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New directions and challenges in engineering biologically-enhanced biochar for biological water treatment.

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New directions and challenges in engineering Biologically-Enhanced Biochar for biological water treatment

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Abstract

Cost-effective, efficient, and sustainable water treatment solutions utilising existing materials and technology will make it easier for low and middle-income countries to adopt them, improving public health. The ability of biochar to mediate and support microbial degradation of contaminants, combined with its carbon-sequestration potential, has attracted attention in recent years. Biochar is a possible candidate for use in cost-effective and sustainable biological water treatment, especially in agrarian economies with easy access to abundant biomass in the form of crop residues and organic wastes. This review evaluates the scope, potential benefits (economic and environmental) and challenges of sustainable biological water treatment using 'Biologically-Enhanced Biochar' or BEB. We discuss the various processes occurring in BEB systems and demonstrate the urgent need to investigate microbial degradation mechanisms. We highlight the need to correlate biochar properties to biofilm development, which can eventually determine process efficiency. We also demonstrate the various opportunities in adopting BEB as a cheaper and more viable alternative in Low and Middle Income Countries and compare it to the current benchmark, 'Biological Activated Carbon'. We focus on the recent advances in the areas of data science, mathematical modelling and molecular biology to systematically and sustainably design BEB filters, unlike the largely empirical design approaches seen in water treatment. 'Sequential biochar systems' are introduced as specially designed end-of-life techniques to lower the environmental impact of BEB filters and examples of their integration into biological water treatment that can fulfil zero waste criteria for BEBs are given.

Keywords: Biologically-Enhanced Biochar, Biological Water Treatment, Biochar-Microbial interactions, Sequential Biochar Systems

1. Introduction

According to the World Health Organisation, half of the world's population will be living in water-stressed areas by 2025[1]. Only 8% of the wastewater in low-income countries is currently being treated and at least 2 billion people use a drinking water source contaminated with faeces leading to 485 000 deaths from diarrhoea each year[1-3]. With climate crisis looming in the distance, there is an urgent need for a massive collaborative effort rooted in inter-disciplinary research to find affordable and sustainable solutions for wastewater treatment. It is in this regard that we review the scope of using specially designed 'Biologically-Enhanced Biochar' or BEB in sustainable biological water treatment.

Biochar is a carbon-rich product resulting from thermochemical conversion of carbonaceous materials in an oxygen-deficient environment at high temperatures and is a carbon-negative technology (300-800°C) [4–7]. Recent developments in biochar research highlight the potential for many applications ranging from water treatment, soil remediation, and agriculture to energy conversion and storage [4,8–10]. There is increasing evidence of biochar mediation in microbial metabolism in several energy and environmental applications such as soil and water remediation, anaerobic digestion, and several Microbial Electrochemical Technologies (METs) such as Microbial Fuel Cells (MFCs) [11–14]. Biochar mediation in microbial degradation of contaminants combined with adsorbent properties make biochar a very attractive option for biological water treatment.

Carbonaceous materials such as biochar can be used in tertiary water treatment which use both adsorption and microbial activity to remove contaminants [5,15,16]. Here, microbes (naturally present in water or externally introduced to suit target contaminants) can immobilise on the surface of porous carbon forming biofilms. Biofilms then metabolise and degrade the adsorbed contaminants in a bio-regeneration process. This bioregeneration process frees up clogged pores of the filter material (such as biochar), regenerating their adsorptive capacity and significantly increasing their lifespan [17,18]. Despite limited experience with the BEBs in biological water treatment, this review discusses the potential and opportunities to deploy biochar for efficient, cost-effective and sustainable water treatment applications, especially for on-site and home-scale water treatment units in Low and Middle-Income Countries (LMICs) where centralised, industrial-scale water treatment units may be too expensive to operate.

Moreover, current water treatment units are largely designed based on empirical research and there is no clear consensus on how, why and to what extent biochar-microbial interactions can affect the efficiency of the water treatment application. Recent technological advancements and a wide array of analytical, statistical, mathematical, and molecular biology techniques allow us to decode biochar-microbial interactions more systematically, making this review timely and important. This review examines the dynamic and complex biofilm-biochar interactions of BEB in water treatment and identifies gaps that require new research. The review provides directions that will allow researchers to navigate through this highly multi-disciplinary area and help bring affordable biochar-based water treatment solutions to everyone, especially to people in LMICs. We discuss opportunities for adopting BEBs and compare biochar with activated carbon (a benchmark material in tertiary water treatment) in economic and environmental aspects. Using the multi-functionality of biochar, we demonstrate how ‘sequential biochar systems’ can be integrated in biological water treatment systems to meet zero waste criteria[19]. In this way, BEB filters have the potential to be safely and effectively used in sequence or combination with various other applications such as MFCs, anaerobic digestion, soil application and gas adsorption.

2. BEB processes for tertiary water treatment integrating adsorption and biodegradation

Most industrial and municipal water treatment processes (drinking water treatment units, waste water treatment units) comprise of primary, secondary and tertiary treatments to remove organic, inorganic and biological contaminants [5,15]. Primary treatments mostly use physical process such as coagulation and flocculation (with aluminium, ferric salts) and chemical precipitation to remove solids, while secondary treatments generally involve chemical and biological processes such as aerobic and anaerobic reactors to remove organic matter [20,21]. Biological Activated Carbon (BAC) is commonly used as part of advanced water treatments for combined adsorption and biodegradation of predominantly organic matter, but also other inorganic and biological pollutants which could not be removed in primary and secondary treatments[5]. In a typical BAC process, activated carbon acts as a support for artificially introduced or naturally occurring microorganisms. These immobilised microbes can reproduce on the activated carbon surface eventually forming BAC and facilitate contaminant removal via a combined adsorption and biodegradation process [5, 17]. Other tertiary treatments such as ion-exchange resins, photocatalysis, membrane processes, disinfection (chlorination, ozonation) are also used [17,21,22]. Ozonation and chlorination steps may precede or succeed BAC treatment depending on the process requirements [23].

Biological water treatment using BEB, like BAC processes, can be used as tertiary water treatment in sequence with other water treatment steps or in stand-alone on-site water treatment units. The biofilms can degrade and remove a wide range of organic, inorganic and biological water-borne contaminants [18,24,25]. This combined adsorption and bio-regeneration process has shown to reduce the BAC costs by a factor of 2-3 in comparison with the use of AC for adsorption-based removal alone [26]. Çeçen et.al. comprehensively detailed the history, mechanisms and mathematical models of this integrated approach, both for attached-growth (BAC) and suspended-growth in Powdered Activated Carbon (PAC) treatment [5]. Here we focus on attached-growth biofilm systems on biochar for water treatment, since powdered carbon processes are more energy intensive owing to the extra step needed to separate the suspended powders from the effluent after water treatment and are therefore less relevant in LMICs [5].

3. Biological water treatment using BEB systems

3.1 Adsorption, Biosorption and Biodegradation:

Removal of contaminants in a biological filter is a multi-step process involving adsorption, biofilm formation, biodegradation, desorption and diffusion of contaminants and nutrients across the biochar-biofilm-water interface [19,20]. **Fig. 1** illustrates the various steps, mechanisms and processes that might occur during the dynamic biochar-biofilm interactions for contaminant removal in BEB filters. Microbes generally attach themselves to the biochar surface by secreting a gluey, Extracellular Polymeric Substances (EPS), made of biopolymers[18,25,27]. The transport of contaminants and other water-borne substances from bulk fluid to this EPS is often dominated by molecular diffusion. Microbes then metabolise these organic and inorganic contaminants via several biochemical and/or bio-electrochemical reactions involved in respiration and cell growth [15,25,28].

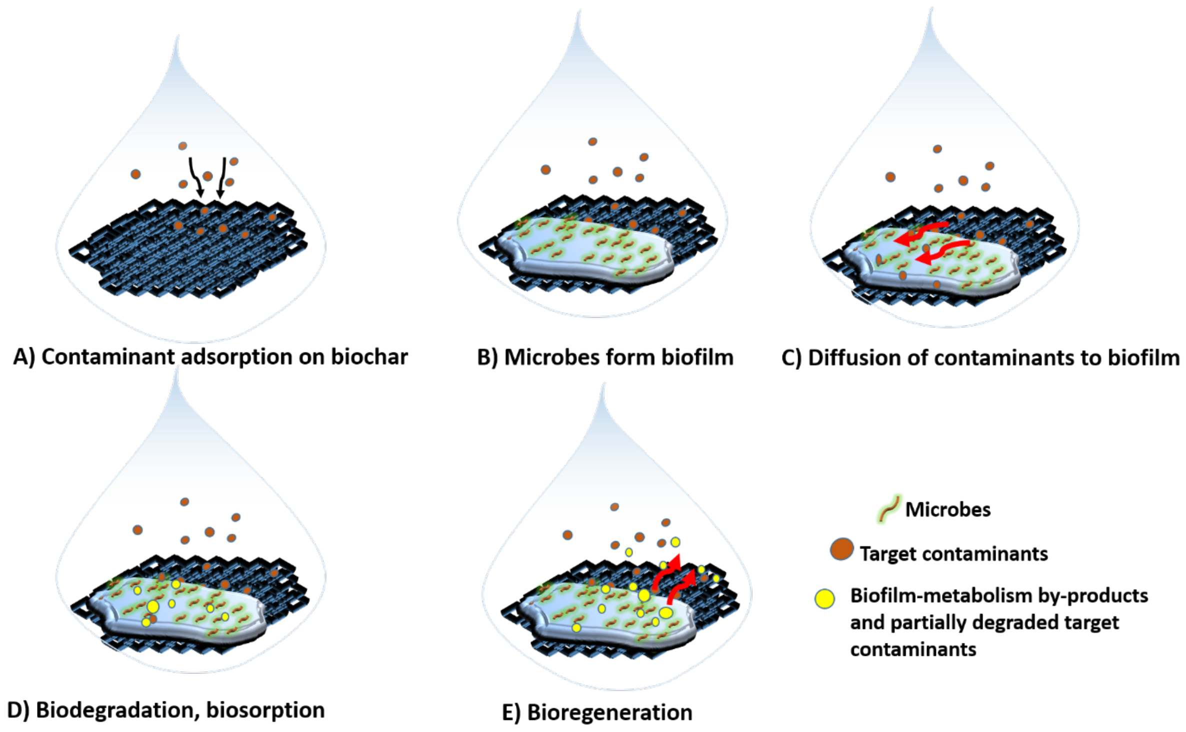

Figure 1: Adsorption, biosorption and biodegradation in Biologically-Enhanced Biochar filters A) Diffusion and adsorption of target contaminants from bulk to biochar surface, B) Microbes forming biofilm with Extracellular Polymeric Substances C) Diffusion of contaminants through Extracellular Polymeric Substances D) Microbes metabolise target contaminants, releasing simpler product molecules; biosorption based on biofilm's adsorption affinity for contaminants/metabolic by-products, E) Spontaneous bio-filter bio-regeneration via desorption of partially degraded contaminants and metabolic products owing to reverse concentration gradients

Researchers have widely studied the adsorptive properties of biochar in water treatment, however studies that investigate the combined adsorption and biological degradation of contaminants using biochar is an emerging area of research[10,29–32]. This use of 'Biologically-Enhanced Biochar', despite a limited number of studies, has promising results for the removal of organic/inorganic/biological substances from wastewater. The biofilm and its biodegradation by-products are also capable of adsorption of several organic-inorganic contaminants via biosorption (adsorption by biomass) [33,34]. Microbial biofilms and their biosorption capabilities for bioremediation form a huge area of research [33,35]. For example, biochar units adsorbed arsenic from wastewater followed by separate periphytic biofilm reactors (containing both heterotrophic and phototrophic microbes), which adsorb the remaining arsenic in the water via biosorption by biofilms [36].

The contaminant removal efficiencies and mechanisms of different BEBs can vary significantly depending on process conditions, type of contaminants and biochar properties. For example, Aspen wood biochar showed a decrease in the degradation of naphthenic acids, while softwood bark biochar showed a significant increase in naphthenic acid degradation; both studied in the presence of metal contaminants[34]. Both biochars supported biofilms of similar thickness. Here, each biochar by virtue of their unique physical and chemical properties, led to a different selection of microbial community. This different biofilm proliferation will lead to different metabolic potential and explains the observed difference in the degradation capacities. This rationale is reinforced by the finding that naphthenic acid degradation capacities were comparable for both biochar types in sterile conditions. Contaminant removal due to adsorption alone was close to 30% when tested on sterile biochar samples compared to up to 87% with biofilm growth. A four-fold increase in metal (Fe, Al, As) uptake from the water phase further confirms the influence of biosorption. This points to other interplaying parameters, such as the physico-chemical properties of the biochar support, microbial community structure and its metabolic potential, type of contaminants, kinetics and mechanisms of biodegradation, which would all dictate the overall contaminant removal capacity of a BEB filter.

Dalahmeh et. al. investigated and compared the degradation of four pharmaceutical compounds (carbamazepine, metoprolol, ranitidine and caffeine) by hardwood biochar and sand filters [37]. The study investigated biochar and sand filters with active, inactive and no biofilms to quantify the effects of adsorption, biodegradation, and a combination of these processes on contaminant removal. The results suggest that the extent of contaminant removal varies greatly with the type of pharmaceutical contaminants, the mechanism of contaminant removal and also the filter material used (biochar or sand). Biochar filters performed better than sand filters for carbamazepine and metoprolol removal with adsorption as the main mechanism of contaminant removal. Both biochar and sand filters had comparable contaminant removal efficiencies for ranitidine and caffeine via a combination of adsorption and biodegradation. Biochar filters with active biofilm performed better than sand filters for the removal of organic matter and nitrogen. The study was extended for the removal of per-and polyfluoroalkyl substances (PFA) using the same model to find that biochar efficiently removed long-chain PFAs with chain length greater than C6[38]. For similar process conditions, the different solubility of contaminants in water, the adsorption affinities of filter for various contaminants and organic matter, and the dissimilar biodegradability of contaminants are the factors which have been identified to result in differences in the efficiency of contaminant removal.

Apart from removing organic/inorganic contaminants, biochar-based water filters are also efficient in removing microbial pathogens such as *Escherichia.coli*, *Staphylococcus aureus* from contaminated water [39–41]. Biochar amendment of conventional sand/compost bio-filters used for storm water treatment is another promising and emerging area of research [42]. These biochar-based interventions can be particularly useful in the light of extreme climate events such as floods, especially for people in LMICs, who are usually the worst-affected.

Studies on removal of *E. coli* in biochar-amended filters show how properties of biochar such as surface area, polarity, particle size and the biofilm properties such as microbial geometries have a very significant effect on capacity of *E. coli* removal [40,43]. Biochar of small particle size with low volatile matter content and low polarity were found to be more beneficial for *E.coli* removal, while the infiltration rates and initial bacterial composition did not have a large influence [40]. The main reasons for the improved *E. coli* removal capacity upon addition of biochar appear to be the improved water-holding capacity of biochar-amended bio-filters and the higher attractive/binding forces of biochar surfaces. A recent meta-analysis relates this improvement in soil structure with biochar amendment to an increased soil porosity, number of pores and pore connectivity [44]. This leads to better *E. coli* attachment on biochar surfaces, increasing their overall removal from infiltrating storm water. The authors also discuss the detrimental effects of increasing dissolved organic content in the system on *E. coli* removal, although a definite explanation for this is yet to be found.

Afrroz. et. al. evaluated the effects of biochar amendment in laboratory-scale bio-filters on two bacterial pathogen removal efficiencies (*Salmonella enterica serovar Typhimurium* and *S. aureus)*, as well as bacterial and viral indicators *E. coli* and *MS2 coliphage* [45]. Biochar amendment resulted in a considerably higher increase in bacterial removal efficiency (9-fold increase) compared to bacteriophage removal efficiency (3-fold increase). The study also finds the experimentally observed microbe removal rates to be 2.8-7 times higher than the theoretically calculated removal rates (using Colloidal Filtration Theory (CFT)). The observed effects are attributed to removal mechanisms such as straining (a sieving effect, where contaminants of large size cannot pass through the small pore size of biochar filter medium) and hydrophobic interactions that are likely to be affected by biochar. These mechanisms are specific to each type of microbe and are not included in CFT. This further highlights the complexities of biochar/microbe interactions and demonstrates how the current models are insufficient in studying these interactions.

3.2 Bio-electrochemical interactions in BEB:

Microbes often use redox active species such as organic matter, ammonium, O_2, NO_3^- etc. as energy sources, energy sinks, energy storage materials, and in cell-to-cell electron transfers[46,47]. Biochar, being electrically conductive and with abundant redox active sites (such as quinone, phenolic groups) can mediate and support microbial metabolism effectively[48,49]. In some cases, the porous carbon support may just act as ideal supports, allowing a good biofilm growth, but not actively taking part in the degradation[50]. While a lot of metabolic biochemical reactions in microbes are intracellular, certain microbes are capable of directly transferring electrons between intracellular redox moieties and external redox sites such as minerals and electrically conducting carbon[51]. Such microbes capable of extracellular electron transfer have been identified as 'electroactive microorganisms' [28,47,52]. The mere presence of electroactive substrates has been shown to improve microbial biodegradation[53]. It is thus highly likely that an immobilized biofilm will proliferate in a way that makes use of the electrical conductivity and redox sites in biochar for bioremediation[11,49]. Biochar can also be carriers of humic substances and redox mediators such as metal composites, which provide additional electron exchange capability for mediation in microbial metabolism [48,54]. This use of electroactive biochar for energy and environmental applications using Microbial Electrochemical Technologies (METs), is another emerging area of research [11,49,55]. Biochar-based METs can make use of microbial catalysis in electrochemical processes and can valorise several forms of waste (solid, liquid, and gas) for sustainable generation of a wide range of products such as electricity, biofuel and biogas [55,56].

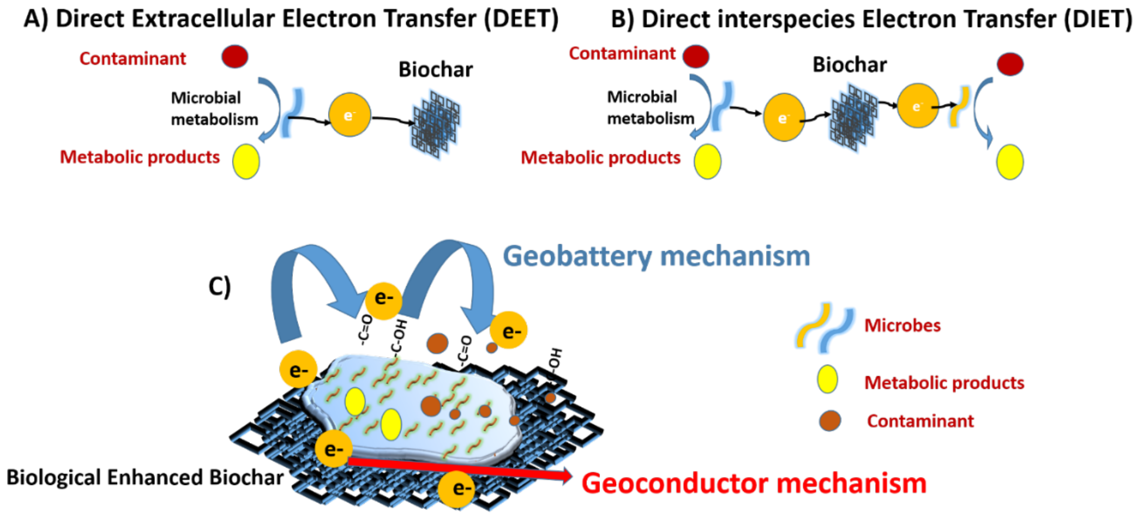

Figure 2 Bio-electrochemical interactions in Biologically-Enhanced Biochar filters A) Direct Extracellular Electron Transfer (DEET) between electroactive microbes and biochar via membrane-bound cytochromes or conductive nanowires manifested in the cells, B) Direct interspecies Electron Transfer (DIET) between two different electroactive microbe species, biochar being both electron acceptor/donor, C) Both DIET and DEET can happen via: Geobattery mechanism- Electron shuttled through the various electron donating/accepting groups –C-OH, C=O or Geo-conductor mechanism- electrons shuttled through the conductive graphitic frameworks of biochar

Electroactive microbes can metabolise contaminants via Direct Extracellular Electron Transfer (DEET) and Direct Interspecies Electron Transfer (DIET) [55,57]. In DEET, microorganisms physically connect to biochar for electron transfer (**Fig. 2**) [13,48,58]. DEET also occurs when microbes utilise nanowires or pili (vesicular extensions of periplasm or outer cell membranes, 2-3 μm long) to connect to the electrodes, especially for interconnections with electrode surfaces from within the deeper layers of the biofilm[59]. In DIET, microorganisms utilise other cells as electron mediators via a syntrophic metabolism[28]. Electrically conducting biochar can promote DIET significantly in processes such as wastewater treatment and anaerobic digestion [14,60,61]. For both DEET and DIET, one way of electron transfer is through the highly conducting graphitic frameworks of biochar, often referred to as the geoconductor mechanism[11,62] **(Fig. 2)**. It is also possible to shuttle electrons by the interaction of surface functional groups such as quinones (electron accepting) and/or phenolic/hydroquinone (electron donating) groups by reversibly accepting and donating electrons, referred to as the geobattery or geocapacitor mechanism[11,62].

Prado et. al. found maximum biodegradation efficiencies by electro-conductive biochar in both batch and continuous operations, compared to coke and graphite, despite graphite showing 40,000 times higher electrical conductivity than biochar[53]. Biochar displayed a larger number of electroactive functional groups compared to coke and graphite. If the geoconductor mechanism was dominant, graphite would be outperforming biochar in biodegradation via geoconductor mechanism, which is not the case. The study thus pinpoints to a possible geobattery mechanism of electron transfer using the abundant functional groups in biochar, which would have enabled it to outperform graphite and coke. However, we point out that the biochar used here had a higher surface area (almost 200 times more) and total pore volume (100 times higher), than the graphite and coke used. It would be interesting to see if biochar with a similar surface area and pore size distribution to coke and graphite would perform well under the same conditions.

The ability of biochar to promote DIET in anaerobic digestion is a very recent discovery and not fully understood yet [12,63]. Even less is known about the role of biochar DIET mediation in biological water treatment. However, due to the prevalence of microbial metabolic activities and biochar-biofilm interactions, it is highly likely that DIET occurs in biological water treatment as well. Previous studies confirmed DIET as the mechanism of syntrophic metabolism, via pili (conductive nanowires) formation in *Geobacter. metallireducens*[60]. However, the process of pili production is slow, and thus, presence of a conductive material, such as biochar can facilitate the electron exchange process by acting as an electrical conduit, by-passing the mechanism relying on pili, thus making the entire metabolic process much faster. A biochar-amended co-culture in a recent study showed a 15x higher ethanol metabolization rate in presence of biochar compared to a co-culture with no biochar[60]. The metabolization rates were comparable to rates reported by Granular Activated Carbon, which had 1000x more electrical conductivity than biochar[60]. However, no direct link between the rates of ethanol metabolism and electrical conductivity of biochar could be established. This indicates that a number of other additional factors such as porosity, aromaticity, and electron exchange capacities may play an important role in DIET. **Table 1** provides more details on the process and experimental conditions for the examples discussed under Section 3.

While it is clear that biochar can support and mediate microbial metabolism leading to removal/degradation of water-borne contaminants, there is no clear consensus on which process conditions and properties of BEB promote particular mechanisms. We also do not know how, why and to what extent each of these mechanisms discussed above can contribute to contaminant removal under a certain set of conditions. Future research should focus on decoding these complex and dynamic interactions of BEBs to be able to systematically engineer BEBs for biological water treatment.

Table 1- Experimental details of Biologically-Enhanced Biochar studies discussed in Section 3

Biochar type and pyrolysis conditions	Biochar properties	Contaminant/Influent	Contaminant removal mechanisms and observations	Process conditions	Ref.
Bismuth-impregnated Wheat straw Production conditions: HTT=500 °C, HR = 1O°C min-^{1}, Residence time= 60 min	SSA: 190.4 m^2 g^{-1}, Avg. pore dia.= 2 nm	Target contaminant: As (III)	• Adsorption by biochar and biosorption by periphytic biofilm, Total As (III) removal rate (90.2–95.4%). • Adsorption removes 60 % As (III) and remaining As by periphytic reactors via calcite, OH and CO groups in biofilm	• Biochar column reactor (565 mL, dia: 6 cm, height: 20 cm), spiral periphyton bioreactor (21.9 m: length, dia: 2.0 mm diameter) • Influent flow rate of = 1.0 mL min-^{1} • Initial As (III) concentrations of 0, 2.0, 5.0, 10.0 and 15.0 mg L^{-1}.	36
Aspen wood (N3) and Softwood (SB) Pyrolysis conditions: n.a	SSA: 4 m^{2}g^{-1} (N3), 189m^2 g-^{1} (SB) Total pore volume: <0.01 mL g^{-1} (N3), 0.12 mL g^{-1} (SB)	Influent: Oil sand process water (OSPW) Target contaminants: Naphthenic acids (NA), Fe, Al, As	. Adsorption, Biosorption, Biodegradation • N3 and SB best-performing among 8 carbon supports; • Biodegradation higher for SB-associated biofilms with NA removal at 87% in presence and 72 % in absence of Fe, Al and As metals •Biosorption enabled up to four times more removal of Fe, Al, and As	• Microbial-biochar attachment assays using 24-well cell culture plates • Initial NA concentration of 200 mg L^{-1}	34
Hardwood (mixture of pine-spruce wood) Pyrolysis conditions: HTT= 800 °C	SSA: 184 m^2 g^{-1}	Influent media: Municipal wastewater Target contaminants: Carbamazepine, Metoprolol, Ranitidine, and Caffeine, Per-and polyfluoroalkyl substances (PFASs)	Adsorption, Biosorption, Biodegradation- • Carbamazepine and metoprolol removal- Biochar> sand-adsorption is dominant. • Ranitidine and caffeine removal- Biochar>Sand-combination of adsorption and biodegradation • Organic matter and nitrogen removal- Biochar with active biofilm> sand filters	• Column filters (diameter 5 cm; height 55 cm) used over a period of 22 weeks Contaminant concentration ranges: • 5400–25000 ng L^{-1} for carbamazepine, • 260-39000 ng L^{-1} for metoprolol, • 2000–10300 ng L^{-1} for ranitidine, • 1800-11000 ng L^{-1} for caffeine • 1500-4900 ng L^{-1} for PFASs	37, 38
Softwood Pyrolysis conditions: HTT= 815-1315 °C. Residence time=1 to 3 s, Used as Biochar-augmented sand filters	Composition: 79% carbon, 12% ash 16% volatile matter	Influent: Synthetic storm water Target contaminant: E.coli	• Small biochar particle size with low volatile matter content and low polarity, low Dissolved Organic Content in media better for E.coli removal • Infiltration rates and initial bacterial composition do not affect E.coli removal • Up to 96 % E.coli removal rate in biochar-augmented sand filters	• Glass chromatography column filters (Kontes, 15 cm length, 2.5 cm diameter) with Teflon fittings at both ends with a built-in mesh (20-µm pore opening) • Initial E.coli concentration- 10^3 to 10^7 colony forming units (CFU) mL^{-1}, infiltration rate constant at 12 cm h^{-1}	40

Feedstock: n.a (commercial biochar) Pyrolysis conditions: n.a	SSA: 104.64±7.80 m^2 g^{-1}	Target contaminant: Bacterial pathogens: Salmonella enterica serovar Typhimurium and Staphylococcus aureus, Bacterial and viral indicators: Escherichia coli and MS2 coliphage	• Electrostatic interactions in bacteriophage removal, Straining and hydrophobic interaction in bacterial removal. • Up to 3.9, 1.9, and 1.8 log10 removal for pathogenic bacteria, E. coli, and MS2, respectively.	• Polyvinyl chloride (PVC) pipes (2.5 × 15 cm) reactors with end fittings and glass wool at both ends • Volumetric flow rate of 1 mL min^{-1} • E.coli and staphylococcus concentration- ~10^5 (CFU) per mL, Salmonella concentration ~10^3 CFU per mL, MS2 ~10^{11} Plaque Forming Units (PFU) mL^{-1} concentrations	45
Quercus wood (commercial biochar) Pyrolysis conditions: n.a	SSA: 210-250 m^{2}g^{-1} Total pore volume 0.12-0.13 cm^{3}g^{-1}	Influent: Synthetic and Urban wastewater Target contaminant: Organic content	• Bio-electrochemical interactions- Biochar outperformed graphite and coke via geobattery mechanisms, • Maximum removal efficiency (92%) and degradation rate (185 g-COD m^{3}d^{-1})) at anodic potential as high as 0.6 V	• Snorkel bio-filters 24 cm high and 3 cm internal dia., total bed volume of 170 cm^3 and a hydraulic volume of 100 mL • Separate three-electrode configuration for electrochemical measurements • Real urban wastewater treated at two different organic loading rates (OLR- 170 mg L^{-1} and 890 mg L^{-1}), two different hydraulic retention times (HRT- 4 and 2 days) and three different anode potentials (short circuit (non-polarized), 0.4 V and 0.6 V vs. Ag/ AgCl	53
Pine wood biochar ESI, BEC and Kiln biochar (KBC) Pyrolysis conditions: ESI- HTT: 500°C for 2 hours BEC- HTT: 700 °C for 30s and 500°C for 15 min KBC - HTT 600°C for 2 hours	SSA: ESI - 167 m^2 g^{-1} BEC - 15 m^2 g^{-1} KBC - 209 m^2 g^{-1} Electrical conductivity: ESI - 2.1 µS cm^{-1} BEC - 4.4 µS cm^{-1} KBC - 4.3 µS cm^{-1}	Biochar mediation and impact on syntrophic associations in ethanol and fumarate media	Direct Interspecies Electron Transfer • Co-cultures of Geobacter metallireducens with Geobacter sulfurreducens (succinate production) or Methanosarcina barkeri (methane production) amended with biochar stimulated syntrophic association leading to higher metabolism of ethanol compared to samples not amended with biochar • Scanning Electron Microscopy suggests electrical connections between the species were through biochar rather than via cell-to-cell electron transfer	•Pure cultures and co-cultures were incubated anaerobically, in 27 mL pressure tubes with 10 mL medium under an anoxic atmosphere of 80:20 of N_2:CO_2	60

***HTT- Highest treatment Temperature, HR- Heating Rate, SSA- Specific Surface Area, Avg. pore dia.- Average Pore Diameter**

4. Design of BEBs

Understanding contaminant removal mechanisms and biochar-biofilm interactions is paramount to design of effective and efficient BEB filters. Producing the right biochar tuned for a specific application, i.e., supporting selected contaminant removal mechanisms (such as adsorption, biodegradation, biosorption, bio-electrochemical interactions) and targeting specific contaminants is of utmost importance for it to be a viable option in water treatment.

Surface area is usually considered to be an important prerequisite for supporting biofilms and contaminant removal. However, there is increasing evidence suggesting that a high surface area alone cannot guarantee high performance of porous-carbon filters, especially where several contaminants are present and multiple dynamic mechanisms in action [34,64,65]. Most microbes measure around 1-2 μm in size and hence cannot directly access micropores (< 2 nm), mesopores (2-50 nm), or macropores (50-200 nm) present in biochar and activated carbon[50,66]. However, while not best suited for direct biofilm formation, the adsorption of nutrients or contaminants in these micro- and mesopores can provide improved conditions for microbial colonisation in adjacent larger pores and interparticle surfaces. At these external surfaces, fluid flow and mass transfer effects play a deciding role in biofilm formation and geometry [67]. Activated carbons displaying high surface areas consist mostly of micropores, making more meso- or macro-porous material such as biochar potentially more suitable for biofilm formation [50,66]. However, the dynamic attachment and growth of microorganisms forming biofilms in three-dimensional porous media such as biochar is not yet fully understood. Thus, an important aspect of BEB design will be to study the effects of biochar pore structure on biofilm establishment and biodegradation. There is growing research in this field to understand and discern this inter-dependence of porous media characteristics (pore size, pore distribution, pore connectivity, roughness, geometry, hydrophobicityhydrophilicity), mass-transfer processes, biofilm proliferation (geometry, homogeneous/heterogeneous distribution of biofilm in porous media), wastewater properties (physico-chemical and biological properties) and cell surface properties [67,68]. This will also help to understand how the competing forces of pore clogging (from the growing biofilm, biodegradation products and contaminants) and biofilm growth and geometry (which needs space for growth and free diffusion of nutrients, oxygen) influence biodegradation, eventually enabling the design of more efficient BEB filters.

Using a biofilm-biochar combination without first considering its specific function may not help in removal/degradation of contaminants in wastewater. The choice of each component and their properties as well as the composition of influent water and operating conditions have to be taken into account. The interplay of these numerous parameters determines whether the presence of a biofilm on biochar enhances or inhibits the rate and extent of contaminant removal. Characterisation techniques and analytical tools suitable to study the complexities of BEB systems are provided in **Table 2**. Control parameters and process conditions which can be used in fine tuning these properties, are shown in **Table 3**. Even though these are properties that have shown to have profound effects on water-borne contaminant removal, additional studies focussing on correlating these properties to their process conditions and BEB properties are necessary to optimise BEBs effectively.

Studying BEB filters with model-based computational tools would be one way to better understand how these various biochar-biofilm properties, process conditions and contaminant efficiency are interdependent. Although a lot of mathematical models and statistical analyses have been used to study biochar properties, microbial biofilms and biofilm reactors individually, studies that integrate all the complexities of biological filters (biochar and biofilm) on a fundamental level are still largely missing in literature[2,53–56].

Table 2 Techniques and analytical tools for BEB characterisation

Techniques and tools	Information
Compositional analysis	
TGA	% (Fixed carbon , volatile matter, ash, moisture)
Elemental analysis	CHNSO composition, biochar stability
ICP-OES/MS, XRF	Mineralogical and elemental composition
XAS (XANES, EXAFS)	Local geometric and/or electronic structure
Surface properties	
Surface profilometer	Topographical analysis
Surface charge analyser	Zeta potential
Contact angle goniometer	Surface wettability
FTIR, Boehm titration	Surface functional groups
XPS	Surface chemistry, bonding information
Morphology and structure	
SEM/EDX	Surface morphology, elemental mapping
XRD, Raman spectroscopy	Crystallinity/amorphous nature
Gas physisorption using N_2, Ar, H_2	Pore-size distribution, surface area
X-ray µ-tomography	Internal 3D structure, pore-connectivity
Electrical and Electrochemical properties	
CV, EIS, other amperometry/potentiometry	Bio-electrochemical interactions, mechanisms
4-point probe resistivity, EIS	Electrical conductivity
Influent/effluent composition, BEB toxicity	
HPLC, LC-MS, GC-MS	Contaminant composition, concentration
Biofilm properties	
16rDNA illumina sequencing, RISE	Biofilm composition-function
SEM, Confocal/Fluorescence microscopy, FISH	Biofilm growth, structure, diversity

TGA- Thermogravimetric analysis, ICP-OES/MS- Inductively Coupled Plasma- Optical Emission Spectroscopy/Mass Spectrometry, XRF- X-ray Fluorescence, XAS-X-ray Absorption Spectroscopy, EXAFS- Extended X-ray Absorption Fine Structure, XANES- X-ray Absorption Near Edge Structure

(XANES), FTIR- Fourier Transform Infrared Spectroscopy, XPS- X-ray Photoelectron Spectroscopy, SEM/EDX- Scanning Electron Microscopy/Energy Dispersive X-ray spectroscopy, XRD- X-ray Diffraction, CV- Cyclic Voltammetry, EIS- Electrochemical Impedance Spectroscopy, HPLC- High Performance Liquid Chromatography, LC-MS- Liquid Chromatography- Mass Spectrometry, GC-MS- Gas Chromatography- Mass Spectrometry, RISE- rRNA intergenic spacer analysis, FISH- Fluorescence In situ Hybridisation

Table 3 Process conditions and biochar production (pyrolysis) conditions crucial for optimising BEB properties, based on literature data

Process conditions	Pyrolysis conditions
Influent concentration, Dissolved Oxygen, Highest Treatment Temperature (HTT), Particle size, pH, Upstream/downstream treatments, Empty bed contact time, Post-treatment	Biomass feedstock, Pre-treatment steps, Temperature, Pressure, Carrier gas, Gas flow rate, Heating/cooling rate

Experimental findings can be used to complement and improve these models and promote a fundamental understanding of how biological filters work under different conditions. Smolin et.al. developed a model to quantify adsorption, biodegradation, and self-bio-regeneration in BAC for organic contaminants[27]. The model allowed a quantitative assessment of cooperative removal (adsorption and biodegradation) of organic matter by taking into account the non-target filling of BAC pores with products of metabolic activity, showcasing potential insights from advanced modelling. This model was applied to evaluate and study the biological filtration of 2-nitrophenol through a BAC filtration unit for 38 months and found that the activated carbon adsorption efficiency was preserved for almost three years as a consequence of bio-regeneration. It was also found that there was an increased non-target porosity loss (metabolic by-products filling pores) with increase in operation time with an estimated porosity loss of 61% of pore volume of fresh sorbent at the end of the experiment.

Correlating changes in microbial community structures and function with changes in biochar properties is an important area requiring more attention as it can help in identifying BEB properties which impact biodegradation capacities. Due to recent technological advancements in molecular biology and data science, there is a fast-growing body of literature on microbial community structures and function in water treatment plants [69–71].

Dalahmeh et. al. found biochar filters to have largely similar microbial community structures and diversity compared to sand filters, but an enhanced diversity in α-proteobacteria and γ-proteobacteria in biochar filters resulted in a larger biodegradation potential for organics[37]. The experiments were performed in column filters of height 55 cm and diameter 5 cm. Additionally, a change in filter media (bark, charcoal and sand filters) for the same process conditions resulted in colonisation by different genera of biofilm-forming bacteria with community structure and potential respiration rate, also changing over time and filter-bed height[72]. It was found that most of the organic biodegradation happened in the top 20 cm for bark and charcoal filters, allowing filter-beds to be shallower compared to sand filters. Similar studies investigated changes in biofilm composition for biological activated carbon supports with different surface chemistry for 4-nitrophenol biotransformation in anaerobic conditons[50]. The results suggest that even though activated carbon cloths with different surface properties induced changes in microbial community structures in the resulting biofilm, the biotransformation efficiencies of 4-nitrophenol for these different types of carbon supports were very similar, i.e. close to 59 %. The microbial composition indicated an enrichment of exoelectrogenic microorganisms (*Geobacter* spp.) over activated carbon cloth surface compared to controls with no support material, as revealed by 16S rRNA amplicon sequencing. These examples reiterate the complexities of biodegradation and the need for more fundamental research decoding these dynamic biochar-microbial interactions.

5. Opportunities for BEBs in water treatment systems

Activated carbon, used for Biological Activated Carbon process, is a benchmark material largely used in tertiary water treatment applications. While biochar is mainly produced from renewable biomass and organic residues, a large fraction of AC production uses non-renewable sources such as coal, a large and cheap carbon source[73,74]. Water treatment and air purification applications alone account for over 77% of the total global AC production[74]. Compared to biochar production, conventional AC production has additional energy requirement which stems from the high-temperature/chemical treatment steps to activate the surface and increase its surface area[75,76]. After use, the exhausted-activated carbon is replaced or regenerated using thermal treatment or steam activation before further reuse [77]. Regeneration is expensive and energy-intensive, accounting for approx. 30% CO_2 equiv. compared to the virgin AC [77–79]. These limitations of AC restrict its widespread use in LMICs, despite its proven efficacy in water treatment applications. This urges us to explore more sustainable options such as BEB for effective water treatment and associated end-of-life treatments.

Countries like India, China, and Indonesia burn agricultural crop residue in the open causing severe air pollution[80–82] . India emitted 211 Tg of CO_2-equivalent GHG emissions in 2017 by burning crop residue alone [83]. Developing biochar-based materials for sustainable water treatment can thus greatly benefit countries with large agrarian sectors. Community-based biochar-based water treatment solutions, ranging from home-scale to large-scale will improve access to potable water while mitigating the impact of burning biomass in the open. There are recent and encouraging studies which demonstrate biochar production using traditional kilns such as 'Kon-Tiki' kilns, especially in rural settings[84,85]. This will mean that there are more sources of potable water, while also ensuring lesser greenhouse gas emissions compared to burning biomass in the open [84,85]. Thus, BEB not only becomes an important material that could become a cheaper and sustianble substitute for BAC in water treatment, but also offers an additional advantage of being a stable carbon sink having the potential to offset the CO_2 emissions from fossil fuels [4,86,87].

5.1 Economic and environmental considerations

To further explore the potential of biochar as a sustainable candidate in biological water treatment, we use published Life Cycle Assessments (LCA) and meta-analysis from literature to compare biochar with activated carbon, a benchmark in tertiary water treatment processes. Since LCAs specific to biological water treatment could not be found in literature, we draw these comparisons using studies which have used a wide range of biochar and AC products, currently used in wastewater treatment. We also point out that possible differences may arise from the interplay of biological mechanisms, which are not accounted for in the selected studies. Special caution is necessary in drawing generalised conclusions from LCAs on specific biochars due to differences in production conditions as well as feedstock sources and therefore biochar properties.

Thompson et al. quantified environmental impacts of biochar produced from wood and biosolids and compared it to various coal-derived AC for use as adsorbents for removal of sulfamethoxazole (SMX), a common antibiotic found in wastewater [76]. The assessment of biochar and AC capacities (low and moderate) was based on the ability of adsorbents to remove SMX, determined as the adsorbent dose required to achieve a 75% SMX adsorption from wastewater effluent following 60 min contact. The results showed that moderate capacity wood biochar had the lowest overall environmental impact, outperforming the other materials on 8 environmental criteria out of the 10 considered. This was largely due to the carbon sequestration potential of biochar and the energy production during pyrolysis of wood biomass. This shows that under specific circumstances, biochar can outperform AC in terms of environmental benefits despite having a lower specific capacity for contaminant removal.

However, biosolid-based biochar had a higher environmental impact compared to both AC and wood-derived biochar due to the high energy requirements in drying biosolids prior to pyrolysis. Here, the specific capacity of biochar to remove SMX was approximately 8.5 times lower than that of activated carbon. Contrarily, when specific capacity of biochar 40 times lower than AC was used in the analysis, activated carbon became an environmentally preferred option[88]. This indicates that not all types of biochar are suitable replacements for activated carbon and conclusions have to be drawn on a case by case basis. It would also be useful to compare sustainability parameters of activated to non-activated biochar, systematic studies of which are currently missing in literature. However, it is important to point out that the contaminant removal in these LCA studies were purely based on adsorption, where biochar has the disadvantage of a lower surface area. The relative performance of biochar and activated carbon is different for biological water treatment, where biofilms regenerate adsorption capacity of the surface and total surface area is less important, and other properties of biochar such as a pore-size distribution may play a more prominent role, making BEBs competitive on performance along with environmental impact.

Results of a meta-analysis study comparing 80 different types of biochar and AC for heavy metal removal showed that biochar had lower energy demands and lower Global Warming Potential (GWP) compared to activated carbon[75]. The average energy demand for production of biochar was considerably lower, at 6.1 MJ/kg, than that for AC, at 97 MJ/kg, mainly due to the energy intensive process of activation and relatively low AC yields, especially when produced from renewable feedstock. Consequently, the average GWP of biochar production was negative at −0.9 kg CO_2-eq/kg due to the carbon-sequestration potential of biochar, while AC had an average GWP of 6.6 kg CO_2-eq/kg [89–91].

In addition to the assessment of environmental performance of biochar *vs* AC as a filter material, the economic perspective is also important to consider. One way to evaluate the economic performance is to compare the cost of removing a certain mass of contaminants. For example, the cost of using biochar as an adsorbent is lower/equal in the case of Ca, Cr, Cd, Zn as compared to AC, with the exception of lead[75]. Average prices cannot reflect the different capacities/efficiencies of biochar materials. This is indicated by the wide price range for biochar from 200-1800 $\$t^{-1}$ as well as for activated carbon from 1100 - 5000 $\$t^{-1}$ [7,75,76,92]. These findings highlight that both environmental and economic performance of biochar will greatly depend on how well the properties of biochar are tuned to suit the final application. Careful evaluation of the water treatment conditions, mechanisms of contaminant removal, properties of contaminants and filter material are crucial in engineering such sustainable water treatment units. Due to a lack of standard production conditions and understanding of contaminant removal mechanisms, comparative economic assessments for biochar and activated carbon are still greatly missing in literature.

5.2 End-of-life for BEBs: Sequential biochar systems

Besides efficiently designing BEBs, research needs to develop novel end-of-life use techniques to reduce any environmental impact. Although the cost of biochar is considerably lower in most cases than that of AC, it is still not negligible, especially for LMICs. Here, we discuss a novel approach to lower the overall cost of biochar in wastewater treatment by considering the whole value chain of the material over the full life cycle.

The concept of sequential biochar systems proposes utilising the multi-functional nature of biochar to integrate different applications into the life cycle of biochar[19]. Due to the diverse applications and variable properties of biochar, it can be economically and environmentally advantageous to recycle biochar and use it for more than one application[93]. As biomass feedstock supply and biochar production are the two highest contributors to the economic costs of biochar, multiple uses of biochar in sequence would offer the benefit of additional revenue streams as well as splitting the production costs and environmental impacts over several use phases[59]. One of the simplest and best-tested approaches is the use of exhausted biochar filter material as a fertiliser in agriculture. This enables the recycling of key nutrients, such as phosphorous and nitrogen from wastewater back to food production[94–96]. However, using exhausted filters for other applications such as agriculture should be done with caution and only after a thorough safety assessment on a case by case basis. This is to avoid any risks associated with potential leaching of toxic molecules (inorganic, organic and biological) incorporated in biochar pores during its prior use in the water treatment process.

More complex sequences are currently under development, and we will discuss two examples here. One feature that all the sequences have in common is the need for the biochar from one application to meet the requirements of the next application. This can be achieved naturally by appropriate sequence of use steps, or by adding an intermediate step to modify biochar properties according to the requirements of the subsequent application.

In the first example, we propose a sequential use of biochar involving biological water treatment and anaerobic digestion (Sequence A in **Fig. 3)**. Here**,** BEB filters are used in biological water treatment in step 2 after pyrolysis. The pyrolysis gas produced in Step 1 in both sequences A and B (**Fig. 3**), could be used to partly provide for other energy requirements of the sequence, such as biomass drying or as source of process heat**.** Biochar is being increasingly used as an additive in anaerobic digestion to increase biogas production [97,98]. Hence, we propose the use of exhausted BEB directly or with physical modifications required to improve biogas production in anaerobic digesters, **Fig. 3**, step 3. The digestate, containing biochar and other nutrients recovered from steps 2 and 3, can be further used in agriculture (Step 4) [99,100]. This sequence might be especially relevant for agrarian communities due to the integration of several spatially related processes within industrial farm settings.

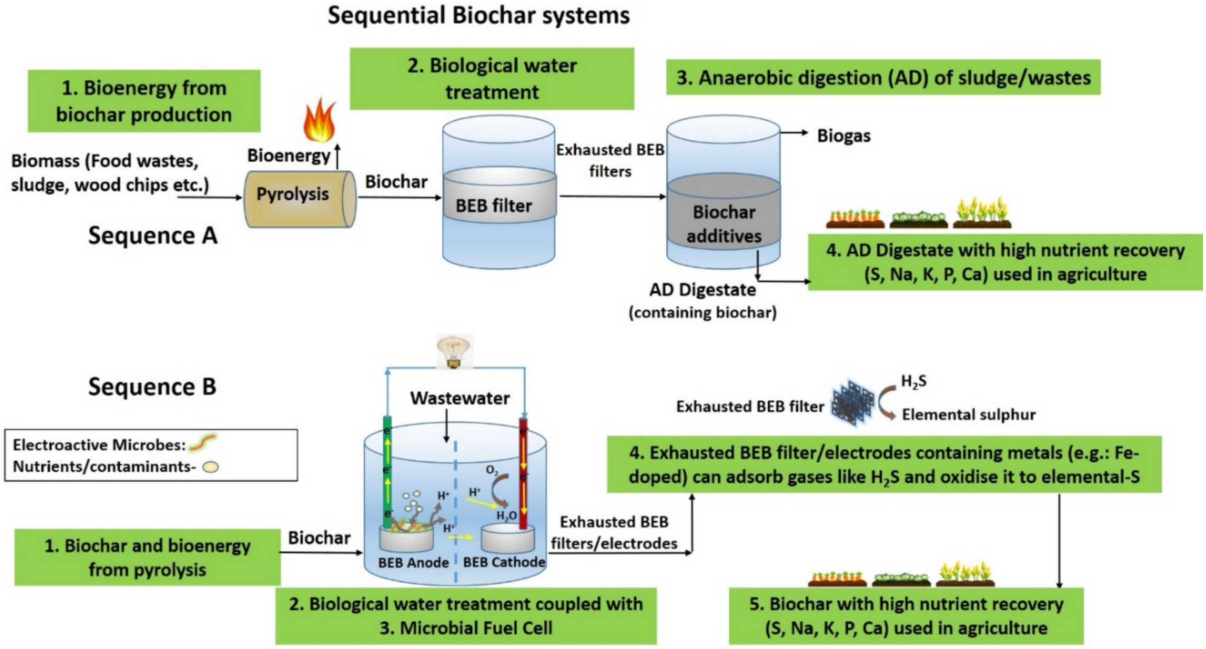

Figure 3 Sequential biochar systems Two examples of sequential biochar systems: Sequence A) Biologically-Enhanced Biochar (BEB) used in wastewater treatment, followed by the use of exhausted-BEB as additive in anaerobic digestion, and the use of digestate containing BEB in agriculture. Sequence B) BEB used in wastewater treatment, coupled with a Microbial Fuel Cell (MFC) for electricity generation using wastewater as the fuel, exhausted-biochar subsequently used in gas adsorption and soil application.

A more advanced example of a sequential biochar system is explained in Sequence B. The electro activity of biochar and electroactive microbes enables its use in Microbial Electrochemical Technologies (METs) such as Microbial Fuel cells for electricity generation and biological water treatment [49,56,101]. Biochar can act as a microbial inoculum carrier and can be used as electrodes (anode and cathode) of a microbial fuel cell, where respiring microbes convert chemical energy to electrical energy [101]. Contaminated water can be used as fuel, where microbes feed on the contaminants in wastewater. This makes them electricity generators as well as biological water treatment units (**Fig. 3**, Sequence B, 2 and 3). Electroactive microbes and the amount of energy they can produce still requires more in-depth study, hence, use of METs are still challenging and in its nascent phase of large-scale development[28]. Biochar works as a strong adsorbent for gases and has the property to filter and convert harmful gases such as H_2S and convert them in to less harmful materials like elemental sulphur [102–104] The exhausted biochar electrodes from MFC/water treatment units can be used with/without additional treatments for use in end applications like gas adsorption/removal **Fig. 3**, step 4) [102–105]. The biochar, with a high nutrient recovery from steps 2, 3 and 4 can then be effectively used as soil additives in agriculture [13,94] (**Fig. 3**, step 5).

6. Challenges and Outlook

Where water treatment with BEB is used as part of a sequence of different treatments, successful establishment of biofilm and its growth during the start-up phase is important. The effects of other upstream treatments such as coagulation, ozonation or chlorination on bacterial growth and density have to be considered, as these treatments can impact biofilm development. For example, residual chlorine in the influent water to a pilot-scale BAC unit reduced the bacterial attachment on its surface, while a pre-ozonation process before BAC treatment improved the biological activity of the biofilm for contaminant degradation in many cases [18,23,106–108]. These aspects should be considered during design of BEB filters for industrial/ municipal applications.

It is important to design filters with pre-specified surface modifications to account for the increasing number and concentration of contaminants in water, including Natural Organic Matter (NOM), Contaminants of Emerging Concern (CECs) such as pharmaceuticals and by-products of biodegradation. This is because high concentration of NOM and variations in nutrition can significantly alter the biofilm growth and efficiencies of biological filters[22,109]. When exhausted filters are used, large amounts of organic matter and nutrients such as ammonia and phosphorus in the effluent water can lead to biofilm formation in the distribution networks[18,26]. It is thus important to adjust the process parameters, frequency and intervals of backwashing and regeneration/change to fresh BEB filters[106]. Competitive adsorption between target contaminants, non-target compounds such as organics and microbial metabolic by-products are a cause of concern for maintaining efficiency of biological filters. Research focusing on the fate and transport of transformation by-products formed during the biofilm metabolism of target contaminants is needed to investigate toxicity risks . The BEB designs should also account for biosorption of these microbial metabolic by-products. We identify several research areas requiring further attention to enable efficient design of BEBs, as illustrated by **Figure 4.**

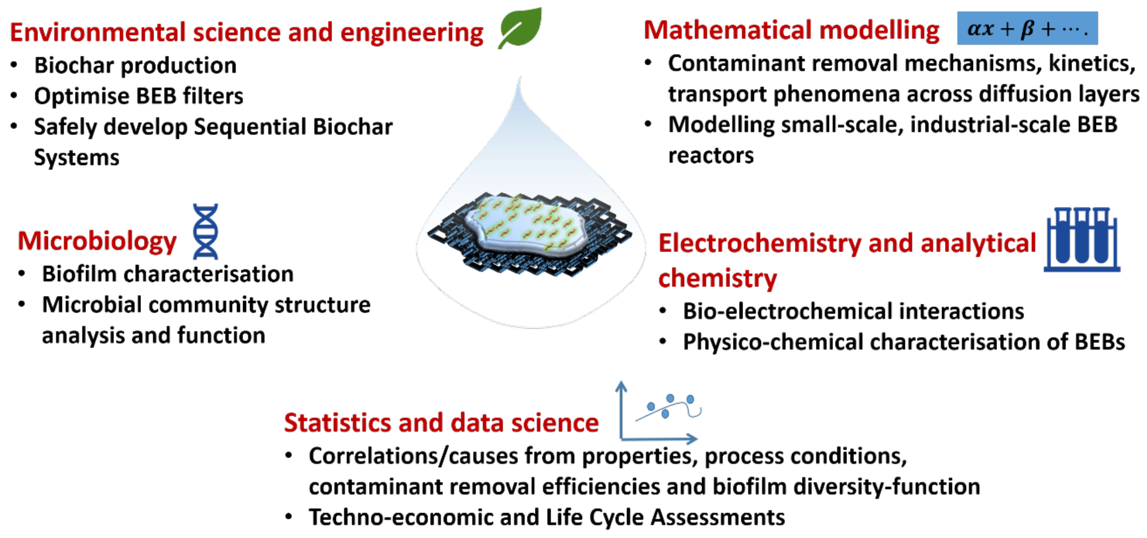

Figure 4 Roadmap to Biologically-Enhanced Biochar (BEB) engineering Schematic identifying the major areas of research vital for systematically engineering BEBs

The presence of toxic and carcinogenic compounds such as Polycyclic Aromatic Hydrocarbons (PAH) and Volatile Organic Compounds (VOCs) that form during pyrolysis and deposit on biochar surface/pores is undesirable[108]. However, production of clean biochar is feasible and can be achieved by tuning a few key process parameters such as peak temperature, feedstock type, residence time and carrier gas flow rate [111–113]. It is important for the biochar production process to be designed and operated in a way that yields biochar with minimal content of these toxic compounds. Biochar production and characterisation should be performed as per EBC and IBI biochar standard guidelines to ensure its safe use, especially in water treatment [114,115]. There is still a lack of research related to standardising biochar production and hence there is an urgent need to have benchmarks and standards for biochar materials, given the large spectrum of feedstock, production conditions and properties of biochar[116].

Carbon pricing is likely to become the norm in the coming years for effective climate change mitigation. Carbon prices will need to be sufficiently high in order to restrict the global warming to less than 2°C. Development of carbon-negative technologies such as biochar can be expected to receive more attention in the future. Apart from having systematic life-cycle assessments and techno-economic analysis specifically for BEBs, these analyses must also account for carbon pricing in correctly estimating the economic and sustainable benefits of biochar for energy and environmental applications, including water treatment. While there are a few studies on biochar stability and aging, more studies should systematically investigate the aging and stability of biochar to truly account for the carbon sequestration potential of different types of biochar[117-119].

7. Conclusions

This review highlights the potential of Biologically-Enhanced Biochar as a sustainable, cost-effective and efficient biological water treatment technology in line with the zero waste concept. Even though BEBs can effectively mediate biodegradation via several complex mechanisms, there is no consensus on why and how certain types of BEBs work better for certain process conditions and contaminant types. A bottom-up approach incorporating research inputs from several disciplines is necessary to fully decode the complex and dynamic biochar-microbial interactions in water treatment. We need to understand how changes in biochar properties and process conditions can bring variations in biofilm microbial diversity. It is also imperative to understand how these changes would reflect in the efficiency and mechanisms of contaminant removal. Research focusing on molecular level interactions of BEB, such as transport phenomena (over several diffusion layers), biodegradation kinetics, biodegradation mechanisms (adsorption, biosorption, biodegradation, bio-electrochemical interaction) and biofilm metabolic potential are necessary. We need more research focusing on toxicity studies of biochar and biodegradation products from BEBs to adopt sequential biochar systems in a safe and cost-effective manner. Mathematical models and statistical tools to support experimental data and accurately correlate the properties and control parameters of an efficient BEB filter are necessary.

If correctly designed, Biologically-Enhanced Biochar filters provide a sustainable solution for water treatment, especially in LMICs with additional economic and societal benefits to local communities. The utilisation of BEB can clearly contribute to meeting many of the United Nations Sustainable Development Goals (SDG) by 2030 and especially SDG 6 (Clean water and Sanitation) and SDG 7 (Affordable and Clean energy).

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Supporting Information

New directions and challenges in engineering Biologically-Enhanced Biochar for biological water treatment

Paper selection for review

Google Scholar Advanced search engine was used to find relevant papers in the subject. Close to 300 papers were screened. When we were faced with choosing papers from multiple options (several papers in the same field), criteria was to choose the paper that best described and discussed the specific point in question. In doing this, care has also been taken to come up with the most recent and highly cited literature that discussed the most recent developments in this field. Journal matrices and following research groups/authors who work in this specific field were very helpful. Missing gaps in literature and the pressing issues associated with these gaps were compiled during this process. This was done using several mind maps and short topic proposals. This was helpful to finalise this specific topic that we chose to review. We were able to find more papers from the reference lists of papers selected in the first screening. The initial screening, paper selection, the specific topic selection, and finally the critical review of relevant literature was all done in a span of 6-8 months, with inputs and discussion from all the contributing authors. The keywords below were rearranged in various combinations, including suggestions from Google scholar after each search, to compile more specific information.

- Water treatment steps
- Biological water treatment
- Activated carbon production
- Biochar production
- Biological activated carbon
- Biochar biofilm interactions

- Microbial biofilms for water treatment
- Biochar characterisation
- Electrical and electrochemical properties of biochar
- Biofilm properties and characterisation
- Modelling biochar properties
- Microbial biofilm modelling
- Biochar Biofilm interactions
- Life cycle analysis of biochar and activated carbon for water treatment
- Environmental comparison of biochar and activated carbon
- Biochar for sustainability
- Nutrient recovery from water treatment
- Biochar for nutrient recovery
- Multifunctional biochar
- Biofilm ecology and biological water treatment
- Biofilm composition and biological activated carbon