instead of freely in the medium. Mixed culture on the other hand seems to grow better on the Typha biochar (205,6 (± 13.5) mg/L). In the presence of ibuprofen, *Geobacter* and mixed culture has similar growth in the presence of activated carbon (133.6 (± 26.5) mg/L for *Geobacter*, 150,4 (± 9.2) mg/L for mixed culture) but also without conductive material (125.6 (± 23.7) mg/L for *Geobacter*, 137.1 (± 0.8) mg/L for mixed culture). In comparison to the previous experiments, the protein concentrations are slightly higher, meaning that the extra spike of Iron(III)citrate is useful in the growth of these bacteria (Appendix 6.4; Table 9).

3.2.5 Gas chromatographic analysis with mass spectroscopy

The GC-MS results show a peak at 11 minutes with a M/z of 1189 that is identified as Ibuprofen (Fig. 7). The relative abundance of Ibuprofen differs between the three samples. A limited relative abundance is observed in the sample with activated carbon (1.3 ppm originally measured with HPLC), while this is more for the sample without material (HPLC: 7.0 ppm) and the highest for the sample with Typha char (HPLC: 12.7 ppm) Due to time constraints no further analysis was performed on the results.

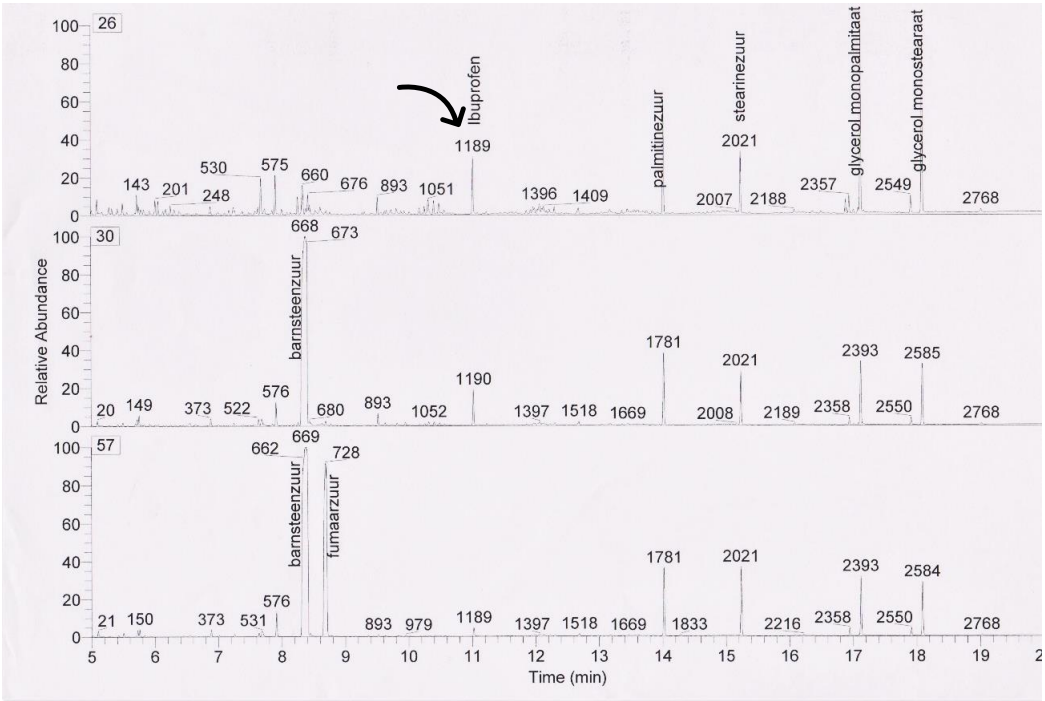


Figure 7: Chromatograms resulting from GC-MS analysis of ibuprofen in presence of Typha char (upper part), no material (middle part) or cyclecarb activated carbon (bottom part). X-axis represent time in minutes, Y-axis the relative abundance, values on peak are M/z values. Arrow indicates ibuprofen peak.

3.3 Anaerobic degradation of acetate in a biochar included microbial electrochemical remediation cell

The concentration of acetate is lower in all conditions when comparing day 1 to day 14 (Fig. 8A). Though only the 25% cells with inoculation has a near significant lower acetate concentration on day 14 (9.4 (± 2.2) mM) compared to day 1 (19.2 (± 1.2) mM) (significant $\alpha = 0.1$, Appendix 7.1; Table 10). The concentration did however not change significantly from the "abiotic" control at day 14. In theory every sample should have an acetate concentration of 20 mM at day 1, this is not met, meaning the results might be incorrect.

Due to the non-sterile environment of the BiMER, keeping the uninoculated cells abiotic was unfeasible. This is also observed in the Bradford results (Appendix 7.2, Fig. 11, Table 11). No significant differences are observed between the biotic and "abiotic" cells. What can be observed from this assay is that the growth in the sand controls is higher than any condition with biochar, which is consistent with the other results concerning the colonization capacity of biochar. In general, the concentrations are slightly on the low side. This might be due to the not-anoxic environment, as well as the colonization of the biochar.

Additionally, thermogravimetric analysis was performed on four cells: 25% biotic and abiotic and 50% biotic and abiotic. The samples were dried, and sand was separated from the biochar by sieving before thermogravimetric analysis for biofilm investigation. The weight loss for the sand samples was negligible with a mean weight loss of 0.2 (± 0.16) percent (Appendix 7.3; Fig. 12). Out of the thermogravimetric results, only one sample showed a clear moment of biomass loss at 450°C, this was biochar from a cell with a biochar mixing ratio of 50% with a mixed culture inoculation (Fig. 8B). The 50% biotic sample showed a relative weight loss of 21.2%. The highest relative weight loss was 27.1% but no peak in the derivative weight loss was observed that could be linked to biomass loss. This might be explained by a water that wasn't initially removed in the drying process.

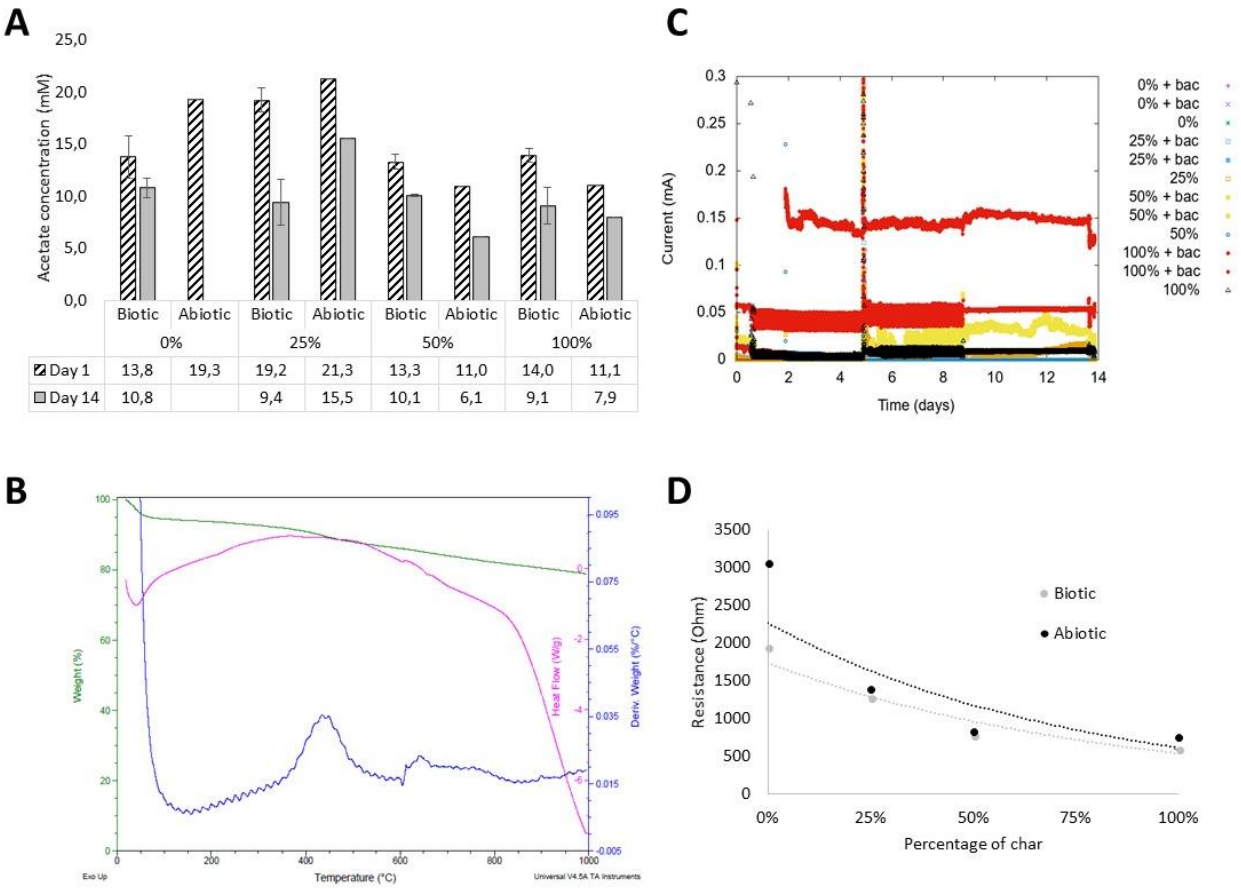


Figure 8: Analysis of the degradation of acetate in a biochar included microbial electrochemical remediation cell. (A) Acetate concentration (mM) compared between conditions and in time. Bacteria were cultured in a microbial electrochemical remediation cell on fresh water medium with 20 mM acetate with different concentrations of biochar (0%,25%, 50%, 100%). Acetate concentrations were measured at day 1 and day 14, both for biotic samples and abiotic control. Error bars represent standard error. **(B)** thermogravimetric analysis of 50% biochar biotic samples. X-axis represents Temperature (°C), Y-axis represents relative weight loss (%, green), the derivative of the weight (%/°C, blue) and the heat flow (W/g, pink). **(C)** Chronoamperic measurements (mA) in time (days) during a 14-day incubation of bacteria to fresh water medium 20 mM acetate with different percentages of biochar. **(D)** Relationship between resistance (Ohm) and percentage of biochar for biotic (grey) and abiotic (black) BiMERs. Bac: bacteria, mA: milliampère, mM: millimolar.

3.3.1 Cyclic voltammetry & Chronoamperometry

The electrochemical analysis of the BiMERs show some promising results. The chronoamperometric analysis (Fig. 8C) indicates a higher current running through the inoculated cells with 100% biochar than all other results. The abiotic control of the former cells shows little current production as well. This data suggests a possible correlation between produced current and biochar percentage.

These findings were confirmed by the results of the cyclic voltammetry tests: for every cell the resistance was calculated out of the cyclic voltammogram, plotted out, this shows a possible inverse correlation between resistance and biochar percentage (Fig. 8D, Appendix 7.4; Fig. 13). Statistical significance was only found when comparing the biotic 25% cells (1273.07 Ohm) to the 100% cells (590.86 (± 0.0002) Ohm) (Appendix 7.4; Table 12).

4 Discussion

4.1 Typha biochar

Biochar can be made of multiple biomass input sources. The idea of biochar made of *Typha latifolia* came from the plant's most frequent use: constructed wetlands. If biochar included microbial electrochemical remediation would be upscaled and implemented into a constructed wetland, the plants mostly found on the wetland could be recycled into biochar. The characteristics of the Typha biochar ascertained its use in foregoing experiments. The high conductivity, especially in comparison with the other biochars was desirable. While its low sorption capacity for ibuprofen was helpful in a way that it lowered the possible interfering factors during anaerobic digestion. The plant did however have a very low yield (2.58%) meaning that a lot of plant material would be needed to be able to carry out bigger experiments and that alternative sources with a higher yield might be interesting as well.

4.2 Anaerobic digestion of pharmaceuticals

The degradation of Diclofenac by electroactive bacteria, in the absence of any conductive material or substrate, is minimal. And although the bacteria grew well (best on medium with both an extra electron acceptor and donor), no breakdown was observed regardless of the medium. When comparing to other studies that used anaerobic digestion for the breakdown of diclofenac, the results are variable. One study by Ternes et al. (6) stated that the biodegradation of diclofenac is rather low. Another study by Lahti et al. (48) investigated the breakdown in a similar set-up and indicated that in the long term (161 days) diclofenac is broken down with 26% in nonsterile conditions, but that in a shorter time frame little to no diclofenac was broken down. Carballa et al (49) showed that diclofenac can indeed be removed from wastewater by anaerobic digestion, though it also mentions that the used sludge needed some adaptation before diclofenac was effectively broken down. The study from Lahti et al. also mentions that the biotransformation of diclofenac is highly dependent on environmental factors, experimental design and the source of inoculum (48). These are considerations that should be kept in mind. More parameters should be controlled and tested. The degradation of ibuprofen on the other hand gave some highly variable results. Interpretation of these may be difficult. Although research has shown that de degradation of ibuprofen by anaerobic digestion is possible, it is considered to be moderate (49). To answer the question 'Are the pharmaceuticals effectively removed by anaerobic digestion in the absence of electron shuttles?': there are no clear indication that diclofenac or ibuprofen is broken down in the used time frame.

What effect has the addition of a conductive substrate on electron exchange, pharmaceutical breakdown and bacterial growth? The main effect that is observed in former experiments is the sorption by the activated carbons. As high amounts of diclofenac are adsorbed, the adsorption may lead to a competition between the carbon and the bacteria for the pharmaceutical compounds. At the same time is there is also the competition between the contaminant and other organic compounds available in the solution (6) for both the activated carbon and the bacteria.

The sonication of the carbon granules to extract the sorbed pharmaceuticals yielded low and variable results, meaning that there either is a strong bond between the pharmaceuticals and the carbon or that the pharmaceuticals are broken down. Since the low concentrations are also the case for the control conditions, the former explanation seems more reasonable. Ternes et al. (6) indicated a removal of 59-75% diclofenac from wastewater that was also mostly due to sorption while indicating that the biodegradation rate was rather low, confirming what was seen in these experiments. The concentration of ibuprofen found in the medium is higher compared to diclofenac, but still lower than the spiked 10 ppm. However, this is also the case in the control conditions. In comparison to diclofenac, a higher concentration is extracted from the cyclecarb activated carbon in FWM_A medium. The concentrations found in the condition with *Geobacter* and with mixed culture could

indicate breakdown of ibuprofen, though no clear conclusion can be drawn with data missing from the control condition. A study by Mugisidi et al. (50) investigated the effect of acetate on the sorption capacity of activated carbon and found out that the sorption capacity was higher when the activated carbon was exposed to acetate. Three milligrams Cu(II) per gram of activated carbon was absorbed by a modified activated carbon (15% sodium acetate), while the unmodified activated carbon only absorbed 1.4 mg/g. Hence, the experiments with activated carbon were performed in fresh water medium with 20 mM of acetate, the sorption might as well be improved by it.

If these results are compared to literature, many things are found. For instance, in 2010 Musson et al published a study (51) where they tested the biodegradation of ibuprofen by activated sludge. They concluded that ibuprofen was not removed from their biotic experiments after 112 days. Indicating that Ibuprofen does not show rapid biodegradation. The explanation they give concerns the chemical structure of ibuprofen: ibuprofen has an aromatic ring with multiple substitutions and the main mechanisms for biodegradation is cleavage of that aromatic ring. Some substitutions on the ring enhance degradation (*e.g*. -COOH or -OH), while other decrease the degradation rate (*e.g*. halogens). The presence of substitutions is reducing the hydroxylation potential due to the spatial interference with degrading enzymes. In the case of ibuprofen: a highly branched structure with substitutions in the *para*-position of the aromatic ring may cause its resistance to biodegradation. The same study also indicated that because of the resistance to biodegradation and the lack of adsorption they assume ibuprofen is removed in WWTP by other mechanisms such as chemical degradation. In 2001 Rivera-Utrilla et al. investigated the effect of bacteria in the presence of activated carbon. Their results showed that colonisation of carbon by bacteria decreased the porosity, but also that the adsorption capacity was increased. Although this was merely the case for metal absorption (52). Bautista-Toledo 2008 agreed to some extend by showing that bacteria on activated carbon can accelerate adsorption of different compounds, mainly due to the higher surface hydrophobicity (53).

The bacterial growth in the presence of activated carbon is also lower than in the absence of any conductive material. As mentioned previously this might be explained by colonization of the granules which isn't considered when performing the Bradford assay. A conformation for this presumption were the observations that were performed with SEM. The colonization was clearly visible on the carbon granule in comparison to the abiotic control. The bacterial growth in the presence of biochar is then again more similar to the growth without any conductive material. The possible explanations for this are either they colonize the biochar granules less or the presence of biochar is beneficial for bacterial growth. This latter presumption is confirmed by a study by Yu et al. (45) that states that the presence of biochar should in fact improve growth..

The concentrations for the biochar experiment are highly variable for both diclofenac and ibuprofen. In comparison to the activated carbon that was included in the experiment, the biochars have a less prone sorption effect resulting in higher concentration in the medium. For diclofenac the concentrations did seem to be lower in the mixed culture conditions than the control conditions. However, the control conditions of two out of three biochars seem to have a lower concentration that the initial 10 ppm spike even after sonication. Meaning again that either the biochars have a high sorption capacity or that there is a deficiency in our measuring technique. The results for ibuprofen displayed highly unusual results where the concentration in the mixed culture conditions was higher in comparison to the control. Only the coffee bean char insinuated breakdown when looking at the total concentration. The coffee bean char does seem to be the superior biochar in this experiment since it showed promising results for both diclofenac and ibuprofen. This finding is a bit contradictory since the coffee bean char had a rather low conductivity in comparison to the Typha char, a welcome characteristic.

Although little research has been performed for biochar in said set-up, research did show that biochar is very similar to activated carbon in the sense that it has a large specific surface area, is very porous, has enriched surface functional groups that make it possible to use it as an adsorbent. The higher the pyrolysis temperature, the higher the surface area. The main mechanisms for adsorption onto biochar are electrostatic interaction, hydrophobic effects, hydrogen bonds and pore-filling (54).

Geobacter sulfurreducens growing in the presence of Iron(iii)citrate results in a higher abundance of electron transport proteins including some cytochromes (55). In other words, the additional spike should improve the anaerobic digestion. However, the effect is mostly seen in the Bradford assay where the protein concentrations rose considerably. Focussing more on the anaerobic digestion of diclofenac with the additional spike: the results confirmed what was observed in the previous experiments. When no conductive material is added no breakdown is observed. When biochar is accessible there is some sorption effect in all conditions, and when activated carbon is added the sorption effect is so substantial that breakdown is unlikely to be seen. The digestion of ibuprofen however, showed less promising results. The mixed culture samples show highly variable results and concentrations are rather low, both for the *Geobacter* as the control.

In general, there is still substantial research that needs to be performed. No breakdown was seen without conductive material in this time frame and with the addition of activated carbon or biochar the results were unclear. The coffee bean char showed the most promising results of all three biochars. Both for ibuprofen and diclofenac. Although the Typha is more conductive than the coffee bean char. These results should be handles with care and the experiment with coffee bean char should be repeated to confirm these results. One of the most promising remarks is that the bacteria need time to adapt to the pharmaceuticals and that longer incubation times are needed. Due to the irregular results and the technical difficulties, it might be interesting to investigate other parameters in the set-up such as the consumption of fumarate or the production of methane if methanogens are present in the mixed culture. The formation of breakdown products is another way of investigating the anaerobic digestion of the pharmaceuticals. This was to some extent investigated with GCMS. No in-depth analyses were performed but these could provide some useful insights. The sorption on the materials should be investigated more deeply, in a more controlled condition. Individual sorption tests of all elements present in the set-up (fumarate, acetate, pharmaceutical etc…) should be performed. Changing the origin of the mixed culture might also be valuable step. For instance, one could use a culture that is present in a WWTP. Though this has some limitation when implementing the technique on a bigger scale such as a METland. Essentially, the most important remark to implement in future research is biomass adaptation.

4.3 Anaerobic degradation of acetate in a biochar included microbial electrochemical remediation cell

What the electrochemical experiments in the BiMER set-ups indicated was that the Typha biochar indeed is a good conductor for electrons. This is in correspondence with the dry and wet conductivity measurements performed for said biochar. The hosting capacity should however be investigated further, hence the protein concentrations in the medium were rather low and the thermogravimetric analysis yielded little results. One explanation for this might be implementation of the thermogravimetric protocol where some of the biofilm might be destroyed. The acetate consumption implied that the consumption does lower after 14 days, though not significantly.

Thus, it is recommended to perform a repetition of the experiment to achieve more uniformity in results. The electrochemical measurements indicated that higher biochar percentages are superior to lower. A study by Arends et al. investigated different mixing ratios for a similar set-up with activated carbon and found an optimal mixing ratio of 67% (36). It would therefore be interesting to expand the experiment to higher percentages. Though some other considerations should also be taken into account such as cost of production and environmental consequences when high biochar percentages are applied in the field.

Further on should there be more control of the conditions, meaning that the abiotic cells should remain sterile. This will create a greater and more accurate contrast between inoculated and uninoculated cells. An additional reference electrode will increase the strength of the electrochemical measurements. Additionally, the acetate concentration would ideally be monitored more closely. The investigation of the biofilm formation should be

checked in more samples, while alternatives to investigate the colony growth should be searched for. If all former factors are clear, new hypotheses could be tested that are more aligning to the degradation of pharmaceuticals.

4.4 Conclusion

Some meaningful knowledge is gained, though there remain multiple question. More research is needed to investigate and explore the subject. Mainly, no substantial evidence of breakdown is detected without conductive material. In the presence of activated carbon or biochar results become questionable. The inclusion of an electrochemical system gives additional insights that can be beneficial if high mixing ratios are adopted. The main remark is that anaerobic digestion of pharmaceuticals is affected by multiple factors with culture adaptation time being the most important one.

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Supplementary materials and methods

Appendix 1: Fresh water medium protocol

Fresh Water (FWNN) Media
1 liter

Ingredient	1 L
Milli-Q H_2O	800 ml
$NaHCO_3$	2.5g
NH_4Cl	0.25g
$NaH_2PO_4 * H_2O$	0.6g
KCl	0.1g
Vitamin mix	10 ml
Mineral mix	10 ml
Complete volume with Milli-Q H_2O to	1000 ml

Use iron-free beaker, stir bar, and canulas

1. Put 800 ml milli-Q water into a beaker with a stir bar
2. Add all ingredients
    a. The $NaH_2PO_4 * H_2O$ concentration can be adjusted between 0.06g and 0.6g depending on the experiment.
3. Allow to mix and dissolve completely
4. Complete volume with milli-Q water in a graduated cylinder
5. Aliquot 10ml per pressure tube or 100ml per 156ml serum bottle
6. Bubble out media with 80:20 of N_2:CO_2
    a. 10ml in Pressure Tubes:
        - 6 minutes open with canulas in liquid
        - 6 minutes with stopper on and canulas in liquid
        - 3 minutes with stopper on and canulas in headspace above liquid
    b. 100mL in 156ml Serum Bottles:
        - 30 minutes open with canulas in liquid
        - 15 minutes with stopper on and canulas in headspace above liquid
    c. 25mL in 60ml Serum Bottles:
        - 15 minutes open with canulas in liquid
        - 15 with stopper on and canulas in liquid
        - 10 minutes with stopper on and canulas in headspace above liquid
    d. 800mL in 1L Pyrex Bottles:
        - at least 1 hour open with diffuser (not canula) in liquid
        - at least 30 minutes with stopper on and canulas in headspace above liquid
7. Crimp to close tightly
8. Autoclave on a fast/wrapped cycle for 30 minutes
9. Before inoculating cells add a reductant, an electron donor, and an electron acceptor.

Vitamin Solution

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

Use iron-free beaker and stir bar

Protect solution from light

1. Put 800 ml milli-Q water into a beaker with a stir bar
2. Add all ingredients
3. Allow to mix and dissolve completely
4. Complete volume with milli-Q water in a graduated cylinder
5. Store at 4°C in a dark bottle

Mineral Mix

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

Use iron-free beaker and stir bar

6. Put 800 ml milli-Q water into a beaker with a stir bar
7. Add all ingredients
8. Allow to mix and dissolve completely
9. Complete volume with milli-Q water in a graduated cylinder
10. Store at 4°C

Appendix 2: Acetate Assay kit protocol

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

76786 *FluoroSELECT*™ Acetate Assay Kit

Product description
Acetate (CH_3COO^-) is a common anion and fundamental to all forms of life. When bound to coenzyme A, it is central to the metabolism of carbohydrates and fats. Its acid form, acetic acid, is produced and excreted by acetic acid bacteria, such as *Acetobacter genus* and *Clostridium acetobutylicum*, which are found universally in foodstuffs, water and soil. Acetic acid is also a component of the vaginal lubrication of humans and other primates, where it appears to serve as a mild antibacterial agent. Acetic acid is the main component of vinegar and extensively used in food, dyes, paints, glue and synthetic fibres. Sigma-Aldrich's assay uses enzyme-coupled reactions to form a colored, fluorescent product. The fluorescence intensity at 530 nm/590 nm is directly proportional to the acetate concentration in the sample.

Detection ranges and limits
Linear detection range: 0.1–1.0 mM.

Equipment required but not included
Z805491-1EA *FluoroSELECT*™ Single channel fluorometer λ_{ex} 530 nm; λ_{em} 590 nm
Z805823-100EA Glass vials for *FluoroSELECT*™ fluorometer

Components
1. 25 mL Assay Buffer
2. Enzyme A (dried)
3. Enzyme B (dried)
4. 120 µL ATP
5. 120 µL Dye Reagent
6. 1 mL Developer
7. 1 mL Standard

The kit is sufficient for approximately 100 assays.

Storage conditions
Store at -20°C

Procedure
Important: Prior to assay, bring the assay reagents to room temperature. Add 650 µL developer to Enzyme A and 120 µL developer Enzyme B tubes. Mix well by pipetting and vortexing. Keep enzyme tubes cold during the assay.

1. Prepare 1 mM acetate standard by mixing of 5 µL provided standard with 995 µL H2O. In separate mini-glass tubes, add 10 µL H2O ("Blank"), 10 µL 1 mM standard ("Std"), and 10 µL sample.
2. Prepare enough working reagent by combining the following per tube: 90 µL assay buffer, 6 µL enzyme A, 1 µL Enzyme B, 1µL ATP, 1 µL Dye reagent. Add 90 µL working reagent to each tube.
Incubate for 30 min at room temperature in the dark.
3. Switch on the reader. To calibrate the reader, place the "Blank" tube into the sample holder. Press "Calibrate", "Assay 1", then "Blank". Reader starts measuring. Press left arrow on "<- Std -> ", until the window shows "1.000". Place the "Std" tube into the sample holder. Press "Measure". The reader shows "Calibrate Finished". The Reader is now calibrated. Press "Return".
4. Measure. Place the sample tube into the sample holder. Press "Measure", "Assay 1", "Measure". The acetate concentration (mM) will be displayed in the window. Note down the data and press "Return" to measure a next sample. Alternatively, press "Save" to save the data for later retrieval, press "Measure" for the next sample. *Note:If the concentration of the sample is higher than the upper limit, dilute sample in H_2O and repeat assay.*

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Appendix 3: Bradford assay protocol

Standard Protocol

1. The standard protocol can be performed in three different formats, 5 ml and a 1 ml cuvette assay, and a 250 µl microplate assay. The linear range of these assays for BSA is 125–1,000 µg/ml, whereas with gamma-globulin the linear range is 125–1,500 µg/ml.
2. Remove the 1x dye reagent from 4°C storage and let it warm to ambient temperature. Invert the 1x dye reagent a few times before use.
3. If 2 mg/ml BSA or 2 mg/ml gamma-globulin standard is used, refer to the tables in the appendix as a guide for diluting the protein standard. (The dilutions in the tables are enough for performing triplicate measurements of the standards.) For the diluent, use the same buffer as in the samples (refer to Troubleshooting section for more information). Protein solutions are normally assayed in duplicate or triplicate. For convenience, the BSA or gamma-globulin standard sets can be used, but blank samples (0 µg/ml) should be made using water and dye reagent.
4. Pipet each standard and unknown sample solution into separate clean test tubes or microplate wells (the 1 ml assay may be performed in disposable cuvettes). Add the 1x dye reagent to each tube (or cuvette) and vortex (or invert). For microplates, mix the samples using a microplate mixer. Alternatively, use a multichannel pipet to dispense the 1x dye reagent. Depress the plunger repeatedly to mix the sample and reagent in the wells. Replace with clean tips and add reagent to the next set of wells.

	Volume of standard and sample	Volume of 1x dye reagent
5 ml	100 µl	5 ml
1 ml	20 µl	1 ml
Microplate	5 µl	250 µl

5. Incubate at room temperature for at least 5 min. Samples should not be incubated longer than 1 hr at room temperature.
6. Set the spectrophotometer to 595 nm. Zero the instrument with the blank sample (not required for microplate readers). Measure the absorbance of the standards and unknown samples. Refer to Section 3 for data analysis.

Note: If the spectrophotometer has a reference and sample holder, the instrument can be zeroed with two blank samples. If the effect of buffer on absorbance is required, zero the instrument with a cuvette filled with water and dye reagent in the reference holder.

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	70	2 mg/ml stock	0	2,000
2	75	2 mg/ml stock	25	1,500
3	70	2 mg/ml stock	70	1,000
4	35	Tube 2	35	750
5	70	Tube 3	70	500
6	70	Tube 5	70	250
7	70	Tube 6	70	125
8 (blank)	–	–	70	0

Appendix 4: Phosphate buffer

Phosphate buffer protocol

1. Add 71.7 ml 1M K_2HPO_4 and 28.3 ml 1M KH_2PO_4
2. Adjust the pH to 7.2
3. Dilute the combined 1M stock solution to 1 litre with distilled H_2O

Supplementary results

Appendix 5: Information sheets of Chemviron activated carbons

Chemviron Carbon

FILTRASORB® 400

Agglomerated Coal Based Granular Activated Carbon

DESCRIPTION

FILTRASORB® 400 is from the renowned **FILTRASORB®** range of Granular Activated Carbons, which are installed in numerous water treatment plants in Europe, the United States and Asia. **FILTRASORB®** carbons are produced by steam activation of selected grades of bituminous coal that have first been pulverised then **agglomerated**.

FILTRASORB® 400 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. In addition, this product is best suited to the removal of total organics such as disinfection by-product precursors, the humic substances, which react with chlorine to form compounds such as trihalomethanes.

FEATURES

Agglomerated coal based granular activated carbons have several properties, which explain their superior performance in a wide range of applications:

- Produced from a pulverised blend, results in a **consistent high quality product**.
- The activated carbon granules are uniformly activated throughout the whole granule, not just the outside. This results in **excellent adsorption properties** and **constant adsorption kinetics** in a wide range of applications.
- High mechanical strength of the coal based carbon gives **excellent reactivation performances**.
- Agglomerated coal based carbon are suitable for **multiple reactivations** compared to other base materials such as peat and wood.
- The agglomerated structure ensures **rapid wetting**. There is no remaining floating material.
- Carbon bed segregation is retained after repeated backwashing, ensuring the **adsorption profile remains unchanged** with time and therefore maximising the bed life before breakthrough.
- **FILTRASORB® 400** complies with EN12915-1 and is approved by the United Kingdom Drinking Water Inspectorate.

SELECTION

FILTRASORB® 400 has a typical effective size of 0.7mm. In general, the smaller the granule size, the better the adsorption performance, therefore **FILTRASORB® 400** should be selected when it offers the optimum performance and pressure drop characteristics. If the pressure drop is too high with **FILTRASORB® 400**, **FILTRASORB® 300** should be selected.

PROPERTIES

SPECIFICATIONS	F400 12x40
Iodine Number, min., mg/g	1000
Abrasion Number, min.	75
Moisture Content, as packed, max., wt%	3
Mesh Size, US Sieve Series > 12 mesh (1.70 mm), max. % < 40 mesh (0.425 mm), max. %	 5 4

(Please refer to the Sales Specification Sheets, which state the Chemviron Carbon test method used to define the above specifications. Copies are available upon request.)

TYPICAL PROPERTIES	F400 12x40
Iodine Number, mg/g	1050
Surface Area, (N_2 BET method[1]), m^2/g	1050
Methylene Blue Number	300
Backwashed and drained Bed Density[2], kg/m^3	425
Floating Content, max., % w/w	0.1
Mean Particle Diameter, mm	1.0
Uniformity Coefficient	1.7
Phenol loading[3] at 1 mg/l, DIN 19603, %	5.2
Detergent (TPBS) loading[3] at 1 mg/l, mg/g	200
Atrazine loading[3] at 1 µg/l, mg/g	40
Toluene loading[3] at 1 mg/l, mg/g	100
Trichloroethylene loading [3] at 50 µg/l, mg/g	20

[1] *Brunauer, Emmett and Teller, J.Am. Chem. Soc. 60. 309 (1938).*
[2] *Backwashed and Drained Density for adsorber sizing;*
[3] *Isotherm loading in distilled water. These are reported for comparison and are unlikely to reflect loadings in practice.*

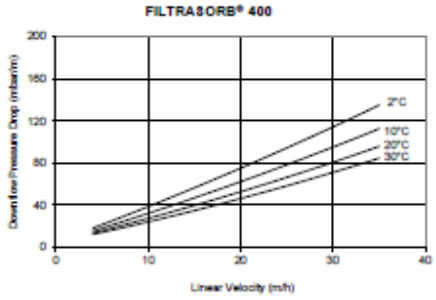
RECYCLING BY THERMAL REACTIVATION

Once granular carbon is saturated, or the treatment objective is reached, it can be recycled, by thermal reactivation, for reuse. Reactivation involves treating the spent carbon in a high temperature reactivation furnace to over 800°C. During this treatment process, the undesirable organics on the carbon are thermally destroyed. Recycling by thermal reactivation is a highly skilled process to ensure that spent carbon is returned to a reusable quality. **Chemviron Carbon** operates Europe's largest reactivation facilities and daily recycles large quantities of spent carbon for a diverse range of customers. Recycling activated carbon by thermal reactivation meets the environmental need to minimise waste, reducing CO_2 emissions and limiting the use of the world's resources.

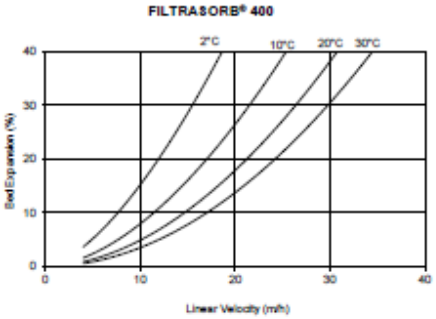
The combined high mechanical strength of **FILTRASORB® 400** with the transport pores gives the carbon **excellent reactivation performance** and **low losses**.

Making Water and Air Safer and Cleaner www.chemvironcarbon.com

TYPICAL PRESSURE DROP CURVE FOR A BACKWASHED AND SEGREGATED BED



TYPICAL BED EXPANSION CURVE FOR A BACKWASHED AND SEGREGATED BED



DESIGN INFORMATION

The following are typical design parameters for **FILTRASORB® 400** installed for the treatment of surface water:

- Superficial contact time 10-30 min.
- Bed depth 1-3 m
- Linear velocity 5-20 m/h
- Backwash bed expansion 20 %

PACKAGING

- 25 kg bags
- Big bags
- Bulk tanker

SAFETY MESSAGE

Wet activated carbon preferentially removes oxygen from air. In closed or partially closed containers and vessels, oxygen depletion may reach hazardous levels. If workers are to enter a vessel containing carbon, appropriate sampling and work procedures for potentially low-oxygen spaces should be followed.

QUALITY

Each of our worldwide operations has achieved **ISO9001:2008** certification for their quality management system related to activated carbon. **Chemviron Carbon** guarantees the specifications against representative sampling. For food grade applications, it is recommended to check the quality of the initial effluent before putting the adsorber into service.

CHEMVIRON CARBON

Pure Water. Clean Air. Better World.

Chemviron Carbon, the European operation of Calgon Carbon Corporation, is a global manufacturer, supplier and developer of activated carbons, innovative treatment systems, value added technologies and services for optimising production processes and safely purifying the environment.

With our experience developed since the early years of the twentieth century, facilities around the world and a world-class team of over 1,200 employees, Calgon Carbon Corporation can provide the solutions to your most difficult purification challenges.

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Chemviron Carbon

CYCLECARB® 201 AND 301

Reactivated Agglomerated Coal Based Granular Carbon

DESCRIPTION

CYCLECARB® is a granular reactivated carbon supplied on a service basis for wastewater treatment and other non-food industrial liquid phase applications. Exhausted carbon from different customers is recycled and pooled together to maximise the flexibility and logistics of operating granular activated carbon adsorption systems. Recycling by thermal reactivation involves processing the exhausted carbon in a special furnace at over 800°C dedicated to non-food grade applications.

FEATURES

CYCLECARB® Service has several advantages for a wide range of non-food grade applications:

- Large quantities are available quickly from inventory and can be supplied at the same time as the pick-up of exhausted carbon, **simplifying the logistics** and associated costs.
- Strict **quality control procedures** are followed on both exhausted and final product to ensure consistent reactivated carbon quality and high performance.
- Recycling **reduces CO_2 emissions** and waste, compared to the re-valorisation in cement kilns or disposal of spent carbon, in a cost effective way.
- **CYCLECARB®** service is in full **compliance with all** regional, national and European Union **regulations** for cross-border movements and recycling of exhausted carbon.
- **CYCLECARB®** can be provided as part of a **total service package** combining reactivation and mobile adsorbers on a rental basis, avoiding capital expenditure.

SELECTION

CYCLECARB® is used in a wide range of applications such as industrial and municipal wastewater treatment, landfill leachate treatment, ground water remediation and treatment of non-food grade liquids. They are used to remove:

- Chemical Oxygen Demand (COD)
- Total Organic Carbon (TOC)
- Aromatic compounds
- Halogenated organics: eg. AOX, chlorinated organics, flame retardants or fluorinated surfactants (PFOS)
- Pesticides and other micropollutants

CYCLECARB® 301 is used in applications with high organic load. Steam activated carbons are slightly alkaline which results in a *p*H rise for the first few bed volumes of water treated depending on the characteristics of the water. Where there are strict *p*H limits, **Chemviron Carbon** recommends **CYCLECARB® 201 *p*H**, a special grade of carbon specially treated to reduce this *p*H rise. In most cases the *p*H with this product is lower than 8 to 9. **CYCLECARB® 301 *p*H** is also available.

PROPERTIES

SPECIFICATIONS	CYCLE CARB® 201	CYCLE CARB® 201 *p*H	CYCLE CARB® 301
Iodine Number, min., mg/g	775	775	900
Molasses Number, min.	175	175	175
Moisture Content, max., % w/w	2	*	2
Modified Contact *p*H, max.	-	8.7	-
Mesh Size, US Sieve Series, max., % w/w	8x40	8x40	8x40
> 8 mesh (2.36 mm)	10	10	10
< 40 mesh (0.425 mm)	5	5	5

(Please refer to the Sales Specification Sheets, which state the Chemviron Carbon test method used to define the above specifications. Copies are available upon request.)

TYPICAL PROPERTIES	CYCLE CARB® 201	CYCLE CARB® 201 *p*H	CYCLE CARB® 301
Bed Density[1], (For adsorber sizing), kg/m³	470	470	450
Mean Particle Diameter, mm	1.1	1.1	1.1
Total Surface Area, (N_2, BET method[2]), m²/g	775	775	900

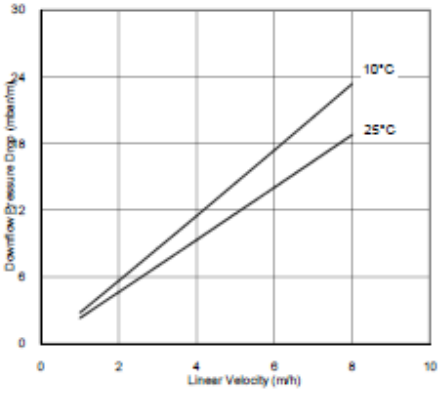
[1] *Bed Density is used for adsorber sizing.*
[2] *Brunauer, Emmett and Teller, J.Am. Chem. Soc. 60. 309 (1938)*
CYCLECARB® 301 pH is also available.

RECYCLING BY THERMAL REACTIVATION

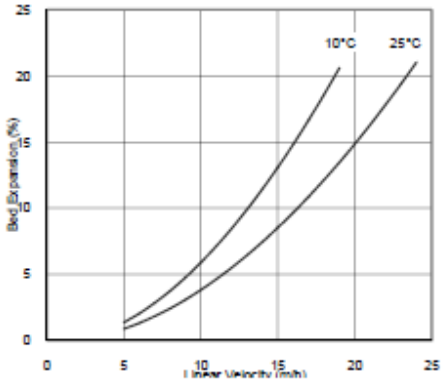
Once granular activated carbon is saturated or the treatment objective is reached, it can be recycled, by thermal reactivation, for reuse. Reactivation involves treating the spent carbon in a high temperature reactivation furnace to over 800°C. During this treatment process, the undesirable organics on the carbon are thermally destroyed. Recycling by thermal reactivation is a highly skilled process to ensure that spent carbon is returned to a reusable quality. **Chemviron Carbon** operates Europe's largest reactivation facilities and daily recycles large quantities of spent carbon for a diverse range of customers. Recycling activated carbon by thermal reactivation meets the environmental need to minimise waste, reducing CO_2 emissions and limiting the use of the world's resources.

CYCLECARB® is a granular reactivated carbon supplied on a service basis. Various test work has shown that in most cases, the performance of reactivated carbon is similar to virgin (new) carbon. Product quality is ensured by strict carbon acceptance procedures, selective blending of the carbon going into the furnace and stringent quality control specifications on the final product to ensure a consistent high performance granular activated carbon.

Typical Pressure Drop curves for CYCLECARB®



Typical Bed Expansion curves for CYCLECARB®



DESIGN INFORMATION

The design of a granular activated carbon wastewater treatment system will depend on the nature of the stream to be treated. The following are typical design parameters for **CYCLECARB®** for wastewater treatment:

- Superficial contact time 1-12 hours
- Bed depth 1-10 m
- Linear velocity 1-5 m/h

Chemviron Carbon can provide a comprehensive service to evaluate wastewater requirements including laboratory test work, pilot testing and engineering. **Chemviron Carbon** also supplies a wide range of adsorption systems, including the **CYCLESORB®** range of mobile service equipment, designed for the treatment of wastewater using **CYCLECARB®** products.

PACKAGING

- Bulk

SAFETY MESSAGE

Wet activated carbon preferentially removes oxygen from air. In closed or partially closed containers and vessels, oxygen depletion may reach hazardous levels. If workers are to enter a vessel containing carbon, appropriate sampling and work procedures for potentially low-oxygen spaces should be followed.

QUALITY

Each of our worldwide operations has achieved **ISO9001:2008** certification for their quality management system related to activated carbon. **Chemviron Carbon** guarantees the specifications against representative sampling.

CHEMVIRON CARBON

Pure Water. Clean Air. Better World.

Chemviron Carbon, the European operation of Calgon Carbon Corporation, is a global manufacturer, supplier, and developer of activated carbons, innovative treatment systems, value added technologies and services for optimising production processes and safely purifying the environment.

With our experience developed since the early years of the twentieth century, facilities around the world, and a world-class team of over 1,100 employees, Calgon Carbon Corporation can provide the solutions to your most difficult purification challenges.

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ISO 9001 ISO 14001 ISO 22000 BUREAU VERITAS Certification

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Chemviron Carbon

HPC SERIES – SUPER 830, MAXX 830, ULTRA 830

Granular Activated Carbon for Industrial Applications

DESCRIPTION

The HPC Series of virgin coal-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. These granular activated carbons are used for purification in a wide range of aqueous and organic liquid systems such as wastewater, liquid chemicals and leachate/remediation applications. With a lower density as compared to typical coal-based activated carbons, the **HPC Series** products have the advantage of a lower cost per unit volume.

FEATURES

The HPC Series has several properties which explain its superior performance in a wide range of industrial applications:

- Reduced contact time due to very fast diffusion kinetics and large volume of transport pores.
- High surface area and large pore sizes provide excellent decolourisation and high loading capacity.
- Subject to the Carbon Acceptance process, spent granular activated carbons can be recycled by thermal reactivation in our dedicated industrial facility.

PACKAGING

- Big bags
- Bulk deliveries

SAFETY MESSAGE

Wet activated carbon preferentially removes oxygen from air. In closed or partially closed containers and vessels, oxygen depletion may reach hazardous levels. If workers are to enter a vessel containing carbon, appropriate sampling and work procedures for potentially low-oxygen spaces should be followed.

QUALITY

Each of our worldwide operations has achieved **ISO 9001:2008** certification for their quality management system related to activated carbon. **Chemviron Carbon** guarantees the specifications against representative sampling.

PROPERTIES

SPECIFICATIONS	SUPER 830	MAXX 830	ULTRA 830
Iodine number, min., mg/g	900	1000	1100
Moisture, as packed, max., wt%	5	5	5
Mesh Size US Sieve Series, max. wt% > 8 mesh (2.36mm) <30 mesh (0.60mm)	 5 5	 5 5	 5 5

(Please refer to the Sales Specification Sheets, which state the Chemviron Carbon test method used to define the above specifications. Copies are available upon request.)

TYPICAL PROPERTIES	SUPER 830	MAXX 830	ULTRA 830
Molasses number	250-300	300	400
Hardness	>80	>80	>80
Apparent Density, kg/m³ Min. Max.	 370 430	 330 400	 300 370

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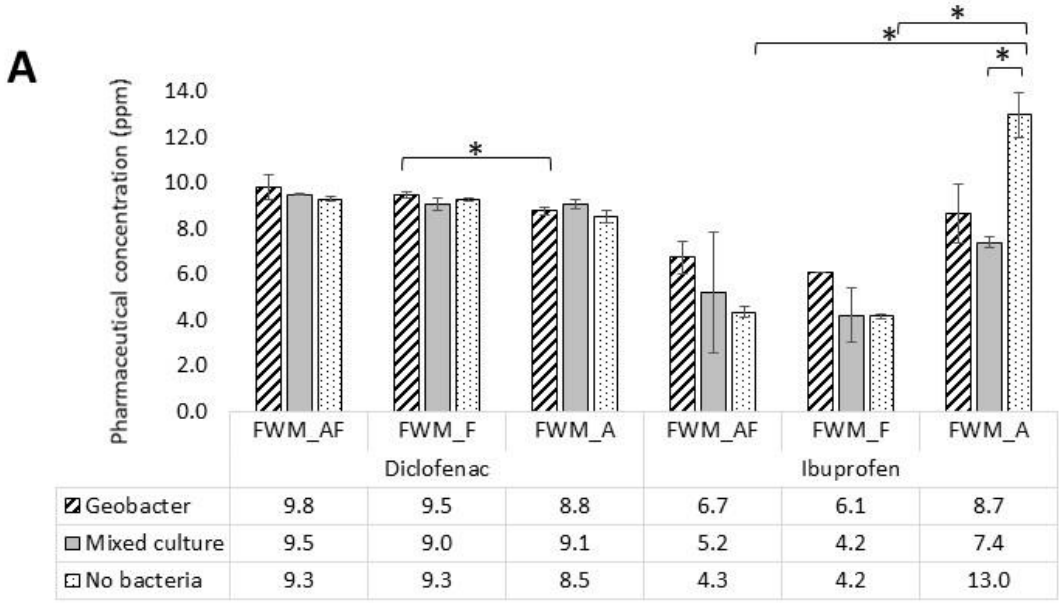
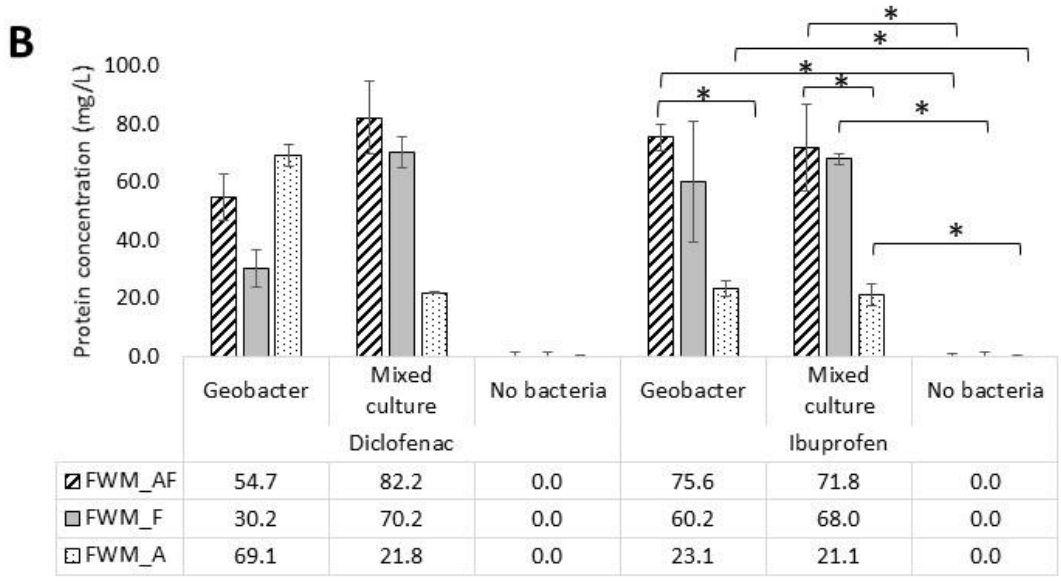
Appendix 6: Additional data anaerobic digestion of pharmaceuticals

6.1 Without biochar or activated carbon



	Diclofenac			Ibuprofen		
	FWM_AF	FWM_F	FWM_A	FWM_AF	FWM_F	FWM_A
Geobacter	9.8	9.5	8.8	6.7	6.1	8.7
Mixed culture	9.5	9.0	9.1	5.2	4.2	7.4
No bacteria	9.3	9.3	8.5	4.3	4.2	13.0



	Diclofenac			Ibuprofen		
	Geobacter	Mixed culture	No bacteria	Geobacter	Mixed culture	No bacteria
FWM_AF	54.7	82.2	0.0	75.6	71.8	0.0
FWM_F	30.2	70.2	0.0	60.2	68.0	0.0
FWM_A	69.1	21.8	0.0	23.1	21.1	0.0

Figure 9: Results of the anaerobic digestion of pharmaceutical in different media. (A) Concentration of diclofenac (left) or Ibuprofen (right) in ppm after a 14-day incubation of bacteria to a 10-ppm pharmaceutical spike in different media. Bacteria (*Geobacter sulfurreducens* or mixed culture) were exposed to 10 ppm pharmaceutical for two weeks in medium with acetate only, fumarate only or both acetate and fumarate. Both were compared to their equivalent abiotic control. Diclofenac concentration in ppm in Y-axis. Error bars represent standard error. **(B)** Protein concentration in mg/L of bacteria incubated for 14 days to 10 ppm diclofenac (left) or ibuprofen (right) grown in different media. Bacteria (*Geobacter sulfurreducens* or mixed culture) are exposed to 10 ppm of diclofenac or ibuprofen and cultured in fresh water medium with acetate and fumarate, acetate only or fumarate only. Protein concentration in mg/L in Y-axis. Error bars represent standard error. All compared and normalized to abiotic control. FWM_A: fresh water medium with acetate, FWM_AF: fresh water medium with acetate and fumarate, FWM_F: fresh water medium with fumarate, mg/L: milligrams per litre, ppm: parts per million

Table 2: P-values concerting the HPLC analysis of bacteria exposed to diclofenac or ibuprofen in different media.

Comparison for Diclofenac	**P-values**		
Within group comparison	**FWM_AF**	**FWM_F**	**FWM_A**
***Geobacter* vs. control**	0.954	0.234	0.478
Mixed culture vs. Control	0.108	0.461	0.168
Mixed culture vs. *Geobacter*	0.507	0.227	0.283
Between group comparison	**FWM_AF vs. FWM_A**	**FWM_AF vs. FWM_F**	**FWM_A vs. FWM_F**
Geobacter	0.699	0.239	0.033
Mixed culture	0.175	0.087	0.916
Control	0.677	0.054	0.058
Comparison for Ibuprofen	**P-values**		
Within group comparison	**FWM_AF**	**FWM_F**	**FWM_A**
***Geobacter* vs. Control**	0.055	0.835	0.055
Mixed culture vs. Control	0.031	0.976	0.005
Mixed culture vs. *Geobacter*	0.605	0.501	0.388
Between group comparison	**FWM_AF vs. FWM_A**	**FWM_AF vs. FWM_F**	**FWM_A vs. FWM_F**
Geobacter	0.230	0.262	0.133
Mixed culture	0.087	0.859	0.060
Control	0.015	0.003	0.001

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA.

Table 3: P-values concerting the bradford assay of bacteria exposed to diclofenac or ibuprofen in different media.

Comparison for Diclofenac	**P-values**		
Within group comparison	**FWM_AF²**	**FWM_F²**	**FWM_A²**
***Geobacter* vs. mixed culture vs. control**	0.043	0,043	0.043
Between group comparison	**FWM_AF vs. FWM_A vs. FWM_F²**		
Geobacter	0,043		
Mixed culture	0.165		
Control	0.043		
Comparison for Ibuprofen	**P-values**		
Within group comparison	**FWM_AF**	**FWM_F**	**FWM_A**
***Geobacter* vs. Control**	0.000	0.046	0.001
Mixed culture vs. Control	0.009	0.000	0.005
Mixed culture vs. *Geobacter*	0.820	0.730	0.683
Between group comparison	**FWM_AF vs. FWM_A**	**FWM_AF vs. FWM_F**	**FWM_A vs. FWM_F**
Geobacter	0.586	0.001	0.163
Mixed culture	0.943	0.038	0.000
Control	0.167	0.016	0.495

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA (no indication) or Kruskal-Wallis (indicated with "2").

6.2 With activated carbon

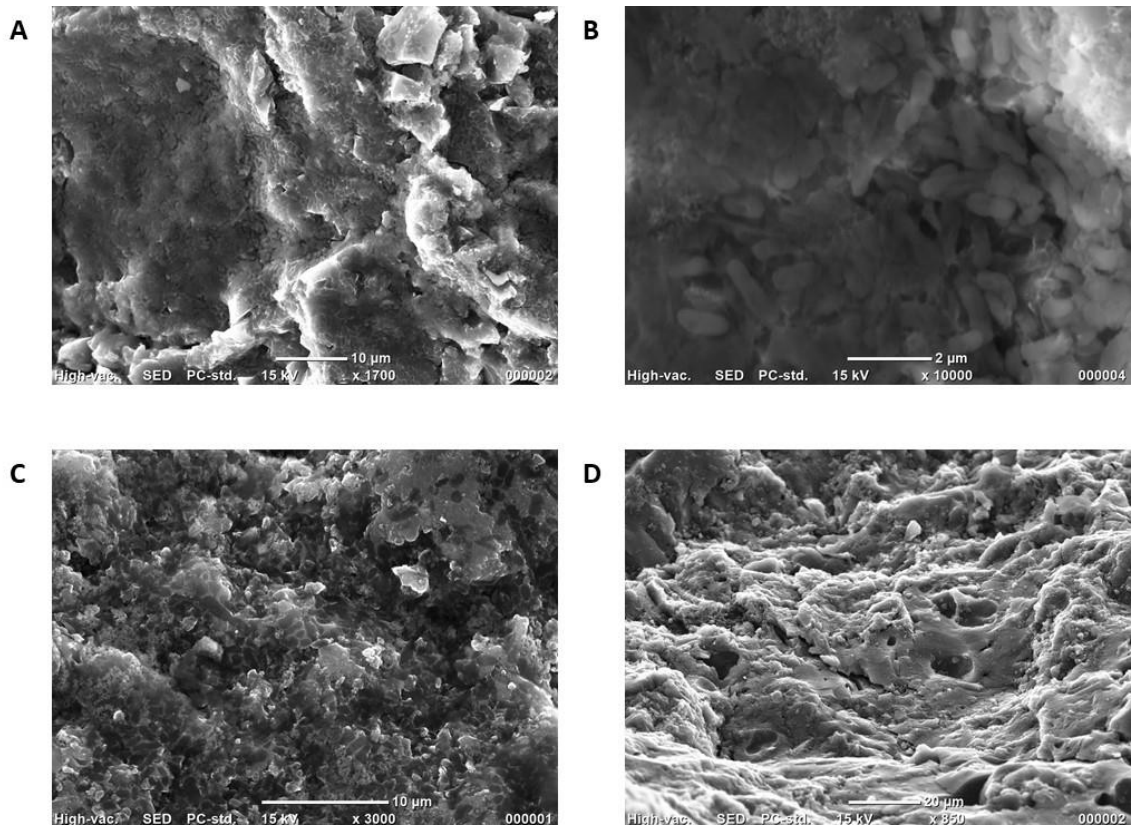


Figure 10: Scanning electron microscopy images of Filtrasorb 400 activated carbon particles exposed to diclofenac and mixed culture (biotic) or control (abiotic). (A) biotic sample, magnification factor: x1700, scale: 10 µm, **(B)** biotic sample, magnification factor: x10 000, scale: 2 µm, **(C)** biotic sample, magnification factor: x3000, scale: 10 µM, **(D)** abiotic sample, magnification factor: x850, scale: 20 µM

Table 4: P-values concerting the HPLC analysis of bacteria exposed to diclofenac or ibuprofen in the presence of activated carbon

P-values for Diclofenac				
Within group comparison	**Filtrasorb**	**HPC MAXX**	**Cyclecarb[3]**	**Cyclecarb_F[3]**
***Geobacter* vs. control**	0.099	0.006	0.475	0.004
Mixed culture vs. control	0.388	0.249	0.405	0.011
***Geobacter* vs. mixed culture**	0.482	0.769	0.402	0.007
Between group comparison	**Filtrasorb vs. HPC MAXX**	**Filtrasorb vs. Cyclecarb[2]**	**HPC MAXX vs. cyclecarb[2]**	**Cyclecarb vs. cyclecarb_F[3]**
Geobacter	0.807	0.050	0.050	0.676
Mixed culture	0.964	0.513	0.564	0.523
Control	0.318	0.827	0.827	0.107
Comparison to no material	**Filtrasorb[2]**	**HPC MAXX[2]**	**Cyclecarb[3]**	**Cyclecarb_F[3]**
Geobacter	0.050	0.050	0	0
Mixed culture	0.050	0.050	0	0.002
Control	0.050	0.050	0.001	0.002
P-values for Ibuprofen				
Within group comparison	**Filtrasorb**	**HPC MAXX**	**Cyclecarb**	**Cyclecarb_F**
***Geobacter* vs. control**	0.338	0.127	No data	0.221
Mixed culture vs. control	0.915	0.805	No data	0.210
***Geobacter* vs. mixed culture**	0.354	0.383	0.846	0.059
Between group comparison	**Filtrasorb vs. HPC MAXX[1]**	**Filtrasorb vs. Cyclecarb[1]**	**HPC MAXX vs. Cyclecarb[1]**	**Cyclecarb vs. cyclecarb_F**
Geobacter	0.213	0.102	0.769	0.007
Mixed culture	0.740	0.724	0.940	0.043
Control	0.179	No data	No data	No data
Comparison to no material	**Filtrasorb[1]**	**HPC MAXX[1]**	**Cyclecarb[1]**	**Cyclecarb_F[1]**
Geobacter	0.397	0.023	0.097	0.003
Mixed culture	0.033	0.013	0.182	0.029
Control	0.001	0.001	No data	0.003

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA (no indication) or Kruskal-Wallis (indicated with "[2]"). "3" indicates the use of total concentration (in medium + extracted from carbon).

Table 5: P-values concerting the bradford assay of bacteria exposed to diclofenac or ibuprofen in the presence of activated carbon

P-values of Bradford assay for diclofenac				
Within group comparison	Filtrasorb	HPC MAXX	Cyclecarb	Cyclecarb_F
***Geobacter* vs. control**	0.116	0.007	0.318	0.614
Mixed culture vs. control	0	0.001	0.021	0.006
***Geobacter* vs. mixed culture**	0.037	0.020	0.512	0.050
Between group comparison	Filtrasorb vs. HPC MAXX	Filtrasorb vs. Cyclecarb	HPC MAXX vs. Cyclecarb	Cyclecarb vs. Cyclecarb_F
Geobacter	0	0	0.721	0.514
Mixed culture	0	0.001	0.577	0.048
Control	0	0	0.043	0.122
Comparison to no material	Filtrasorb	HPC MAXX	Cyclecarb	Cyclecarb_F
Geobacter	0.406	0.001	0	0.006
Mixed culture	0	0	0	0.129
Control	0	0	0	0
P-values of Bradford assay for ibuprofen				
Within group comparison	Filtrasorb	HPC MAXX	Cyclecarb	Cyclecarb_F
***Geobacter* vs. control**	0.639	0.001	0.015	0.077
Mixed culture vs. control	0.332	0	0.005	0.002
***Geobacter* vs. Mixed culture**	0.526	0.008	0.853	0.015
Between group comparison	Filtrasorb vs. HPC MAXX	Filtrasorb vs. Cyclecarb	HPC MAXX vs. Cyclecarb	Cyclecarb vs. Cyclecarb_F[3]
Geobacter	0.213	0.146	0.354	0.990
Mixed culture	0.472	0.650	0.015	0.004
Control	0.388	0.344	0.449	0.487
Comparison to no material	Filtrasorb	HPC MAXX	Cyclecarb[3]	Cyclecarb_F[3]
Geobacter	0.006	0	0	0
Mixed culture	0.011	0	0	0.002
Control	0	0	0	0.742

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA. "3" indicates total concentration used (in medium + extracted from carbon).

6.3 With biochar

Table 6: P-values concerting the HPLC analysis of bacteria exposed to diclofenac or ibuprofen in the presence of biochar.

P-values for Diclofenac				
Within group comparison	**Typha**	**Wood mix**	**Coffee beans**	**Cyclecarb**
Mixed culture vs. control	0.081	0.016*	0.040	0.180
Between group comparison	**Typha vs. Wood mix**	**Typha vs. Coffee beans**	**Typha vs. cyclecarb**	**Typha vs. no material**
Mixed culture	0.894*	0.027	0.196	0
Control	0.293	0.030	0.015	0
Between group comparison	**Wood mix vs. coffee beans**	**Wood mix vs. Cyclecarb**	**Wood mix vs. no material**	**Coffee beans vs. cyclecarb**
Mixed culture	0*	0.116*	0*	0
Control	0.245	0.055	0.022	0
Between group comparison	**Coffee beans vs. no material**	**Cyclecarb vs. no material**		
Mixed culture	0.001	0		
Control	0.398	0		
P-values for Ibuprofen				
Within group comparison	**Typha**	**Wood mix**	**Coffee beans**	**Cyclecarb**
Mixed culture vs. control	0.503	0.001	0.005	0.553
Between group comparison	**Typha vs. Wood mix**	**Typha vs. Coffee beans**	**Typha vs. cyclecarb**	**Typha vs. no material**
Mixed culture	0.233	0.218	0.063	0.061
Control	0.023	0.031	0.007	0.045
Between group comparison	**Wood mix vs. coffee beans**	**Wood mix vs. Cyclecarb**	**Wood mix vs. no material**	**Coffee beans vs. cyclecarb**
Mixed culture	0.836	0.003	0.061	0.000
Control	0.000	0.144	0.112	0.000
Between group comparison	Coffee beans vs. no material	Cyclecarb vs. no material		
Mixed culture	0.059	0.389		
Control	0	0.001		

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA. Total concentration was used (medium + biochar extraction) unless indicated with "*", which indicates use of only the concentration in medium.

Table 7: P-values concerting the bradford assay of bacteria exposed to diclofenac or ibuprofen in the presence of biochar.

P-values for Diclofenac				
Within group comparison	**Typha**	**Wood mix**	**Coffee beans**	**Cyclecarb**
Mixed culture vs. control	0.018	0.009	0.012	0.001
Between group comparison	**Typha vs. Wood mix**	**Typha vs. Coffee beans**	**Typha vs. cyclecarb**	**Typha vs. no material**
Mixed culture	0.0574	0.155	0.113	0.154
Control	0.793	0.644	0.191	0.167
Between group comparison	**Wood mix vs. coffee beans**	**Wood mix vs. Cyclecarb**	**Wood mix vs. no material**	**Coffee beans vs. cyclecarb**
Mixed culture	0.291	0.080	0.198	0.842
Control	0.817	0.238	0.337	0.371
Between group comparison	**Coffee beans vs. no material**	**Cyclecarb vs. no material**		
Mixed culture	0.723	0.763		
Control	0.724	0.057		
P-values for Ibuprofen				
Within group comparison	**Filtrasorb²**	**HPC MAXX²**	**Cyclecarb²**	**Cyclecarb_F²**
Mixed culture vs. control	0.050	0.050	0.050	0.050
Between group comparison	**Typha vs. Wood mix²**	**Typha vs. Coffee beans²**	**Typha vs. cyclecarb²**	**Typha vs. no material²**
Mixed culture	0.050	0.050	0.050	0.050
Control	0.500	0.513	0.127	0.050
Between group comparison	**Wood mix vs. coffee beans²**	**Wood mix vs. Cyclecarb²**	**Wood mix vs. no material²**	**Coffee beans vs. cyclecarb²**
Mixed culture	0.513	0.050	0.080	0.513
Control	0.827	0.513	0.050	0.261
Between group comparison	**Coffee beans vs. no material²**	**Cyclecarb vs. no material²**		
Mixed culture	0.050	0.050		
Control	0.050	0.050		

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA (no indication) or Kruskal-Wallis (“2”) .

6.4 With one biochar, one activated carbon or no material

Table 8: P-values concerting the HPLC analysis of bacteria exposed to diclofenac or ibuprofen in the presence of biochar, activated carbon or no material.

P-values for Diclofenac			
Within group comparison	**No material**	**Typha[2]**	**Cyclecarb**
***Geobacter* vs. control**	0.338	0.827	0.277
Mixed culture vs. control	0.307	0.050	0.002
***Geobacter* vs. mixed culture**	0.007	0.127	0
Between group comparison	**No material vs. Typha[2]**	**No material vs. Cyclecarb**	**Typha vs. Cyclecarb[2]**
Geobacter	0.050	0	0.050
Mixed culture	0.050	0	0.050
Control	0.050	0	0.050
P-values for Ibuprofen			
Within group comparison	**No material**	**Typha**	**Cyclecarb**
***Geobacter* vs. control**	0.309	0.445	0.311
Mixed culture vs. control	0.004	0.001	0.932
***Geobacter* vs. mixed culture**	0.796	0.025	0.026
Between group comparison	**No material vs. Typha**	**No material vs. Cyclecarb**	**Typha vs. Cyclecarb**
Geobacter	0.941	0.970	0.918
Mixed culture	0.003	0	0.011
Control	0.015	0.010	0.774

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA.

Table 9: P-values concerting the bradford assay of bacteria exposed to diclofenac or ibuprofen in the presence of biochar, activated carbon or no material.

P-values for Diclofenac			
Within group comparison	**No material**	**Typha**	**Cyclecarb**
***Geobacter* vs. control**	0.022	0.054	0.056
Mixed culture vs. control	0.107	0.235	0.068
***Geobacter* vs. mixed culture**	0.422	0.004	0.007
Between group comparison	**No material vs. Typha**	**No material vs. Cyclecarb**	**Typha vs. Cyclecarb**
Geobacter	0.753	0.310	0.520
Mixed culture	0.863	0.564	0.789
Control	0.946	0.933	0.995
P-values for Ibuprofen			
Within group comparison	**No material²**	**Typha**	**Cyclecarb**
***Geobacter* vs. control**	0.006	0.064	0.030
Mixed culture vs. control	0	0	0.001
***Geobacter* vs. mixed culture**	0.652	0.040	0.030
Between group comparison	**No material vs. Typha²**	**No material vs. Cyclecarb²**	**Typha vs. Cyclecarb**
Geobacter	0.112	0.775	0.090
Mixed culture	0.154	0.155	0.074
Control	0.161	0.104	0.712

P-values below 0.05 were consideren significant and are colored in green. Significance tested with One-way ANOVA (no indication) or Kruskal-Wallis (“2”).

Appendix 7: Additional data BiMER

7.1 Acetate assay

Table 10: P-values concerning the acetate assay.

Biotic vs. Abiotic	**0%**	**25%**	**50%**	**100%**
Day 1	0.269	0.380	0.225	0.170
Day 14	No date	0.266	0.020	0.680
Day 1 vs. Day 14	**0%**	**25%**	**50%**	**100%**
Biotic	0.269	0.083	0.189	0.342

Significance was determined with One-sample T-test, P-values lower than 0.05 were considered significant.

7.2 Bradford assay

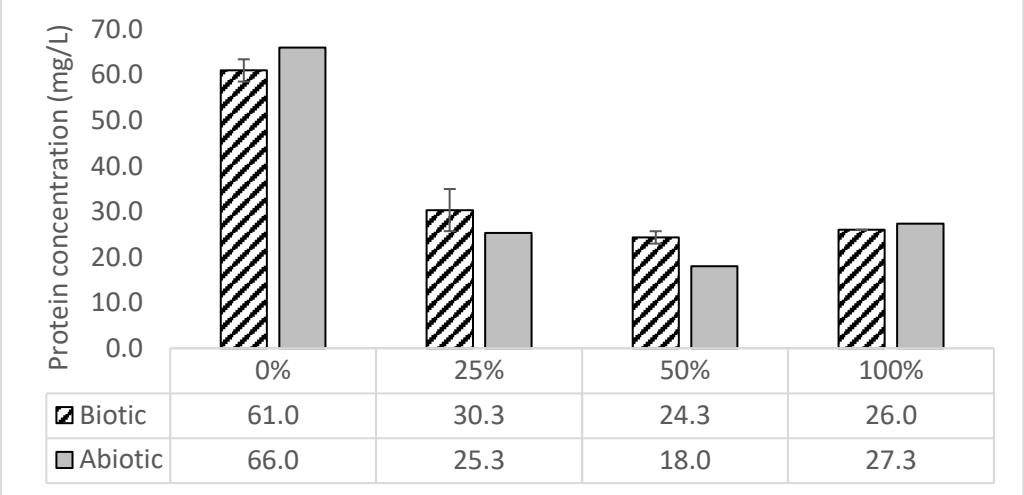


Figure 11: Protein concentration in mg/L of bacteria used in a microbial electrochemical remediation cell. Mixed culture samples were grown in a microbial electrochemical remediation cell for two weeks on fresh water medium with acetate. As electroconductive material the cells had 25%, 50% or 100% biochar compared to a 100% sand control. Protein concentration in ppm on Y-axis. All compared uninoculated (abiotic) control. Error bars represent standard error. Mg/L: milligram per litre.

Table 11: P-values concerning the Bradford assay of the BiMER experiment.

P-values for Bradford assay				
Within groups	0%	25%	50%	100%
Day 14 biotic vs. abiotic	0.344	0.540	0.164	No analysis possible
Between groups	0% vs. 25%	0% vs. 50%	0% vs. 100%	25% vs. 50%
Biotic	0.041	0.009	0.007	0.417
Between groups	25% vs. 100%	50% vs. 100%		
Biotic	0.524	0.423		

All experiments used one-way ANOVA to determine significance, P-values < 0.05 indicated significance.

7.3 Thermogravimetric analysis

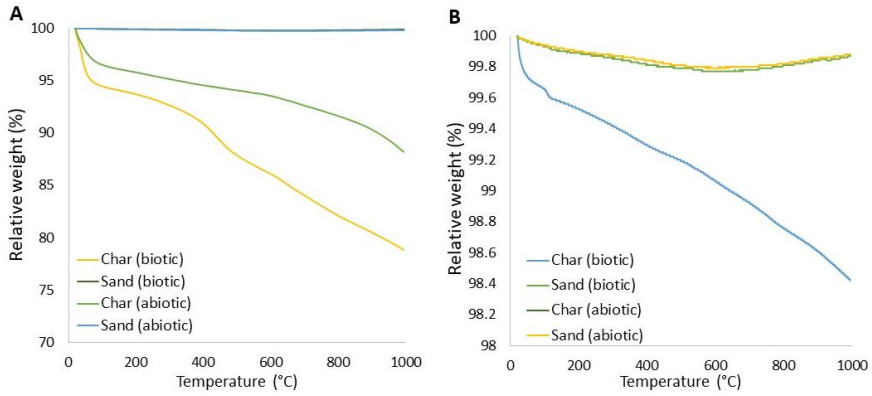


Figure 12: Thermogravimetric analysis of samples with a biochar:sand mixing ratio of 25:75 and 50:50. (A) Relative weight in % per rise in temperature (°C) for biotic and abiotic sand or biochar samples previously in a mixing ratio of 25% biochar. **(B)** relative weight (%) compared to rise in temperature (°C) for biotic and abiotic sand or biochar samples previously in a mixing ratio of 50%.

7.4 Cyclic voltammetry

Table 12: P-values concerning the differences in resistance of BiMERs determined through cyclic voltammetry.

Within group comparison	**0%**	**25%**	**50%**	**100%**
Biotic vs Abiotic	0.362	No statistical analysis possible	No statistical analysis possible	0.302
Between group comparison	0% vs. 25%	0% vs. 50%	0% vs. 100%	25% vs. 50%
Biotic	0.398	0.271	0.131	No statistical analysis possible
Between group comparison	25% vs. 100%	50% vs. 100%		
Biotic	0.075	0.268		

All significance (P-value < 0.05) was determined with One-Sample T-tests.

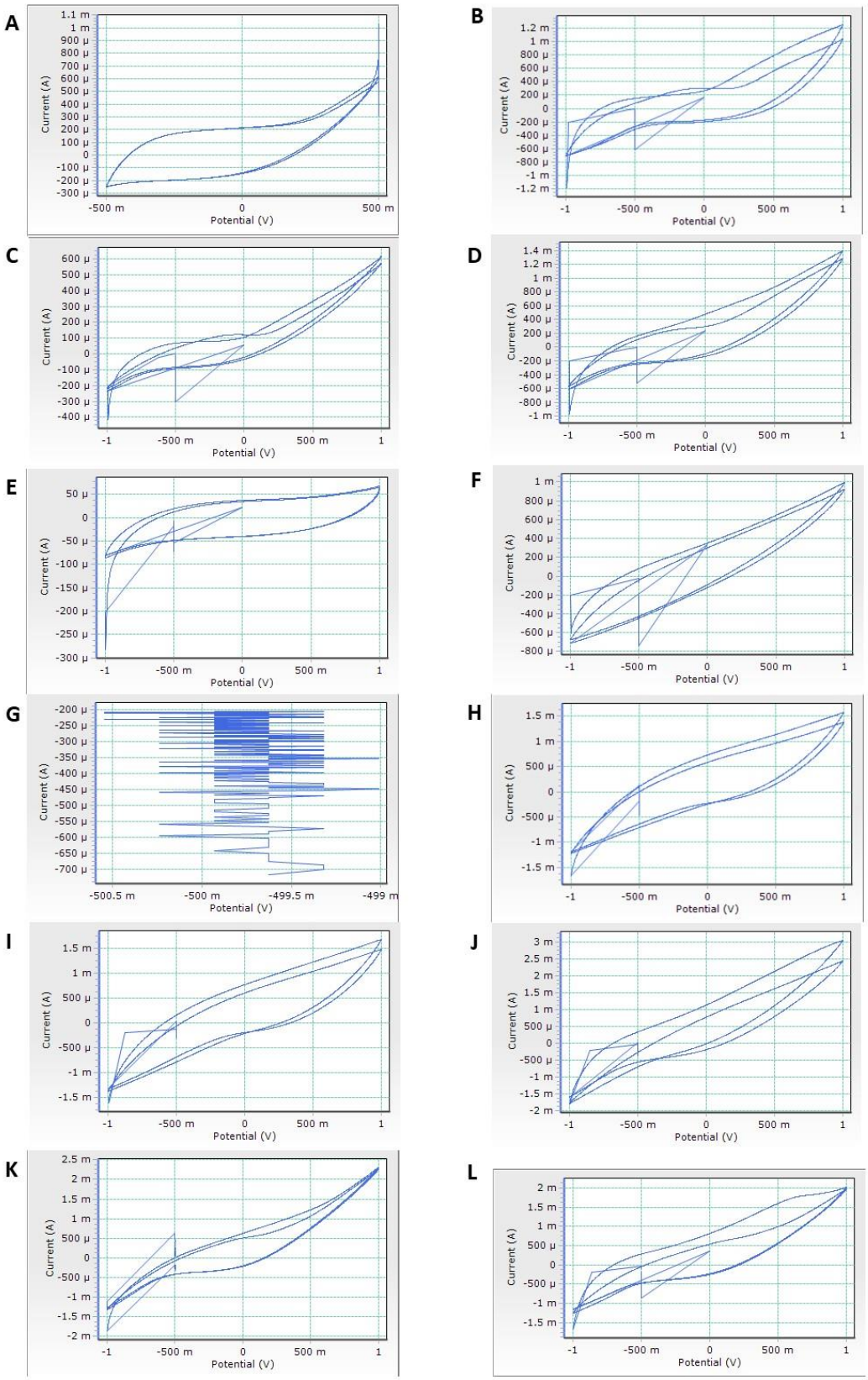


Figure 13: Cyclic voltammograms of the remediation cells. Cyclic voltammogram was established through two cycles with a voltage range of -1V to +1V, with steps of 0.1V/s and -500mV as starting point (exception: (A) from -0.5V to + 0.5V). X-axis represents the shift in potential (V), Y-axis represents the measured current (A). **(A)-(B)** 0% biochar, inoculated, **(C)** 0% biochar, uninoculated, **(D)-(E)** 25% biochar, inoculated, **(F)** 25% biochar, uninoculated, **(G)-(H)** 50% biochar, inoculated, **(I)** 50% biochar uninoculated, **(J)-(K)** 100% biochar, inoculated, **(L)** 100% biochar uninoculated. A: ampere, V: volt

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**

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

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Van Limbergen, Thessa

Datum: **11/06/2018**