



Review Article

Synthesis, modification and antimicrobial potential of biochar and its modifications against water-borne pathogens: A review

Dipro Mukherjee^a, Moumita Sil^b, Arunava Goswami^b, Debasmita Bhattacharya^{c,**},
Moupriya Nag^{d,***}, Dibyajit Lahiri^{d,*}, Kuldeep Sharma^e, Rajan Verma^e

^a Department of Bioengineering, Indian Institute of Technology, Jodhpur, Rajasthan, India

^b Biological Sciences Division, Indian Statistical Institute, Kolkata, West Bengal, India

^c Department of Basic Science and Humanities, Institute of Engineering and Management, Kolkata, University of Engineering and Management, Salt Lake Sector, Kolkata, West Bengal, India

^d Department of Biotechnology, Institute of Engineering and Management, Kolkata, University of Engineering and Management, New Town Sector, Kolkata, West Bengal, India

^e Centre of Research Impact and Outcome, Chitkara University Institute of Engineering and Technology, Rajpura, Punjab, 140401, India

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ABSTRACT

Biochar is a solid substance with a charcoal-like appearance. It is highly flammable and is made from the burning of agricultural and forest-based organic wastes by various controlled processes like pyrolysis. Biochar is rich in carbon and storage of the same in soil is highly recommended to ease off climate change by sequestration of carbon along with enhancing agricultural yield and production of energy. According to the World Health Organization, one of the biggest threats to human life in the present century is livestock water contamination. Among different contaminants, microbial contamination is responsible for several harmful diseases many of which are fatal. The current disinfectant methods are quite useful but they produce harmful by-products which can cause more hazards to human health. Magnetic biochar which is a modification of normal biochar is a green, facile, and cost-effective bactericide that has immense antimicrobial potential against water-borne pathogens. Magnetic biochar in conjugation with quaternary phosphonium salt produces Magnetic Biochar-Quaternary phosphonium salt [MBQ], which is a further modification of magnetic biochar that holds much better antimicrobial properties than biochar or magnetic biochar. It can successfully undergo inhibition of water-borne pathogens like *Escherichia coli* and *Staphylococcus aureus*. MBQ can disrupt the bacterial membrane and induce oxidative damage inside the bacteria, causing their inactivation and inhibition. MBQ also shows biocidal effects. In this review, we will discuss biochar, its properties, various methods of synthesis of biochar, different methods of modification of biochar, antimicrobial and antibacterial properties of biochar, magnetic biochar, and MBQ. Synthesis, Characterization, and antimicrobial properties of MBQ against waterborne microorganisms are also discussed in detail.

1. Introduction

Burning of fossil fuels releases CO₂, which contributes to global temperature rise and impacts global climate. Fossil fuel combustion also produces air pollutants including sulfur and nitrogen oxides (Zhang et al., 2007). The requirement for developing an alternate energy supply to take the place of traditional fossil fuels is being driven by the depletion of fossil fuels, rising oil prices, and the escalation of global

environmental issues. Biomass is the general term for combinations of organic as well as inorganic components derived from living life (Tripathi et al., 2016). In addition to living things like animals as well as plants, animal dung, sludge, and scrap wood are also considered to be a part of biomass. Biomass materials are converted into syngas, biochar as well as bio-oil by thermochemical breakdown processes. Numerous studies are being carried out on the development, improvement, and uses of gas products which include syngas as well as liquid products

* Corresponding author.

** Corresponding author.

*** Corresponding author.

E-mail addresses: debasmita.bhattacharya@iem.edu.in (D. Bhattacharya), moupriya.nag@uem.edu.in (M. Nag), dibyajit.lahiri@uem.edu.in (D. Lahiri).

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including bio-oil, both of which are considered best as fossil fuel alternatives (Ko et al., 2012). Since carbon absorption takes place during photosynthesis, biomass is regarded as a carbon-neutral substance as it offsets the CO₂ that it emits. As it comprises less S and N biomass is also recognized to have a reduced impact on the environment emitting relatively a smaller number of SO_x and NO_x compared to fossil fuels (No, 2014). According to the International Biochar Initiative biochar is described as a solid material generated when biomass is carbonized. Multiple researches have been conducted to produce novel biomass uses because biochar is affordable, environmentally safe, and effective for a wide range of purposes such as soil restoration, managing waste, reducing greenhouse gas, and energy generation (Zhang et al., 2013). Aside from carbon [C], which makes up most of the biochar, it also comprises oxygen [O], ash, hydrogen [H], and minute amounts of nitrogen [N] as well as sulfur [S]. The elements contained in the product depend on the properties of the carbonization process as well as on the raw biomass that was used to produce the biochar (Abdoulmoumine et al., 2015). Since biochar is rich in minerals and has high porosity with relatively large surface area and functional groups, it has been employed as an accelerator for removing tar or producing biodiesel. It serves as a soil amendment as well as an adsorbent for contaminants in water and air. There have also been recent publications concerning the application of biochar in fuel cells, supercapacitors, as well as several other devices (Richardson et al., 2012). Despite various uncertainties of using biochar like its unknown capacity, impact, and sustainability at a global scale; biochar remains a highly suggested and researched way towards net zero emissions (Lehmann and Joseph, 2009). There are multiple focuses on biochar as a novel strategy towards agricultural net zero emission. Various research works are carried out on the properties, preparation, and modification strategies of biochar, different methods by which biochar removes pollutants, applications of biochar with special emphasis on agricultural fields of India, and the limitations of biochar (Liu et al., 2015). Before discussing examples of possible uses of biochar, this article reviews past research on modifying biochar to enhance its qualities as well as the thermochemical degradation processes for producing biochar (Duku et al., 2011). Most of the reviews regarding the uses of biochar focus on utilizing biochar for removing contaminants from soil as well as from water (Ahmad et al., 2014a). Biochar approaches for removing hazardous pollutants, which include air contaminants and tar, are described in depth. This is in contradiction to several other newly emerging application sectors, like fuel cells, hydrogen storage, and supercapacitors (Kambo and Dutta, 2015). This article briefly discusses the emerging research advancements in such domains.

Waterborne pathogens are a major health problem globally which is responsible for over 485,000 deaths annually, causing diseases like cholera, typhoid, and dysentery affecting low-income regions severely. Traditional methods of water purification, that includes chlorination, filtration, and UV sterilization, have multiple limitations like inability against a number of pathogens, economic burden, membrane fouling, and secretion of harmful by-products. Moreover, emergence of antibiotic-resistant bacteria complicates the existing treatment strategies even further. Biochar, a carbon-rich material derived from biomass pyrolysis, gives us with a sustainable and cost-effective natural method that removes pathogens, heavy metals, and organic pollutants along with being effective against antibiotic resistance. Its antimicrobial potential, low cost, and scalability makes it a promising solution to overcome the challenges of traditional water treatment, especially in resource-limited conditions (Qiu et al., 2022).

2. Biochar and its various methods of synthesis

Biochar, a carbon-rich material formed by pyrolysis of biomass, carries certain unique properties which makes it highly effective for antimicrobial applications against waterborne pathogens. The high porosity and surface area of biochar is extremely beneficial for microbial

adsorption. Moreover, the surface functional groups (e.g., hydroxyl, carboxyl, and carbonyl) of biochar contribute significantly to pathogen removal by disrupting their microbial metabolism. The negatively charged surface of biochar allows electrostatic interactions with the pathogens, causing aggregation and inactivation. Again, biochar form reactive oxygen species (ROS) that can damage their cell membrane and disrupt their nucleic acids, enhancing its antimicrobial efficacy even more. Its strong hydrophobicity and adsorption property allows it to remove essential nutrients and toxins, limiting pathogen growth. These characteristics of biochar, along with its modifications using metal nanoparticles or chemical activation, makes biochar an excellent natural alternative for water treatment and environmental (Batista et al., 2018).

The properties of various distinct carbonization techniques used for producing biochar are outlined in this section [Fig. 1].

2.1. Pyrolysis

Organic substances can be broken down by a method called pyrolysis at temperatures between 300 and 900 °C in the absence of oxygen (Shen, 2015a). Biomass molecules such as cellulose, lignin, and hemicellulose each go through a distinct set of reactions throughout thermal degradation, comprising of cross-linking, depolymerization, as well as fragmentation at their respective temperature, resulting in the production of solid, gaseous, and liquid by-products (Mohan et al., 2014). The end products are known as char and bio-oil, respectively. The gaseous mixture comprising CO, H₂, CO₂, and C1–C2 hydrocarbons are known as syngas (Konwar et al., 2014). The characteristics of the raw biomass source as well as the pyrolysis procedures influence the pyrolysis product yields (Lone et al., 2015). The residence time, reaction temperature, and heating rate are factors affecting how the pyrolysis procedures develop their by-products. As temperature during pyrolysis increases, the production of biochar generally declines while the amount of syngas turnout increases (Yu et al., 2014). According to studies conducted by several scientists, during pyrolysis the amounts of biochar as well as acidic functional groups as outputs fell when the temperature was raised however pH, and carbon stability, ash content rose. It was determined that reducing organic functional groups, including –COOH as well as –OH, was what caused the pH to rise when temperature was raised during pyrolysis (Elleuch et al., 2013). Because cracking occurs at elevated temperatures, the bio-oil output was maximum at about 500 °C. The elemental contents of biochar are generated at various pyrolysis temperatures. Given how quickly the temperature rises, pyrolysis reactions might be classified as slow or fast. Continuous vapor-phase responses boost the char output in slow pyrolysis, where the pyrolyzed vapors stay in the reactor for a prolonged time during low temperatures (Gupta et al., 2015). The features of the chars formed at two

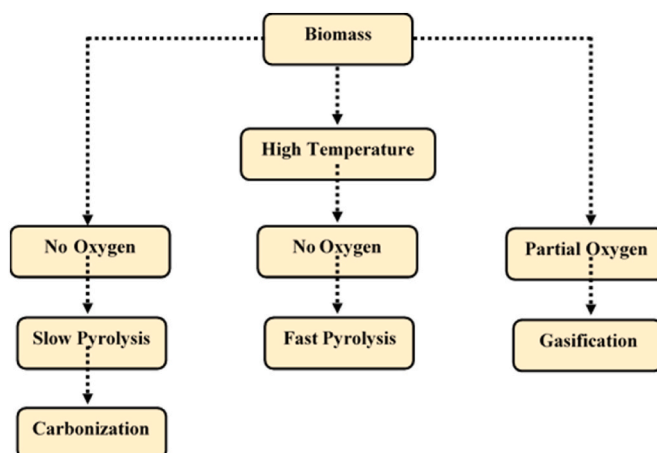


Fig. 1. Various ways for biochar production.

distinct temperatures rapidly increasing with rates ranging from 5 °C per minute and 60 °C per minute were evaluated by several researchers. They stated that the char formed at the relatively high temperature with a rising rate exhibited a lesser volatile matter percentage while having a greater content of ash [which includes fixed carbon], and they concluded that an elevated temperature increasing rate is preferred with regards to the quality biochar produced (Inyang and Dickenson, 2015). An elevated temperature during pyrolysis. Fast pyrolysis hence typically aims to produce a liquid product with a high yield. According to scientists, who studied the impacts of residence time on the constituents' resultant products in a pyrolysis procedure, concluded that the production of biochar dropped with longer residence times while maintaining the constant pyrolysis temperature (Tan et al., 2015). According to a study conducted by numerous researchers on the impacts of residence time on unique surface area as well as on pore properties of biochar, these characteristics rose with rising residence time up to 2 h at 500–900 °C (Tan et al., 2015). but declined after the residence time reached 2 h. Once the residence time at extreme temperatures was longer than 2 h, specifically, the surface area as well as pore area significantly dropped (Gul et al., 2015). It was reported that when the residence period was raised from 30 min to 1 h at a pyrolysis temperature of 950 °C, the specific surface area was reduced from 141 m²/g to 125 m²/g and the pore volume was reduced from 0.209 cm³/g to 0.187 cm³/g (Lehmann et al., 2006). The findings of several researchers concluded that constriction and closing of the pore entrances caused by the sintering of char, decreased the specific surface area (Beesley et al., 2011).

2.2. Gasification

Carbonaceous components, including biomass, coal, and plastic materials, are transformed into gaseous products employing gasification substances [air, CO₂, steam, O₂, or gas mixture] through the thermochemical partial oxidation process known as gasification (Jeffery et al., 2011). Gasification generates liquid [includes tar & oil], gaseous [H₂, carbon monoxide, carbon dioxide, N₂, etc.], as well as solid [char & ash] by-products. Although gasification is designed to produce gaseous compounds only as products, it produces relatively lower yields when compared to that of fast pyrolysis [15–20%] and is only around 5–10 percent of the mass of the raw biomass material (Lehmann et al., 2011).

As stated below, the process of gasification may be broken down into several stages, although every stage cannot be easily distinguished from the rest with respect to temperature as well as pressure [Fig. 2] (Lee et al., 2016).

2.3. Drying

In this process, the moisture in the biomass is evaporated without any energy being retrieved for drying (Han et al., 2016). Depending on the biomass material that is employed during gasification, the moisture content of the biomass fluctuates. An additional drying step must be performed when the moisture level of the biomass is relatively high before the biomass is added to the gasifier (Heidari et al., 2014).

2.4. 2nd Pyrolysis

The thermally vulnerable components of biomass, like lignin, are broken down during pyrolysis, which takes place between temperatures ranging from 150 to 400 °C resulting in the production of char, gases,

and liquids [Fig. 3] (Shafaghat et al., 2016).

During pyrolysis, gases like H₂, carbon monoxide, CO₂, water, and small-molecular-mass hydrocarbons like CH₄ are produced (Inguanzo et al., 2001a). Most of the liquid result is mucous tar made of organic material with relatively small molecular weights (Demirbas, 2004). The reaction temperature, rise in rate of temperature, and pressure all affect the composition of the product.

2.5. Oxidation/combustion

A significant source of the energy needed for gasification reactions is the oxidation as well as combustion of certain gas species, as well as char (Chen et al., 2003). The gasification agent added to the gasifier interacts with combustible materials producing carbon monoxide, CO₂, and water, and with the char created during the pyrolysis process yielding carbon dioxide (Mohammad et al., 2013).



2.6. Gasification

Through several gasification processes, the char generated during the pyrolysis stage is transformed into carbon monoxide, CH₄, and hydrogen (Ayllon et al., 2006). Depending on how the gasification agent, as well as biomass, come into contact, the gasifiers—where the gasification reactions occur—are split into fixed bed reactors, fluidized bed reactors, moving bed reactors, and entrained bed reactors (Zhang et al., 2015). The reaction temperature, pressure gasification agent-biomass ratio, and gasification agent type are all factors influencing gasification reactions. Many researchers claimed that as the temperature rose, more hydrogen, carbon monoxide, and carbon were produced while less tar, carbon dioxide, CH₄, and hydrocarbons were produced (Lu et al., 1995). In addition, a group of scientists showed that the content of tar in gas was elevated and carbon conversion decreased when the gasification temperature was lowered in fluidized bed gasification (Lee et al., 2013a). The gasification agent employed in the gasification process affects not only the conversion of carbon but also the constitution and heating value of the resulting gas (Inguanzo et al., 2001b). When [H₂O] and [CO₂ and O₂] were used as reducing and oxidizing agents respectively during gasification a research team investigated how gasification agents affect the properties of the char (Abu El-Rub et al., 2008). When water was employed, the ratio of small to massive aromatic rings present in the aromatic compounds making up the char was relatively low and the amounts of alkaline metals like Magnesium and Calcium were higher (Boucher, 2000).

2.7. Hydrothermal carbonization [HTC]

When the moisture content of biomass is relatively low, dry procedures like pyrolysis as well as gasification can give significant product yields while wasting little energy (Bandosz and Block, 2006). However, since the majority of biomass materials have significant moisture contents, an additional drying phase is necessary to produce end products with higher yields while using less energy during the process (Lee et al., 2013b). It is predicted that hydrothermal procedures will be capable of overcoming this weakness of dry processes. The word “hydro-char” is



Fig. 2. The process of gasification.

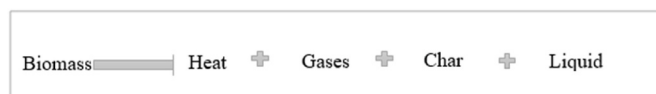


Fig. 3. Principal components of biomass.

frequently applied for differentiating biochar generated during hydrothermal procedures from biochar generated through dry processes (Brewer et al., 2009). Throughout this article, hydro-char is referred to as "biochar".

Inside a closed reactor, H₂O, as well as biomass, are combined, and after a predetermined amount of time, the temperature is adjusted to stabilize the process (Taba et al., 2012). Additionally, more pressure is required to keep water in a liquid form above 100 °C. The primary by-products of a hydrothermal process rely on the temperature under saturated pressure and are, accordingly, biochar, gaseous products, such as carbon monoxide, CO₂, hydrogen and CH₄, and bio-oil (Gomez-Barea et al., 2013).

As a result, the hydrothermal process is referred to as hydrothermal gasification [HTG], hydrothermal carbonization [HTC], and hydrothermal liquefaction [HTL], depending on the range of temperature (Tay et al., 2013). According to reports, the primary factors affecting the characteristics of products include residence time, reaction temperature, the water-to-biomass ratio, pressure, and residence duration (Roman et al., 2012). The char made using the HTC process possesses a higher amount of carbon compared to the char formed using dry techniques (Xiao et al., 2012).

2.8. Other thermochemical technologies

Along with the previously studied hydrothermal, pyrolysis, carbonization, gasification, and numerous other thermochemical conversions of biomass processes include flash carbonization as well as torrefaction. To convert biomass into gas-as well as solid compounds, flash fire is started on a dense bed of biomass where a high pressure [1–2 MPa] is maintained (Chana et al., 2014). The typical temperature as well as reaction time employed in a flash carbonization ranges from 300 to 600 °C and 30 min (Kruse, 2009). According to reports, at 1 MPa, roughly 40% of biomass is turned into a compound [biochar], while carbonization time shortens with pressure (Sabio et al., 2016). However, there aren't as many publications regarding flash carbonization as there are for different biochar synthesis methods (Antal et al., 2003). Torrefaction is the removal of moisture, CO₂, and O₂ from biomass while maintaining an inert environment ranging from 200 to 300 °C. Long strands of polysaccharides are then depolymerized to create a hydrophobic solid possessing a low O/C ratio (Nunoura et al., 2006). Mild pyrolysis is the term used to describe this process because it is often carried out at a delayed heating rate. Torrefaction temperature, as opposed to residence time, has been found by several researchers to have a greater impact on the chemical as well as on the thermophysical characteristics of the product. It also contributes to decreasing biomass weight (Wade et al., 2006). The primary goal of research on torrefaction is to increase the thermochemical characteristics of biomass utilized in co-combustion, combustion, and gasification with coal. Table 1 describes the advantages and limitations of the different synthesis methods of biochar.

3. Modifications of biochar

Thermally treated biochar is frequently activated before use to improve its specific surface area as well as pore fraction or to create functional groups within itself. Physical activation as well as chemical activation are the two categories for the activation techniques (Chen et al., 2016). The kind as well as amount of biomass combined with activating gas have an impact on the pore fraction and pore size distribution of the activated biochar (Benavente and Fullana, 2015). The different methods of biochar modification are as follows.

3.1. Physical activation

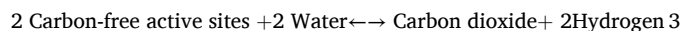
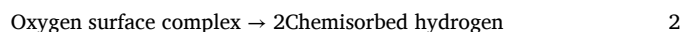
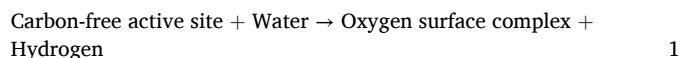
At temperatures above 700 °C, biochar is activated physically [also known as gas-activated] using gases like steam, carbon dioxide, and

Table 1
Advantages and limitations of the synthesis method of biochar.

Synthesis Method	Advantages	Limitations
Pyrolysis	<ul style="list-style-type: none"> - Produces biochar, bio-oil, and syngas. - High carbon content and stability. - Scalable process. 	<ul style="list-style-type: none"> - Requires controlled conditions (temperature 300–900 °C). - Efficiency depends on feedstock and heating rate. - Residue ash content varies.
Gasification	<ul style="list-style-type: none"> - Generates syngas with lower tar content. - Produces biochar as a minor product. 	<ul style="list-style-type: none"> - Low biochar yield (5–10%). - Higher complexity in maintaining conditions. - Not ideal for high biochar production.
Hydrothermal carbonization (HTC)	<ul style="list-style-type: none"> - Suitable for high-moisture feedstocks. - Produces hydrochar with high carbon content. - No drying step is required. 	<ul style="list-style-type: none"> - Requires high pressure and temperature control. - May produce liquid by-products requiring disposal.
Flash carbonization	<ul style="list-style-type: none"> - Rapid process. - Produces biochar with a high surface area. 	<ul style="list-style-type: none"> - Limited research and scalability. - High-pressure requirements (1–2 MPa).
Torrefaction	<ul style="list-style-type: none"> - Mild pyrolysis. - Produces hydrophobic biochar with a low oxygen-to-carbon ratio. 	<ul style="list-style-type: none"> - Lower yields compared to pyrolysis. - Limited to specific applications like co-combustion.

ozone (Chioua et al., 2016). There are two steps in the physical activation process (Agarwal et al., 2003). In the first phase, the interior surface area is increased by selectively decomposing the unstructured sections of the carbonized material while opening tiny pores encased in the carbon framework (Nimlos et al., 2003). The second step comprises activation reactions depleting crystalline carbon comprising of carbonized material or C with small pores, creating enlarged pores. Pore development during physical activation is deeply linked to the removal of carbon through reactions (Meyer et al., 2011).

The following describes the reaction mechanism of steam-based carbon activation:



The amount of ash present in the carbonized material and the condition of the pores affect how quickly the pores are formed when they are physically activated (Jung and Kim, 2014).



The adhesion to the solid carbon surface is depicted by the parentheses in Equations (4) and (5). When oxygen bound to the surface of carbon combines with the C thus generating carbon monoxide, water vapor decomposes to release H₂ gas from the carbon surface (Manocha et al., 2010). The H₂ gas produced may stay close to the carbon surface and slow down the reactions in the following ways:



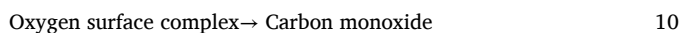
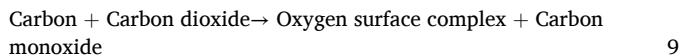
The below equation can be used to illustrate the rate of activation by water in this situation:

$$\text{Rate}_{\text{C-H}_2\text{O}} = \frac{k_1 P_{\text{H}_2\text{O}}}{1 + k_2 P_{\text{H}_2}^{1/2} + k_3 P_{\text{H}_2\text{O}}} \quad 7$$

According to the following reaction equation, carbon dioxide is used to activate carbon:



Like the steam-based activation process, the CO produced by the reaction illustrated in Equation (Duku et al., 2011) – (Kambo and Dutta, 2015) may stay close to the surface of the carbon to hinder additional reactions (Hui and Zaini, 2015).



The removal of the oxide complex by carbon monoxide produced during CO₂ activation from the surface of carbon results in non-uniform gasification, which reduces the rate of C–CO₂ reaction (Cha et al., 2010). However, when uniform gasification is taken under consideration, the reaction rate of C–CO₂ can be represented as:

$$\text{Rate}_{\text{C-CO}_2} = \frac{k_1 P_{\text{CO}_2}}{1 + k_2 P_{\text{CO}} + k_3 P_{\text{CO}_2}} \quad 12$$

The biochar that has been activated, the reaction parameters, and the activating gas, all affect the characteristics of physical activation (Shen et al., 2015). Utilizing steam and Carbon dioxide, a group of scientists were able to activate the biochar produced by the pyrolyzing coffee endocarp (Kastner et al., 2009a). They claimed that using steam increased the activation rate while using CO₂ increased the specific surface area as well as pore size. A research team also employed Carbon dioxide and steam as the activating gases in an experiment of the carbonizing olive seed to obtain activated carbon (Gonzalez et al., 2009). They also compared the outcomes of these experiments. Pure steam-produced activated carbon exhibited a relatively small micropore volume compared to carbon dioxide-produced activated carbon [CO₂-activated carbon] (Gonzalez et al., 2009).

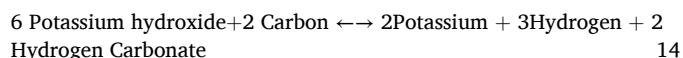
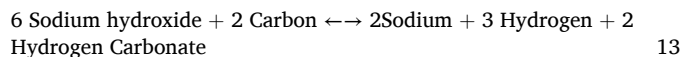
However, when employed at elevated heat, diluted steam acquired a microporosity resembling that of CO₂-activated carbon (Shim et al., 2015). Furthermore, they claimed that steam activation was more effective than CO₂ activation at creating meso-as well as macro-porosity, resulting in larger pores (Azargohar and Dalai, 2008). A bunch of researchers also investigated how CO₂-activation temperature as well as time affects the properties of biochar obtained from the rapid pyrolysis of corn stover, waste oak wood, and corn hulls (Nabais et al., 2008). With rising activation temperature, all three forms of chars demonstrated an increased specific surface area as well as micropore volume. However, based on the initial biomass material, the activation duration had varying impacts (Toles et al., 2000). Compared to the other 2 chars that exhibited a decrease in specific surface area when the activation duration was increased, the char made from oak wood waste demonstrated an increasing specific surface area when the activation duration was increased (Ros et al., 2006). To study how the activation temperature, duration, and Carbon dioxide flow rate affect the micropore volume, specific surface area, carbon fraction, and total pore volume of char, a group of scientists produced char at 600 °C by pyrolyzing coconut shell and then it was activated by utilizing carbon dioxide keeping a temperature at a range of 750–950 °C (Zhang et al., 2014). In cases where the activation temperature was less than 900 °C, the specific surface area as well as micropore volume of the char produced showed a rapid increase when the activation temperature rose (Zhang et al., 2004). But, when the activation temperature reached 900 °C, the specific surface area as well as micropore volume declined while there was an increase in the mesopore volume when activation temperature rose. All three mesopore volumes, specific surface area, and micropore volume were increased, within 5 h of activation (Guo et al., 2009). Unfortunately, activation for longer than 5 h resulted in the pores

contracting, which reduced the specific surface area as well as pore volume. The char production dropped as the carbon dioxide flow rate increased owing to the carbon burn-out (Kastner et al., 2009b). The results showed that the parameters influencing the process showed an impact on char activation since an elevated carbon dioxide flow rate below a particular threshold encouraged pore formation while an excess flow over that threshold reduced the specific surface area as well as pore volume (Jimenez-Cordero et al., 2015).

3.2. Chemical activation

In chemical activation, char is doped with a chemical agent and micropores are formed by subsequent dehydration and oxidation (Reinoso et al., 1995).

During chemical activation, char is loaded with a chemical agent, and then subsequently dehydrated and oxidized to create micropores (Ncibi et al., 2009). The activation effectiveness of chemical activation is significantly greater compared to that of physical activation, even though it has more disadvantages than physical activation, including the chemical corrosion of the apparatus, difficulties in recovering chemicals, as well as expensive chemical costs (Tay et al., 2009). The typical chemical agents utilized for the chemically activating char include basic substances like potassium hydroxide, sodium hydroxide, NH₃, Potassium carbonate, and ZnCl₂, along with acids like phosphoric acid, sulphuric acid, and Hydrochloric acid (Wu et al., 2005). The interaction between carbon and alkali metal activating agents is shown in equations [n] and [o].



The kind and dosage of the activating agent as well as the activation temperature are factors that affect the production of biochar when chemically activated (Foo and Hameed, 2012). To investigate how the char derived from sewage sludge is affected by activating agents, a research team employed Carbon dioxide as the activating agent during physical activation and Phosphoric acid, sodium, and potassium hydroxide, as the activating agents in the chemical activation method (Park et al., 2013). To their research, Phosphoric acid failed to enhance the specific surface area as well as pore volume of sludge char, using carbon dioxide during the activation process resulted in a significantly lesser rise in specific surface area as well as pore volume compared to activating agents derived from alkali metals (Hayashi et al., 2002). When sodium or potassium hydroxide was utilized, there was an increase in the specific surface area as well as in the pore volume with increasing doses of activating agent; specifically, when the sodium hydroxide: char ratio was raised from 1:1 to 1:3, there was an increase in the specific surface area as well as pore volume from 689 m²/g to 1224 m²/g as well as from 0.29 cm³/g to 0.44 cm³/g, respectively (Angin et al., 2013). The specific surface area as well as pore volume rose from 853 m²/g to 1686 m²/g and from 0.34 cm³/g to 0.64 cm³/g, respectively, when the potassium hydroxide: char ratio was raised from 1:1 to 1:3 (Zhang et al., 2005). Thus, KOH performed better when compared to NaOH at enhancing the properties of sludge char.

A research group examined how activation temperature [600–800 °C], as well as activating agents [Potassium carbonate and Potassium hydrogen], affect the production of activated carbon from soybean oil cake (Diergaardt et al., 2004). They claimed that compared to Potassium hydroxide, K₂CO₃ produced activated carbon with a greater yield, reduced ash, and sulfur levels, and a higher pore fraction (Pernak et al., 2003). When potassium carbonate was employed, pore volume, and microporosity, increased with increasing activation temperature, however when KOH was used, microporosity dropped when activation temperature was increased (Zhang et al., 2019a). Even

though both activating agents yielded a greater increase in specific surface area at when the activation temperature was increased, the impacts of the activation temperature were substantially bigger for potassium carbonate compared to that of KOH. K_2CO_3 was used to achieve the highest specific surface area of the activated carbon, which was $1352.86 \text{ m}^2/\text{g}$, like those that are commercially available activated carbon (Winward et al., 2008). $ZnCl_2$ was employed as the activating agent by a group of researchers for activating biochar produced when safflower seed press cake was pyrolyzed for studying how the activation temperature as well as impregnation ratio affect the properties of biochar and methylene blue dye adsorption (Lee and Westerhoff, 2009). In that investigation, the porosity, adsorption ability, as well as surface area of the activated biochar, rose when the activation temperature [600–900 °C], as well as the impregnation ratio, was raised [1:1–4:1] (Sousa et al., 2017). The biochar derived from pyrolyzing cotton stalk was activated in a study by two distinct activating agents, carbon dioxide and NH_3 , and the carbon dioxide adsorption capabilities of both the two activated biochar were evaluated (Zhang et al., 2019b). After the adsorption temperature reached 20 °C, CO_2 adsorption rose as the activation temperature was raised, and the biochar activated through carbon dioxide adsorbed more Carbon dioxide when compared to NH_3 - or $CO_2 + NH_3$ -activated biochar (Zhu et al., 2017). This was explained by the capacity of carbon dioxide to improve micropore structure as well as to generate heteroatom groups, such as C–O as well as C=O, especially when activation temperatures were high, which are more potent active sites compared to any nitrogen functional groups (Cai et al., 2011). Table 2 below, elaborates on the recent work on the physical activation, and chemical activation methods for biochar production found in literature (see Table 3).

4. Antimicrobial potential of biochar

Microbial contamination and microbial infections are a serious problem in the present century. According to the World Health Organization, approximately 25% of the total population of the world does not have access to clean water (Chang et al., 2014). The contaminated water consists of a lot of microorganisms like *Salmonella enteritidis*, *Bacillus dysenteriae*, *Vibrio cholera*, *Escherichia coli*, and others. Use of this contaminated water may lead to several waterborne diseases some of which are even fatal (Freiberg and Zhu, 2004). The conventional methods for disinfecting the contaminated water involve the use of free chlorine, chloramines, and ozone. To some extent, these disinfectants can stop the pathogens and inhibit their further growth (Lei et al., 2018). But at the same time, these disinfectants tend to form several

by-products; which are harmful for the human body. Moreover, they cannot carry out the complete inhibition of these pathogens.

Modifications can be done on biochar through various chemical, physical, and biological methods to increase its antimicrobial properties. Increasing its active surface sites, introducing functional groups, and improving adsorption capacity are some of them. Chemical modifications like acid/base treatments, oxidation, and metal doping can include new functional groups and antimicrobial ions for disrupting bacterial membranes and induce oxidative stress. Physical modifications such as steam activation, thermal treatment, and plasma exposure increases porosity, surface area, and charge interactions, and improves the microbial retention time and biofilm inhibition. Biological modifications such as microbial functionalization and enzyme immobilization, forms antimicrobial agents which inhibit growth of several pathogens. All these modifications improves the antimicrobial potential of biochar as a whole by increasing the microbial adsorption, causing oxidative damage, releasing toxic metal ions, and changing the surface charge to restrict bacterial adhesion, making biochar an ideal alternative material for wastewater treatment and microbial control (Hu et al., 2019).

Biochar possesses a number of antimicrobial properties across bacteria, viruses, and protozoa, due to its high surface area, porosity, and chemical functionality (Xiang et al., 2022). Against bacteria, biochar can adsorb bacterial cells, damage cell membranes, and up-regulate reactive oxygen species (ROS) levels, inhibiting a number of bacterial pathogens like *Escherichia coli* and *Staphylococcus aureus*. Against viruses, biochar can immobilize viral particles and denature viral capsids. Metal-doped biochar has the ability to degrade viral proteins or genomes, showing high potential against SARS-CoV-2-like enveloped viruses. The anti-protozoal properties of biochar is often used in treating wastewater, where biochar captures and neutralizes protozoan cysts and trophozoites, for example in *Giardia lamblia* and *Cryptosporidium parvum*. All these showcases the potential of biochar for bioremediation and wastewater treatment. However, further research is needed for conducting optimizations and modifications against specific pathogens.

In comparison to various other water treatment materials, such as activated carbon and nanoparticles, biochar offers some unique advantages and some limitations. Just like activated carbon, biochar has an extremely high surface area as well as porosity for adsorbing contaminants, however it is more cost-effective and sustainable because it is formed from biomass waste. But it should also be noted that activated carbon normally has a more consistent surface chemistry and enhanced adsorption capacity for a number of pollutants and nanoparticles provide highly targeted antimicrobial effects and can be synthesized for specific pathogens. However, all these have their own challenges,

Table 2
Recent studies on physical and chemical activation methods for biochar production.

Sl No	Type of Biomass	Method of Activation	Chemicals	Operating Conditions	Key Findings	Ref.
1.	Pinewood	Physical	None	Pyrolysis: 700 °C Activation: CO_2 (800 °C, 2 h)	Surface area: $800 \text{ m}^2/\text{g}$ Function: Adsorption	Sajjadi et al. (2019)
2.	Coconut shell	Physical	None	Pyrolysis: 600 °C Activation: Steam (800 °C, 2 h)	Surface area: $950 \text{ m}^2/\text{g}$ (microporous) Function: Gas adsorption	Gale et al. (2021)
3.	Rice husk	Chemical	Potassium hydroxide	Impregnation: KOH (1:1 ratio) Pyrolysis: 600 °C, 1 h	Surface area: $1500 \text{ m}^2/\text{g}$ Function: Heavy metal removal	Siipola et al. (2018)
4.	Sawdust	Chemical	Phosphoric acid	Impregnation: H_3PO_4 (1:2 ratio) Pyrolysis: 450 °C, 2 h	Surface area: $1200 \text{ m}^2/\text{g}$ Function: Dye adsorption	Díaz et al. (2024)
5.	Corn stover	Physical	None	Pyrolysis: 600 °C Activation: CO_2 (900 °C, 1 h)	Surface area: $700 \text{ m}^2/\text{g}$ (mesoporous) Function: Catalyst support	ATEŞ, (2025)
6.	Bamboo	Chemical	Zinc chloride	Impregnation: $ZnCl_2$ (1:1.5 ratio) Pyrolysis: 550 °C, 1.5 h	Surface area: $1100 \text{ m}^2/\text{g}$ Function: Adsorbing organic pollutants	Johnston (2018)
7.	Olive stones	Physical	None	Pyrolysis: 600 °C Activation: Steam (800 °C, 2 h)	Surface area: $850 \text{ m}^2/\text{g}$ Function: CO_2 capturing	Wang et al. (2022)
8.	Peanut shells	Chemical	Sulphuric acid	Impregnation: H_2SO_4 (1:1 ratio) Pyrolysis: 500 °C, 1 h	Surface area: $900 \text{ m}^2/\text{g}$ Function: Adsorption for ammonia nitrogen	Dalai and Azargohar (2007)

Table 3
Different ways for preparation of biochar, its modification strategies, properties, and conditions of production.

Sl	Biochar Base	Methods of Biochar Modification	Required Temp.	pH	Adsorption Capacity [approx.]	Removal efficiency [approx.]	Ref.
1.	Rice husk biochar	Magnetic alkali and acid	300 °C	8–9	98.6 mg g ⁻¹	N.A.	Liu et al. (2015)
2.	Biochar from residues of peanut shell	Copper nitrate	450 °C	6–8	68.8 mg g ⁻¹	93%	Ahmad et al. (2014a)
3.	Palm biochar	Goethite	600 °C	6.8–7.0	8132.9 mg g ⁻¹	N.A.	Ahmad et al. (2014a)
4.	Soot biochar	Iron or zinc	600 °C	N.A.	101.00 mg g ⁻¹	90%	Diao et al. (2014)
5.	Wheat husk biochar	Ball milling	700 °C	7.0–7.3	268.1 mg g ⁻¹	N.A.	Cheng et al. (2019)
6.	Biochar from residues of palm	Nano-dimensional iron	600 °C	4.8–5.0	89.00 mg g ⁻¹	100%	Wang et al. (2020)
7.	Biochar from residues of palm	Silica	600 °C	4.5	45.60 mg g ⁻¹	70%	Ahmad et al. (2014a)
8.	Vinasse biochar	Iron or manganese	800 °C	4.8	133.00 mg g ⁻¹	80–90%	Bradford (1976)
9.	Biochar from residues of vinasse	Iron or manganese	800 °C	5.0	210 mg g ⁻¹	N.A.	Deng et al. (2013)
10.	Biochar from residues of palm	Zeolite	600 °C	4.5–5.0	30.40 mg g ⁻¹	40%	Hu et al. (2019)
11.	Hay biochar from alfalfa	Sodium hydroxide	300 °C	9–10	302.36 mg g ⁻¹	N.A.	Zhang et al. (2017)
12.	Vinasse biochar	Iron or manganese	800 °C	4.8–5.0	251 mg g ⁻¹	55–60%	Jia et al. (2018)
13.	Biochar from residues of municipality	Magnetic alkali and acid	800 °C	7–8	N.A.	90%	Chen et al. (2011)
14.	Camphor biochar	Zinc oxide nanoparticles	650 °C	N.A.	N.A.	70–80%	Singh et al. (2008)
15.	Biochar from residues of sludge	Chitosan nanoparticles	750 °C	8.7–9.0	183.00 mg g ⁻¹	N.A.	Pena-Bahamonde et al. (2017)
16.	Bamboo biochar	Activation by steam	500 °C	5.0	N.A.	96%	Ahmad et al. (2014a)
17.	Bamboo biochar	Ball milling	450 °C	N.A.	N.A.	90%	Bradford (1976)
18.	Biochar from residues of dung	N.A.	700 °C	10–11	N.A.	78%	Pena-Bahamonde et al. (2017)
19.	Grass biochar	π-π ectodysplasin A	800 °C	N.A.	N.A.	62–65%	Wu et al. (2011)
20.	Cassava biochar	Potassium hydroxide	500 °C	10.2–11.0	N.A.	87–97%	Singh et al. (2008)
21.	Biochar from aerobic sludge	Zinc chloride	700 °C	7.0	93.40 mg g ⁻¹	N.A.	Shen (2015a)
22.	Coffee biochar	Magnetite	250 °C	N.A.	N.A.	96%	Pena-Bahamonde et al. (2017)
23.	Biochar from residues of cotton	Water particles	350 °C	N.A.	N.A.	50%	Diao et al. (2014)
24.	Biochar from residues of municipality	Magnetic alkali and acid	800 °C	6.8–7.0	287.00 mg g ⁻¹	N.A.	Deng et al. (2013)
25.	Cassava biochar	Potassium hydroxide	500 °C	10.5–10.9	N.A.	12–28%	Yang et al. (2014)
26.	Grape biochar	Chitosan	450 °C	2.8–3.0	36.70 mg g ⁻¹	N.A.	Ma et al. (2015)
27.	Soot biochar	Cobalt or iron	500 °C	5.0	N.A.	99%	Jung et al. (2017)
28.	Bagasse biochar	Anaerobic digestion	600 °C	8–9	8.60 mg g ⁻¹	N.A.	Azargohar et al. (2014)
29.	Biochar from manure	Phosphoric acid	700 °C	8–9	365.40 mg g ⁻¹	95%	Bradford (1976)
30.	Pinus biochar	Sodium hydroxide	N.A.	N.A.	274.80 mg g ⁻¹	N.A.	Cheng et al. (2019)
31.	Biochar from residues of wood	Nano-dimensional hydroxyapatite	700 °C	6.0	118.80 mg g ⁻¹	N.A.	Liu et al. (2015)
32.	Rice husk biochar	Phosphoric acid	700 °C	9.0	552.00 mg g ⁻¹	95–100%	Chen et al. (2015)
33.	Camphor biochar	Zinc oxide	650 °C	4.0	449.40 mg g ⁻¹	75–80%	Diao et al. (2014)
34.	Biochar from residues of wood	N.A.	700 °C	5.8–6.0	149.30 mg g ⁻¹	N.A.	Hegab et al. (2016)
35.	Biochar from residues of date	No modification	600 °C	5–6	28.190 mg g ⁻¹	36%	Ahmad et al. (2014a)
36.	Cassava biochar	Potassium hydroxide	500 °C	10–11	N.A.	66–96%	Liu et al. (2015)
37.	Corn biochar	Manganese oxide	350 °C	4.0	39.92 mg g ⁻¹	N.A.	Pena-Bahamonde et al. (2017)
38.	Potato biochar	Potassium hydroxide	500 °C	N.A.	23.40 mg g ⁻¹	N.A.	Zhang et al. (2017)
39.	Bagasse biochar	Anaerobic digestion	600 °C	2.0	54.20 mg g ⁻¹	N.A.	Cheng et al. (2019)

majorly related to high cost, potential toxicity, and environmental persistence. Biochar on the other hand, stands out from the others due to its better efficacy, sustainability, and especially low cost benefits (Ambaye et al., 2021).

For this reason, the synthesis of alternative natural products which will not only carry out complete inhibition of harmful waterborne pathogens but also devoid themselves of forming any harmful by-products, is very important. For example, QPS or Quaternary phosphonium salt is a newly synthesized cationic biocide (Ilker et al., 2004). It possesses a disinfection efficiency that is higher than the existing chemicals. It can also function equally in varied pH ranges from a pH range of 2–12. It also possesses a broad-spectrum activity. Again, Quaternary phosphonium salt usually stays inert towards alkali, acid, and normal redox agents; which drives away any chances for the synthesis of disinfecting by-products that are carcinogenic (Dong et al., 2014). But at

the same time, high levels of water solubilization are usually not associated with targeted specificity and might cause uncontrolled release. These might lead to a decrease in the duration of their effectiveness on increasing the dosage and on specific target surfaces (Malmsten, 2011). Not too long ago, various kinds of antimicrobial polymers were applied to solve these issues. Antimicrobial polymers not only provide effectiveness for a long duration and give an environment-friendly assurance (Zheng et al., 2014). They are also associated with various antimicrobial moieties which are bonded covalently. These moieties eliminate the chances of massive biocidal diffusion increase the antimicrobial efficiency and at the same time drive away any chances of accumulation of residual toxicity (Lehmann, 2007). However, a lot of modifications are still required for synthesizing an antibacterial polymer with slow-releasing and long-acting efficiency for the application of Quaternary phosphonium salt.

Biochar is formed from the pyrolysis of biomass which is rich in carbon and is regarded as a very useful porous substance associated with a lot of functional groups and increased surface areas with specificity (Inyang et al., 2016). Biochar is seen to be an extraordinary adsorbent and is applied to water cleaning applications [cleaning of water-related contaminations like organic pollutants, inorganic pollutants, heavy metals; etc] for the same (Zhang et al., 2018). Multiple research works have proven biochar is an efficient medium for immobilizing different bacterial species or assembling various catalytic materials through sequential immobilization to remove various organic contaminants from wastewater (Liu et al., 2019a). Biochar is also considered a multifunctional material. Biochar has numerous applications in the synthesis of both benign and efficient antimicrobial composites (Lou et al., 2019). But it is to be noted that, till now no reports are found on the synergistic activities of biochar and Quaternary phosphonium salt in their bacteriostatic effectivity. So, the recent research works carried on mainly focus on the development of an eco-friendly antibacterial agent using the synergistic effectiveness of both biochar and Quaternary phosphonium salt which is cost-effective (Lou et al., 2019). It also focuses on the characterization of the antibacterial material that is developed due to their synergistic activities. It should also focus on finding out the antibacterial efficiency and the exact mode of action of their bactericidal activity (Hu et al., 2019). A commonly known Quaternary phosphonium salt, C24H52BrP or Dodecyl tributyl phosphonium bromide exhibits high antibacterial activity. Various research works conducted in recent times tried to find out the bactericidal activity and efficiency of Dodecyl tributyl phosphonium bromide (Yang et al., 2019). The incorporation of iron oxide was done in Dodecyl tributyl phosphonium bromide to increase its magnetic properties and for easier separation (Liu et al., 2019b). In a study, *Staphylococcus aureus* causing nosocomial diseases in humans, and *Escherichia coli* responsible for various human health-related diseases, were taken as model organisms to check the antibacterial effectiveness (Jia et al., 2013). It was seen that biochar not only established itself as an antibacterial agent by inhibiting both the microorganisms from the contaminated water and making the water contamination-free but also provided a long-lasting, recyclable, and

effective functionalization [Table 2] (Deng et al., 2013).

5. Magnetic biochar-quaternary phosphonium salt (MBQ)

To apply the synergistic activities of biochar and quaternary phosphonium salt for increasing antimicrobial efficiency, the development of a biochar-based polymer: MBQ or Magnetic Biochar-Quaternary Phosphonium Salt was done for its application in the treatment of livestock wastewater (Hegab et al., 2016). It was also seen that Magnetic Biochar-Quaternary Phosphonium Salt not only exhibits a higher level of effectiveness in the inhibition of various bacterial species from water but also carries out the deactivation of antimicrobial-resistant genes or ARG from the same (Chen et al., 2015). Antimicrobial efficiency can be found in both quaternary phosphonium salt as well as from MBQ; but when looked upon precisely, it is seen that MBQ could change the protein folds of the antimicrobial-resistant genes and disseminate them way more compared to quaternary phosphonium salt (Diao et al., 2014). Along with this, MBQ has the potential to decrease the microbiological diversity in livestock wastewater and disrupt the cell membranes of the wastewater bacterial species. Revealing the presence of bacterial mobile genetic elements and various microbial communities in wastewater is seen through redundancy, network as well as variation partitioning analyses (Bradford, 1976). The bacterial mobile genetic elements and the different microbial communities are the two things that determine the type and pattern of antibiotic resistance associated with the water (Zhang et al., 2017). As MBQ can target, disrupt, alter, or inhibit both these factors that is mobile genetic elements and microbial communities, it holds high potential in decontamination of wastewater treatment [Fig. 4] (Pena-Bahamonde et al., 2017).

5.1. Synthesis of magnetic biochar-quaternary phosphonium salt

The process of formation of Magnetic Biochar is quite simple. The base used for the formation of MBQ can be different (Eroglu et al., 2009).

Maize straw biochar is used a lot as a base for the synthesis of MBQ. Rice husk biochar is also a reliable base for MBQ synthesis. These are



Fig. 4. Complete process of preparation of magnetic biochar-quaternary phosphonium salt [MBQ].

mainly used as bases because of their efficiency, abundance, and easy availability as agricultural wastes (Jia et al., 2018). The synthesis of biochar is carried out at a temperature of about 500 °C. During the formation process, an anoxic environment is maintained. Several physicochemical parameters should be taken care of during the synthesis process (Yu et al., 2004). The biochar is usually suspended in ultra-pure water and then it is sonicated from 25 to 30 min. This is done to homogenize the biochar suspension. Now, maintaining a nitrogen atmosphere, crystallized ferric chloride and ferrous sulphate normally in a molar ratio of 2:1 or 1.5:1 are added along with water and stirred vigorously preferably through a magnetic stirrer. The addition of ammonia to this solution generates an orange solution which is pellucid. After all these processes, the suspended biochar is added to the solution. The stirring should be continuously done throughout the process. A temperature in the range of 80 °C–100 °C is usually maintained during the process (Chen et al., 2011). After all this, the solution is cooled down and separation of the desired product is carried out. Usually, magnetic separation is done in most cases. Additional steps can be performed for the removal of impurities like washing the desired product with pure water or with absolute ethanol (Singh et al., 2008). After the removal of all the undesired impurities, the final product is dried and given a powdered form for market use.

For the formation of Magnetic Biochar-Quaternary Phosphonium Salt, some additional steps are to be followed in the synthesis of magnetic biochar (Wu et al., 2011). For the synthesis of MBQ, a dodecyl tributyl phosphonium bromide aqueous solution is usually prepared. Under continuous stirring, magnetic biochar, and soluble dodecyl tributyl phosphonium bromide are added to prepare a mixture. The stirring may be continued almost for 24 h, to get a homogeneous mixture (Cheng et al., 2019). Once this is done, Quaternary Phosphonium Salt undergoes self-assembly with magnetic biochar. This occurs at a moderate temperature of about 60 °C–70 °C (Wang et al., 2020). The resultant solution undergoes washing multiple times for removal of contamination as well as the Quaternary Phosphonium Salts that did not go self-assembly with magnetic biochar. Mainly this is done with the help of metal salts like silver nitrate.

5.2. Characterization of magnetic biochar-quaternary phosphonium salt

The morphology and physical properties of Magnetic Biochar-Quaternary Phosphonium Salt are normally done by various analytics tools (Azargohar et al., 2014).

Scanning Electron Microscopy is very popular in seeing the morphological features of MBQ. Many studies have also used Transmission Electron Microscopy for the morphological analysis of MBQ (Jung et al., 2017). FTIR or Fourier transform infrared spectrometric studies are performed on each Biochar, Magnetic Biochar [MB], Quaternary Phosphonium Salt [QPS], and Magnetic Biochar-Quaternary Phosphonium Salt [MBQ] to identify and analyze the functional groups present in them. This is mainly done in the range of 4000 to 400 cm⁻¹. Raman Spectroscopic analysis is also done on MBQ (Cobos et al., 2019). The Raman spectra are usually generated to probe the unstructured and disordered structures of various carbon-based materials like graphite. Here, unstructured, and disordered structures of biochar are analyzed by obtaining their Raman Spectra. An X-ray diffractometer is used for the synthesis of the crystallographic structures of Magnetic Biochar-Quaternary Phosphonium Salt (Ma et al., 2015). For the analysis of the loading amount for monitoring the mass change of Magnetic Biochar-Quaternary Phosphonium Salt, TGA or Thermo-gravimetric analysis is done. This analysis is strictly carried on under an argon atmosphere and under a temperature ranging from 40 °C to 800 °C (Cai et al., 2010). The scanning rate of 10 C/min to 20 C/min is also maintained throughout this process. The magnetic properties are analyzed too. Normally, the magnetic properties of Magnetic Biochar-Quaternary Phosphonium Salt and Magnetic Biochar are analyzed before they are applied to any contaminated water bodies (Yang et al., 2014).

5.3. Antimicrobial activity of magnetic biochar-quaternary phosphonium salt

Several research works have been conducted to see the antimicrobial efficacy of MBQ. The antimicrobial efficiency of MBQ is seen against several model bacterial organisms which are normally found in wastewater (Qiu et al., 2014). Some of these are *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*; etc. As all these organisms are associated with medical infections and health-related diseases, germicidal testing of these organisms is crucial. Both Magnetic biochar and Magnetic Biochar-Quaternary Phosphonium Salt are usually seen to inhibit these bacterial species. However, the inhibition is seen to be way more for Magnetic Biochar-Quaternary Phosphonium Salt because of their property of inhibiting and altering antimicrobial-resistant genes (Shao et al., 2018). It is to be noted that in these antimicrobial studies of MBQ, the residual composites of the MBQ are usually fixed with the help of an external magnet. Once the Quaternary Phosphonium Salt is completely released from the MBQ, the remaining composites of the MBQ can be extracted or re-purified to regenerate and re-construct the MBQ for using them in further processes (Zhang et al., 2019c). These studies also proved the reusable properties of MBQ. It was seen that the MBQ which is used against colonies of *Escherichia coli* can again be re-utilized against other bacterial colonies once their usage is over and they are purified (Yoo et al., 2016).

6. Key challenges and future prospects

Current biochar applications for pathogen removal face significant challenges, including limited adsorption capacity, variability in antimicrobial efficacy, and environmental trade-offs associated with production and disposal. Conventional biochar often exhibits inconsistent performance due to its dependency on feedstock properties, pyrolysis conditions, and post-treatment modifications. Additionally, while modifications such as metal doping or polymer functionalization enhance biochar's antimicrobial properties, they may introduce environmental concerns, such as toxicity or secondary pollution. To address these limitations, future research should focus on optimizing biochar synthesis through sustainable methods, such as hydrothermal carbonization, to reduce emissions and enhance efficiency (Shen, 2015b). Advances in functionalization strategies, including the incorporation of nanoparticles or bio-polymers, hold promise for improving biochar's pathogen adsorption and inactivation capabilities (Ahmad et al., 2014b; Tan et al., 2016). Biochar-based composites, such as Magnetic Biochar-Quaternary Phosphonium Salt (MBQ), demonstrate the potential for disrupting antimicrobial resistance by targeting genetic elements and altering microbial community dynamics in wastewater. Furthermore, integrating biochar with advanced treatment systems, such as UV disinfection or ozonation, could enhance its pathogen removal efficacy while maintaining environmental safety. Comprehensive life cycle assessments are critical to evaluate the long-term impacts of biochar production, application, and regeneration, ensuring it remains a sustainable solution. Future efforts should also focus on elucidating the molecular mechanisms underlying biochar's antimicrobial activity to develop targeted and efficient materials capable of addressing both microbial contamination and antibiotic resistance in water systems.

Regardless of all the promising aspects of biochar and its antimicrobial potential, there are a number of knowledge gaps in the current research scenario on biochar. Studies on antiviral activity and virus removal is very limited, especially regarding non-enveloped viruses and the mechanisms involved. Moreover, inconsistent optimizations in the methodologies involving finding out antimicrobial activities, like variations in biochar preparation, testing conditions, and pathogen strains, limit the comparability and reproducibility of all the findings. Also, there lies a significant gap in the long-term performance and potential environmental impacts of biochar use in water treatment. Addressing these gaps will be critical to fully realizing the potential of biochar as a

versatile antimicrobial material.

7. Conclusion

Biochar as an antimicrobial agent in wastewater treatment is a fascinating concept that is gaining more and more scientific attention. Livestock wastewater is contaminated with a lot of harmful microorganisms which causes a lot of harmful diseases. The available techniques for the decontamination of wastewater and inhibition of microorganisms are not specific, not environment-friendly, and not completely effective. Also, they generate a lot of by-products that can be toxic to human health. Biochar products are an ideal replacement for them as it is not only highly specific and efficient but also eco-friendly and reusable. Biochar formed by various ways of pyrolysis provides us with an option to vary or change its various interacting and removal effectiveness. Biochar can interact with the microorganisms through various surface functionalities and undergo their inhibition. Some of these are electrostatic interactions, π - π stacking, ion-exchange interactions, Hydrogen bonding interactions; etc. Magnetic biochar and Magnetic Biochar-Quaternary Phosphonium Salt are seen to have better efficiency than native biochar in antibacterial properties. Magnetic Biochar-Quaternary Phosphonium Salt is the best-enhanced version of biochar in terms of livestock wastewater treatment and inhibition of bacterial species in contaminated water. Biochar and its modifications demonstrate antimicrobial effects through several interconnected mechanisms, including adsorption, reactive oxygen species (ROS) generation, and membrane disruption, each of which contributes to the inactivation of microorganisms. Biochar's high surface area, porosity, and functional groups (such as hydroxyl and carboxyl) enable efficient adsorption of microbial cells and their essential nutrients. This adsorption disrupts the environment necessary for microbial survival, involving interactions like electrostatic attractions between negatively charged biochar surfaces and positively charged microbial membranes, hydrogen bonding, and π - π interactions with membrane proteins or lipids. These processes immobilize microbes, impair nutrient access, and reduce microbial proliferation. Modifications to biochar, such as metal doping or magnetic incorporation, enhance its antimicrobial properties by facilitating the catalytic generation of ROS, including hydroxyl radicals (\bullet OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2). These ROS cause oxidative stress, damaging microbial cell components like proteins, lipids, and DNA, and ultimately leading to cell death. In addition to adsorption and oxidative stress, biochar disrupts microbial cell membranes through physical and chemical means. The sharp edges and surface structure of biochar particles physically damage membranes, causing leakage of cellular contents, while interactions with the lipid bilayer alter membrane permeability and disrupt ion gradients. These effects are amplified in modified biochar composites, such as Magnetic Biochar-Quaternary Phosphonium Salt (MBQ), which combine adsorption and ROS effects with targeted biochemical actions. For instance, MBQ targets antimicrobial resistance genes (ARGs) by altering protein folding and disrupting genetic elements responsible for resistance dissemination. This helps prevent the horizontal gene transfer that enables the spread of resistance among microbial populations. Moreover, biochar-based modifications influence microbial community dynamics by selectively adsorbing specific species, thereby reducing the prevalence of harmful microorganisms and altering the environmental conditions that favor pathogen survival. These synergistic mechanisms, especially when enhanced by biochar modifications, make biochar a powerful, eco-friendly tool for applications like water treatment and microbial contamination control. The synergy of biochar and microbial remediation for efficient pollutant removal, emphasizes biochar's role in enhancing microbial activity and protection. It explores mechanisms, adaptability, and challenges in commercializing this cost-effective and sustainable approach to environmental cleanup (Saravanan et al., 2023). Previous studies have shown that biochar can remove polycyclic aromatic hydrocarbon, a carcinogen, and mutagen (Yaashikaa et al.,

2024). A study demonstrated neem-shell-derived biochar's high efficiency in removing methylene blue dye from wastewater, achieving a maximum adsorption capacity of 139.59 mg/g at 35 °C through a monolayer Langmuir mechanism, making the biochar a sustainable, cost-effective solution for dye remediation (Karce et al., 2024). So, in conclusion, it can be said that Biochar especially Magnetic Biochar-Quaternary Phosphonium Salt [MBQ] can be the replacement for existing harmful water decontamination technologies and can be the reason for people using microorganism-free livestock water in the future.

CRedit authorship contribution statement

Dipro Mukherjee: Methodology. **Moumita Sil:** Methodology. **Arunava Goswami:** Methodology. **Debasmita Bhattacharya:** Supervision. **Moupriya Nag:** Conceptualization. **Dibyajit Lahiri:** Writing – original draft. **Kuldeep Sharma:** Project administration. **Rajan Verma:** Conceptualization.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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