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A critical review on using biochar as constructed wetland substrate: Characteristics, feedstock, design and pollutants removal

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(Article begins on next page)

Ecological Engineering

A critical review on using biochar as constructed wetland substrate: characteristics, feedstock, design and pollutants removal mechanisms

--Manuscript Draft--

Manuscript Number:	ECOLENG-D-22-01018R1
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Keywords:	Natural based solutions; Sorbent materials; Wastewater treatment; Biomass thermal conversion; Configuration of constructed wetlands; Emerging contaminants .
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Order of Authors:	Sofiane El Barkaoui, Mr Laila Mandi Faissal AZIZ Massimo Delbubba Naaila Ouazzani, Ph.D
Abstract:	<p>Constructed wetland systems (CWs) are physically and biologically constructed systems that simulate natural wetlands and they can be used to treat wastewater from several sources of pollution. The present review aims to synthesise the updated literature on constructed wetlands integrating biochar in the substrate. The study focuses on the biochar characteristics that are generally integrated into this treatment ecotechnology and the feedstocks generally used (sewage sludge, agricultural waste and wood, food waste, and marine feedstock). The biochar quality is affected by the conditions involved in preparing such biochar (pyrolysis temperature, heating time and rate, etc.). The properties of biochar used for wastewater treatment, the effect of its implementation on CW substrate and its treatment efficiency have also been described. Several factors alter the removal efficiency of pollutants in CWs, such as substrate chemical and physical properties, hydraulic retention time, oxygenation and redox conditions. In addition, the implementation level of biochar in the filter and the choice of the macrophytic plant are crucial to the efficiency of the treatment system. Different configurations of implementing the biochar in CW substrate have been reported and compared. Constructed wetlands (CWs) are constructed systems that simulate natural wetlands and can be used to treat wastewater from several sources of pollution through physical, chemical and biological depuration processes. This work aims to critically review the updated literature on constructed wetlands (CWs) integrating biochar in the substrate. In detail, the study focuses on the characteristics of biochar that are generally integrated into this treatment ecotechnology and the processes used to prepare the materials, including conditions of thermal conversion and the kind of feedstock used (e.g., agricultural, food, and wood wastes, sewage sludge, and argal marine feedstock). Based on the literature review, it is found that the feedstock must be rich in carbon (c) and low in the mineral matter to produce good quality biochar, i.e. large pore volume and high specific surface area, thus allowing to effectively remove pollutants from wastewater. The biochar quality is affected by the conditions involved in preparing biochars (e.g., pyrolysis temperature, heating rate and carbonization time). The properties of biochar used for wastewater treatment, the effect of its implementation as CW substrate and its treatment efficiency have also been described. Several factors alter the removal efficiency of pollutants in CWs, such as substrate chemical and physical properties, hydraulic retention time, oxygenation, and redox conditions in the reed bed. In addition, the mode by which biochar is implemented in the filter and the choice of macrophyte are crucial for regulating the efficiency of the treatment system. <i>Phragmites australis</i> was the most used plant in the previous studies because of its large advantages. Different configurations of CWs integrating biochar into the wetland as a filling medium, were reported and compared.. In vertical flow CWs (VF-CWs), which are the system mostly investigated, several</p>

	<p>studies have shown that the optimal position for the biochar substrate is the intermediate one between two layers of inert materials, to avoid clogging of the filtration system or biochar flotation.</p>
<p>Suggested Reviewers:</p>	<p>andreas Angelakis professor, National foundation of agricultural research, Iraklio, Greece angelak@cc.uh.gr specialiste of wastewater treatment by constructed wetland.</p> <hr/> <p>ALi El Hanandeh Grifit university Brisbane, Australia a.elhanandeh@griffith.edu.au have already published papers in the treatment of Wastewater by Constructed wetlands amended by biochar.</p>

Le 03/10/2022

Dear editor of Ecological Engineering Journal

I have a great pleasure to send my paper to your honorable journal and hope that it will be taken in consideration for publication; our paper is entitled

<Review on using biochar in constructed wetland substrate: characteristics, feedstock, configurations and pollutants removal mechanisms>. Prepared by El Barkaoui Sofiane, Mandi Laila, Aziz Faissal, DelBubba Massimo et Ouazzani Naaila.

The present review aims to synthesise the updated literature on constructed wetlands integrating biochar in the substrate. The study focuses on the biochar characteristics that are generally integrated into this treatment ecotechnology and the feedstocks generally used (sewage sludge, agricultural waste and wood, food waste, and marine feedstock). The biochar quality is affected by the conditions involved in preparing such biochar (pyrolysis temperature, heating time and rate, etc.). The properties of biochar used for wastewater treatment, the effect of its implementation on CW substrate and its treatment efficiency have also been described. The review was emphasizing on recent literature from respected peer-reviewed journals.

Finally, I certify that the paper is original and has not been sent to any other journal for publication.

Best regards

Naaila OUAZZANI

The `Corresponding author

Dear Editor,

Dear Reviewers,

Thank you for giving us the opportunity to submit a revised version of our manuscript entitled “Review on using biochar in vertical flow constructed wetland substrate: characteristics, feedstock, configurations and pollutants removal mechanisms.” to *Ecological engineering*.

We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on our manuscript. We are grateful to the reviewers for their insightful comments on our paper. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers. We have used Microsoft Word’s “track changes” to indicate changes within the manuscript.

Here is a point-by-point response to the reviewers’ comments and concerns.

Response to Reviewer 1 Comments

Point 1: The use of biochar obtained from waste materials can be a contribution to increasing the sustainability of constructed wetlands. The proposed review work is interesting but there are recent review works on this topic, such as "The performance and mechanism of biochar-enhanced constructed wetland for wastewater treatment" (DOI 10.1016/j.jwpe.2021.102522), "Preparation of straw biochar and application of constructed wetland in China: A review" (DOI 10.1016/j.jclepro.2020.123131), and "Incorporating Biochar into Wastewater Eco-treatment Systems: Popularity, Reality, and Complexity" (DOI 10.1021/acs.est.9b01101). I recommend that authors evaluate those works and present the improvements and advances achieved by their own work. In my opinion, a clear justification of the contribution of the present work is critical for accepting it for publication.

Response 1: We thank the reviewer for this comment, it’s done, we added these references. And we have presented the improvements and advances achieved by our work

Point 2: The title refers to the use of biochar as CW substrate, but the work deals only with vertical flow configuration. Besides the recommendation to change the title accordingly, a justification to exclude horizontal flow CW must be presented.

Response: we have included the horizontal CW in the section 3.1.2

Point 3: The main results obtained must be referred to in the abstract.

Response: The authors thank the reviewer for figuring out this comment, it's fixed (please see the abstract)

Point 4: Please check "proprieties" in the highlights, abstract, and text.

Response: we want to thank the reviewer for this observation. The "proprieties" was checked and corrected in the highlights, abstract, and text.

Point 5: Please rewrite highlights 3, 4, and 5. The "pollutants adsorption capacity" refers to the substrate and not to the CW; CW is not defined (the same comment for the abstract); The "middle" of technology definition is unclear; The "&" must be replaced by "and"; Finally, check the number of characters.

Response: - We have fixed the sentence "pollutants adsorption capacity"

- We have rewritten highlights 3, 4, and 5,

- The word "middle" was changed in the whole manuscript as suggested by the reviewer to "interlayer".

Point 6: The name of genera and species must be written in italics. Please check the entire document.

Response: It's checked and fixed

Point 7: I recommend replacing the reference "Abedi and Mojiri, 2019" with a more recent work reviewing the sustainability of constructed wetlands (on page 3).

Response: The reference "Abedi and Mojiri, 2019" was changed by "Younas et al., 2022".

Point 8: I also recommend replacing the references "Guittonny-philippe et al, 2015" and "Guo et al. 2020" with more recent documents dedicated to constructed wetland substrates. Some available reviews focus on CW substrates can be referenced to.

Response: The references "Guittonny-philippe et al, 2015" and "Guo et al. 2020" was changed by "(Addo-Bankas et al., 2021) and (Ohore et al., 2022)".

Point 9: In addition, the reference Deng et al. 2021 is too specific to be used as reference work for substrates.

Response: Deng et al. 2021 removed from the part of the substrates.

Point 10: More references and recent works on plant contribution can also be added together with the works of Guittonny-philippe et al, 2015 and Srivastava et al., 2008).

Response: We have added other references such as “Kataki et al., 2021; Karungamye et al., 2022”.

Point 11: The method applied to survey and select the literature, if any, must be described to support the review presented.

Response: the methods used are cited in the text as follow: SciFinder, Elsevier ScienceDirect, and Google Scholar.

Point 12: There are available several reviews on biochar feedstocks and preparation. Section 2.1 must be based on those review works.

Response: We have added other references such as “(Berslin et al., 2022) (Garcia et al., 2022) (Zhuang et al., 2022) (Abdelhafez et al., 2021)” and other recent studies in table 2.

Point 13: table 1 can be reorganized avoiding blank lines with scarce data.

Response: we have reorganized the table (see the table 1)

Point 14: Furthermore, the goal of section 2 is unclear. I suggest shortening section 2 to focus on biochar production processes, main raw materials, and main relevant properties for using it as substrate in CWs.

Response: it's done

Point 15: For the first time in the document, section 3 refers to vertical flow CW. As suggested above, I recommend a clarification of the option for this type of configuration.

Response: It's fixed, we have devised this section and we included another section 3.1.2 about horizontal CW.

Point 16: The inclusion of "substrate nature" and "medium used in the bed" in the same sentence seems to be redundant.

Response: Corrections were added to the main text as requested, and the sentence “in the same sentence seems to be redundant” was removed.

Point 17: Sections 3.1 and 3.2 seem to be irrelevant to the work carried out, and I recommend their remotion unless a clear relationship between the different plants and biochar granulometry with the CW's effectiveness can be provided.

Response: we have modified both sections (3.1 and 3.2) and their titles as requested.

Point 18: I recommend merging Figures 1 to 3 into one figure.

Response: It's done as requested

Point 19: Please clarify the statement "It can be bound to the soil as an alteration and expel toxins from wastewater."

Response: It's clarified in the text that wastewater supplements may be connected in the soil as an alteration. Still, using the biochar substrate allows the removal of this supplement from wastewater.

Point 20: In addition, check "poisons".

Response: the word poisons is changed by "pollutants" in the text

Point 21: Please clarify "greater in biochar-added wastewater compared to non-biochar wastewater".

Response: It's clarified and corrected "The average N₂O and CO₂ fluxes were significantly lower, while CH₄ fluxes were significantly greater in the biochar-added and non-biochar CW".

Point 22: Please check/clarify "Similarly, COD was increased with an increasing biochar addition ratio".

Response: It's removed

Point 23: Table 3 can be the core of the work. I recommend adding data on CW size, HLR, fraction and order of substrates, and plant species.

Response: we have merged table 2 and 3, and we have added data on CW size, HLR, fraction and order of substrates, and plant species.

Point 24: Please check "CAO et al., 2009).

Response: It's checked and fixed

Point 25: Please check the sentence "Multiple pathways remove nitrogen from wastewater plant uptake, substrate adsorption, ..."

Response: It's corrected, "Multiple pathways are used to remove nitrogen from wastewater in CW, substrate adsorption, ammonia volatilization, plant uptake and microbial processes".

Point 26: I recommend referring to P as "Phosphorus compounds".

Response: It is referred in the whole manuscript.

Point 27: Sections 5 and 6 can be shortened and should be merged.

Response: It is merged as requested

Point 28: Please check the apparent contradiction "Due to its low cost, availability, and high commercial potential, the preparation of biochar has been developed rapidly in recent years ..." with "However, biochar is rarely used in water treatment due to its high cost, high ash content, and difficulty in ash removal..."

Response: It's checked and corrected, "low- cost, availability of the raw materials"

Point 29: The relationship with CW of some data in table 4 is not clear, such as "raise weed growth during lentil culture", and "Improve the retention of water".

Response: We thank the reviewer for this comment, we have removed these sentences (See table 4).

Point 30: The main "conclusions" of the work must be presented in the conclusion section, such as the improvements in the efficiency of pollutant removal, the typical volume fraction and position of the biochar in the substrate, the contribution to hydraulics, the main advantages, and disadvantages, ...

Response: We have added the main conclusion (please see the conclusion)

Point 30: Finally, please check the reference list. For example, in Chand et al. 2021, Chang et al. 2022, and in other references the journal name appears before the paper title; Cao et al. 2009, Pignatello 2011, and other references are out of alphabetical order.

Response: Many thanks, we have checked the reference list and it's fixed. (Please see reference list)

Response to Reviewer 2 Comments

Point 1: There are no line numbers, which make hard to review, and hard to list a completed comment which link to each specific problem. Therefore, some issues may appear in different parts, which are needed to be carefully checked and revised for the whole manuscript.

Response: we want to thank the reviewer for this observation about line numbers, it's done.

Point 2: How did the results mentioned in the part 2.1 Biochar feedstock come to be reached (Page 5)? Is the data listed in Table 1 comprehensively and accurately represent the results reached in this part? (e.g., "Bamboo is widely used as a raw material for biochar" (Page 5) was mentioned.) After all, lots of characteristics of biochar feedstock (e.g., bamboo, hardwood) have not been recorded.

Response: - we have enriched table 1 with more characteristics data in line with the text of part 2.1.

Point 3: In the terms of the part 3.1 Types of macrophytes used in CWs implemented with biochar, is it more valuable to summarize different macrophytes types to discuss their role in CW implemented with biochar? (Page 8)

Response: it's done, thanks

Point 4: Undeniably, this study has done a lot of statistical work in this respect, (for instance, the characteristics, the role of macrophytes and categories of plants used, the location of biochar in the substrate, its dimensions, and the effectiveness of biochar in removing various pollutants from wastewater), but the data in the full text is a little scattered. Is it necessary to conduct in-depth analysis and discussion through mathematical statistics to quantify the data?

Response: The authors thank the reviewer for raising this interesting remark, it's fixed.

1 **A critical review on using biochar as constructed wetland substrate:** 2 **characteristics, feedstock, design and pollutants removal mechanisms**

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16

17

18 Highlights:

19 -Investigations on constructed wetland integrating biochar (CWB) were reviewed

20 -Pyrolysis time, heat and feedstock origin determine prepared biochar properties

21 -Biochar substrate (BS) improves CW efficiency and pollutants adsorption capacity

22 -Biochar in the substrate interlayer is the optimal ecotechnology configuration

23 -In situ experiments are needed to test effectiveness and current effect of CWB

24

25 Abstract

26 Constructed wetlands (CWs) are constructed systems that simulate natural wetlands and can be used to
27 treat wastewater from several sources of pollution through physical, chemical and biological depuration
28 processes. This work aims to critically review the updated literature on constructed wetlands (CWs) integrating
29 biochar in the substrate. In detail, the study focuses on the characteristics of biochar that are generally integrated
30 into this treatment ecotechnology and the processes used to prepare the materials, including conditions of
31 thermal conversion and the kind of feedstock used (e.g., agricultural, food, and wood wastes, sewage sludge,,
32 and argal marine feedstock). Based on the literature review, it is found that the feedstock must be rich in carbon
33 (c) and low in the mineral matter to produce good quality biochar, i.e. large pore volume and high specific
34 surface area, thus allowing to effectively remove pollutants from wastewater. The biochar quality is affected by
35 the conditions involved in preparing biochars (e.g., pyrolysis temperature, heating rate and carbonization time).

1 The properties of biochar used for wastewater treatment, the effect of its implementation as CW substrate and its
2 treatment efficiency have also been described. Several factors alter the removal efficiency of pollutants in CWs,
3 such as substrate chemical and physical properties, hydraulic retention time, oxygenation, and redox conditions
4 in the reed bed. In addition, the mode by which biochar is implemented in the filter and the choice of macrophyte
5 are crucial for regulating the efficiency of the treatment system. *Phragmites australis* was the most used plant in
6 the previous studies because of its large advantages. Different configurations of CWs integrating biochar into the
7 wetland as a filling medium, were reported and compared.. In vertical flow CWs (VF-CWs), which are the
8 system mostly investigated, several studies have shown that the optimal position for the biochar substrate is the
9 intermediate one between two layers of inert materials, to avoid clogging of the filtration system or biochar
10 flotation.

11 Keywords: Natural based solutions; Sorbent materials; Wastewater treatment; Biomass thermal
12 conversion; Configuration of constructed wetlands; Emerging contaminants .

13 **1. Introduction**

14 Constructed wetlands (CWs) are a kind of green technology that can be considered as sustainable nature
15 based solution for wastewater treatment (Younas et al., 2022). In such systems, the plant and the substrate play
16 an important role in the removal of pollutants (Addo-Bankas et al., 2021;Ohore et al., 2022). The substrate is an
17 essential component of CWs since it can mediate and promote the implementation of mechanical, physical and
18 biological mechanisms for reducing pollutants concentration in CW effluents, allowing for the direct removal of
19 contaminants, making available reactive agents for transforming pollutants, promoting plant growth, and
20 ensuring biofilm adhesion (Deng et al., 2021). Furthermore, plants uptake nutrients, directly increase biological
21 activity in the substrate by supplying oxygen through their roots, and play an important role in the hydraulic
22 conductivity within the filter. Hence, choosing the most appropriate plant species is important for obtaining the
23 best performance ; (Srivastava et al., 2008; Guittonny-philippe et al., 2015; Kataki et al., 2021; Karungamye,
24 2022).

25 The CWs have been widely tested for urban wastewater treatment, while the purification of sewage
26 from industrial or mixed urban-industrial origin has been investigated with lesser extent (Stefanakis, 2018;
27 Kataki et al., 2021). CWs demonstrated high efficiency in removing conventional pollutants such as suspended
28 solids, nutrients, biodegradable organic matter, and heavy metals (Huong et al., 2020; Zhuang et al., 2022).
29 However, in most cases, CWs have shown a lower efficiency against various ecotoxic pollutants, such as
30 detergents, heavy metals, plasticizers, disinfectants, pesticides, and pharmaceutical residues, which remain
31 largely unremoved in CWs effluents (Gosset et al., 2020). To improve CWs efficiency, various materials, other
32 than those conventionally used in CWs (i.e., gravel and sand) (Zhang et al., 2021; Fu et al., 2020), have been
33 tested as substrates, namely pozzolan (El Ghadraoui et al., 2020), charcoal (Hamada et al., 2021), zeolite (Du et
34 al., 2020), and biochar (Vymazal et al., 2021). Among them, biochar has recently gained an increasing interest
35 (Rozari et al., 2016) as a stable, porous, carbon-rich, and originated from inexpensive material obtained by
36 thermochemical conversion of waste biomass through various thermochemical processes such as. hydrothermal

1 carbonization (HTC), hydrothermal liquefaction (HTL), gasification, and pyrolysis (Deng et al., 2021). Slow
2 pyrolysis (i.e., thermal conversion in the absence of oxygen and with contact time from minutes to hours) is
3 commonly used as it is cheaper than other processes and/or gives rise to a higher yield of the solid fraction(i.e.,
4 biochar) with low syngas and bio-oil production (Enaime et al., 2020; Wang et al., 2020a). Various renewable
5 and locally available waste biomaterials, such as compost, agricultural by-products, sludge, manure, and
6 shellfish, have been used to produce biochar (Zhuang et al., 2022). In addition, biochar may also be produced
7 from wetland plant straws and then reintroduced into wastewater treatment environments, thereby facilitating
8 wetland plant management and sustainable exploitation of wastewater treatment systems (Wang et al., 2020a;
9 Deng et al., 2021). Introducing biochar as a substrate in CWs can significantly increase the system's efficiency
10 since it may have a high sorption capacity for organic and inorganic pollutants (Srivastava et al., 2008; Wang
11 and Wang, 2019). However, the sorption capacity of biochar depends on the kind of feedstock used and its
12 preparation conditions (Tan et al., 2015). The location of the biochar substrate in the filter can also affect the
13 efficiency of the treatment system. Recently, several existing studies have investigated the effect of biochar used
14 in CWs. Nevertheless, each study focused on one of the aforementioned aspects separately, while no review
15 exists to date that critically evaluates all parameters involved in the treatment and how they might interact to
16 improve the treatment efficacy of CWs (Wu and Wu, 2019; Wang et al., 2020a; Ambaye et al., 2021; Cui et al.,
17 2022; Zhuang et al., 2022). and, no synthetic review exists until now discussing the optimal position of substrate
18 biochar in the CW. We tried to collect all this aspects to enrich our synthetic review. In addition very few
19 reviews have described the emergent pollutants removal capacities of CWB.

20 According to a literature overview performed using the search engines SciFinder, Elsevier
21 ScienceDirect, and Google Scholar, this paper critically reviewed data and information on (i) the characteristics
22 and properties of biochars used in constructed wetlands (e.g. the conditions of thermal conversion and the type of
23 feedstock used for the preparation of biochars, as well as the specific surface area (SSA) and environmental
24 compatibility of the material), (ii) the methods of integrating the biochar within the CWs, and (iii) the results
25 obtained in terms of removal of macro-parameters, as well as conventional and emerging micropollutants.

26 **2. Biochar incorporated into CWs**

27 **2.1. Biochar feedstock**

28 Biochar can be made from a wide variety of feedstocks (Gabhane et al., 2020; Berslin et al., 2022;
29 Garcia et al., 2022; Zhuang et al., 2022). The composition of the feedstock and its availability are essential
30 factors in the production of efficient and cost-effective biochar. Therefore, proper classification and
31 characterization of feedstocks are required for their successful application.

32 Biochar feedstock used in the literature comes from various materials that can be classified into sewage
33 sludge, agricultural waste and wood, food waste, and marine feedstock (**Table 1**).

1 *Table 1: Feedstocks used for the production of biochars intended to be used in CWs, preparation*
 2 *conditions and characteristics of the material obtained.*

Feedstock	Pyrolysis temperature	Surface characteristics (SA, PV,PS) and pH	Composition	Reference
Bamboo	500 °C	SA(335 m ² /g)	C (68%)	(Zhang et al., 2021)
Bamboo	tubular furnace 500 °C - 10 °C/min - 2 h	SA(116.24 m ² /g)	C (74.56%); H (1.12%); O (6.28%); N (1.06%)	(Xin et al., 2021)
Bamboo	600 °C	SA (2.5 × 10 ⁸ m ² /m ³)	C (59.44%); H (2.06%); O (15.89%); N (0.40%); P (0.34%)	(Jia et al., 2020)
Bamboo chips	500 °C - 2h - N ₂	PS(10 µm)	C (56.4%); O (6.3%)	(Feng et al., 2021a)
Bamboo	700 °C - 10 °C/min - 6 h	SA(228.26 m ² /g); PV(0.086 cm ³ /g)pH(9.5)	-	(Ajibade et al., 2020)
<i>Arundo donax</i>	600 °C- 1h	SA(281.15 m ² /g)	C (63.18%); H (1.80%); N (1.13%)	(Li et al., 2018b)
<i>Arundo donax</i>	Muffle furnace 500 °C - 10 °C.min ⁻¹ - 1h - N ₂	SA(1272.67 m ² /g) ; PV(1.021 cm ³ /g)	C(79.9 %) ;N(2.27 %) ; O(17.84 %)	(Shen et al., 2020)
Agricultural waste	500 °C	SA(809 m ² /g); PV(0.22 cm ³ /g)	-	(Abedi and Mojiri, 2019)
Lodgepole Pine Wood	1000 °C	SA(152 m ² /g); PS(1 - 40 µm) pH(9.66)	-	(Huggins et al., 2016)
Oak woody (Quercus Sp)	600°C - 10h -10°C/min	PS(1 - 10 µm)	O (8%); C (90%); P (0.54%); K (0.38%); S (0.1%); Ca (0.38%)	(Gupta et al., 2016)
Wood	600 °C - 10 °C/min - 10h	SA(147 m ² /g); PV(0.176 cm ³ /g); PS(5.3 nm) pH(9.8)	C (90%); H (1.5%); O (8.3%); N (0.5%); S (0.3%)	(Kizito et al., 2017)
Wood dust	700 °C	SA(488.60 m ² /g); PV(0.286 cm ³ /g)	C (81.50%); H (1.87%); O (15.63%); N (0.07%)	(Lun, L. Chen, 2018)
Cattail (<i>Typha latifolia</i>)	600 °C - 2h - 10 °C/min	SA(6.14 m ² /g); PV(0.02 cm ³ /g)pH(8.9)	-	(Zheng et al., 2022)
Tree branches	550 °C - 2h - N ₂	SA(32.09 m ² /g); PV(2.31 mm ³ g ⁻¹)	-	(Ji et al., 2020)
Softwoods	700 °C – (gasification)	SA(485 m ² /g) pH(7.8)	C (89.2%); H (1.6%); O (1.9%); N (1%); S (0.04%); P (4.3%)	(Kaetzel et al., 2018)
Corn on the cob	600 °C - 10 °C/min - 10h	SA(123 m ² /g); PV(0.098 cm ³ /g); PS(6.2 nm) pH(8.9)	C (69%); H (3.4%); O (17.6%); N (6.1%); S (4.4%)	(Kizito et al., 2017)
Corn cob	600 °C -2h	SA(263.0 m ² /g)	-	(Gotore et al., 2022)
Giant reed straw	500 °C - 2h	SA(345.92 m ² /g); PV(0.2467 cm ³ /g); PS(1.95 nm)	-	(Deng et al., 2019)
Corn straw	450 °C, 2 h -	SA(232.715 m ² /g); PV(0.098 cm ³ /g);	C (77.30%) H (2.35%) N	(Wang et al.,

	10 °C min ⁻¹ - N ₂	PS(1.286 nm)	(0.87%) O (11.26%) S (0.02%) P (1.43%) Cl (10.38%)	2022)
Nut shells	450 °C - 2h	SA(14.76 m ² /g)-pH(8.1)	C (68.6%); K (5.1%); Ca (4.0%)	(Chang et al., 2022)
Sludge	600 °C - 2h - 10 °C/min	SA(13.13 m ² /g); PV(0.12 cm ³ /g); PS(18.71 nm) pH(7.9)	-	(Zheng et al., 2022)
Walnut shells	450 °C - 2h- N ₂	SA(14.76m ² /g)	C (68.6%); K (5.1%); Ca (4.0%)	(Chang et al., 2022)

1 SA: Surface area; PV: Pore volume; PS: Particle size.

2 Agricultural waste and wood-derived biochar have been recently employed for the application in CWs.
3 Bamboo is widely used as a raw material for biochar production, due to its abundance and high carbon content
4 (>50%), which gives a good quality of biochar (Zhou et al., 2017; Jia et al., 2020; Gao et al., 2018; Zhang et al.,
5 2021; Xin et al., 2021). Furthermore, plants such as *Arundo donax* and *cattail* can absorb phosphorus and
6 nitrogen from wastewater through their roots and transport them to the shoot, which may then be harvested and
7 converted into biochar that can be reused as functional substrates in CWs, thus thus achieving a virtuous circular
8 approach in this fiels. (Guo et al., 2020; Li et al., 2018). Other vegetal materials have been transformed into
9 biochar and used for wastewater treatment, such as cut residues of *Alnus* (Kasak et al., 2018), *Acacia*
10 *auriculiformis* (Nguyen et al., 2020), *Gliricidia* (Yasaratne, 2017), coconut shell (You et al., 2019), and various
11 agricultural waste (Abedi and Mojiri, 2019), because of their wide availability and high productivity. However,
12 terrestrial macroplants have so far been the primary source of biochar used in CWs(Aghoghovwia et al., 2020;
13 Du et al., 2020). The biochar performance derived from sewage sludge or marine life (e.g. macroalgae) may
14 differ from terrestrial plants (Zhuang et al., 2022). In addition, Deng et al. (2021) stated that the biochars used in
15 the CW treatment systems are generally made from *Arundo donax* straw, corn/straw cobs, bamboo, shells, tree
16 branches and wooden containers (Deng et al., 2021). Finally, the feedstock must be rich in carbon and low in the
17 mineral matter to produce good quality biochar.

18 2.2. Biochar production conditions

19 Pyrolysis is commonly performed to prepare biochar used in CWs because of its advantages generally
20 consisting in higher yields of biochar and lower content of bio-oil and syngas (Enaime et al., 2020; Abdelhafez
21 et al., 2021; Pereira and Astruc, 2021; Zhuang et al., 2022)..The temperature range between 400 and 600 °C were
22 the most commonly adopted to prepare the biochar used in the filters (**Table 1**) (Abedi and Mojiri, 2019; Chand
23 et al., 2021; Zheng et al., 2022). . The time and the temperature of pyrolysis are determining factors of the
24 biochar characteristics (e.g., density, carbon content, pH, porosity) (Gong et al., 2019; Xiao et al., 2020) and,
25 consequently, the performance of wastewater treatment (Alsewaileh et al., 2019; Hsu et al., 2019). Even though
26 the kind of feedstock used for biochar preparation affects the characteristics of the material, it has been
27 demonstrated that the increase in temperature generally produces higher percentages of ash, which is regulated
28 by the EN 12915-1 standard (Comite Europeen de Normalisation (CEN), 2009) in materials intended for water
29 filtration, since a high ash content in filtering media is expected to reduce adsorption activity (Castiglioni et al.,
30 2022). Also the presence of polycyclic aromatic hydrocarbons (PAHs), themselves regulated by the EN 12915-1,

1 depends on the conversion temperature adopted, which plays a main role in PAH formation up to about 500 °C,
2 but also in their degradation beyond this value (Castiglioni et al., 2022). The conversion temperature is also
3 crucial in determining the SSA of the biochar and its microporosity/mesoporosity distribution, being the
4 highest SSA values obtained at the highest temperatures, due to the increase of both pore size classes (Del Bubba
5 et al., 2020). This result is also related to the progressive loss of the functional groups present in the material as
6 the temperature increases (Del Bubba et al., 2020). However, the yield of fabricated biochar decreases with the
7 rise of pyrolysis temperature (Apolin and Conceptualization, 2020).

8 Based on the above considerations, the adsorption performance of biochars obtained under different
9 experimental conditions (e.g., different feedstock, conversion temperature, and contact time) will be better or
10 worse depending on the contaminant to be removed. Accordingly, researchers used materials produced at very
11 different temperatures for achieving the removal of their target contaminants. For example, the pyrolysis
12 temperature of the sludge-based biochar at 400°C showed optimal ammonia adsorption, while pyrolysis
13 temperatures at 350 °C or 550 °C were not favorable for the biochar's adsorption capability (Tang et al., 2018),
14 i.e., without any clear consistent effect of pyrolysis temperature on biochar adsorption performance towards
15 ammonia (Tang et al., 2018). However, Ajibade et al. (2020) and Huggins et al. (2016) were prepared the
16 biochar at high pyrolysis temperature (700 and 1000 °C) and justified the choice of these temperatures to their
17 high surface area and pore volume that will serve as a niche for microbes for the effective treatment of pollutants
18 (Ajibade et al., 2020).

19 **2.3. Biochar characteristics for wastewater treatment**

20 The physicochemical properties of biochar, such as pore distribution and size, surface functional
21 groups, alkalinity, SSA, etc., which strongly depend on the feedstock and thermal conversion conditions, are
22 responsible for pollutant adsorption capacity, and biofilm adhesion (Wang et al., 2019; Tan et al., 2015). As a
23 result, biochar's ability to remove inorganic and organic contaminants is determined by its characteristics as well
24 as the characteristics of the molecules to be eliminated, such as the size, charge and chemical moieties. As
25 mentioned above, biochar produced at low temperatures has more oxygen-containing functional groups,
26 favorable for the adsorption of polar compounds, and may show a higher mechanical strength for being used
27 preferably in CWs. In contrast, biochar produced at high temperatures has a larger porosity and SSA, a higher
28 aromaticity, a higher carbon content, and overall a higher hydrophobic character (Del Bubba et al., 2020;
29 Castiglioni et al., 2021). The net surface charge of the chars (commonly evaluated by the pH of the point of zero
30 charge and/or Boehm's titration), which mainly depends on the surface functional groups of the material and is
31 often related to its ash content, is a further crucial parameters to explain the adsorption behaviours of biochars,
32 particularly towards ionized or ionisable compounds (Castiglioni et al., 2022). c Accordingly, best performing
33 biochars can be obtained a lower or higher temperatures, depending on the target molecule to be removed. For
34 example, phenol adsorption was higher for biochars produced at 900 °C than for those prepared at lower
35 temperature 600 °C, probably due to the relative increase in SSA at the higher pyrolysis temperature(Mohammed
36 et al., 2018). Similarly, Xu and Lu. (2019) reported an increasing removal efficiency of biochar towards
37 bisphenol from aqueous solutions with increasing the preparation temperature. However, Del Bubba et al.,

1 (2020), studying the removal of 16 alkylphenols and alkylphenol ethoxylates from real wastewater, with biochar
2 produced at 450, 650 and 850°C, observed higher absolute absorption maxima for materials produced at the two
3 highest temperatures, depending on of the investigated molecule.

4 The biochar can be modified chemically, physically or biologically to increase its properties and
5 achieve greater adsorption and catalysis capacities for the target pollutants (Xu and Lu, 2019). In addition, the
6 pH of the solution played a key role in controlling the deprotonation and hydrophobicity of the compounds,
7 which is in agreement with the correlation analysis of the maximum sorption capacity. The pH of biochar
8 produced to be used as a substrate in CWs was generally alkaline and varied between 7.9 and 9.8 (**Table 1**)
9 (Enaime et al., 2020; Kizito et al., 2017; Zheng et al., 2022).

10 The carbon content can give an early indication of biochar quality. Generally carbon (C) was the main
11 compositional element of biochar, varying approximately from 50% to 90%, followed by oxygen (O) and
12 nitrogen (N) and other elements that were present at much lower percentages (**Table 1**) (Gupta et al., 2016;
13 Kizito et al., 2017). In Kizito's study, element C was found at 69% in biochar derived from corn cobs and 90% in
14 wood, confirming that biochar characteristics are feedstock dependent (Kizito et al., 2017). The biochar
15 generally had a high surface area of several hundreds m²/g (Abedi and Mojiri, 2019; Deng et al., 2019); for
16 example, in Abedi's study, the BET surface area of biochar was around 809 m²/g (Abedi and Mojiri, 2019).
17 However, other investigations have found it as low as a few tens of m²/g (Ji et al., 2020; Zheng et al., 2022). For
18 example, the study by Zheng, who works on two feedstocks, the cattail (*Typha latifolia*) and sludge, shows that
19 the two feedstocks give low specific surfaces of 6.14 and 13.13 m²/g, respectively (Zheng et al., 2022). With
20 increasing pyrolysis temperature, the porosity, surface area and carbon content of biochar increased. However,
21 bio-assimilation decreased. The percentage of carbon in biochar grew from 57.8% to 63.2% as the pyrolysis
22 temperature increased from 300 to 500 °C. On the other hand, the surface area increased by more than one
23 magnitude from 10.0 m²/g to 281 m²/g (Li et al., 2018a). This shows that the porosity is extremely sensitive to
24 temperature variation compared to the percentage of carbon. These properties will probably influence their
25 function in CWs. According to Liao et al. (2022), the biochar must have a large pore volume and surface area to
26 adsorb pollutants and provide adhesion of microorganisms (Liao et al., 2022). In most cases the biochar used in
27 CWs has a higher specific surface area (>200 m²/g) to provide a higher number of adsorption sites (Shen et al.,
28 2020; Zhang et al., 2021; Gotore et al., 2022).

29 **3. Configurations of biochar-based CWs and their removal efficiency**

30 The performance of a CW depends on the type of CW, temperature, vegetation, water flow regime
31 (hydraulic regime), dissolved oxygen (DO), substrate nature, redox potential (Eh) and applied hydraulic load
32 (Parde et al., 2021; Malyan et al., 2021). Table 2 shows the order, dose, dimension of substrates, different plants
33 used in CW and the removal efficiency of pollutants of each configuration.

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Table 2: Characteristics of CWs integrated with biochar.

Implementation mode of the substrate (by order)	Plant species and density	Wastewater	CW size	Aeration	Feeding	HLR	HRT	Experiment duration	Removal efficiency	Reference
- Sand (0.5–2 mm) h= 50 mm - Biochar (2.95%) + gravel: h= 300 mm - Gravel (10–20 mm) h= 50 mm	<i>Acorus calamus L.</i> 4 rhizomes	Tail water	VF-CW h=450 mm d=160 mm	No	-	0.055 $\text{m}^3 \cdot (\text{m}^2 \cdot \text{d})^{-1}$	3 days	2 months	COD (76%) - TP (52%) - TN (82%) – NH_4^+ (84%) – NO_3^- (89%)	(Wang et al., 2022)
- Zeolite (d=2mm–4 mm) h=30 cm - Biochar (d=3mm–5 mm) h=30 cm - Cobblestone (d=20mm–30 mm) h=5 cm	<i>Phragmites australis</i>	Synthetic wastewater	VF-CW h=75 cm d=14 cm V= 2 L	No	-	260 $\text{L} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$	12 h	4 months	NH_4^+ (95.49%) - NO_3^- (83.24%) – TN (83%)	(Zhong et al., 2021)
- Clay ceramite (d= 2-5 mm) h=7 cm - Biochar (d= 2-5 mm) h= 14 cm - Clay ceramite (d= 2-5 mm) h=7 cm	<i>Lythrum salicaria</i>	Domestic wastewater	HF-CW l= 30 cm w= 15 cm h= 30 cm	Yes	Manually 4 L	-	24h	6 months	COD (75.5%) - TP (76.2%) - TN (59.2%) – NH_4^+ (62.5%)	(Ji et al., 2020)
- Gravel (d=7-8 mm) h = 3 cm - Biochar (d= 6-8 mm) h=10 cm - Gravel (d= 7-8 mm) h = 3 cm	<i>Plants hydroponics</i>	Synthetic wastewater	VF-CW d = 12 cm	-	-	-	-	6 months	COD (99.84 %) – NH_4^+ (92.00 %) – TP (88.63 %)	(Liao et al., 2022)
- Gravel (d=1-3 cm) - Biochar (d=1-2 cm) h=3-6-9 cm - Gravel (d=1-3 cm)	<i>Acorus calamus</i> 30 rhizomes· m^{-2}	Synthetic Wastewater	VF-CW h=35 cm d=33 cm	-	Manually 10 L	0.05 $\text{m}^3 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$	48 h	6 months	COD (89.88%) TN (86.36%) - NH_4^+ (63.51%)	(Deng et al., 2019)
- Pebbles (d= 90 mm) h=5 cm - Biochar (d=10 cm) -Gravel (d= 15 mm) h=17 cm - Gravel (d= 10 mm) h=5 cm	<i>Canna sp</i>	Synthetic wastewater	HF-CW 1m x 0.3m x 0.3m	Yes	32 L	-	72 h	-	COD (91.3%) - TN (58.3%) - NH_3^- (58.3%) – NO_3^- (92%) - TP (79.5%) - PO_4^{3-} (67.7%)	(Gupta et al., 2016)
- Pebbles (d=5-7mm); h=5 cm - Coke (d=3-5 mm); h=74 cm - Fe-modified biochar (50 mm×10 mm×5 mm)	<i>Canna</i>	River water	VF-CW h=100 cm d=30 cm	-	-	-	-	5 months	Abamectin (99%) – COD (98%) - NH_4^+ (65%) – TP (80%)	(Sha et al., 2020)

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- Pebbles (d=5-7mm); h=5 cm										
- Sandy soil h=10 cm - Sand (d= 2 mm) h=20 cm - Biochar (d=1-3 cm) h=40 cm - Gravel (d=2-3 cm) h=10 cm	<i>Colocasia esculenta</i> 64 seedlings/m ²	Domestic wastewater	VF-CW h=1.0 m d=0.5 m	Yes	-	-	-	6 months	COD (73%) - DBO ₅ (79%) - NH ₄ ⁺ (91%) – TSS (71%) - Total coliforms (70%)	(Nguyen et al., 2020)
- Sand (d < 2 mm) h= 15 cm - Gravel + Biochar (v/v=1:1): (d=1-2 cm) h=15 cm - Gravel + Biochar (v/v=1:1): (d=2-4 cm) h=25 cm - Gravel (d=5-7 cm) h=10 cm	<i>Iris pseudacorus</i> 6 rhizomes	Swine wastewater	VF-CW h=65 cm d=20 cm	Yes	-	33.74 g.m ⁻³ .d ⁻¹	72 h	2 months	COD (77.18 %) – NH ₄ ⁺ (96.54 %) - TN (40.12 %) - ARGs (99.3%)	(Feng et al., 2021a)
- Sand (d= 1-2 mm) h=150cm - Biochar + fine gravel (v/v=3:1): (d= 10-20 mm) h=150 mm - Gravel (d= 20-40 mm) h=250 mm - Gravel (d= 50-70 mm) h=100 mm	<i>Oenanthe Javanica</i> 12 rhizomes	Domestic wastewater	VF-CW h=65 cm d=20 cm	Yes	5.5 L	-	72 h	3 months	COD (91.80%) - NH ₄ ⁺ (50.05%) - TN (49.90%)	(Zhou et al., 2018)
- Gravel (d= 5-8 mm) h= 0.1 m - Biochar (sludge) + gravel (v/v=1:4) h= 0.2 m - Gravel (d= 5-8 mm) h= 0.1 m	<i>Typha latifolia</i>	Synthetic wastewater	VF-CW h= 0.5 m d= 0.2 m	No	-	-	72 h	60 batches	COD (90.99%) – NO ₃ ⁻ (99.50%) – NH ₄ ⁺ (99.59%) - TN (90.94%) - TP (51.59%)	(Zheng et al., 2022)
- Gravel (d= 5-8 mm) h= 0.1 m - Biochar (cattail) + gravel (v/v=1:4) h= 0.2 m - Gravel (d= 5-8 mm) h= 0.1 m	<i>Typha latifolia</i>	Synthetic wastewater	VF-CW h= 0.5 m d= 0.2 m	No	-	-	72 h	60 batches	COD (77.41%) - NO ₃ ⁻ (84.72%) - NH ₄ ⁺ (96.12%) - TN (80.73%) - TP (43.95%)	(Zheng et al., 2022)
- Gravel (d=2-6 mm) h=0.05 m - Biochar (v/v=1%) + sand (d=2-10 mm) h=0.2 m - Gravel (d=2-6 mm) h=0.05 m - Gravel (d=2-10 mm) h=0.05 m	<i>Iris pseudacorus</i> 5 rhizomes	Synthetic wastewater	VF-CW h=0.45 m d=0.15 m	No	-	-	72 h	4 months	COD (89.1%) - TN (90.2%) - NH ₄ ⁺ (81%)	(Ajibade et al., 2020)

- Soil h=10 cm - Quartz sand h=5 cm - Zeolite d=8–10 mm + biochar d=2–4 mm (v/v=1:1): h=30 cm - Cobblestones (d=7–10 cm): h=10 cm	<i>Phragmites communis</i> 6 plants	Synthetic wastewater	VF-CW l=50 cm w=40 cm d=60 cm	Yes	30 L	0.050 m ³ .m ⁻² .d ⁻¹	72 h	4 months	TN (62.98%) - NH ₄ ⁺ (93.93%) - NO ₃ ⁻ (93.28%) - COD (86.64%) – CIPH (88.05%) – SMZ (56.57%)	(Yuan et al., 2020)
- Sand (d = 2-4 mm) h = 2 cm - Biochar (2%) + Sand (98%): (d=5-10 mm) h= 15 cm - Sand (d = 2-4 mm) h = 3 cm	<i>Phragmites australis</i>	Synthetic stormwater	VF-CW h = 25 cm d = 11 cm	-	-	10-40 cm/h	5 days	3 months	TSS (71.1%) – TOC (29.3%) - NH ₄ ⁺ (13.5%) - TN (11.7%) - TP (8%) - <i>E.coli</i> (87.1%)	(Chen, 2018)
- Sand - Biochar + gravel: v/v = 50%. - Gravel	<i>Iris pseudacorus</i> 6 rhizomes	Synthetic wastewater	VF-CW h = 50 cm d = 10 cm	Yes	-	-	72 h	5 months	COD (93.21 %) - NH ₄ ⁺ (98.30 %) - TN (72.22 %) – TP (53.32%)	(Li et al., 2019)
- Gravel (d=8-10 mm) h=0.1 m - Biochar + gravel (v/v=4:1): h=0.2 m - Gravel (d=8-10 mm) h=0.1 m	<i>Typha latifolia</i>	Synthetic wastewater	VF-CW l= 0.3 m w= 0.3 m h = 0.5 m	-	-	-	5 days	60 batches	NH ₄ ⁺ (66.3%) – TN (65.4%) – COD (90%)	(Guo et al., 2020)
- Biochar (d=2-3 cm) h=25 cm - Zeolite (d=2-3 cm) h=25 cm - Gravel (d=2-3 cm) h=25 cm	<i>Phragmites australis</i>	Synthetic Wastewater	VF-CW h=80 cm d=40 cm	Yes	-	-	57.4 h	3 months	COD (99.9%) - NH ₃ ⁻ (99.9%) - Phenols (99.9) - Pb (99.9%) – Mn (99.9%)	(Abedi and Mojiri, 2019)
- Biochar (20%) + sand (80%): h=20 cm - Gravel: h=5 cm	<i>O. javanica</i> 12 rhizomes	Synthetic wastewater	VF-CW h = 50 cm d = 25 cm	NO	-	0.13 m ³ .m ⁻² .batch ⁻¹	7 days	8 months	COD (78.71%) - NO ₃ ⁻ (92.72%) - TN (93.26%) - NH ₄ ⁺ (94.26%)	(Li et al., 2018a)
- Biochar + sand: (d=0.5-1 mm) h=15cm - Gravel (d=4-6 mm) h=10cm - Gravel (d=8-12 mm) h=10cm - Rocks (d=20-21 mm) h=5cm	<i>Colocasia esculenta</i> 10 rhizomes	Domestic wastewater	VF-CW h=37cm d=33.5cm	Yes	-	-	10 days	40 days	COD (96.8%) - NO ₃ ⁻ (57.85%) - TN (68.02%) - NH ₄ ⁺ (88.16%) - PO ₄ ³⁻ (75.26%) - SO ₄ ²⁻ (80.50)	(Chand et al., 2021)
- Biochar (corn cobs) (d= 2-10 mm) h= 0.6 m - Gravel (d=50 mm); h=0.1 m	-	Industrial wastewater	VF-CW h = 0.9 m d = 0.2 m	No	-	-	-	5 months	COD (59%) - BOD ₅ (75%) - TN (37%) – NH ₄ ⁺ (76%) - PO ₄ ³⁻ (71%)	(Kizito et al., 2017)
- Biochar (wood) (d= 2-10 mm) h= 0.6 m - Gravel (d=50 mm); h=0.1 m	-	Industrial wastewater	VF-CW h = 0.9 m d = 0.2 m	No	-	-	-	5 months	COD (72%) - BOD ₅ (83%) - TN (47%) – NH ₄ ⁺ (83%) - PO ₄ ³⁻ (85%)	(Kizito et al., 2017)

- Biochar (d=2–4 mm) h=120 mm	<i>Salicaria seedling</i>	Synthetic wastewater	VF-CW d=110 mm h=150 mm	Yes	550 ml	-	24 h	> 3 months	Hg (>94%) – COD (>88%) – NH ₄ ⁺ (92.1) – TP (74.7%)	(Chang et al., 2022)
Mixture of Quartz rock d=2 - 4 mm (v/v=25 %), Bioceramic d=3 - 6 mm (v/v=25 %), and biochar d=1 - 7 mm (v/v= 50%) h=200 mm	<i>Cyperus alternifolius</i>	Synthetic wastewater	HF-CW l=670 mm h=310 mm w=300 mm	NO	30 L	-	25 h	-	NO ₃ ⁻ (67.16%) – TP (74.25%) – TN (64.31%) - NO ₂ ⁻ (51.6%) - PO ₄ ³⁻ (96.73%)	(Gao et al., 2018)
Mixture of quartz sand + soil (v/v=1:1) and Fe-modified biochar (v/v:10%)	<i>Iris hexagonus</i> 13 plants/m ²	Tailwater	VF-CW l= 100 cm w= 60 cm d= 75 cm	-	-	-	96 h	-	NO ₃ ⁻ (95.30 %) - TN (86.68 %) - NH ₄ ⁺ (86.33 %) - NO ₂ ⁻ (79.35 %) - COD (63.36 %)	(Jia et al., 2020b)
Mixture of biochar (v/v=10%) (d<20mm) and LECA (d=2-4 mm)	<i>Typha latifolia</i> 10 plants/mesocosm	Municipal wastewater	HF-CW l=1.5 m w=0.6 m d=0.6 m	-	-	60 L/d	48 h	4 months	TN (20.0 %) - TP (22.5 %)	(Kasak et al., 2018)
- Gravel (d=2-6 mm) h=0.05 m - Biochar (v/v=1%) + sand (d=2-10 mm) h=0.2 m - Gravel (d=2-6 mm) h=0.05 m - Gravel (d=2-10 mm) h=0.05 m	<i>Iris pseudacorus</i> 5 rhizomes	Synthetic wastewater	VF-CW h=0.45 m d=0.15 m	No	-	-	72 h	4 months	COD (75.9%) – TN (69.2%) – NH ₄ ⁺ (70.8%) – NO ₃ ⁻ (74.7%) – SMX (65.3%)	(Ajibade et al., 2021)
- Biochar + sand (d=0.25–1 mm) h=6 cm - Gravel (d=4-6 mm) h=10 cm - Gravel (d= 8-12 mm) h=10 cm - Boulders (d= 20-21 mm) h=5 cm	<i>Colocasia</i>	Synthetic wastewater	VF-CW d=33.5 cm h=37 cm V=30 L	No	-	-	-	-	COD (88.8%), NH ₄ ⁺ (83.1%), and NO ₃ ⁻ (64.9%) AMX (75.51%) - CF (87.53%) - IBU (79.93%)	(Chand et al., 2022)
Sand h=15 cm Biochar h= 20 cm Gravel h=15 cm	<i>G. maxima</i>	Synthetic wastewater	VF-CW d=15 cm h=55 cm	No	-	2 L/ 4d	-	3 months	PPCPs (99.99 %)	(Kang et al., 2019)
Stones (d= 5-10mm) h=0.05 m Biochar (d= 5-10mm) h=0.76 m Stones (d= 5-10mm) h=0.05 m	<i>Phragmites</i>	Municipal wastewater	VF-CW h=0.91 m d=0.15 m	No	-	-	-	-	NH ₄ ⁺ (89.8%) - NO ₂ ⁻ (38.5%) - TN (82.5%) – TP (91%) –BOD (95%) - COD (96.2%) – TSS (99.7%)	(Saeed et al., 2020)
Gravel (d=2 cm) Biochar v/v=30% (d=2 cm) Gravel (d=2 cm)	<i>Cyperus alternifolius L</i>	Synthetic wastewater	VF-CW h=35 cm S=0.1 m ²	Yes	-	-	24 h	-	COD (93.4%) - TN (94.9%) - NH ₄ ⁺ (99.4%)	(Liang et al., 2020)

Fe-modified biochar v/v=1/3 (d=1–2 mm) + gravel (diameter of 2–4 mm) h=50 cm	<i>Acorus calamus</i>	Synthetic wastewater	VF-CW h=60 cm d=25 cm	-	-	-	3 days	-	NH ₄ ⁺ (44.8%) – NO ₃ ⁻ (51.8%)	(Kang et al., 2023)
Cu-Biochar (40%) + sand (60%): h= 50 cm	<i>Iris pseudacorus</i> 6 plants/unit	Synthetic wastewater	VF-CW h= 75 cm d=25 cm	No	-	-	3 days	2 months	COD (75.33%) – NO ₃ ⁻ (91.11%) – Phenanthrene (94.09%)	(Shen et al., 2020)
Two cells: first one with gravel and second with biochar	<i>Melaleuca quinquenervia</i>	Domestic wastewater	HF-CW 1.2 m × 0.76 m × 0.4 m	No	-	0.023 m/day	5.1 days	7 months	PO ₄ ³⁻ (97%)	(Bolton et al., 2019)
Gravel (v/v=80% ; d=1–2 cm) + soil (v/v=10%) + biochar (v/v=10% ; d=0.1–0.5 mm)	<i>Hydrocotyle verticillata</i> + <i>Iris germanica</i> 100 clumps/m ²	Tail wastewater	HF-CW S= 900 m ²	No	-	-	1 day	3 months	TN(62.62%) - TP(52.99%) - NO ₃ ⁻ (73.28%) - NH ₃ ⁻ (53.11%) - PO ₄ ³⁻ (67.58%)	(Gao et al., 2019)
Zeolite (d=20 cm) Biochar (d=10 cm) Gravel (d=20 cm)	<i>Canna indica</i> 16 plant/m ²	Synthetic wastewater	HF-CW 110 cm ×40 cm ×60 cm	No	-	-	-	11 months	NH ₄ ⁺ (89.1%) – TN(88.1%) – TP(75.9%)	(Wu et al., 2022)

1 HRL: Hydraulic loading rate, HRT: Hydraulic retention time

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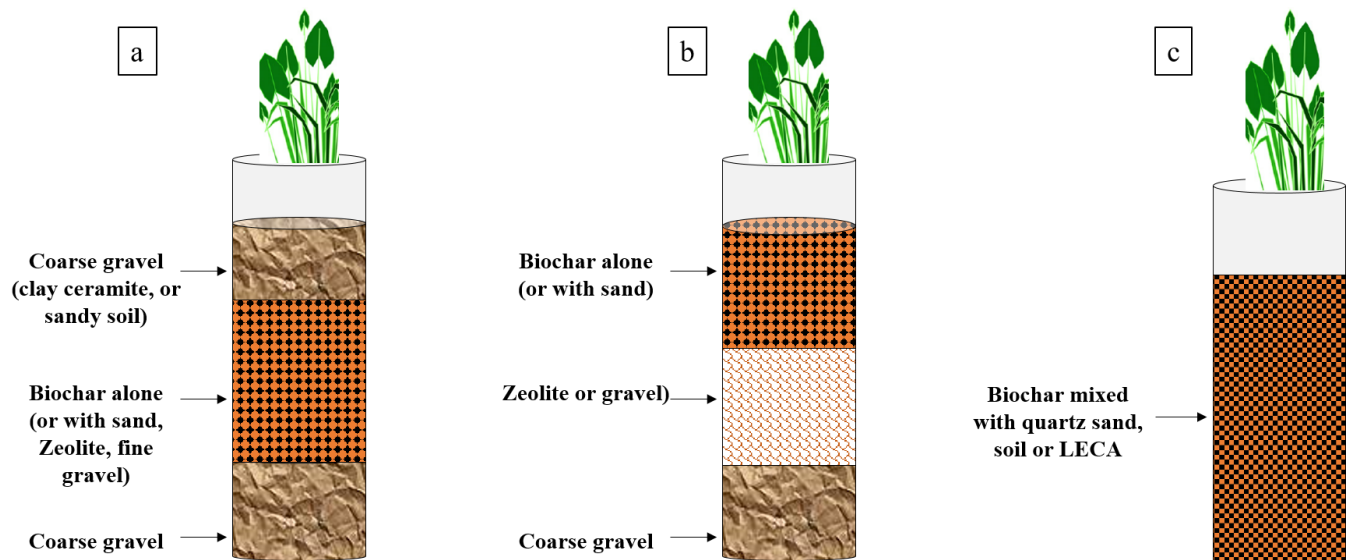
3.1. Integration mode of biochar in CWs

3.1.1. Biochar in vertical flow CW

When used as substrate in VF-CWs, biochar can potentially promote contaminant removal. As illustrated in Fig. 1-a, most CWs are implemented by positioning the biochar between two layers of inert material (see Table 2), thereby avoiding the clogging of the filtration system (Ji et al., 2020; Liang et al., 2020; Liao et al., 2022). In this interlayer, the biochar is used alone or mixed with other materials, namely sand, gravel, etc. (Table 2) (Ajibade et al., 2020; Liao et al., 2022; Zhong et al., 2021; Zhou et al., 2018).

Several authors have used the biochar substrate alone as an interlayer of the filter system in order to increase the removal rate of different pollutants. For example, in the study of Nguyen et al. (2020), the biochar substrate is used under two sand and sandy soil layers. This distribution increases the removal efficiencies of total coliforms up to 70% (Nguyen et al., 2020). Moreover, using biochar substrate under a coarse stone substrate allows the removal of total phosphorus up to 91% and organic matter such as BOD and TSS up to 95% and 99.7%, respectively, from municipal wastewater (Saeed et al., 2020). Another study placed the biochar substrate under a coarse pebble layer to improve nitrate removal performance up to 92% and orthophosphate up to 67.7% (Gupta et al., 2016). However, using gravel substrate over biochar increases the removal performance up to 94.9% TN, 99.4% NH_4^+ and 99.84% COD (Liang et al., 2020; Liao et al., 2022). On the other hand, the modification of biochar with iron shows high removal performance of pollutants such as Abamectin (99%), COD (98%), NH_4^+ (65%) and TP (80%) (Sha et al., 2020).

Biochar can be mixed with gravel (Feng et al., 2021a), sand (Ajibade et al., 2020), or zeolite (Yuan et al., 2020) to form a single substrate to filter various micropollutants from wastewater. Zheng et al. (2022) found that mixing biochar with gravel at a volume ratio of 1:4 resulted in high removal efficiency of COD (90.99%), NO_3^- (99.50%), TN (90.94%), NH_4^+ (99.59%), and TP (51.59%). On the other hand, mixing biochar with sand with a low volume ratio of biochar (2%) gave low removal rates (TOC (29.3%); NH_4^+ (13.5%); TN (11.7%); TP (8%)) except for *E.coli*, TSS and coliforms, which show high removal efficiency, coming up to 87.1% and 71.1% for *E.coli* and TSS, respectively (Chen, 2018). Similarly, Ajibade et al. (2020), also mixed biochar with sand. Still, this time gave a high performance compared to the study of Lun and Chen. (2018), where the removal efficiency of some pollutants reached 89.1% for COD, 90.2% for TN and 81% for NH_4^+ (Ajibade et al., 2020). The ratio of biochar can explain the difference between these two studies that is higher in the second one. Yuan et al. (2020) reported that mixing biochar with zeolite can improve the removal percentage up to 63% for TN, 94% for NH_4^+ , 93% for NO_3^- and 87% for COD. This result may be justified by the fact that the biochar inhibited the formation of quinolone resistance genes and enhanced the COD removal efficiency by increasing the abundance of bound microorganisms (Yuan et al., 2020). In most studies, biochar substrates mixed with gravel showed higher removal efficiency of various pollutants compared to biochar substrates mixed with sand (Table 2).



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2 *Figure 1: Position of biochar substrate (a): as interlayer of VF-CW, (b): on top of the VF-CW, (c):*
3 *filling all the VF-CW*

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6 Biochar can also be placed at the top (**Fig. 1-b**) (**Table 2**) of the filtration system with large grain size
7 (2-30 mm) in order to avoid the clogging phenomenon (Abedi and Mojiri, 2019; Kizito et al., 2017). In Abedi
8 and Mojiri. (2019), the top biochar layer played an important role in decreasing the content of various pollutants
9 such as COD, NH_4^+ , phenols, Pb, and Mn. This study showed the best removal performance compared to the
10 literature, since the removal efficiency was quantitative for COD, NH_4^+ , phenols, Pb and Mn (Abedi and Mojiri,
11 2019). This result can be explained because biochar is mainly attributed to the greater adsorption capacity and
12 microbial culture in the porous medium of biochar (Kizito et al., 2017). Furthermore, the use of biochar at the
13 upper filter level revealed that adding biochar in VF-CWs improves the oxidative removal of $\text{NH}_4^+\text{-N}$, SO_4^{2-} , and
14 PO_4^{3-} and contributes to the uptake of other plants (Chand et al., 2021). Another study conducted by Chand et al.
15 (2021) used biochar on top of a system with small grain size ($d = 0.5\text{-}1\text{ mm}$), but to avoid clogging, they mixed
16 the biochar with sand, which allowed them to increase the treatment efficiency and thus removed up to 97%
17 COD, 58% NO_3^- , 68% TN, 88% NH_4^+ , 75.26% PO_4^{3-} and 80% SO_4^{2-} (Chand et al., 2021).

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20 Sometimes the whole filter is filled from top to bottom with biochar (**Fig. 1-c**) (**Table 2**) mixed at low
21 rate (10%) with another material (quartz sand, soil, LECA), to avoid the clogging of the system. For example, Jia
22 et al. (2020) mixed 10% biochar with quartz sand and soil to fill the entire filter and obtained an increase of the
23 removal efficiency of pollutants (NO_3^- (95.30%); TN (86.68%); NH_4^+ (86.33%); NO_2^- (79.35%); COD (63.36%))
24 (Jia et al., 2020).

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3.1.2. Biochar substrate in the horizontal flow CW

The use of biochar in horizontal flow CWs (HF-CWs) is still limited, and a little number of articles was found (Gao et al., 2018; Bolton et al., 2019; Gao et al., 2019; Jia and Yang, 2021; Wu et al., 2022). For example Bolton et al., (2019) implemented two small pilot- scale HF-CWs planted with *Melaleuca quinquenervia* trees, each one consisting in two cells separated by a polyethylene baffle. The first wetland contained two cells in series filled with gravel (control wetlands), while in the other wetland the first cell was filled with gravel to trap sediments, thus avoiding blockages in the downstream cell, the latter filled with an enriched biochar cell (biochar wetlands). This study showed that the removal efficiencies of PO_4^{3-} - P in the biochar wetland was up to 97% probably due to the higher number of adsorption sites in the substrate. In contrast, the control achieved only an average PO_4^{3-} - P removal of 91%, indicating a rapid saturation of the gravel. Another study realized by Gupta et al., (2016) revealed that HF-CWs with biochar were more efficient to reduce various pollutants (organic and inorganic) as compared to the wetland with gravels alone. Hence, the removal efficiencies achieved were around 58% of TN, 79% of TP, 92% of NO_3 -N, 58% of NH_3 -N, 68% of PO_4^{3-} -P and 91 % of COD. The high removal of NH_4^+ -N obtained in HF-CWs is probably related to the enhanced microbial nitrification when adding biochar (Gupta et al., 2016). The improved NO_3 -N removal efficiency is attributed to a higher denitrification, due to the anoxic conditions in HF-CWs. These results indicate clearly that integrating of biochar in HF-CW can be primarily used for a secondary treatment of municipal and domestic wastewaters leading to nutrients removal. In general, the use of biochar in HF-CWs can be a cost-effective and sustainable wastewater treatment option with a smaller energy footprint (Wu et al., 2022; Gupta et al., 2016).

3.2. Effect of substrate nature, biochar dose and granulometry on CWs efficiency

The fundamental element of the CW system is the substrate or media, which is essential for removing contaminants from wastewater. It serves as a platform for biofilm development, macrophyte root growth, and a reaction site for pollutants' immobilization and supporting matrix (Wu et al., 2015). Therefore, the choice of bed materials is highly important in a CW. Inexpensive and locally available materials can be used depending on the size of the media, its hydraulic conductivity, texture, porosity, and other factors (Wu et al., 2015). Gravel, biochar, zeolite, composite materials and activated carbon have been used as CW substrates (Kataki et al., 2021). Substrates such as sawdust, light expanded clay aggregate (LECA), zero-valent iron, and gravel can effectively remove phosphorus, organic matter, arsenates, and sulfates (Parde et al., 2021).

Biochar-based CWs show promising wastewater treatment efficiency (Enaime et al., 2020). However, granular biochar is more suitable for applications than powdered ones. This can be explained by its good pore size distribution, low abrasion index, durability, high bulk density, and ability to regenerate (Louarrat, 2019). In addition, this type of biochar has sufficient mechanical strength and is suitable for ensuring the stability and

1 hydraulic permeability of the matrix (Deng et al., 2021). In addition, particle size has a significant effect on
2 pollutants adsorption. Nitrate-nitrogen content, ammonia nitrogen content, and denitrification intensity of the
3 wetland substrate decreased by 51%, 47%, and 35%, respectively, after the introduction of biochar with a
4 particle size ranging from 1-2 mm in CW (Zhou et al., 2018), when compared to biochar with a particle size
5 lower than 1 mm. Biochar with a 1-3 cm diameter is widely used as a substrate in CWs to avoid clogging (**Table**
6 **2**) (Nguyen et al., 2020). Other factors influence the adsorption of pollutants, such as increasing of the contact
7 time, pH, temperature, and concentration of NH_3 . But adsorption is decreasing with increasing the size of biochar
8 particles (Kizito et al., 2015). According to these results we can state that the biochar granulometry has a
9 significant effect on the efficiency of the treatment of the pollutants.

10 On the other hand, the biochar dose in CW substrate strongly influences the removal performance of
11 various pollutants. However, a study conducted by Deng et al. (2019) was built based on different volumes of
12 biochar in common gravel (0%, 10% (h=3), 20% (h=6), and 30% (h=9)) to see the effect of increasing biochar
13 substrate depth on the characteristics of metabolites and microbes. This experiment found that increasing the
14 biochar dose in the gravel medium enhanced the contaminant removal efficiency in CWs. Hence, Illumina
15 MiSeq sequencing reported that the microbial community showed some obvious variations. The relative
16 abundances of *Candidatus competibacter*, *Thauera*, *Dechloromonas*, *Chlorobium*, *Thiobacillus* and
17 *Desulfobulbus* were significantly improved with the biochar dose. On the other hand, the content of total Extra
18 Polymeric Substances (EPS) decreased with increasing the biochar percentage.

19 Furthermore, the increase in biochar dose in CWs substrate reflects an improvement in the
20 biodegradation of EPS and the richness of microbial communities, which promotes the removal of organic and
21 nitrogenous substances (Deng et al., 2019). Similarly, Liang et al. (2020) used 4 CW microcosms with different
22 volume ratios of biochar (0%, 10%, 20%, and 30%) to analyze the improvement of pollutant removal
23 performance. The results showed that the increase in biochar dose increased the average removal efficiencies of
24 total nitrogen (TN) and ammonium ($\text{NH}_4^+\text{-N}$). At the same time, nitrous oxide (N_2O) emissions were reduced.
25 The increase in biochar dose can explain this change in the diversity and similarity of the microbial community.
26 In addition, the relative abundance of functional microorganisms such as Nitrospira, Nitrosomonas,
27 Pseudomonas, and Thauera increased due to the increase in biochar content, which favored nitrogen cycling and
28 reduced N_2O emissions.

29 **3.3. Effect of macrophytes used and its role in CWs implemented with biochar**

30 Plants are essential in removing pollutants, as they generally play an indirect role in the wastewater
31 treatment performance in CWs (Fu et al., 2022). The choice of appropriate plant species is crucial for the best
32 performance (Guittonny-philippe et al., 2015; Srivastava et al., 2008; Kulshreshtha et al., 2022). Hence, the right
33 choice was based on several parameters; the species that are preferred are characterized by high ecological
34 adaptability, adaptation to local climatic and nutritional conditions, high biomass productivity, resistance to pests
35 and diseases; having good coverage with high prospects of successful establishment, tolerance to pollutants and
36 hypertrophic waterlogging conditions, low tendency to dominate or forming monocultures, a high capacity for

1 pollutant removal, easy propagation, and rapid establishment (Nuamah et al., 2020; Kataki et al., 2021).
2 According to literature the *Phragmites australis* was the most used plant in the studies (**Table 2**), due to its effect
3 on the efficiency of CW, resistance to pests and diseases, tolerance to pollutants and hypertrophic waterlogging
4 conditions, high capacity for pollutant removal, easy propagation and adaptation to local climatic and nutritional
5 conditions (Zhong et al., 2021; Yuan et al., 2020; Chen, 2018). However, a comparative study done by Qadiri et
6 al., (2021) has demonstrated that the CWs transplanted with *Phragmites* has more capacity in removing TN,
7 COD, TP and TSS than *Sagittaria latifolia* and *Iris kashmiriana*, due to its well developed roots in the substrates
8 which gives a better remediation effect. Furthermore, the presence of a biochar substrate in the CW promotes
9 plant growth, microbial metabolism and substrate characteristics in many aspects (Qadiri et al., 2021). Another
10 key parameter in selecting CW species is the higher water use efficiency index (Stefanakis, 2020). Several
11 studies have shown that plants with fibrous root systems provide a greater surface area for biofilm enhancement,
12 sedimentation, and particulate matter trapping. They show higher photosynthesis and radial oxygen loss levels
13 and are more effective in removing contaminants than plants with thick roots (Kataki et al., 2021); (Borne et al.,
14 2013; Lai et al., 2012). In addition, previous studies have shown that plant density affects CWs performance at 5
15 to 50 plants/m². A low density (16 m²) CW planting may result in lower nitrogen removal than a CW with a high
16 plant density (32 m²) (reduced by almost half) (Hernández et al., 2017). Another factor to consider is the age of
17 the plant, as oxygen release and contaminant uptake are lower in older plants due to the presence of older
18 lignified roots (Valipour and Ahn, 2015).

19 **3.4. Effectiveness of biochar in removing various pollutants**

20 Biochar is a solid material with high porosity, a high surface area, and diverse surface functional groups
21 and properties, making it an attractive option for wastewater treatment. Biochar has been proposed as an
22 effective substrate for capturing wastewater supplements that may be connected to soil alteration.. The
23 adsorption properties and high porosity allow pollutants to accumulate on its surfaces, resulting in supplement-
24 rich biochar and a clean effluent (Peiris et al., 2017; Yaashikaa et al., 2020). Biochar adsorbents have been used
25 to remove various contaminants (**Table 2**) such as antibiotics (Ahmed et al., 2017), pesticides (Mandal et al.,
26 2021), pharmaceuticals (Masrura et al., 2021; Solanki and Boyer, 2017), and personal care products from aquatic
27 environments (Keerthanan et al., 2020). The use of biochar for wastewater treatment is becoming more viable
28 due to the low cost of the raw material and the ease of the manufacturing process, as well as the various
29 improved physicochemical characteristics of biochar, which have been successfully used in a diverse range of
30 applications for the contaminated wastewater remediation, including toxic heavy metals adsorption (the
31 following techniques have been used: chemisorption, physical sorption, ion exchange, and precipitation) and
32 dyes from aqueous solutions, as immobilization support for microorganisms, as a support for catalysts, and as an
33 adsorbent for inhibiting substances during anaerobic digestion, thanks to its unique and very versatile
34 characteristics. Overall, it is clear that biochar has multiple potential economic and environmental benefits, and
35 its effectiveness in removing various contaminants on a laboratory scale has been widely reported (Ahmad et al.,
36 2021; Enaime et al., 2020; Chen et al., 2022).

1 Biochar added to CW substrate can considerably enhance the wastewater purification effect (Kizito et
2 al., 2017), as biochar can remove more nutrients and reduce greenhouse gas (GHG) emissions than other
3 substrates, e.g., ceramite, while promoting more diverse bacterial communities and greater abundances of
4 available taxa (Ji et al., 2020). The average N₂O and CO₂ fluxes were significantly lower, while CH₄ fluxes were
5 significantly greater in the biochar-added and non-biochar CWs (Guo et al., 2020). Biochar combined with sand,
6 zeolite, and other artificial CW substrates can enhance microbial activity and compensate for the lack of carbon
7 sources (Wang et al., 2020b). Abedi and Mojiri. (2019) reported that CW containing three substrate layers,
8 namely biochar, gravel and zeolite layers, showed high performance in wastewater treatment compared to the
9 other CWs containing gravel as a substrate; the first CW can remove pollutants from wastewater better than the
10 second one. At an optimum retention time (57.4 h) and pH (6.3), this biochar integrated CW can remove up to
11 99.9% of COD (1000 mg/L), ammonia (1000 mg/L), phenols (50 mg/L), Pb (50 mg/L) and Mn (50 mg/L). In
12 addition, the emission of nitrous oxide was lower in gravel CW than in the integrating biochar CW (Abedi and
13 Mojiri, 2019). These results can explain that the introduction of biochar considerably improved the abundance of
14 biological bacteria in CW, consequently increasing the efficiency of removing various contaminants in
15 wastewater (Li et al., 2018a). This agrees with the results of Liang's study (**Table 2**), which explains the increase
16 in nitrogen removal efficiency and the decrease in N₂O emissions resulting from the increase in biochar addition
17 ratio. This shows that biochar addition changed the diversity and similarity of the microbial community (Liang et
18 al., 2020).

19 In general, the removal efficiency of pollutants was increased due to biochar adsorption (Meng et al.,
20 2019). In addition, the total amount of extracellular polymeric substance (EPS) decreased significantly with the
21 addition of biochar, which is explained by the change in the functional groups of EPS, including amide,
22 carbonyl, and hydroxyl groups of proteins. Furthermore, biochar has the potential to convert metabolized high
23 molecular weight compounds into low molecular weight compounds (Deng et al., 2019).

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27 The biochar can be used at various stages of the wastewater treatment process to increase treatment
28 capacity and recover value-added by-products. The adsorption, buffering, and immobilization mechanism of
29 microbial cells may influence the use of biochar in the wastewater treatment system. For example, properly
30 modified biochar could effectively adsorb nutrients such as phosphorus and nitrogen from treated effluent,
31 allowing it to be used for soil rehabilitation as a nutrient-enriched material. In addition, biochar could help
32 develop activated sludge's treatment and settling capacity by adsorbing inhibitors and hazardous chemicals or
33 providing a surface for microbial immobilization when used in the treatment process. The introduction of
34 biochar to the biological system can also help increase the soil amendment capabilities of biosolids, extend the
35 value chain, and provide other economic benefits as interest in its use in soil applications increases (Mumme et
36 al., 2014). The following sections discuss biochar's role in removing various contaminants from wastewater.

3.4.1. Removal of organic pollutants

Numerous studies have been conducted in recent years to test the effectiveness of biochar in removing various organic substances from water, such as antibiotics, drugs, agrochemicals, polycyclic aromatic hydrocarbons (PAHs), cationic aromatic dyes, and volatile organic compounds (VOCs) (see **Table 2**) (Adeel et al., 2016; Mondal et al., 2016).

3.4.1.1. Removal of conventional pollutants

Organic pollutants are another important type of pollutant in the aquatic environment, the biochar has shown a high removal efficiency towards this kind of pollutants. Based on the literature, the biochar prepared at a higher pyrolysis temperature will improve non-polar organic compounds' removal efficiencies due to higher microporosity and surface area (Mohamed et al., 2016; Mohanty et al., 2013). On the other hand, the biochar prepared at a temperature below 500 °C comprises a higher amount of hydrogen and oxygen-containing functional groups, so it is more likely to have a high affinity for polar organic molecules (Suliman et al., 2016). For example, biochar derived from rice husk and pyrolyzed soybeans at 600-700 °C facilitates the removal of trichloromethylene (VOC) and non-polar carbofuran (pesticide) from contaminated water (Suliman et al., 2016). In addition, at T >700 °C, red gum wood chips and chicken litter-derived biochar efficiently removed pyrimethanil and diesopropylatrazine (fungicide/pesticide), whereas the same biochar at T <500 °C proved ineffective (Chen and Chen, 2009; Yu et al., 2010). And for the removal of polar insecticides and herbicides such as norflurazon, 1-naphthol and fluridone was performed using biochar produced at <300 °C, as a result of the pollutant's interaction with the biochar's functional groups (Li et al., 2016; Sun et al., 2011). On the other hand, the biochar with more O and H functional groups (<400 °C) showed higher sorption of aromatic cationic dyes such as methyl-blue and methyl-violet. Still, the process strongly depended on pH (Adeel et al., 2016; Teixid et al., 2011). In addition, the polar antibiotic sulfamethazine (SMZ) exhibits pH-dependent interactions when sorbed to softwood/hardwood-derived biochars (pyrolyzed at 300-700 °C) (Mohan et al., 2014). Therefore, it can be considered an important parameter for biochar interactions and polar organic contaminant removal.

Generally, organic matter from wastewater may be removed by filtration, adsorption, hydrolysis, chemical reduction or oxidation by microbial degradation, etc. (Vymazal and Tereza, 2015). The degradation by the microbiota attached to the substrates is responsible for the elimination of organic matter in aqueous solutions (Faulwetter et al., 2009). Conventional organic compounds such as chemical oxygen demand (COD) and biological oxygen demand (BOD₅) can be removed effectively due to the coupling role of anaerobic and aerobic degradation in CW systems (Saeed and Sun, 2017; Zhao et al., 2020). Thus, the integration of biochar into CWs plays an important role in COD removal, even though organic matter can be leached from biochar (Zhou et al., 2019). However, Several studies have shown that biochar amendment promotes COD removal in CWs (Deng et al., 2019; Guo et al., 2020). This result can be explained by the good adsorption capacity of biochar toward organic molecules and provides a heterogeneous surface with very high porosity for oxygen filling and habitation by various organic degradation microbes. Moreover, biochar can promote plant growth, releasing additional oxygen into CW substrates for aerobic COD decomposition. A recent finding by some researchers

1 show that the introduction of biochar into CWs can reduce the quantity of microbial extracellular polymeric
2 substances (EPS) accumulated in the wastewater matrix and induce their metabolization of heavy molecular
3 weight EPS metabolites into lower molecular weight compounds because biochar increases the metabolic and
4 abundance activities of heterotrophic bacteria, thus reflecting organic decomposition, which is conducive to
5 mitigating the clogging of wastewater treatment substrate.

6 **3.4.1.2. Emerging pollutants**

7 Emerging hazardous organic pollutants that can be contained in stormwater, livestock wastes,
8 agricultural waters, and industrial wastewaters, etc., such as dyes, pesticides, herbicides, endocrine disruptors
9 (e.g., phthalic acid esters, polycyclic aromatic hydrocarbons, and bisphenol A), and antibiotics (**Table 2**), pose
10 serious long-term threats to ecosystems and public health, even at minute concentrations (Vymazal and Tereza,
11 2015). Hydrophobic effects, electrostatic attraction, conjugation of aromatic-donors and cationic-acceptors, pore
12 filling, and hydrogen bonding are all processes that biochar can use to adsorb these contaminants (Xiang et al.,
13 2020; Zhang et al., 2019). Most importantly, biochar possesses catalytic and redox-reactive activities, allowing it
14 to accept/donate electrons or promote generate ROS and electrical conduction, thus accelerating the abiotic
15 decomposition of adsorbed organic pollutants (Devi and Saroha, 2015; Zhang et al., 2019). In addition, biochar
16 substrates may stimulate the reproduction and development of microbes involved in decomposing organic
17 pollutants. However, this augmentation role of biochar has only been studied profoundly so far (Yan et al., 2017;
18 You et al., 2020). The mechanisms involved depend mainly on biochar properties, operating conditions and
19 contaminants. Due to the exceptional ability of biochar to adsorb bisphenol A, Lu and Chen. (2018) found that
20 the integrating biochar into CWs improved the elimination of bisphenol A from stormwater and increased the
21 life of CW systems. According to the same authors, the biochar prepared at 700 °C performed significantly
22 better than biochar prepared at 300 and 500 °C. In addition, the biochar substrate supported the increase of
23 functional microbes and served as an excellent biofilm carrier to indirectly enhance the decomposition of
24 bisphenol A. Improved plant growth in CWs also facilitates the removal of organic pollutants (Chen, 2018).
25 Tang et al. (2016) used plant-derived biochar that was planted in a *Cyperus alternifolius* CW and then modified
26 with Fe(NO₃)₃ solution to achieve higher removal efficiencies (>99%) and constant rate for four pesticides in
27 wastewater than the non-biochar control (64 - 99%) (Tang et al., 2016). The cause is that biochar adsorbs the
28 pesticides and promotes their microbial decomposition. The use of biochar derived from fruit pits in zeolite-
29 based CWs significantly increased antibiotic removal rates (sulfamethazine and ciprofloxacin) while also
30 decreasing the production of sulfonamide and quinolone resistance genes, which was attributed to the biochar's
31 ability to facilitate antibiotic biodegradation and adsorption (Yuan et al., 2020). Biochar is a good attachment
32 medium for microbes that degrade organic matter. For example, Mahmood et al. (2015) used corn-derived
33 biochar manufactured at 400 °C as a biofilm support for *Pseudomonas putida* cells to adsorb and reduce dyes
34 and Cr (VI) in a continuous flow bioreactor for the efficient treatment of tannery wastewater containing azo
35 dyes, aniline and Cr (VI).

36 Other organic compounds, such as pharmaceuticals and pesticides, are considered emerging
37 contaminants because of their effects on human health, and have been detected in municipal wastewater

1 treatment plants (Firouzsalar et al., 2019; Shi et al., 2021). Wastewater from the pharmaceutical industry
2 contains pharmaceutical intermediates used in production (Karunanayake et al., 2017), antibiotics and active
3 ingredients such as hormones (Rashid et al., 2021). However, pesticides are found in industrial wastewater
4 through pesticide production (Pinto et al., 2018), washing of commercial containers used to store or transport
5 pesticides (Zapata et al., 2010), and agri-food industries (Lopes et al., 2020). The biochar as adsorbent promote
6 the degrade antibiotic and antibiotic resistance genes (ARGs) from wastewater, and dissolved organic carbon
7 release in CWs indicated that water and alkaline media portray the optimum conditions for SMX and ARGs
8 removal, this shows the feasibility of using biochar for regulated sulfamethoxazole (SMX) removal and ARG
9 accumulation (Ajibade et al., 2021). However, the study of Feng et al., (2021) showed the relation between
10 ARGs removal and dissolved organic matter (DOM). They, noted that the photosensitized DOM is responsible
11 for producing reactive intermediates to remove ARGs. Hence incorporating biochar under forced aeration into
12 CWs could remove ARGs up to 99.3% and DOM 72% effectively from swine wastewater. Abas et al., (2022)
13 confirmed that the integration of biochar substrate has an effect in improving Chlorantraniliprole (CAP) removal,
14 CAP mass removal was very high in biochar (99%). The biochar also enhance the efficiency of the treatment
15 pharmaceuticals and personal care products (PPCPs) form wastewater, the presence of the colonization of
16 arbuscular mycorrhizal fungi (AMF) in CWs enhanced the best removal performance for PPCPs in biochar
17 added systems (more than 99.99%). These results can be attributed to the higher adsorption capacity of PPCPs of
18 biochar, due to its large surface area and porous structures of biochar substrate, which could also promote the
19 development and growth of microbes and the adsorption of PPCPs, thus enhancing its biodegradation (Hu et al.,
20 2022; Hu et al., 2022).

21 Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic compounds (Gaurav et al., 2021),
22 with at least two aromatic rings (Kang et al., 2019). They include compounds such as phenanthrene, naphthalene,
23 anthracene, pyrene, fluorine and benzofluoranthene (Jain et al., 2020; Kong et al., 2021). Several studies have
24 used biochar as an adsorbent substrate to remove this pollutant, because biochar may provide a reproduction
25 habitat for microbes and enhance the microbial community to improve denitrification and PAHs removal
26 performance (Cao et al., 2021). Furthermore, the biochar was also tested to remove benzofluoranthene (BbFA), a
27 typical PAH in CWs, and has shown higher BbFA with its removal efficiency exceeding 99%, which could be
28 attributed to enhanced PAH biodegradation (Guo et al., 2020). In the same way Kang et al., (2023), was studying
29 removal efficiency of representative PAH, benzofluoranthrene (BbFA), using biochar modified by iron as a
30 supplement to the CW substrate. They reached to increase the performance of BbFA removal by 20.4 %, because
31 the biochar may increase dissolved organic carbon content, particularly low-aromaticity, which contributed to
32 PAH degradation by microorganisms. In addition, the presence of functional groups on the biochar surface may
33 improve the electron interactions between microorganisms and PAHs.

34 **3.4.2. Removal of inorganic pollutants**

35 Inorganic contaminants in wastewater include compounds such as nitrite (NO_2^-), ammonium (NH_4^+),
36 nitrate (NO_3^-), hydrogen sulfide (H_2S), phosphorus (PO_4^{3-}) and heavy metals (Cu, Cr, Cd, Pb, Fe, Hg, Zn and As
37 ions) (**Table 2**) that cause a dangerous risk to human health and the environment (CAO et al., 2009). Generally,

1 biochar produced at low pyrolysis temperature (about 500°C) is used to remove inorganic contaminants. The
2 nature of biochar sorption is influenced by the morphological structure and chemical composition (Abdelhafez
3 and Li, 2016).

4 **3.4.2.1. Nitrogen removal**

5 Multiple pathways are used to remove nitrogen from wastewater in CW, substrate adsorption, ammonia
6 volatilization, plant uptake and microbial processes (Saeed and Sun, 2017). Classical microbial nitrification,
7 followed by denitrification, and finally converting N to N₂O or N₂, is considered the most common mechanism
8 (Jia et al., 2020b; Vymazal, 2011). However, the insufficient ability of sand, and gravel to adsorb nitrogen and
9 provide habitable microsites for denitrifying microorganisms remains a major challenge in conventional CW
10 systems filled with gravel, ceramite, or sand (Kizito et al., 2017; Yang et al., 2018), although ceramite gives
11 better results than gravel or sand which are widely used (Vohla et al., 2011). In addition, low dissolved oxygen
12 (DO) due to inadequate reoxygenation may limit nitrification in flooded streams, and/or denitrification can be
13 limited by electron donors deficient for nitrate reduction (Lu et al., 2020; Vymazal, 2011). Therefore, several
14 solutions are being investigated to improve nitrogen removal from wastewater, including introducing substrates
15 with high nitrogen removal capacity (Jia et al., 2020b; Shen et al., 2018).

16 Cation exchange can keep cations in biochars with a high surface charge density. Consequently, the
17 internal porosity, high biochar surface, and presence of polar and non-polar sites on the biochar surface promote
18 nitrifier growth and nutrient adsorption and simpler and easier atmospheric aeration and oxygen replenishment at
19 the bottom of the CW matrix. As well as, the addition of the biochar substrate can increase the rate of
20 nitrification, resulting in a great improvement in total nitrogen (TN) and NH₄⁺ removal in CW (Kizito et al.,
21 2017; Rozari et al., 2018; Zhou et al., 2019). However, the leaching of dissolved organic matter (DOM) can be
22 done with the help of biochar, which is mainly based on humic acid, which allows it to temporarily trap the
23 influent DOM in the pores as a carbon source to stimulate denitrification after desorption (Li et al., 2018a; Zhou
24 et al., 2019). Denitrifier proliferation may also be enhanced, resulting in nitrate denitrification for low C/N
25 effluents (Zhou et al., 2019). On the other hand, biochar acts as a chemically redox-active material with
26 electroactive functional groups on its surface (e.g. phenols and quinones), which promotes the biochemical
27 transfer of the material into wastewater (Yuan et al., 2018; Zhang et al., 2019). According to Wu et al. (2018),
28 biochar derived from cattail stalks prepared at 300°C can increase the electron conversion efficiency between the
29 metabolism of carbon and nitrate reduction by modulating the electron shuttle mechanism and increasing the
30 activities of denitrifying enzymes, which can increase the rate of denitrification in wastewater, in contrast,
31 biochar made at 800 °C inhibits these mechanisms. As a result, many studies have reported that biochar addition
32 to domestic, swine, anaerobic, and secondary wastewater effluents improved nitrogen removal efficiency (by
33 more than 20% on average). Removal efficiency increased proportionally with biochar dosage, although the
34 performance improvement depended on biochar loading and preparation conditions, wastewater properties, and
35 wastewater operating conditions. Biochar substrates in settling ponds showed better nitrogen removal than
36 conventional gravel or sand and some functional fillers, such as zeolite and ceramite (Ji et al., 2020; Yuan et al.,
37 2020).

1 **3.4.2.2. Phosphorus removal**

2 Phosphorus compounds (P) in wastewater may be eliminated by a variety of processes, including
3 substrate precipitation, adsorption, plant uptake, and microbial uptake into wastewater, with substrate retention
4 generally being the most widely used process (Kumar and Dutta, 2019; Saeed and Sun, 2017). Elements such as
5 Fe, Ca, Mg, and Al in CW fillers can bind phosphorus stably; therefore, materials rich in these elements (Fe, Ca,
6 Al, Mg) are preferable as CW substrates enable phosphorus removal efficiently and also increase the lifetime of
7 CW systems. Conventional CW substrates consisting of sand or gravel can only effectively remove total
8 phosphorus (TP) from wastewater for a short time (Chang et al., 2016; Shi et al., 2017). In some studies,
9 biochar-based filters (CWs) were found to have higher phosphorus removal efficiencies than control systems
10 filled with zeolite or gravel. Still, the improved impact for Phosphorus compounds removal was much lower than
11 for N removal. The biochar substrates could trap more phosphorus from wastewater than gravel, especially from
12 wastewater with a high phosphorus concentration (e.g., anaerobic digestion effluent) (Kizito et al., 2017). In
13 addition, the incorporation of biochar into CWs can enhance plant growth and the proliferation of Phosphorus
14 compounds accumulating microorganisms (PAOs), thereby improving biotic Phosphorus removal pathways (Ji
15 et al., 2020; Shi et al., 2017). However, this ameliorative effect cannot be easily maintained. The chemical
16 properties of biochar and wastewater, especially the biochar's surface charge, are important factors in removing
17 anionic phosphates (Wichern et al., 2018). However, other studies have shown that adding biochar to gravel-
18 filled CW did not improve phosphorus removal (Zhou et al., 2019). Mixed biochar and sand substrates are even
19 less efficient than sand alone in phosphorus removal (Rozari et al., 2016). These results can be explained
20 because biochar has a negative surface charge and a low affinity for phosphate. Other negatively charged
21 molecules in the wastewater (organic matter) can compete with phosphate for exchange sites in biochar (Rozari
22 et al., 2016). Biochar substrates made from /Fe/Al/Ca-rich feedstocks, such as crab shells, can improve P's
23 recovery/removal capacity from wastewater (Dai et al., 2017). Biochar can be modified with metal salts (iron,
24 magnesium, and aluminum compounds) to make metallic biochar before filling (Wang., 2019; Zheng et al.,
25 2019), or combined with other fillers with high Phosphorus compounds adsorption efficiency (crab shells) to
26 prepare biochar (Shi et al., 2017; Yang et al., 2018). There is still a need for further research and relevant
27 applications in phosphorus removal using biochar substrates.

28 **3.4.2.3. Metals removal**

29 Heavy metals are generally non-biodegradable and are found in large quantities in rainwater, mining
30 effluents, and industrial wastes. Biochar with a unique pore structure, a high percentage of organic carbon, and
31 many functional groups have a high chance of interacting with heavy metals in several ways (Oliveira et al.,
32 2017). Heavy metals are absorbed by biochar mainly through complexation and ion exchange between heavy
33 metal ions and functional groups of biochar (e.g., COOH, OH, R-OH) (Hsu et al., 2009; Lu et al., 2011).
34 Additionally, the coordination of metal ions with π -electrons (C=C) of biochar (Yu et al., 2010) and the
35 formation of metal precipitates with inorganic constituents (Ippolito et al., 2012; Lu et al., 2011) could play a
36 role in the P removal by biochar. Adsorption through the biochar matrix is affected by its chemical properties,
37 which are affected by feedstock type, pyrolysis temperature, application rate, pH, and other factors. For example,

1 copper (Cu^{2+}) had a high affinity for OH⁻ and COOH⁻ groups in hardwood and crop biochars, which varied with
2 pH and feedstock type (Lima et al., 2010). Similarly, biochars derived from soybean straw, guayule shrub,
3 hermaphrodite sida, and wheat straw effectively removed Ni^{2+} , Cu^{2+} , Zn^{2+} , and Cd^{2+} (Lu et al., 2017). The higher
4 biochar efficiency was attributed to the high O and C contents, polarity index and high O/C molar ratio, which
5 were regulated mainly by pH (Bogusz et al., 2015; Peng et al., 2016). In addition, the removal of mercury (Hg^{2+})
6 was effectively performed using alkaline biochar prepared from both manure and various agricultural residues
7 (corn stover, soybean straw, cocoa husks, switchgrass, and corn stover). Due to its high sulfur content (SH and
8 sulfate groups), biochar produced from cocoa hulls and animal manure was particularly effective in removing
9 Hg^{2+} , precipitating up to 90% of the Hg^{2+} as HgCl_2 or $\text{Hg}(\text{OH})_2$, mainly by co-precipitation with the anions (O,
10 S, Cl) in the biochar (Baltrenaite, 2015; Mohamed et al., 2016). Similarly, the biochar dosage affected the
11 removal of heavy metals such as Cd^{2+} , Zn^{2+} , Pb^{2+} and Cu^{2+} . Thus, the removal efficiency was higher with rising
12 biochar loading in the aqueous system, due to the increase in surface area and pH (Laird et al., 2010; Xu et al.,
13 2013).

14 Dissolved heavy metals in wastewater, such as hydroxides and sulfides, can be removed mainly by
15 precipitation, adsorption from the abiotic substrate, and microbial reduction of sulfates for hydroxides and
16 sulfides precipitation (Kosolapov et al., 2004). Adding biochar can help gravel ponds improve metal holding
17 capacity by increasing abiotic pathways. Under ideal conditions, a study was conducted in a gravel-filled pond to
18 remove just 58% Mn and 51.6% Pb from synthetic industrial wastewater. In comparison, adding biochar and
19 zeolite increased the removal efficiency of both metals up to 99.9%. These results can be explained because both
20 metals have high adsorption capacities toward biochar and zeolite (Abedi and Mojiri, 2019). In addition, the
21 inorganic components of the biochar impart an alkaline nature to the biochar, allowing it to raise the pH value of
22 acidic mine wastewater and subsequently reduce the metal ions solubility by inducing the formation of metal
23 hydroxide precipitates (Gwenzi et al., 2017). Biochar substrates can be modified before amendment with
24 heteroatoms and oxidizing agents, acids, or anionic moieties (e.g., HSO_3 , OH, S_2 , etc.) to enhance the metal
25 retention capacity of CWs (Wang et al., 2019).

26 **3.4.2.4. Pathogens removal**

27 The removal of pathogens from wastewater is essential for protecting human health. Removal was
28 accomplished by filtration, predation, adsorption, oxidation, and inactivation by exposure-several regulatory
29 standards for pathogens in wastewater effluent for reuse (Wu et al., 2016). The high porosity of biochar, high
30 specific surface area, numerous pores with a wide range of sizes, hydrophobicity and organic leaching may make
31 biochar more suitable for removing microbial contaminants than gravel or sand. However, there has been
32 relatively little research on removing pathogens from wastewater using biochar-enhanced CWs. According to
33 Mohanty et al. (2014) and Lau et al. (2017), the introduction of biochar into sand-based biofilters (FBs)
34 significantly increased the presence of *Escherichia coli* in stormwater. In addition, it decreased the
35 remobilization of sequestered nuisance bacteria during intermittent influx and highlighted the high potential of
36 using biochar substrate in CWs for wastewater disinfection. Furthermore, biochar with volatile content and
37 polarity had a higher removal efficiency for *E. coli* (Mohanty et al., 2014). This improvement effect may be

1 explained by the fact that biochar can produce antimicrobials that significantly adsorb viruses and bacteria
2 mainly using hydrophobic interactions and reduce the driving forces that detach pathogens.

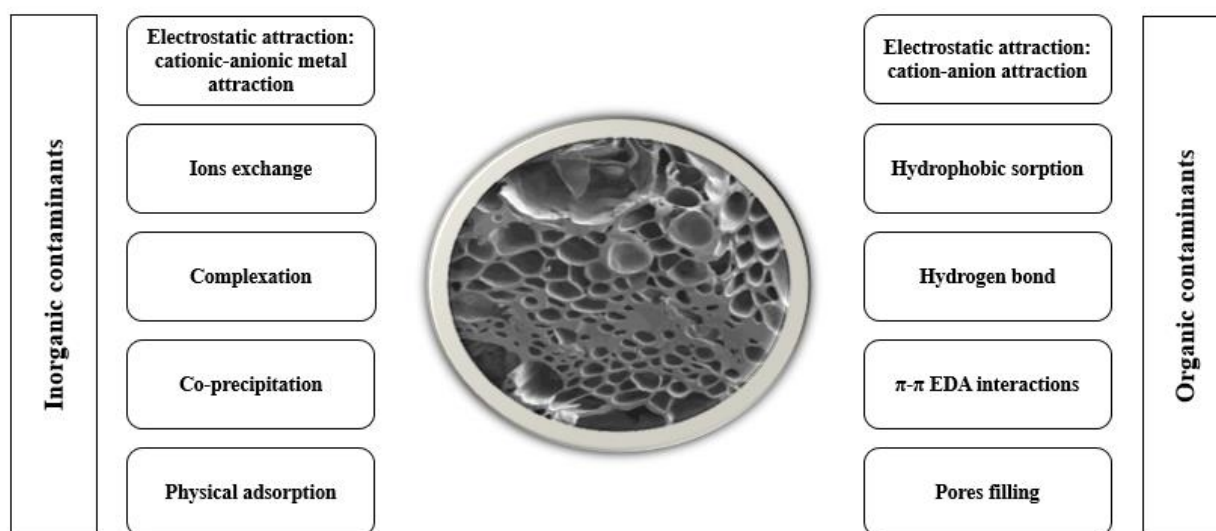
3 On the other hand, another recent study by Kaetzl et al. (2019) found that CWs filled with rice husk-
4 derived biochar can remove bacteriophages and fecal indicator bacteria (FIB) from pretreated municipal
5 wastewater much better or as much as CWs filled with sand or original rice husk (Kaetzl et al., 2019). The ability
6 of biochar to remove pathogens varies with preparation conditions and feedstock (Mohanty et al., 2014).
7 Modifying biochar with H₂SO₄ increases the surface area of biochar prepared from wood, reflecting a significant
8 improvement in *E. coli* elimination in bioretention systems and reducing remobilization during drainage and
9 intermittent flow (Lau et al., 2017). Even though biochar-based filters show high FIB removal efficiency
10 comparable to sand-based filters (Wichern et al., 2018), biochar remains an attractive feedstock in CW systems
11 for pathogen removal due to its economic production and performance, using locally available biological waste,
12 and can be reused as a soil amendment.

13 **4. Mechanisms and factors influencing the pollutants adsorption on biochar**

14 The heterogeneity of the biochar surface allows a variety of sorption processes to occur. The chemical
15 characteristics of the adsorbent surface and the nature of the contaminants determine the adsorption mechanism
16 (Rosales et al., 2017). The three main adsorption mechanisms, according to Pignatello (Pignatello., 2011), are
17 the precipitation mechanism, in which the adsorbent forms layers on the adsorbent surface, and the physical
18 mechanism, in which the adsorbate (e.g., pollutants) is deposited on the adsorbent surface (e.g., biochar), and the
19 pore-filling mechanism, in which the adsorbate (e.g., pollutants) condenses in the adsorbent pores (e.g., biochar).
20 The adsorption process of organic pollutants is generally carried out by electrostatic attraction, complex
21 adsorption, electron-acceptor- donor interaction, pore filling, hydrophobic interactions and hydrogen bonding
22 (see Fig. 4) (Pignatello., 2011). For example, the sorption of organic contaminants by the biochar surface via the
23 pore filling process is influenced by the total volume of the mesopores and micropores; so that the penetration of
24 the pollutant into the internal structure of the biochar is all the more favored when its ionic radius is small, which
25 reflects an increase in the biochar adsorption efficiency (Ahmad et al., 2014; Rosales et al., 2017). Soluble
26 pollutants may attach to the alkaline surface of the hydrophobic biochar using their hydrophobic functional
27 group or be precipitated. Due to the dissociation of oxygen-containing functional groups on the biochar surface,
28 the biochar is generally negatively charged, causing an electrostatic attraction between the positively charged
29 molecules and biochar (Ahmad et al., 2014; Qambrani et al., 2017).

30 The biochar produced at high temperatures lost its functional group-containing hydrogen and oxygen,
31 making it more aromatic and less polar and, consequently, less suitable for removing polar organic pollutants.
32 However, the electrostatic repulsion between the biochar and the negatively charged anionic organic molecules
33 could favor the production of hydrogen bonds, leading to adsorption. On the other hand, if there is no hydrogen
34 interaction, non-polar pollutants are more likely to penetrate hydrophobic areas (Ahmad et al., 2014). On the
35 other hand, many mechanisms can be involved in removing inorganic pollutants such as heavy metals, such as
36 ion exchange and complexation, surface precipitation under alkaline circumstances, and anionic and cationic

1 electrostatic attraction (Fig. 4). Similarly, Lu et al. (2011) examined the relative contributions of different Pb
 2 adsorption mechanisms on sludge-derived biochar. They arrived at the following mechanisms: (i) co-
 3 precipitation and complexation with mineral oxides and organic matter in the biochar, (ii) electrostatic
 4 complexation due to the exchange of the metal with cations (sodium and potassium) present in the biochar, (iii)
 5 surface precipitation as lead silicate- phosphate ($5\text{PbO}\cdot\text{P}_2\text{O}_5\cdot\text{SiO}_2$), and (iv) surface complexation with free
 6 carboxyl and mineral oxides in the biochar.



7

8 *Fig. 2: Mechanisms for biochar's elimination of organic and inorganic contaminants.*

9 The variation in these removal mechanisms and the physicochemical properties of biochar greatly
 10 implicates its suitability and efficacy for the remediation of the targeted pollutants. Several factors such as
 11 biochar characteristics, dosage of biochar, solution pH and temperature of the medium greatly influence the
 12 biochar's overall adsorption capacity by modifying the removal mechanisms involved in the remediation of
 13 specific pollutants aqueous systems (Abbas et al., 2018; Ambaye et al., 2021).

14 **4.1. Characteristics of biochar**

15 The volume of micropores in an adsorbent controls its ability to absorb an adsorbate (Lowell, 2004;
 16 Zabaniotou et al., 2008). Pores of different sizes are found in adsorbent materials, and classified into macropores,
 17 micropores, and mesopores based on the width of the opening (Mosher, 2011). The experimental conditions
 18 strongly influence the distribution and size of the pores during the preparation of the biochar, and especially the
 19 pyrolysis temperature has the greatest influence (Zhou et al., 2010). The micropores are the most abundant in the
 20 biochar structure and would be responsible for their high adsorption capacity and surface area. Zabaniotou et al.
 21 (2008) reported that biochar prepared at a high pyrolysis temperature contains a very high volume of micropores
 22 that varies between 50%-78% of the total pores. The sorption rate of the biochar is controlled by the size of the
 23 adsorbate, such that larger particles can cause blockage or exclusion of sorption sites. In comparison, smaller
 24 particles increase the van der Waal force of penetration of the adsorbate into the adsorbent and decrease the mass

1 transfer limitation (Daifullah and Girgis, 1998). It also depends on the surface functional groups' levels and types
2 (Qambrani et al., 2017). The carbonization process, the feedstock's chemical composition, and the carbonization
3 temperature all influence the distribution of surface functional groups (Ahmad et al., 2012). Gascó et al. (2018)
4 compared the properties of hydrochar and biochar produced from pig manure using HTC and pyrolysis.

5 The results showed that when the pyrolysis temperature is high, the broad peak around 3400 cm^{-1} ,
6 corresponds to the -OH stretching vibration in the hydroxyl and carboxyl groups and becomes less visible for
7 biochars compared to the feedstock. Due to the decarboxylation and dehydration reactions during the HTC
8 process, the HTC hydrochars revealed broadband at 3400 cm^{-1} with less intensity than the feedstock. Several
9 scientists agreed that a high aromatic structure characterizes biochar prepared at a high temperature of around
10 $600\text{ }^{\circ}\text{C}$. On the other hand, hydrochar prepared using the HTC method at a temperature between 200 and $240\text{ }^{\circ}\text{C}$
11 for 2 h favors biochar with more aliphatic structures. According to Qambrani et al. (2017), the functional groups
12 (-CH₂, O-H, C=O, C=C and -CH₃) of biochar have changed due to the pyrolytic conditions, which promote the
13 hydrophobic interactions of biochar. The hydrophobic character of biochar is determined by the amount of
14 oxygen and nitrogen-containing functional groups; the lower the nitrogen and oxygen-containing functional
15 groups in the biochar, the higher hydrophobic the biochar (Moreno-castilla, 2004). Hence, the presence of
16 oxygen-containing functional groups on the hydrophilic biochar surface facilitates water to penetrate through
17 hydrogen bonds, resulting in competition between the adsorbate and water on the available sites of the biochar
18 surface. Hydrophobic biochars are expected to contribute to insoluble adsorbate adsorption, while hydrophilic
19 biochars are considered less effective due to water sorption. Adsorbates that are less soluble or insoluble are
20 most likely to be absorbed into the biochar pores in aqueous solutions (Li et al., 2002).

21 **4.2. Dosage of the adsorbent**

22 The adsorbent dosage significantly impacts the sorbent-sorbate balance of an adsorption system. Hence,
23 using a high adsorbent dosage increases the removal efficiency of inorganic and organic contaminants due to the
24 availability of a larger number of sorption sites (Chen, 2013; Chen et al., 2011). On the other hand, the
25 application of a dosage rate that is too high leads to a reduction of the adsorption capacity of the biochar and
26 consequently, an overlapping of the adsorption layers will be produced, which protects the accessible active sites
27 on the sorbent surface (Kizito et al., 2015; Linville et al., 2017). Therefore, the adsorbent dosing must be well
28 optimized to achieve high elimination capacity and make the process cost-effective.

29 **4.3. pH of the solution**

30 The pH of the solution is a crucial factor that controls the adsorption process by influencing the
31 ionization degree and charge of the adsorbate, the adsorbent surface charge and the speciation (Kılıc et al.,
32 2013). The competition between protons and cationic pollutants decreases as the pH of the solution is above the
33 point of zero charges, and a negative charge appears on the adsorbent surface as a result of the deprotonation of
34 carboxylic groups and phenolic on the surface. Basic functional groups, such as amines, are protonated and
35 positively charged at low pH, improving anions' adsorption (Kumar et al., 2011). This means that deprotonation
36 of the functional groups and the pH of the medium influences the biochar adsorption behavior. Kizito et al.

1 (2015) and Hu et al. (2019) studied the effect of pH on the adsorption capacity of biochar towards ammonium
2 (NH_4^+). They showed that the adsorption capacity of NH_4^+ increased with the initial solution pH between 4 and 8
3 and then decreased when the pH was above 9.

4 **4.4. Temperature of the medium**

5 The medium temperature in which the biochar is applied impacts its adsorption capacity. Most studies
6 showed that adsorption efficiency increased with temperature, confirming that the adsorption process is
7 endothermic. The study by Enaime et al. (2017) indicated that the indigo carmine sorption on potassium
8 hydroxide (KOH) activated biochar rises with temperature due to the endothermic nature of the sorption process.
9 The increase in temperature leads to an increase in the mobility of the dye molecule and the possibility of an
10 increase in the adsorbent porosity. This can be explained by the swelling effect of the adsorbent internal structure
11 when the temperature increases, allowing more dye to penetrate further. Another study, Kizito et al. (2015)
12 found that increasing the temperature above 300 °C to 450 °C is beneficial for maximum removal efficiency.

13 **5. Advantages and limitations of biochar as a CW substrate**

14 The use of biochar as a substrate in CWs solves the problem of environmental pollution (**Table 3**). Due
15 to the low-cost availability of the raw materials, and the high commercial potential of biochar. The preparation of
16 biochar has developed rapidly in recent years (Lili et al., 2017). Due to its adsorption capacity and porous
17 structure, biochar is commonly used as a slow-release fertilizer filler (Xu and Lu, 2019). However, biochar is
18 rarely used in water treatment due to its high cost, high ash content, and difficulty in ash removal (Kasak et al.,
19 2018). Theoretically, biochar may considerably enhance the purification of wastewater (Deng et al., 2019), as an
20 additional carbon source for CWs (Kasak et al., 2018), and their surface allows the adsorption of various
21 pollutants.

22 Furthermore, biochar may improve the activity of the microorganisms in CWs (Tang et al., 2017).
23 Therefore, biochar could improve the degradation of high molecular weight compounds in low molecular weight
24 compounds in CW (Deng et al., 2019). The biochar's main objective is to increase the adsorption efficiency of
25 the substrate and provide the carbon source to enhance the denitrification efficiency. However, the application of
26 the CW substrate is easy to generate a blockage due to the low structural strength of the biochar and the ease of
27 generating a powder (Saeed et al., 2019).

28

29 *Table 3: Limitations and advantages of biochar as a CW substrate.*

Advantages	Reference	Disadvantages	Reference
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- Sustainable and abundant resources, cheap and more oxygen groups present in biochar improves pollutants adsorption.	(Houben et al., 2013)	- Elimination pollutants efficiency is undetermined and heavy metals retain in soil.	(Houben et al., 2013)
- Effective medium for capturing pollutants from wastewater which can connect to the soil and result in an alteration -Reduce greenhouse gas emissions	(Yaashikaa et al., 2020)	- High cost, high ash content, and difficulty in ash removal	(Kasak et al., 2018)
- Improve the activity of microorganisms in CWs	(Tang et al., 2017)	- Easy to generate a blockage and the ease of generating a powder	(Saeed et al., 2019)
- Provide reactive sites for microbes	(Li et al., 2019)		
- Adsorb NO ₃ -N, NH ₄ ⁺ and PO ₄ ³⁻ - Remove suspended solids, BOD ₅ , metals and coliforms	(Gao et al., 2018)	Substance release (e.g. N, P, salt, alkaline)	(Zhuang et al., 2022)

1

2 **6. Conclusion and perspectives**

3 The present review highlighted the constructed wetlands (CWs) a natural system that are largely
4 investigated for different kind of wastewater (urban, industrial, mixture) treatment throw physical (porosity of
5 substrate), chemical (adsorption, precipitation and biological processes (biodegradation, nitrification
6 denitrifications), under vertical or horizontal flow regime. The constructed wetland has proven good
7 performances for the elimination of organic matter (99 %), nutrients especially phosphates (88 %) and nitrogen
8 (96 %). However, constructed wetlands still very limited on removing recalcitrant or emergent pollutant such as
9 heavy metals, pesticides, drugs, PAHs, volatile organic compounds (VOCs) etc., According to previous
10 literature, removal capacity of CW depends on the type of macro-phytic plant and the substrate of the bed.
11 According to the analyzed references, different plants can be used in CW. Nevertheless, phragmites australis and
12 Around donax have been the most applied that are considered as the most resistant or high organic load and
13 present the capacity to oxygenate the substrate and enhance the hydraulic conductivity in the filter. The substrate
14 plays also an important role in constructed wetland depuration efficiency that could reach NH₄⁺-N (40.23%),
15 NO₃-N (48.94 %), TN (52%), and COD (35%) when sand or gravel substrate are used. Any improvement of
16 the CW efficiency must be performed via the integration of a good substrate in the filter. Among several
17 materials generally tested as substrate for CW such as zeolite, pozzolan, charcoal, and biochar is gaining big
18 interest recently, due to its promising characteristics as an optimal adsorbent having the ability to remove not
19 only conventional pollutants but owing to good removal performances for even emergent ones that are very toxic

1 and recalcitrant. Furthermore, biochar could bring carbon to the substrate and have a great impact on the
2 pollutants biodegradation by giving a good niche of functional group of microorganisms. The removal
3 percentage could reach COD (99 %), TP (88 %), NH₄⁺ (96 %), Abamectin (99 %), TSS (71 %), Total coliforms
4 (70 %), TN (40 %), and ARGs (99 %).

5 These interesting characteristics of the biochar are obviously dependent on the processes used to
6 prepare the material, and the conditions of the preparation including conditions of thermal conversion and the
7 kind of feedstock used. Based on the literature review, it was found that the optimum pyrolysis temperature must
8 be around 400 and 600 °C, with a possibility to have an oriented prepared biochar depending of the targeted
9 pollutants basing on the temperature. Furthermore, feedstock must have some specific characteristics to give a
10 good quality of the biochar that depends of the feedstock richness in carbon (c) and low quantity of mineral
11 matter. The large pore volume and high specific surface area reaching 200 m²/g, thus allowing to effectively
12 remove pollutants and pathogens from wastewater. The biochar quality is affected by the conditions involved in
13 preparing biochars (e.g., pyrolysis temperature, heating rate and carbonization time).

14 Several factors alter the removal efficiency of pollutants in CWs, such as substrate chemical and
15 physical properties, hydraulic retention time, the oxygenation conditions, and redox conditions. In addition,
16 configuration where the biochar is implemented as interlayer between two inert layers (sand, gravel, zeolite) has
17 been reported as optimal design for CW integrating biochar to avoid clogging of the filtration system or biochar
18 flotation.

19 Overall, the use of biochar in horizontal flow CW is still limited, and a few papers discussed this aspect.
20 Similarly, there is only limited information on the removal of emerging organics, and pathogens from
21 wastewaters by biochar CWs, that mean the involved mechanisms and potential capability of biochar CWs in the
22 removal of these pollutants should be further explored and elucidated. Moreover, it is undeniable that biochar
23 offers various economic and environmental benefits and advantages, and its effectiveness in removing various
24 contaminants at the laboratory scale has been widely reported. However, more in situ experiments should be
25 conducted to test the effectiveness of biochar using real effluents and to examine the actual effect of biochar on
26 the environment before its large-scale application. Furthermore, the biochar stability after many use cycles and
27 its regeneration should be further studied.

28
29
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1 **A critical review ~~Review~~ on using biochar ~~asin~~ —constructed wetland**
 2 **substrate: characteristics, feedstock, design configurations and pollutants**
 3 **removal mechanisms**

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 20 **Highlights:**

21 -Investigations on constructed wetland ~~integrating biochar~~ ~~(CWB)~~ ~~(CWB)~~ were reviewed

22 -Pyrolysis time, heat and feedstock origin -determine prepared biochar ~~properties~~ ~~proprieties~~

23 ~~Integrating b~~ Biochar substrate (BS) improves CW efficiency and ~~its~~ pollutants adsorption capacity

24 ~~Biochar in the substrate interlayer is the optimal ecotechnology configuration~~ Optimal CWB
 25 configuration set biochar in the middle of the ecotechnology

26 -In situ experiments are needed to test ~~the effectiveness~~ ~~and~~ ~~&~~ current actual-effect of CWB ~~biochar~~

27
 28 **Abstract**

29 Constructed wetlands ~~systems~~ (CWs) are ~~physically and biologically~~ constructed systems that simulate
 30 natural wetlands and ~~they~~ can be used to treat wastewater from several sources of pollution through physical,
 31 chemical and biological depuration processes. ~~This worke present review~~ aims to critically review synthesise the
 32 updated literature on ~~constructed wetlands~~ (CWs) integrating biochar in the substrate. In detail, ~~t~~ The study
 33 focuses on the ~~biochar~~ characteristics of biochar that are generally integrated into this treatment ecotechnology
 34 and the ~~processes feedstocks generally used to prepare the materials, including conditions of thermal conversion~~
 35 and the kind of feedstock used (e.g., agricultural, food, and wood wastes, sewage sludge, ~~sewage sludge,~~
 36 agricultural waste and wood, food waste, and argal marine feedstock). Based on the literature review, it is found

1 ~~that the feedstock must be rich in carbon (c) and low in the mineral matter to produce good quality biochar, i.e.~~
2 ~~large pore volume and high specific surface area, thus allowing to effectively remove pollutants from~~
3 ~~wastewater.~~ The biochar quality is affected by the conditions involved in preparing ~~such~~ biochars (e.g., pyrolysis
4 temperature, heating ~~rate and carbonization~~ time ~~and rate, etc.~~). The properties of biochar used for wastewater
5 treatment, the effect of its implementation ~~as a~~ CW substrate and its treatment efficiency have also been
6 described. Several factors alter the removal efficiency of pollutants in CWs, such as substrate chemical and
7 physical ~~properties~~ ~~properties~~, hydraulic retention time, oxygenation, and redox conditions ~~in the reed bed.~~ ~~In~~
8 ~~addition, the mode by which biochar is implemented~~ ~~In addition, the implementation level of biochar~~ in the filter
9 and the choice of ~~the~~ macrophyte ~~ie plant~~ are crucial ~~for regulating~~ ~~to~~ the efficiency of the treatment system.
10 ~~*Phragmites australis* was the most used plant in the previous studies because of its large advantages. Different~~
11 ~~configurations of CWs integrating biochar into the wetland as a filling medium, were reported and compared.~~
12 ~~Different configurations of implementing the biochar in CW substrate have been reported and compared. In~~
13 ~~vertical flow CWs (VF-CWs), which are the system mostly investigated, several studies have shown that the~~
14 ~~optimal position for the biochar substrate is the intermediate one between two layers of inert materials, to avoid~~
15 ~~clogging of the filtration system or biochar flotation.~~

16 Keywords: ~~Natural based solutions; Sorbent materials~~ ~~Constructed wetland;~~ Wastewater ~~treaitment;~~
17 ~~Biochar properties;~~ Biomass thermal conversion; Configuration of constructed wetlands; Emerging
18 ~~contaminants~~ ~~Substrate.~~

19 1. Introduction

20 Constructed wetlands (CWs) are a kind of green technology that can be considered as sustainable ~~nature~~
21 ~~based solution to treating for~~ wastewater ~~treatment~~ (Abedi and Mojiri, 2019) (Younas et al., 2022). In such
22 systems, the plant and the substrate ~~play an important role are decisive~~ in the ~~removal of~~ pollutants
23 ~~removal~~ (Addo-Bankas et al., 2021) (Ohore et al., 2022) (Guitttonny philippe et al., 2015; Guo et al., 2020). ~~The~~
24 ~~substrate is an essential component of CWs since it can mediate and promote the implementation of mechanical,~~
25 ~~physical and biological mechanisms for reducing pollutants concentration in CW effluents, allowing for the~~
26 ~~direct~~ ~~The substrate involves mechanical, physical, and biological mechanisms to remove various pollutants. In~~
27 ~~addition, the substrate is an essential component of CWs because it allows them to remove~~ ~~ale of~~ contaminants,
28 ~~making available~~ ~~ensure~~ reactive agents for transforming pollutants, ~~promot~~ ~~inge~~ plant growth, and ~~ensur~~ ~~inge~~
29 biofilm ~~adhesion~~ ~~fixation~~ (Deng et al., 2021). Furthermore, plants ~~uptake~~ ~~absorb~~ nutrients, directly increase
30 biological activity in the substrate by supplying oxygen through their roots, and play an important role in the
31 hydraulic conductivity within the filter. ~~Hence~~ ~~So~~, choosing the ~~most~~ appropriate plant species is ~~important~~ ~~ueial~~
32 ~~to for~~ obtaining the best performance. (Karungamye, 2022); (Guitttonny philippe et al., 2015; (Srivastava et al.,
33 2008; Guitttonny-philippe et al., 2015; Katakai et al., 2021; Karungamye, 2022).

34 The CWs have been widely tested for urban wastewater treatment, ~~while the purification of sewage~~
35 ~~from industrial or mixed urban-industrial origin has been investigated with lesser extent~~ (Stefanakis, 2018;
36 ~~Katakai et al., 2021)~~ ~~and showed good efficiency in removing organics, nutrients, and pathogens~~ (Angassa et al.,

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1 ~~2020). Other investigations have tested the CWs to treat industrial wastewater or a mixture of urban and~~
2 ~~industrial wastewater (Mateus and Pinho, 2020). CWs demonstrated high efficiency in removing classical~~
3 ~~conventional pollutants such as suspended solids, organic matter, nutrients, biodegradable organic matter, and~~
4 ~~some heavy metals (Huong et al., 2020; (L. L. Zhuang et al., 2022). However, in most cases, CWs have shown~~
5 ~~a lower efficiency against various ecotoxic pollutants, such as detergents, heavy metals, plasticizers,~~
6 ~~disinfectants, pesticides, and pharmaceutical residues, which remain largely unremoved in CWs~~
7 ~~effluents. However, in most cases, CWs only remove part of the pollutants in the influent. Therefore, the treated~~
8 ~~effluent may include a complex mixture of different ecotoxic pollutants, such as detergents, heavy metals,~~
9 ~~plasticizers, disinfectants, pesticides and pharmaceutical residues that could escape to the CWs capacities~~
10 ~~(Gosset et al., 2020). To improve CWs efficiency, various materials, other than those conventionally used in~~
11 ~~CWs (i.e., gravel and sand) (Zhang et al., 2021; Fu et al., 2020), have been tested as substrates, namely, pozzolan~~
12 ~~(El Ghadraoui et al., 2020). To improve the efficiency of these constructed wetlands, various materials have been~~
13 ~~tested as substrates, namely charcoal (Hamada et al., 2021), gravel (Zhang et al., 2021), sand (Fu et al., 2020),~~
14 ~~zeolite (Du et al., 2020), and biochar (Vymazal et al., 2021) etc. Among them, Recently, biochar has recently~~
15 ~~gained an increasing interest (Rozari et al., 2016) as Biochar, a stable, porous, carbon-rich, and originated from~~
16 ~~inexpensive organic material, is obtained by thermochemical transformation conversion of waste biomass under~~
17 ~~no or low oxygen conditions through various thermochemical processes such as (Deng et al., 2021). It could be~~
18 ~~produced by several thermal transformation processes such as hydrothermal carbonization (HTC), gasification,~~
19 ~~hydrothermal liquefaction (HTL), gasification, and pyrolysis (Deng et al., 2021). Slow pyrolysis (i.e., thermal~~
20 ~~conversion in the absence of oxygen and with contact time from minutes to hours) is commonly used as it is~~
21 ~~cheaper than other processes and/or gives rise to a higher good yield of the solid fraction matter (i.e., biochar)~~
22 ~~with low syngas and bio-oil and production low off gassing (Enaime et al., 2020; (Wang et al., 2020a). Various~~
23 ~~renewable and locally available waste biomaterials wastes, such as compost, agricultural by-products (crop~~
24 ~~residues), sludge, manure, and shellfish, have been are raw materials used to produce biochar (L. L. Zhuang et~~
25 ~~al., 2022). In addition, biochar may also be produced from wetland plant straws and then reintroduced into~~
26 ~~wastewater treatment environments, thereby facilitating wetland plant management and sustainable exploitation~~
27 ~~of wastewater treatment systems (Wang et al., 2020a); (Deng et al., 2021). Introducing biochar as a substrate in~~
28 ~~CWs can significantly increase the system's efficiency since it may have has a high sorption capacity affinity for~~
29 ~~organic and inorganic pollutants adsorption (Srivastava et al., 2008; Wang and Wang, 2019). However, the~~
30 ~~sorption biochar capacity of biochar is dependsent on the kind of feedstock used and its preparation conditions~~
31 ~~(Tan et al., 2015). The location of the biochar substrate in the filter can also affect the efficiency of the treatment~~
32 ~~system. Recently, several existing studies have investigated the effect of biochar used in constructed~~
33 ~~wetlands CWs. Nevertheless, each study focused on one of the aforementioned aspects separately, while no~~
34 ~~review exists to date that critically evaluates all parameters involved in the treatment and how they might interact~~
35 ~~to improve the treatment efficacy of CWs. However, each study focused on one of the aforementioned aspects~~
36 ~~separately (Wu and Wu, 2019); (Wang et al., 2020a); (Ambaye et al., 2021); (Cui et al., 2022); (L. L. Zhuang et~~
37 ~~al., 2022). No synthetic review exists until now about all these parameters involved in the treatment and how~~
38 ~~they could interact to improve the CWs treatment efficacy, and, no synthetic review exists until now discussing~~

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1 the optimal position of substrate biochar in the CW. We tried to collect all this aspects to enrich our synthetic
 2 review. In addition very few reviews have described the emergent pollutants removal capacities of CWB.

3 According to a literature overview performed using the search engines SciFinder, Elsevier
 4 ScienceDirect, and Google Scholar, this paper critically reviewed data and information on (i) the characteristics
 5 and properties of biochars used in constructed wetlands (e.g. the conditions of thermal conversion and the type of
 6 feedstock used for the preparation of biochars, as well as the specific surface area (SSA) and environmental
 7 compatibility of the material), (ii) the methods of integrating the biochar within the CWs, and (iii) the results
 8 obtained in terms of removal of macro-parameters, as well as conventional and emerging micropollutants. This
 9 review objective is to report updated information on the various properties of biochar generally integrated into a
 10 constructed wetland as a substrate, such as the feedstock origin, conditions used for its preparation, the best
 11 design of biochar amended filter, and how the implementation of the biochar in the CW substrate could enhance
 12 the depuration efficiency of such nature-based technology, emphasizing recent literature from respected peer-
 13 reviewed journals.

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14 2. Biochar incorporated into ~~a~~ Constructed wetland CWs

15 2.1. Biochar feedstock

16 The composition of the feedstock and its availability are two essential factors in producing efficient and
 17 cost-effective biochar. Even though feedstocks are widely available, adequate classification and characterization
 18 are required for their adequate application. Biochar can be made from a wide variety of feedstocks (Gabhane et
 19 al., 2020; (Berslin et al., 2022); (Garcia et al., 2022); (L. L. Zhuang et al., 2022) (Gabhane et al., 2020), The
 20 composition of the feedstock and its availability are essential factors in the production of efficient and cost-
 21 effective biochar. Therefore, proper classification and characterization of feedstocks are required for their
 22 successful application.

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23 Biochar feedstock used in the literature comes from various materials, that can be, classified into sewage
 24 sludge, agricultural waste and wood, food waste, and marine feedstock (Table 1).

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25 Table 1: ~~Different~~ Feedstocks used for the production of biochars intended to be used in CWs,
 26 ~~preparation~~ production under varying conditions and ~~obtained~~ characteristics of the material obtained.

Feedstock	Pyrolysis temperature	Surface Characteristics (SA, PV, PS) and pH	Composition	Refs
Bamboo	500 °C Muffle furnace—600 °C—3 h	SA(335 m ² /g)- - -	C (68%)-	(Zhang et al., 2021)(Sha et al., 2020)

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Bamboo	tubular furnace 500 °C - 10 °C/min - 2 h	SA(116.24 m²/g)46.24	C (74.56%); H (1.12%); O (6.28%); N (1.06%)	(Xin et al., 2021)
		-		
		-		
Bamboo	600 °C 500 °C - 2h	SA (2.5 × 10⁸ m²/m³)	C (59.44%); H (2.06%); O (15.89%); N (0.40%); P (0.34%)	(Jia et al., 2020a)(Liang et al., 2020)
		-		
		-		
		-		
Bamboo chips	500 °C - 2h - N ₂	PS(10 μm)	C (56.4%); O (6.3%)	(Feng et al., 2021a)
		-		
		10 μm		
		-		
Bamboo	500 °C without O ₂	-	-	(Zhou et al., 2017)
		-		
		-		
		-		
Bamboo	800 °C - 48 h	-	-	(Gao et al., 2018)
		-		
		-		
		-		
Bamboo	Pyrolysis at 600 °C and modified by Fe	-	-	(Jia et al., 2020)
		-		
		-		
		-		
Bamboo	700 °C - 10 °C/min - 6 h	228.26 0.086 cm ³ /g	-	(Ajibade et al., 2020)

		<u>SA(228.26 m²/g); PV(0.086 cm³/g)-</u>		
		<u>pH(9.5)</u>		
<u>Arundo donax</u>	<u>600 °C- 1h</u>	<u>SA(281.15 m²/g)</u>	<u>C (63.18%); H (1.80%); N (1.13%)</u>	(Li 2018b) Formatted: Font: Italic
<u>Arundo donax</u>	<u>Muffle furnace</u>	<u>SA(1272.67 m²/g) ; PV(1.021 cm³/g)</u>	<u>C(79.9 %) ;N(2.27 %) ; O(17.84 %)</u>	(Shen 2020) Formatted: Font: Italic
	<u>500 °C - 10 °C.min⁻¹ - 1h</u> <u>- N₂</u>			
Agricultural waste	500 °C	809 0.22 - <u>- SA(809 m²/g); PV(0.22 cm³/g)</u>	-	(Abedi and Mojiri, 2019)
Lodgepole Pine Wood	1000 °C	<u>SA(152 m²/g); PS(1 - 40 μm)452</u> - <u>1 - 40 μm</u> <u>pH(9.66)</u>	-	(Huggins et al., 2016)
Oak woody (Quercus Ssp)	600°C - 10h -10°C/min	<u>PS(1 - 10 μm)-</u> - <u>1 et 10 μm</u>	O (8%); C (90%); P (0.54%); K (0.38%); S (0.1%); Ca (0.38%)	(Gupta et al., 2016)
Wood	600 °C - 10 °C/min - 10h	<u>SA(147 m²/g); PV(0.176 cm³/g); PS(5.3 nm)447</u> 0.176 cm ³ /g 5.3 nm <u>pH(9.8)</u>	C (90%); H (1.5%); O (8.3%); N (0.5%); S (0.3%)	(Kizito et al., 2017)
Wood dust	700 °C	<u>SA(488.60 m²/g); PV(0.286 cm³/g)488.60</u> 0.286 cm ³ /g -	C (81.50%); H (1.87%); O (15.63%); N (0.07%)	(Lun, L. Chen, 2018)

Cattail (<i>Typha latifolia</i>)	600 °C - 2h - 10 °C/min	6.14 0.02 cm ³ /g SA(6.14 m ² /g); PV(0.02 cm ³ /g)- pH(8.9)	-	(Zheng et al., 2022)
Tree branches	550 °C - 2h - N ₂	SA(32.09 m ² /g); PV(2.31 mm ³ g ⁻¹) 2.31 mm ³ g ⁻¹ - -	-	(Ji et al., 2020)
Softwoods	Gasifier-700 °C= (gasification)	485 - SA(485 m ² /g)- pH(7.8)	C (89.2%); H (1.6%); O (1.9%); N (1%); S (0.04%); P (4.3%)	(Kaetzel et al., 2018)
Corn on the cob	600 °C - 10 °C/min - 10h	123 0.098 cm ³ /g 6.2 nm SA(123 m ² /g); PV(0.098 cm ³ /g); PS(6.2 nm) pH(8.9)	C (69%); H (3.4%); O (17.6%); N (6.1%); S (4.4%)	(Kizito et al., 2017)
Corn cob	600 °C -2h	SA(263.0 m ² /g)	=	(Gotore et al., 2022)
Giant reed straw	500 °C - 2h	345.92 0.2467 1.95 nm - SA(345.92 m ² /g); PV(0.2467 cm ³ /g); PS(1.95 nm)	-	(Deng et al., 2019)
Corn straw	450 °C, 2 h - 10 °C min ⁻¹ - N ₂	SA(232.715 m ² /g); PV(0.098 cm ³ /g); PS(1.286 nm)	C (77.30%) H (2.35%) N (0.87%) O (11.26%) S (0.02%) P (1.43%) Cl (0.38%)	(Wang et al., 2022)
Hardwood	500 °C	- -	-	(Rezari et al., 2018)

		-		
		-		
Bark of Acacia auriculiformis	500 °C - 10 °C/min - 2 h	-	-	(Nguyen et al., 2020)
		-		
		-		
Nut shells	450 °C - 2h	14.76	C (68.6%); K (5.1%); Ca (4.0%)	(Chang et al., 2022)
		-		
		SA(14.76 m ² /g)-		
		pH(8.1)		
Cattails	slow pyrolysis - 300 °C - 5 h - 10 °C/min - N ₂ (99.9%)	-	-	(Guo et al., 2020)
		-		
		-		
		-		
Sludge	600 °C - 2h - 10 °C/min	SA(13.13 m ² /g); PV(0.12 cm ³ /g); PS(18.71 nm)13.13	-	(Zheng et al., 2022)
		0.12 cm ² /g		
		-		
		pH(7.9)		
Walnut shells	450 °C - 2h - N ₂	SA(14.76m ² /g)	C (68.6%); K (5.1%); Ca (4.0%)	(Chang et al., 2022)
Fresh cattle dung	400 °C - 4h - 26.5 °C/min	-	-	(Chand et al., 2021)
		-		
		-		
		9.5		

- 1 SA: Surface area; PV: Pore volume; PS: Particle size.
- 2 Agricultural waste and wood-derived biochar have been recently were commonly employed for the
- 3 application in CWs. Bamboo is widely used as a raw material for biochar production, due to its abundance and

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1 high carbon content (>50%), which gives a good quality of biochar (Jia et al., 2020b; Zhou et al., 2017; Jia et al.,
2 2020; Gao et al., 2018; Zhang et al., 2021; Xin et al., 2021). Furthermore, plants such as *Arundo donax* and
3 cattail can absorb phosphorus and nitrogen pollutants from wastewater through their roots, and transport them to
4 the shoot, which may then be harvested and converted into biochar that can be reused as functional substrates
5 of CWs, thus achieving a virtuous circular approach in this field for wastewater treatment to achieve the
6 transformation of plant waste into bio-resources (F. Guo et al., 2020; Li et al., 2018a). Other vegetal
7 materials have been transformed into biochar and used for wastewater treatment, such as cut residues
8 of including *Alnus alder* (Kasak et al., 2018), coconut shell (You et al., 2019), *Acacia auriculiformis* (Nguyen et
9 al., 2020), *Gliricidia* (Yasaratne, 2017), coconut shell (You et al., 2019), and various agricultural waste (Abedi
10 and Mojiri, 2019), because of their wide availability and high productivity. However, terrestrial macroplants
11 have so far been the primary source of biochar used in CWs thus far, given various biomass diverse features
12 (Aghoghovwia et al., 2020; Du et al., 2020). The biochar performance derived from sewage sludge or marine life
13 (e.g. macroalgae) may differ from that of terrestrial plants (L. Zhuang et al., 2022). In addition, Deng et al.
14 (2021) stated that the biochars used in the CW treatment systems are generally made from *Arundo donax* straw,
15 corn/straw cobs, bamboo, shells, tree branches and wooden containers (Deng et al., 2021). Finally, the feedstock
16 must be rich in carbon and low in the mineral matter to produce good quality biochar.

17 2.2. Biochar production conditions

18 Pyrolysis is commonly performed to prepare biochar used in CWs because of its advantages generally
19 consisting in higher yields of biochar and lower content of bio-oil and syngas (Enaime et al., 2020; b)
20 (Abdelhafez et al., 2021; Pereira and Astruc, 2021; L. L. Zhuang et al., 2022). Pyrolysis is a thermal
21 conversion process that takes place generally at temperatures between 300 and 900 °C (Wang and Wang, 2019),
22 with the temperature range between 400 and 600 °C being were the most commonly adopted used to prepare
23 the biochar used in the filters (Table 1) (Abedi and Mojiri, 2019; Chand et al., 2021; Zheng et al., 2022). Chand
24 et al. (2021) prepared biochar, dried and fresh cattle dung, which were collected from a local animal farm; this
25 feedstock was dried for 24 h at 80 °C to eliminate moisture, and then pyrolyzed 4 h at 400 °C with a heating rate
26 26.5 °C min⁻¹, under anaerobic conditions in a muffle furnace. Similarly, Deng et al. (2019) prepared biochar
27 from giant reed straw. They pyrolyzed for two hours at 500 °C, resulting in a specific surface area of 345.92
28 m²·g⁻¹, a pore volume of 0.2467 cm³·g⁻¹, and a pore diameter of 1.95 nm. The pyrolysis temperature was steadily
29 increased at a rate of 10 °C/min until it reached the desired temperature (e.g., 600 °C) and then maintained at a
30 maximum temperature for 2-10 h (F. Guo et al., 2020; Kizito et al., 2017; Rozari et al., 2018). The time and the
31 temperature of pyrolysis are determining factors of the biochar characteristics (e.g., density, carbon content, pH,
32 porosity) (Gong et al., 2019; Xiao et al., 2020) and, consequently, the performance of wastewater treatment
33 (Alsewaleh et al., 2019; Hsu et al., 2019). Even though the kind of feedstock used for biochar preparation
34 affects the characteristics of the material, it has been demonstrated that the increase in temperature generally
35 produces higher percentages of ash, which is regulated by the EN 12915-1 standard (Comite Europeen de
36 Normalisation (CEN), 2009) in materials intended for water filtration, since a high ash content in filtering media
37 is expected to reduce adsorption activity (Castiglioni et al., 2022). Also the presence of polycyclic aromatic
38 hydrocarbons (PAHs), themselves regulated by the EN 12915-1, depends on the conversion temperature

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1 adopted, which plays a main role in PAH formation up to about 500 °C, but also in their degradation beyond this
2 value (Castiglioni et al., 2022). The conversion temperature is also crucial in determining the SSA of the biochar
3 and its microporosity/mesoporosity distribution, being the highest SSA values obtained at the highest
4 temperatures, due to the increase of both pore size classes (Del Bubba et al., 2020). This result is also related to
5 the progressive loss of the functional groups present in the material as the temperature increases (Del Bubba et
6 al., 2020). However, the yield of fabricated biochar decreases ~~from 42.9% to 19.6%~~ with the rise of pyrolysis
7 temperature ~~from 300°C to 900°C~~ (Apolin and Conceptualization, 2020).

8 Based on the above considerations, the adsorption performance of biochars obtained under different
9 experimental conditions (e.g., different feedstock, conversion temperature, and contact time) will be better or
10 worse depending on the contaminant to be removed. Accordingly, researchers used materials produced at very
11 different temperatures for achieving the removal of their target contaminants. For example, The pyrolysis
12 temperature of the sludge-based biochar at 400°C showed optimal ammonia adsorption, and while pyrolysis
13 temperatures at 350 °C or 550 °C were not favorable for the biochar's adsorption capability (Tang et al., 2018),
14 i.e., without any clear. There was no consistent effect of pyrolysis temperature on biochar adsorption
15 performance towards ammonia pollutants (Tang et al., 2018). However, For example, Ajibade et al. (2020)
16 worked at pyrolysis temperatures up to 700 °C. Similarly, and Huggins et al. (2016) were prepared the converted
17 lodgepole pine wood to biochar at high pyrolysis temperature (700 and 1000 °C) and justified the choice of these
18 temperatures to their high surface area and pore volume that will serve as a niche for microbes for the effective
19 treatment of pollutants (Ajibade et al., 2020).

20 **2.3. Biochar characteristics for wastewater treatment**

21 The physicochemical properties of biochar, such as pore distribution and size, surface functional
22 groups, alkalinity, ~~specific surface area~~SSA, etc., which strongly depend on the feedstock and thermal
23 conversion conditions of production, are responsible for pollutant adsorption capacity, ~~soil improvement~~, and
24 biofilm adhesion attachment (Wang et al., 2019; Tan et al., 2015). As a result, biochar's ability to remove
25 inorganic and organic contaminants is determined by its characteristics as well as the characteristics of the
26 molecules to be eliminated, such as and the size, charge and chemical moieties of the molecules to be eliminated.
27 As mentioned above, Biochar produced at low temperatures has more oxygen-containing functional
28 groups, hydrophobic and polar functional fractions favorable for the adsorption of polar compounds, and may
29 show it can have a higher mechanical strength for being used preferably in CWs. In contrast, biochar produced at
30 high temperatures has a larger porosity size and specific surface area SSA, a higher aromaticity, more π-bonds
31 and a higher carbon content, and overall a higher hydrophobic character but lower surface polarity (Enaime et
32 al., 2020a) (Del Bubba et al., 2020; Castiglioni et al., 2021). The net surface charge of the chars (commonly
33 evaluated by the pH of the point of zero charge and/or Boehm's titration), which mainly depends on the surface
34 functional groups of the material and is often related to its ash content, is a further crucial parameters to explain
35 the adsorption behaviours of biochars, particularly towards ionized or ionisable compounds (Castiglioni et al.,
36 2022). In addition, according to Enaime et al. (2020), high-temperature pyrolysis produces hydrophobic
37 biochars with higher micropore volume and surface area, which are better for the sorption of organic pollutants.

1 Accordingly, best performing biochars can be obtained a lower or higher temperatures, depending on the target
2 molecule to be removed. For example, phenol adsorption was higher for biochars produced at 900 °C than for
3 those prepared at lower temperature 600 °C, probably due to the relative increase in SSA at the higher pyrolysis
4 temperature-(Mohammed et al., 2018).

5 In contrast, biochars prepared at low temperatures have a lower surface area, smaller pores, and a higher
6 content of oxygen-containing functional groups, which are better for removing inorganic pollutants. For
7 example, organic contaminants adsorption was higher for biochars produced at 900 °C than for those prepared at
8 lower temperature 600 °C due to the relative increase in surface area at the higher pyrolysis temperature
9 (Mohammed et al., 2018; Tang et al., 2018). Heavy metal adsorption is affected by the nature of the metals, and
10 their competitive behavior towards the properties of the biochar and the sorption sites of the biochar. Similarly,
11 Xu and Lu. (2019) reported an increasing removal efficiency of biochar towards bisphenol from aqueous
12 solutions with increasing the preparation temperature. However, Del Bubba et al., (2020), studying the removal
13 of 16 alkylphenols and alkylphenol ethoxylates from real wastewater, with biochar produced at 450, 650 and
14 850°C, observed higher absolute absorption maxima for materials produced at the two highest temperatures,
15 depending on of the investigated molecule, confirmed that the biochar removal efficiency of bisphenol from
16 aqueous solutions increased in parallel with the preparation temperature. This is explained by the increase in
17 pore volume and specific surface area. On the other hand, the yield and economic viability of the biochar
18 decreased.

19 The biochar can be modified chemically, physically or biologically to increase its properties and
20 achieve greater adsorption and catalysis capacities for the target pollutants (Xu and Lu, 2019). In addition, the
21 pH of the solution played a key role in controlling the deprotonation and hydrophobicity of the compounds,
22 which is in agreement with the correlation analysis of the maximum sorption capacity. The pH of biochar
23 produced to be used as a substrate in CWs was generally alkaline and varied between 7.9 and 9.8 (Table 1)
24 (Enaime et al., 2020a; Kizito et al., 2017; Zheng et al., 2022).

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25 According to Huggins et al. (2016) study, The medium pore size of biochar used for wastewater
26 treatment can reach 40.0 µm. Generally, The carbon content can give an early indication of biochar quality.
27 Generally carbon (C) was the main compositional element of biochar, varying approximately from 50.0% to
28 90.0%, followed by oxygen (O) and nitrogen (N) and other elements that were presented in low at much lower
29 percentages (Table 1) (Gupta et al., 2016; Kizito et al., 2017). In Kizito's study, element C was found at 69% in
30 biochar derived from corn cobs and 90% in wood, confirming that biochar characteristics are feedstock
31 dependent (Kizito et al., 2017). The biochar generally had a high surface area of several hundreds m²/g (Abedi
32 and Mojiri, 2019; Deng et al., 2019); for example, in Abedi's study, the BET surface area of biochar was around
33 809 m²/g (Abedi and Mojiri, 2019). However, other investigations have found it as low as a few tens of m²/g
34 (Ji et al., 2020; Zheng et al., 2022). For example, the study by Zheng, who works on two feedstocks, the cattail
35 (*Typha latifolia*) and sludge, shows that the two feedstocks give low specific surfaces of 6.14 and 13.13 m²/g,
36 respectively (Zheng et al., 2022). With increasing pyrolysis temperature, the porosity, surface area and carbon
37 content of biochar increased. However, bio-assimilation decreased. The percentage of carbon in biochar grew

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1 from 57.8% to 63.2% as the pyrolysis temperature increased from 300 to 500 °C. On the other hand, the surface
2 area increased by more than one magnitude from 10.0 m²/g to 281 m²/g (Li et al., 2018a). This shows that the
3 ~~surface property was porosity is~~ extremely sensitive to temperature variation compared to the percentage of
4 carbon. These properties will probably influence their function in CWs. According to Liao et al. (2022), the
5 biochar must have a large pore volume and surface area to adsorb pollutants and provide adhesion of attachment
6 ~~points for~~ microorganisms (Liao et al., 2022). In most cases the biochar used in CWs has a higher specific
7 surface area (>200 m²/g) to provide a higher number of adsorption sites (Shen et al., 2020; Zhang et al., 2021;
8 Gotore et al., 2022).

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9 3. Configurations of biochar-based CWs~~the vertical flow constructed wetland built~~ 10 based on biochar and their removal efficiency

11 The performance of a CW depends on the type of CW, temperature, vegetation, water flow regime
12 (hydraulic regime), dissolved oxygen (DO), substrate nature, redox potential (Eh) and, applied hydraulic load
13 ~~and the medium used in the bed~~ (Parde et al., 2021; Malyan et al., 2021). Table 2 shows the order, dose,
14 dimension of substrates, different plants ~~and substrates~~ used in CW ~~and their dimensions and the removal~~
15 efficiency of pollutants of each configuration.

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16 3.1. Types of macrophytes used in CWs implemented with biochar

17 Plants are essential in removing pollutants, as they generally play an indirect role in the wastewater
18 treatment performance in CWs. The choice of appropriate plant species is crucial for the best performance
19 (Guittonny philippe et al., 2015; Srivastava et al., 2008). Hence, the right choice was based on several
20 parameters; the species that are preferred are characterized by high ecological adaptability, adaptation to local
21 climatic and nutritional conditions, high biomass productivity, resistance to pests and diseases; having good
22 coverage with high prospects of successful establishment, tolerance to pollutants and hypertrophic waterlogging
23 conditions, low tendency to dominate and form monocultures, high capacity for pollutant removal, easy
24 propagation, and rapid establishment (Kataki et al., 2021). Another key parameter in selecting CW species is the
25 higher water use efficiency index (Stefanakis, 2020). Several studies have shown that plants with fibrous root
26 systems provide a greater surface area for biofilm enhancement, sedimentation, and particulate matter trapping.
27 They show higher photosynthesis and radial oxygen loss levels and are more effective in removing contaminants
28 than plants with thick roots (Borne et al., 2013; Lai et al., 2012). Previous studies have shown that plant density
29 affects CWs performance at 5 to 50 plants/m². A low density (16 m²) CW planting may result in lower nitrogen
30 removal than a CW with a high plant density (32 m²) (reduced by almost half) (Hernández et al., 2017). Another
31 factor to consider is the age of the plant, as oxygen release and contaminant uptake are lower in older plants due
32 to the presence of older lignified roots (Valipour and Ahn, 2015).

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2
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Table 2: Characteristics of Constructed wetland CWs integrated with biochar with different plants and substrates in the literature.

<u>Implementation mode of the substrate (by order)</u>	<u>Plant species and density</u>	<u>Wastewater</u>	<u>CW size</u>	<u>Aeration</u>	<u>Feeding</u>	<u>HLR</u>	<u>HRT</u>	<u>Experi- me nt duration</u>	<u>Removal efficiency</u>	<u>Reference</u>
<u>- Sand (0.5–2 mm) h= 50 mm</u> <u>- Biochar (2.95%) + gravel: h= 300 mm</u> <u>- Gravel (10–20 mm) h= 50 mm</u>	<u><i>Acorus calamus L.</i></u> <u>4 rhizomes</u>	<u>Tail water</u>	<u>VF-CW</u> <u>h=450 mm</u> <u>d=160 mm</u>	<u>No</u>	<u>-</u>	<u>0.055</u> <u>m³·(m²· d)⁻¹</u>	<u>3</u> <u>days</u>	<u>2 months</u>	<u>COD (76%) - TP (52%) -</u> <u>TN (82%) - NH₄⁺ (84%) -</u> <u>NO₃⁻ (89%)</u>	<u>(W)</u>
<u>- Zeolite (d=2mm–4 mm) h=30 cm</u> <u>- Biochar (d=3mm–5 mm) h=30 cm</u> <u>- Cobblestone (d=20mm–30 mm) h=5 cm</u>	<u><i>Phragmites australis</i></u>	<u>Synthetic wastewater</u>	<u>VF-CW</u> <u>h=75 cm</u> <u>d=14 cm</u> <u>V= 2 L</u>	<u>No</u>	<u>-</u>	<u>260</u> <u>L·m⁻²· d⁻¹</u>	<u>12 h</u>	<u>4 months</u>	<u>NH₄⁺ (95.49%) - NO₃⁻</u> <u>(83.24%) - TN (83%)</u>	<u>(C)</u> <u>a</u>
<u>- Clay ceramite (d= 2-5 mm) h=7 cm</u> <u>- Biochar (d= 2-5 mm) h= 14 cm</u> <u>- Clay ceramite (d= 2-5 mm) h=7 cm</u>	<u><i>Lythrum salicaria</i></u>	<u>Domestic wastewater</u>	<u>HF-CW</u> <u>l= 30 cm</u> <u>w= 15 cm</u> <u>h= 30 cm</u>	<u>Yes</u>	<u>Manually</u> <u>4 L</u>	<u>-</u>	<u>24h</u>	<u>6 months</u>	<u>COD (75.5%) - TP (76.2%)</u> <u>- TN (59.2%) -</u> <u>NH₄⁺ (62.5%)</u>	
<u>- Gravel (d=7-8 mm) h = 3 cm</u> <u>- Biochar (d= 6-8 mm) h=10 cm</u> <u>- Gravel (d= 7-8 mm) h = 3 cm</u>	<u><i>Plants hydroponics</i></u>	<u>Synthetic wastewater</u>	<u>VF-CW</u> <u>d = 12 cm</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>6 months</u>	<u>COD (99.84 %) - NH₄⁺</u> <u>(92.00 %) - TP (88.63 %)</u>	<u>(L)</u>

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<p>- Gravel (d=1-3 cm) - Biochar (d=1-2 cm) h=3-6-9 cm - Gravel (d=1-3 cm)</p>	<p><i>Acorus calamus</i></p> <p>30 rhizomes·m⁻²</p>	<p>Synthetic Wastewater</p>	<p>VF-CW</p> <p>h=35 cm</p> <p>d=33 cm</p>	<p>-</p>	<p>Manually</p> <p>10 L</p>	<p>0.05</p> <p>m³·m⁻²·d⁻¹</p>	<p>48 h</p>	<p>6 months</p>	<p>COD (89.88%) TN (86.36%) - NH₄⁺ (63.51%)</p>	<p>(D)</p> <p>Formatted: Font: Italic</p> <p>Formatted: Font color: Auto</p>
<p>- Pebbles (d= 90 mm) h=5 cm - Biochar (d=10 cm) -Gravel (d= 15 mm) h=17 cm - Gravel (d= 10 mm) h=5 cm</p>	<p><i>Canna sp</i></p>	<p>Synthetic wastewater</p>	<p>HF-CW</p> <p>1m x 0.3m</p> <p>x 0.3m</p>	<p>Yes</p>	<p>32 L</p>	<p>-</p>	<p>72 h</p>	<p>-</p>	<p>COD (91.3%) - TN (58.3%) - NH₃ (58.3%) - NO₃⁻ (92%) - TP (79.5%) - PO₄³⁻ (67.7%)</p>	<p>(G)</p> <p>Formatted: Font: Italic</p> <p>Formatted: Font color: Auto</p>
<p>- Pebbles (d=5-7mm); h=5 cm - Coke (d=3-5 mm); h=74 cm - Fe-modified biochar (50 mm×10 mm×5 mm) - Pebbles (d=5-7mm); h=5 cm</p>	<p><i>Canna</i></p>	<p>River water</p>	<p>VF-CW</p> <p>h=100 cm</p> <p>d=30 cm</p>	<p>-</p>	<p>-</p>	<p>-</p>	<p>-</p>	<p>5 months</p>	<p>Abamectin (99%) - COD (98%) - NH₄⁺ (65%) - TP (80%)</p>	<p>(S)</p> <p>Formatted: Font: Italic</p> <p>Formatted: Font color: Auto</p>
<p>- Sandy soil h=10 cm - Sand (d= 2 mm) h=20 cm - Biochar (d=1-3 cm) h=40 cm - Gravel (d=2-3 cm) h=10 cm</p>	<p><i>Colocasia esculenta</i></p> <p>64 seedlings/m²</p>	<p>Domestic wastewater</p>	<p>VF-CW</p> <p>h=1.0 m</p> <p>d=0.5 m</p>	<p>Yes</p>	<p>-</p>	<p>-</p>	<p>-</p>	<p>6 months</p>	<p>COD (73%) - DBO₅ (79%) - NH₄⁺ (91%) - TSS (71%) - Total coliforms (70%)</p>	<p>(A)</p> <p>Formatted: Font: Italic</p> <p>Formatted: Font color: Auto</p>
<p>- Sand (d < 2 mm) h= 15 cm - Gravel + Biochar (v/v=1:1): (d=1-2 cm) h=15 cm - Gravel + Biochar (v/v=1:1): (d=2-4 cm) h=25 cm - Gravel (d=5-7 cm) h=10 cm</p>	<p><i>Iris pseudacorus</i></p> <p>6 rhizomes</p>	<p>Swine wastewater</p>	<p>VF-CW</p> <p>h=65 cm</p> <p>d=20 cm</p>	<p>Yes</p>	<p>-</p>	<p>33.74</p> <p>g·m⁻³·d⁻¹</p>	<p>72 h</p>	<p>2 months</p>	<p>COD (77.18 %) - NH₄[±] (96.54 %) - TN (40.12 %) - ARGs (99.3%)</p>	<p>(F)</p> <p>Formatted: Font: Italic</p> <p>Formatted: Font color: Auto</p>

<p>- Sand (d= 1-2 mm) h=150cm - Biochar + fine gravel (v/v=3:1): (d= 10-20 mm) h=150 mm - Gravel (d= 20-40 mm) h=250 mm - Gravel (d= 50-70 mm) h=100 mm</p>	<p><i>Qenanthe Javanica</i> 12 rhizomes</p>	<p>Domestic wastewater</p>	<p>VF-CW h=65 cm d=20 cm</p>	<p>Yes</p>	<p>5.5 L</p>	<p>-</p>	<p>72 h</p>	<p>3 months</p>	<p>COD (91.80%) - NH₄⁺ (50.05%) - TN (49.90%)</p>	<p>(Z) Formatted: Font: Italic Formatted: Font color: Auto</p>
<p>- Gravel (d= 5-8 mm) h= 0.1 m - Biochar (sludge) + gravel (v/v=1:4) h= 0.2 m - Gravel (d= 5-8 mm) h= 0.1 m</p>	<p><i>Typha latifolia</i></p>	<p>Synthetic wastewater</p>	<p>VF-CW h= 0.5 m d= 0.2 m</p>	<p>No</p>	<p>-</p>	<p>-</p>	<p>72 h</p>	<p>60 batches</p>	<p>COD (90.99%) - NO₃⁻ (99.50%) - NH₄⁺ (99.59%) - TN (90.94%) - TP (51.59%)</p>	<p>(Z) Formatted: Font: Italic (a) Formatted: Centered Formatted: Font color: Auto Formatted: Font color: Auto</p>
<p>- Gravel (d= 5-8 mm) h= 0.1 m - Biochar (cattail) + gravel (v/v=1:4) h= 0.2 m - Gravel (d= 5-8 mm) h= 0.1 m</p>	<p><i>Typha latifolia</i></p>	<p>Synthetic wastewater</p>	<p>VF-CW h= 0.5 m d= 0.2 m</p>	<p>No</p>	<p>-</p>	<p>-</p>	<p>72 h</p>	<p>60 batches</p>	<p>COD (77.41%) - NO₃⁻ (84.72%) - NH₄⁺ (96.12%) - TN (80.73%) - TP (43.95%)</p>	<p>(Z) Formatted: Font color: Auto (a) Formatted: Font: Italic, Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto</p>
<p>- Gravel (d=2-6 mm) h=0.05 m - Biochar (v/v=1%) + sand (d=2-10 mm) h=0.2 m - Gravel (d=2-6 mm) h=0.05 m - Gravel (d=2-10 mm) h=0.05 m</p>	<p><i>Iris pseudacorus</i> 5 rhizomes</p>	<p>Synthetic wastewater</p>	<p>VF-CW h=0.45 m d=0.15 m</p>	<p>No</p>	<p>-</p>	<p>-</p>	<p>72 h</p>	<p>4 months</p>	<p>COD (89.1%) - TN (90.2%) - NH₄⁺ (81%)</p>	<p>(A) Formatted: Font: Italic (a) Formatted: Font color: Auto</p>
<p>- Soil h=10 cm - Quartz sand h=5 cm - Zeolite d=8-10 mm + biochar d=2-4 mm (v/v=1:1): h=30 cm - Cobblestones (d=7-10 cm): h=10 cm</p>	<p><i>Phragmites communis</i> 6 plants</p>	<p>Synthetic wastewater</p>	<p>VF-CW l=50 cm w=40 cm d=60 cm</p>	<p>Yes</p>	<p>30 L</p>	<p>0.050 m³.m⁻².d⁻¹</p>	<p>72 h</p>	<p>4 months</p>	<p>TN (62.98%) - NH₄⁺ (93.93%) - NO₃⁻ (93.28%) - COD (86.64%) - CIPH (88.05%) - SMZ (56.57%)</p>	<p>(Y) Formatted: Font: Italic Formatted: Font color: Auto</p>

- Sand (d = 2-4 mm) h = 2 cm - Biochar (2%) + Sand (98%): (d=5-10 mm) h= 15 cm - Sand (d = 2-4 mm) h = 3 cm	<i>Phragmites australis</i>	Synthetic stormwater	VF-CW h = 25 cm d = 11 cm	-	-	10-40 cm/h	5 days	3 months	TSS (71.1%) – TOC (29.3%) - NH ₄ ⁺ (13.5%) - TN (11.7%) - TP (8%) - <i>E.coli</i> (87.1%)	(C) Formatted: Font: Italic Formatted: Font color: Auto
- Sand - Biochar + gravel: v/v = 50%. - Gravel	<i>Iris pseudacorus</i> 6 rhizomes	Synthetic wastewater	VF-CW h = 50 cm d = 10 cm	Yes	-	-	72 h	5 months	COD (93.21 %) - NH ₄ ⁺ (98.30 %) - TN (72.22 %) - TP (53.32%)	(C) Formatted: Font: Italic Formatted: Font color: Auto
- Gravel (d=8-10 mm) h=0.1 m - Biochar + gravel (v/v=4:1): h=0.2 m - Gravel (d=8-10 mm) h=0.1 m	<i>Typha latifolia</i>	Synthetic wastewater	VF-CW l= 0.3 m w= 0.3 m h = 0.5 m	-	-	-	5 days	60 batches	NH ₄ ⁺ (66.3%) – TN (65.4%) – COD (90%)	(A) Formatted: Font: Italic Formatted: Font color: Auto
- Biochar (d=2-3 cm) h=25 cm - Zeolite (d=2-3 cm) h=25 cm - Gravel (d=2-3 cm) h=25 cm	<i>Phragmites australis</i>	Synthetic Wastewater	VF-CW h=80 cm d=40 cm	Yes	-	-	57.4 h	3 months	COD (99.9%) - NH ₃ ⁻ (99.9%) - Phenols (99.9%) - Pb (99.9%) – Mn (99.9%)	(A) Formatted: Font: Italic Formatted: Font color: Auto Formatted: Font color: Auto
- Biochar (20%) + sand (80%): h=20 cm - Gravel: h=5 cm	<i>O. javanica</i> 12 rhizomes	Synthetic wastewater	VF-CW h = 50 cm d = 25 cm	NO	-	0.13 m ³ m ⁻² batch ⁻¹	7 days	8 months	COD (78.71%) - NO ₃ ⁻ (92.72%) - TN (93.26%) - NH ₄ ⁺ (94.26%)	(C) Formatted: Font: Italic Formatted: Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto
- Biochar + sand: (d=0.5-1 mm) h=15cm - Gravel (d=4-6 mm) h=10cm - Gravel (d=8-12 mm) h=10cm	<i>Colocasia esculenta</i> 10 rhizomes	Domestic wastewater	VF-CW h=37cm d=33.5cm	Yes	-	-	10 days	40 days	COD (96.8%) - NO ₃ ⁻ (57.85%) - TN (68.02%) - NH ₄ ⁺ (88.16%) - PO ₄ ³⁻	(C) Formatted: Font: Italic Formatted: Font color: Auto

- <u>Rocks (d=20-21 mm) h=5cm</u>										(75.26%) - SO ₄ ²⁻ (80.50)	
- <u>Biochar (corn cobs) (d= 2-10 mm) h= 0.6 m</u> - <u>Gravel (d=50 mm); h=0.1 m</u>		Industrial wastewater	VF-CW	No	-	-	-	5 months		COD (59%) - BOD ₅ (75%) - TN (37%) - NH ₄ ⁺ (76%) - PO ₄ ³⁻ (71%)	(K) Formatted: Font color: Auto Formatted: Font: Italic Formatted: Font color: Auto
- <u>Biochar (wood) (d= 2-10 mm) h= 0.6 m</u> - <u>Gravel (d=50 mm); h=0.1 m</u>		Industrial wastewater	VF-CW	No	-	-	-	5 months		COD (72%) - BOD ₅ (83%) - TN (47%) - NH ₄ ⁺ (83%) - PO ₄ ³⁻ (85%)	(K) Formatted: Font color: Auto Formatted: Font: Italic, Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto
- <u>Biochar (d=2-4 mm) h=120 mm</u>	<i>Salicaria seedling</i>	Synthetic wastewater	VF-CW	Yes	550 ml	-	24 h	> 3 months		Hg (>94%) - COD (>88%) - NH ₄ ⁺ (92.1) - TP (74.7%)	(d) Formatted: Font: Italic a Formatted: Font color: Auto
<u>Mixture of Quartz rock d=2 - 4 mm (v/v=25 %), Bioceramic d=3 - 6 mm (v/v=25 %), and biochar d=1 - 7 mm (v/v= 50%) h=200 mm</u>	<i>Cyperus alternifolius</i>	Synthetic wastewater	HF-CW	NO	30 L	-	25 h	-		NO ₃ ⁻ (67.16%) - TP (74.25%) - TN (64.31%) - NO ₂ ⁻ (51.6%) - PO ₄ ³⁻ (96.73%)	(G) Formatted: Font: Italic Formatted: Font color: Auto
<u>Mixture of quartz sand + soil (v/v=1:1) and Fe-modified biochar (v/v:10%)</u>	<i>Iris hexagonus</i> 13 plants/m ²	Tailwater	VF-CW	-	-	-	96 h	-		NO ₃ ⁻ (95.30 %) - TN (86.68 %) - NH ₄ ⁺ (86.33 %) - NO ₂ ⁻ (79.35 %) - COD (63.36 %)	(d) Formatted: Font: Italic Formatted: Font color: Auto

<u>Mixture of biochar (v/v=10%) (d<20mm) and LECA (d=2-4 mm)</u>	<u><i>Typha latifolia</i></u>	<u>Municipal wastewater</u>	<u>HF-CW</u>	<u>-</u>	<u>-</u>	<u>60 L/d</u>	<u>48 h</u>	<u>4 months</u>	<u>TN (20.0 %) - TP (22.5 %)</u>	(K)	Formatted: Font: Italic Formatted: Font color: Auto
	<u>10 plants/mesocosm</u>		<u>l=1.5 m w=0.6 m d=0.6 m</u>								
<u>- Gravel (d=2-6 mm) h=0.05 m</u> <u>- Biochar (v/v=1%) + sand (d=2-10 mm) h=0.2 m</u> <u>- Gravel (d=2-6 mm) h=0.05 m</u> <u>- Gravel (d=2-10 mm) h=0.05 m</u>	<u><i>Iris pseudacorus</i></u>	<u>Synthetic wastewater</u>	<u>VF-CW</u>	<u>No</u>	<u>-</u>	<u>-</u>	<u>72 h</u>	<u>4 months</u>	<u>COD (75.9%) - TN (69.2%) - NH₄⁺ (70.8%) - NO₃⁻ (74.7%) - SMX (65.3%)</u>	(A)	Formatted: Font color: Auto Formatted: Font: Italic, Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto
	<u>5 rhizomes</u>		<u>h=0.45 m d=0.15 m</u>								
<u>- Biochar + sand (d=0.25-1 mm) h=6 cm</u> <u>- Gravel (d=4-6 mm) h=10 cm</u> <u>- Gravel (d= 8-12 mm) h=10 cm</u> <u>- Boulders (d= 20-21 mm) h=5 cm</u>	<u><i>Colocasia</i></u>	<u>Synthetic wastewater</u>	<u>VF-CW</u>	<u>No</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>COD (88.8%), NH₄⁺ (83.1%), and NO₃⁻ (64.9%) AMX (75.51%) - CF (87.53%) - IBU (79.93%)</u>	(C)	Formatted: Font color: Auto Formatted: Font: Italic, Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto
			<u>d=33.5 cm h=37 cm V=30 L</u>								
<u>Sand h=15 cm</u> <u>Biochar h= 20 cm</u> <u>Gravel h=15 cm</u>	<u><i>G. maxima</i></u>	<u>Synthetic wastewater</u>	<u>VF-CW</u>	<u>No</u>	<u>-</u>	<u>2 L/ 4d</u>	<u>3 months</u>	<u>-</u>	<u>PPCPs (99.99 %)</u>	(K)	Formatted: Font color: Auto Formatted: Font: Italic, Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto
			<u>d=15 cm h=55 cm</u>								
<u>Stones (d= 5-10mm) h=0.05 m</u> <u>Biochar (d= 5-10mm) h=0.76 m</u> <u>Stones (d= 5-10mm) h=0.05 m</u>	<u><i>Phragmites</i></u>	<u>Municipal wastewater</u>	<u>VF-CW</u>	<u>No</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>NH₄⁺ (89.8%) - NO₂⁻ (38.5%) - TN (82.5%) - TP (91%) -BOD (95%) - COD (96.2%)</u>	(S)	Formatted: Font: Italic, Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto Formatted: Font color: Auto
			<u>h=0.91 d=0.15 m</u>								

Biochar (d=10 cm) 16 plant/m² wastewater 110 cm ×40 - TP(75.9%)
Gravel (d=20 cm) cm ×60 cm

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2 HRL: Hydraulic loading rate, HRT: Hydraulic retention time

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3.2. Biochar granulometry in constructed wetlands

The fundamental elements of the CW system are the substrates or media, which are essential for removing contaminants from wastewater. They serve as a platform for biofilm development, macrophyte root growth, and a reaction site for pollutants' immobilization and supporting matrix (Wu et al., 2015). Therefore, the choice of bed materials is highly important in a CW. Inexpensive and locally available materials can be used depending on the size of the media, its hydraulic conductivity, texture, porosity, and other factors (Wu et al., 2015). Gravel, biochar, zeolite, composite materials and activated carbon have been used as CW substrates (Kataki et al., 2021). Substrates such as sawdust, light expanded clay aggregate (LECA), zero-valent iron, and gravel can effectively remove phosphorus, organic matter, arsenates, and sulfates (Parde et al., 2021). Biochar-based CWs show promising wastewater treatment efficiency (Enaime et al., 2020). However, granular biochar is more suitable for applications than powdered ones. This can be explained by its good pore size distribution, low abrasion index, durability, high bulk density, and ability to regenerate (LOUARRAT, 2019). In addition, this type of biochar has sufficient mechanical strength and is suitable for ensuring the stability and hydraulic permeability of the matrix (Deng et al., 2021). Because particle size has a significant effect on pollutant adsorption, the nitrate nitrogen content, ammonia nitrogen content, and denitrification intensity of the wetland substrate decreased by 51.1%, 46.6%, and 35.4%, respectively, after the introduction of biochar with a particle size ranging from 1-2 mm in CW (Zhou et al., 2018), when compared to biochar with a particle size lower than 1 mm. Biochar with a 1-3 cm diameter is widely used as a substrate in CWs to avoid clogging (Nguyen et al., 2020). Other factors influence the adsorption of pollutants, such as adsorption increasing with the contact time, pH, temperature, and concentration of NH_3 but decreasing with the size of biochar particles (Kizito et al., 2015).

On the other hand, CW substrate size strongly influences the removal performance of various pollutants. However, a study conducted by Deng et al. (2019) was built based on different volumes of biochar in common gravel (0%, 10% (h=3), 20% (h=6), and 30% (h=9)) to see the effect of increasing biochar substrate depth on the characteristics of metabolites and microbes. This experiment found that increasing the depth of biochar substrate in the gravel medium enhanced the contaminant removal efficiency in CWs. Hence, Illumina MiSeq sequencing reported that the microbial community showed some obvious variations. The relative abundances of *Candidatus* *competibacter*, *Thauera*, *Dechloromonas*, *Chlorobium*, *Thiobacillus* and *Desulfobulbus* were significantly improved with the biochar. On the other hand, the total Extra Polymeric Substances (EPS) content decreased with increasing biochar substrate depth.

Furthermore, the increase in biochar substrate in CWs reflects an improvement in the biodegradation of EPS and the richness of microbial communities, which promotes the removal of organic and nitrogenous substances (Deng et al., 2019). Similarly, Liang et al. (2020) used 4 CW microcosms with different volume ratios of biochar (0%, 10%, 20%, and 30%) to analyze the improvement of pollutant removal performance. The results showed that the increase in biochar substrate increased the average removal efficiencies of total nitrogen (TN) and ammonium (NH_4^+ -N). At the same time, nitrous oxide (N_2O) emissions were reduced. The increase in

1 biochar substrate depth can explain this change in the diversity and similarity of the microbial community. In
2 addition, the relative abundance of functional microorganisms such as Nitrospira, Nitrosomonas, Pseudomonas,
3 and Thauera increased due to the increase in biochar content, which favored nitrogen cycling and reduced N₂O
4 emissions.

5 **3.3.3.1. Integration mode** ~~Position of biochar implementation in the CWs~~ **Substrate**

6 **3.3.1. Biochar at the middle substrate of CW**

7 **3.1.1. Biochar in vertical flow CW**

8 When used as substrate in VF-CWs, biochar can potentially promote contaminant removal. As
9 illustrated in Fig. 1-a, most CWs are implemented by positioning the biochar between two layers of inert
10 material (see Table 2), thereby avoiding the clogging of the filtration system (Ji et al., 2020; Liang et al., 2020;
11 Liao et al., 2022). In this interlayer, Biochar as a substrate can potentially promote contaminant removal
12 performance in vertical flow CWs. Recently, most of the CWs installations have placed the biochar substrate in
13 the middle of the system (Fig. 1). As a result, the biochar is either used alone or mixed with other materials,
14 namely sand, gravel, etc. (Table 2) (Ajibade et al., 2020; Liao et al., 2022; Zhong et al., 2021; Zhou et al., 2018).
15 The reason for which the biochar substrate is placed in the middle can be explained by the fact that it avoids
16 clogging the filtration system (Deng et al., 2019; Ji et al., 2020; Liang et al., 2020; Liao et al., 2022).

17 Several authors have ~~us~~placed the biochar substrate alone ~~in the middle~~ as an interlayer of the filter
18 system in order to increase the removal rate of different pollutants. For example, in the ~~study of~~ Nguyen et al.
19 (2020) ~~study~~, the biochar substrate is used under two sand and sandy soil layers. This distribution increases the
20 removal efficiencies of total coliforms up to 70% (Nguyen et al., 2020). Moreover, using biochar substrate under
21 a coarse stone substrate allows the removal of total phosphorus up to 91% and organic matter such as BOD and
22 TSS up to 95% and 99.7%, respectively, from municipal wastewater (Saeed et al., 2020). Another study placed
23 the biochar substrate under a coarse pebble layer to improve nitrate removal performance ~~by~~ up to 92% and
24 orthophosphate ~~by~~ up to 67.7% (Gupta et al., 2016). However, using gravel substrate over biochar ~~substrate~~
25 increases the removal performance up to 94.9% TN, 99.4% NH₄⁺ and 99.84% COD (Liang et al., 2020; Liao et al., 2022).
26 On the other hand, the modification of biochar with iron shows high removal performance of
27 pollutants such as Abamectin (99%), COD (98%), NH₄⁺ (65%) and TP (80%) (Sha et al., 2020).

28 Biochar can be mixed with gravel (Feng et al., 2021a), sand (Ajibade et al., 2020), or zeolite (Yuan et
29 al., 2020) to form a single substrate to filter various micropollutants from wastewater. Zheng et al. (2022) found
30 that mixing biochar with gravel at a volume ratio of 1:4 resulted in high removal efficiency of COD (90.99%),
31 NO₃⁻ (99.50%), TN (90.94%), NH₄⁺ (99.59%), and TP (51.59%). On the other hand, mixing biochar with sand
32 with a low volume ratio of biochar (2%) gave low removal rates (TOC (29.3%); NH₄⁺ (13.5%); TN (11.7%); TP
33 (8%)) except for *E.coli*, TSS and coliforms, which show high removal efficiency, coming up to 87.1% and
34 71.1% for *E.coli* and TSS, respectively (Lun, L., Chen, 2018). Similarly, Ajibade et al. (2020), also mixed
35 biochar with sand. Still, this time gave a high performance compared to the study of Lun and Chen. (2018),

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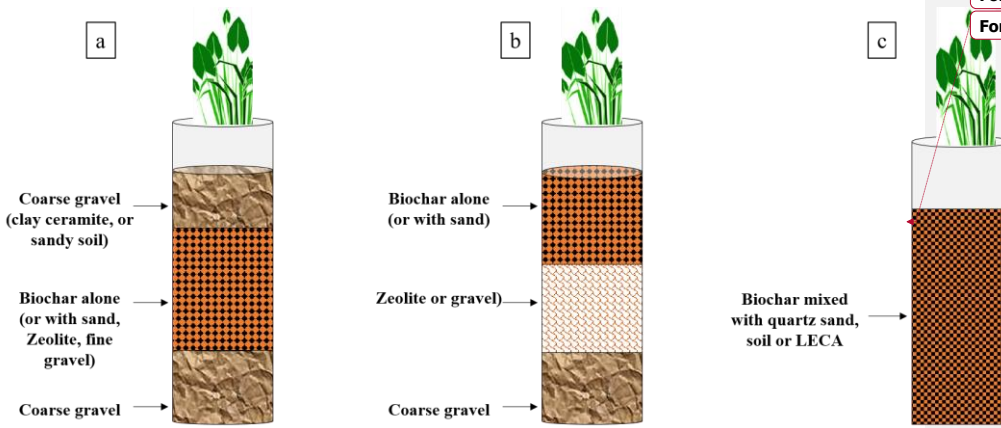
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1 where the removal efficiency of some pollutants reached 89.1 % for COD, 90.2% for TN and 81% for NH_4^+
 2 (Ajibade et al., 2020). The ratio of biochar can explain the difference between these two studies that is higher in
 3 the second one. Yuan et al. (2020) reported that mixing biochar with zeolite can improve the removal percentage
 4 up to 632.98% for TN, 943.93% for NH_4^+ , 93.28% for NO_3^- and 876.64% for COD. This result may be justified
 5 by the fact that the biochar inhibited the formation of quinolone resistance genes and enhanced the COD removal
 6 efficiency by increasing the abundance of bound microorganisms (Yuan et al., 2020). In most studies, biochar
 7 substrates mixed with gravel showed higher removal efficiency of various pollutants compared to biochar
 8 substrates mixed with sand (Table 2).



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9
 10 *Figure 1. Position of biochar substrate (a): as interlayer of VF-CW, (b): on top of the VF-CW, (c):*
 11 *filling all the VF-CW*

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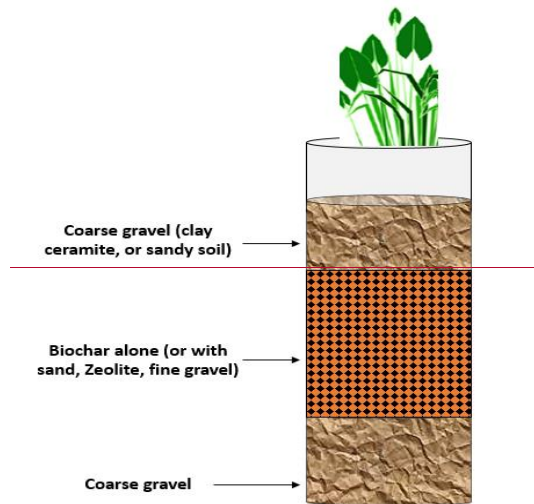


Fig. 1: Position of biochar substrate in the middle of CW.

3.3.2. Biochar at the top substrate of CW

Biochar can also be placed at the top (Fig. 1-b2) (Table 2) of the filtration system with large grain size (2-30 mm) in order to avoid the clogging phenomenon (Abedi and Mojiri, 2019; Kizito et al., 2017). In Abedi and Mojiri. (2019), the top biochar substrate-layer played an important role in decreasing the content of various pollutants such as COD, NH_4^+ , phenols, Pb, and Mn. This study showed the best removal performance compared to the various study literature, where sinve the removal efficiency was quantitative reaches up to 99.9% of for COD, 99.9% of NH_4^+ , 99.9% of phenols, 99.9% of Pb and 99.9% of Mn (Abedi and Mojiri, 2019). This result can be explained because biochar is mainly attributed to the greater adsorption capacity and microbial culture in the porous medium of biochar (Kizito et al., 2017). Furthermore, the use of biochar at the upper filter level revealed that adding biochar in VF-CWs improves the oxidative removal of NH_4^+ -N, SO_4^{2-} , and PO_4^{3-} and contributes to the uptake of other plants (Chand et al., 2021). Another study conducted by Chand et al. (2021) used biochar on top of a system with small grain size ($d = 0.5\text{-}1\text{ mm}$), but to avoid clogging, they mixed the biochar with sand, which allowed them to increase the treatment efficiency and thus removed up to 976.8% COD, 587.85% NO_3^- , 68.92% TN, 88.16% NH_4^+ , 75.26% PO_4^{3-} and 80%.50 SO_4^{2-} (Chand et al., 2021).

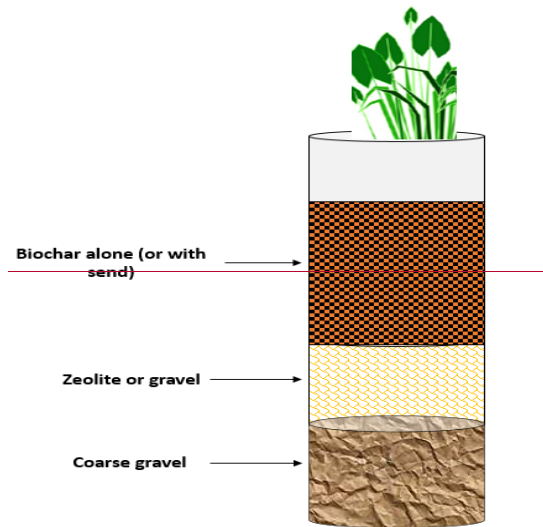


Fig. 2: Position of the biochar substrate on top of the CW.

3.3.3. Biochar substrate filling all the CW

Sometimes the whole filter is filled from top to bottom with biochar ~~is filled with the whole filter from top to bottom~~ (Fig. 1-c3) (Table 2) mixed at low rate (10%) with another material (quartz sand, soil, LECA), but with a low volume ratio of biochar (10%) to avoid the clogging of the system. For example, Jia et al. (2020) mixed 10% biochar with quartz sand and soil to fill the entire filter and obtained an increase of the removal efficiency of pollutants (NO_3^- (95.30%); TN (86.68%); NH_4^+ (86.33%); NO_2^- (79.35%); COD (63.36%)) (Jia et al., 2020b).

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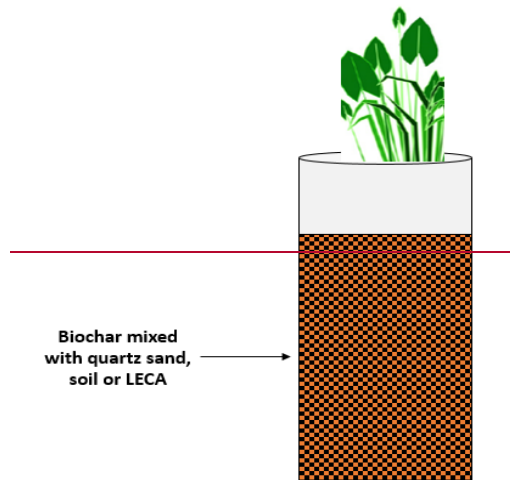


Fig. 3- Biochar substrate filling all the CW.

3.1.2. Biochar substrate in the horizontal flow CW

The use of biochar in horizontal flow CWs (HF-CWs) is still limited, and a little number of articles was found (Gao et al., 2018; Bolton et al., 2019; Gao et al., 2019; Jia and Yang, 2021; Wu et al., 2022). For example Bolton et al., (2019) implemented two small pilot-scale HF-CWs planted with *Melaleuca quinquenervia* trees, each one consisting in two cells separated by a polyethylene baffle. The first wetland contained two cells in series filled with gravel (control wetlands), while in the other wetland the first cell was filled with gravel to trap sediments, thus avoiding blockages in the downstream cell, the latter filled with an enriched biochar cell (biochar wetlands). This study showed that the removal efficiencies of PO_4^{3-} -P in the biochar wetland was up to 97% probably due to the higher number of adsorption sites in the substrate. In contrast, the control achieved only an average PO_4^{3-} -P removal of 91%, indicating a rapid saturation of the gravel. Another study realized by Gupta et al., (2016) revealed that HF-CWs with biochar were more efficient to reduce various pollutants (organic and inorganic) as compared to the wetland with gravels alone. Hence, the removal efficiencies achieved were around 58% of TN, 79% of TP, 92% of NO_3 -N, 58% of NH_3 -N, 68% of PO_4^{3-} -P and 91% of COD. The high removal of NH_4^+ -N obtained in HF-CWs is probably related to the enhanced microbial nitrification when adding biochar (Gupta et al., 2016). The improved NO_3 -N removal efficiency is attributed to a higher denitrification, due to the anoxic conditions in HF-CWs. These results indicate clearly that integrating of biochar in HF-CW can be primarily used for a secondary treatment of municipal and domestic wastewaters leading to nutrients removal. In general, the use of biochar in HF-CWs can be a cost-effective and sustainable wastewater treatment option with a smaller energy footprint (Wu et al., 2022; Gupta et al., 2016).

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3.2. Effect of substrate nature, biochar dose and granulometry on CWs efficiency

The fundamental element of the CW system is the substrate or media, which is essential for removing contaminants from wastewater. It serves as a platform for biofilm development, macrophyte root growth, and a reaction site for pollutants' immobilization and supporting matrix (Wu et al., 2015). Therefore, the choice of bed materials is highly important in a CW. Inexpensive and locally available materials can be used depending on the size of the media, its hydraulic conductivity, texture, porosity, and other factors (Wu et al., 2015). Gravel, biochar, zeolite, composite materials and activated carbon have been used as CW substrates (Kataki et al., 2021). Substrates such as sawdust, light expanded clay aggregate (LECA), zero-valent iron, and gravel can effectively remove phosphorus, organic matter, arsenates, and sulfates (Parde et al., 2021).

Biochar-based CWs show promising wastewater treatment efficiency (Enaime et al., 2020a). However, granular biochar is more suitable for applications than powdered ones. This can be explained by its good pore size distribution, low abrasion index, durability, high bulk density, and ability to regenerate (Louarrat, 2019). In addition, this type of biochar has sufficient mechanical strength and is suitable for ensuring the stability and hydraulic permeability of the matrix (Deng et al., 2021). In addition, particle size has a significant effect on pollutants adsorption. Nitrate-nitrogen content, ammonia nitrogen content, and denitrification intensity of the wetland substrate decreased by 51%, 47%, and 35%, respectively, after the introduction of biochar with a particle size ranging from 1-2 mm in CW (Zhou et al., 2018), when compared to biochar with a particle size lower than 1 mm. Biochar with a 1-3 cm diameter is widely used as a substrate in CWs to avoid clogging (Table 2) (Nguyen et al., 2020). Other factors influence the adsorption of pollutants, such as increasing of the contact time, pH, temperature, and concentration of NH₃. But adsorption is decreasing with increasing the size of biochar particles (Kizito et al., 2015). According to these results we can state that the biochar granulometry has a significant effect on the efficiency of the treatment of the pollutants.

On the other hand, the biochar dose in CW substrate strongly influences the removal performance of various pollutants. However, a study conducted by Deng et al. (2019) was built based on different volumes of biochar in common gravel (0%, 10% (h=3), 20% (h=6), and 30% (h=9)) to see the effect of increasing biochar substrate depth on the characteristics of metabolites and microbes. This experiment found that increasing the biochar dose in the gravel medium enhanced the contaminant removal efficiency in CWs. Hence, Illumina MiSeq sequencing reported that the microbial community showed some obvious variations. The relative abundances of *Candidatus competibacter*, *Thauera*, *Dechloromonas*, *Chlorobium*, *Thiobacillus* and *Desulfobulbus* were significantly improved with the biochar dose. On the other hand, the content of total Extra Polymeric Substances (EPS) decreased with increasing the biochar percentage.

Furthermore, the increase in biochar dose in CWs substrate reflects an improvement in the biodegradation of EPS and the richness of microbial communities, which promotes the removal of organic and nitrogenous substances (Deng et al., 2019). Similarly, Liang et al. (2020) used 4 CW microcosms with different volume ratios of biochar (0%, 10%, 20%, and 30%) to analyze the improvement of pollutant removal performance. The results showed that the increase in biochar dose increased the average removal efficiencies of

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1 total nitrogen (TN) and ammonium (NH₄⁺-N). At the same time, nitrous oxide (N₂O) emissions were reduced.
2 The increase in biochar dose can explain this change in the diversity and similarity of the microbial community.
3 In addition, the relative abundance of functional microorganisms such as Nitrospira, Nitrosomonas,
4 Pseudomonas, and Thauera increased due to the increase in biochar content, which favored nitrogen cycling and
5 reduced N₂O emissions.

6 **3.3. Effect of macrophytes used and its role in CWs implemented with biochar**

7 Plants are essential in removing pollutants, as they generally play an indirect role in the wastewater
8 treatment performance in CWs (Fu et al., 2022). The choice of appropriate plant species is crucial for the best
9 performance (Guitttonny-philippe et al., 2015; Srivastava et al., 2008; Kulshreshtha et al., 2022). Hence, the right
10 choice was based on several parameters; the species that are preferred are characterized by high ecological
11 adaptability, adaptation to local climatic and nutritional conditions, high biomass productivity, resistance to pests
12 and diseases; having good coverage with high prospects of successful establishment, tolerance to pollutants and
13 hypertrophic waterlogging conditions, low tendency to dominate or forming monocultures, a high capacity for
14 pollutant removal, easy propagation, and rapid establishment (Nuamah et al., 2020; Kataki et al., 2021).
15 According to literature, the *Phragmites australis* was the most used plant in the studies (Table 2), due to its effect
16 on the efficiency of CW, resistance to pests and diseases, tolerance to pollutants and hypertrophic waterlogging
17 conditions, high capacity for pollutant removal, easy propagation and adaptation to local climatic and nutritional
18 conditions (Zhong et al., 2021; Yuan et al., 2020; Chen, 2018). However, a comparative study done by (Qadiri et
19 al., 2021) has demonstrated that the CWs transplanted with *Phragmites* has more capacity in removing TN,
20 COD, TP and TSS than *Sagittaria latifolia* and *Iris kashmiriana*, due to its well developed roots in the substrates
21 which gives a better remediation effect. Furthermore, the presence of a biochar substrate in the CW promotes
22 plant growth, microbial metabolism and substrate characteristics in many aspects (Qadiri et al., 2021). Another
23 key parameter in selecting CW species is the higher water use efficiency index (Stefanakis, 2020). Several
24 studies have shown that plants with fibrous root systems provide a greater surface area for biofilm enhancement,
25 sedimentation, and particulate matter trapping. They show higher photosynthesis and radial oxygen loss levels
26 and are more effective in removing contaminants than plants with thick roots (Kataki et al., 2021); (Borne et al.,
27 2013; Lai et al., 2012). In addition, previous studies have shown that plant density affects CWs performance at 5
28 to 50 plants/m². A low density (16 m²) CW planting may result in lower nitrogen removal than a CW with a high
29 plant density (32 m²) (reduced by almost half) (Hernández et al., 2017). Another factor to consider is the age of
30 the plant, as oxygen release and contaminant uptake are lower in older plants due to the presence of older
31 lignified roots (Valipour and Ahn, 2015).

32 **4.3.4. Effectiveness of biochar in removing various pollutants**

33 Biochar is a solid material with high porosity, a high surface area, and diverse surface functional groups
34 and properties, making it an attractive option for wastewater treatment. Biochar has been proposed as an
35 effective substrate for capturing wastewater supplements that may be connected to soil alteration. ~~It can be~~
36 ~~bound to the soil as an alteration and expel toxins from wastewater.~~ ~~The~~Its adsorption properties and high

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1 porosity allow ~~pollutantsoisons~~ to accumulate on its surfaces, resulting in supplement-rich biochar and a clean
2 effluent (Peiris et al., 2017; Yaashikaa et al., 2020). Biochar adsorbents have been used to remove various
3 contaminants **(Table 2)** such as antibiotics (Ahmed et al., 2017), pesticides (Mandal et al., 2021),
4 pharmaceuticals (Masrura et al., 2021; Solanki and Boyer, 2017), and personal care products from aquatic
5 environments (Keerthanana et al., 2020). The use of biochar for wastewater treatment is becoming more viable
6 due to the low cost of the raw material and the ease of the manufacturing process, as well as the various
7 improved physicochemical characteristics of biochar, which have been successfully used in a diverse range of
8 applications for the contaminated wastewater remediation, including toxic heavy metals adsorption (the
9 following techniques have been used: chemisorption, physical sorption, ion exchange, and precipitation) and
10 dyes from aqueous solutions, as immobilization support for microorganisms, as a support for catalysts, and as an
11 adsorbent for inhibiting substances during anaerobic digestion, thanks to its unique and very versatile
12 characteristics. Overall, it is clear that biochar has multiple potential economic and environmental benefits, and
13 its effectiveness in removing various contaminants on a laboratory scale has been widely reported (Ahmad et al.,
14 2021; Enaime et al., 2020); (Chen et al., 2022).

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15 Biochar added to CW substrate can considerably enhance the wastewater purification effect (Kizito et
16 al., 2017), as biochar can remove more nutrients and reduce greenhouse gas (GHG) emissions than other
17 substrates, e.g., ceramite, while promoting more diverse bacterial communities and greater abundances of
18 available taxa (Ji et al., 2020). ~~The a~~Average N₂O and CO₂ fluxes were significantly lower, while CH₄ fluxes
19 were significantly greater in the biochar-added and non-biochar CWsin biochar-added wastewater compared to
20 non-biochar wastewater (E-Guo et al., 2020). Biochar combined with sand, zeolite, and other artificial CW
21 substrates can enhance microbial activity and compensate for the lack of carbon sources (Wang et al., 2020b).
22 Abedi and Mojiri. (2019) reported that CW containing three substrate layers, namely biochar, gravel and zeolite
23 layers, showed high performance in wastewater treatment compared to the other CWs containing gravel as a
24 substrate ~~layer~~; the first CW can remove pollutants from wastewater better than the second one. At an optimum
25 retention time (57.4 h) and pH (6.3), this biochar integrated CW can remove up to 99.9% of COD (1000 mg/L),
26 ammonia (1000 mg/L), phenols (50 mg/L), Pb (50 mg/L) and Mn (50 mg/L). In addition, the emission of nitrous
27 oxide was lower in gravel CW than in the integrating biochar CW (Abedi and Mojiri, 2019). These results can
28 explain that the introduction of biochar considerably improved the abundance of biological bacteria in CW,
29 consequently increasing the efficiency of removing various contaminants in wastewater (Li et al., 2018a). This
30 agrees with the results of Liang's study **(Table 23)**, which explains the increase in nitrogen removal efficiency
31 and the decrease in N₂O emissions resulting from the increase in biochar addition ratio. This shows that biochar
32 addition changed the diversity and similarity of the microbial community (Liang et al., 2020).

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33 ~~Similarly, COD was increased with an increasing biochar addition ratio.~~In general, the removal
34 efficiency of pollutants was increased due to biochar adsorption (Meng et al., 2019). In addition, the total amount
35 of extracellular polymeric substance (EPS) decreased significantly with the addition of biochar, which is
36 explained by the change in the functional groups of EPS, including amide, carbonyl, and hydroxyl groups of

1 proteins. Furthermore, biochar has the potential to convert metabolized high molecular weight compounds into
 2 low molecular weight compounds (Deng et al., 2019).

3

4 *Table 3: Removal rate of pollutants in different CW systems containing biochar.*

Type of substrate in vertical flow CWs	Treated wastewater	Removal efficiency of pollutants	Reference
Gravel Biochar	Synthetic wastewater	COD (89.88%)—TN (86.36%)—NH ₄ ⁺ (63.51%)	(Deng et al., 2019)
Sandy soil Sand Biochar Gravel	Domestic wastewater	COD (73%)—DBO ₅ (79%)—NH ₄ ⁺ (91%)— TSS (71%)—Total coliforms (70%)	(Nguyen et al., 2020)
Biochar Large stones	Municipal wastewater	NH ₄ ⁺ (89.8%)—NO ₂ ⁻ (38.5%)—TN (82.5%) TP (91%)—BOD (95%)—COD (96.2%) —TSS (99.7%)	(Saeed et al., 2020)
Pebbles Coke Fe-Biochar	River water	Abamectin (99%)—COD (98%)—NH ₄ ⁺ (65%)— TP (80%)	(Sha et al., 2020)
Pebbles Biochar Gravel	Synthetic wastewater	COD (91.3%)—TN (58.3%)—NH ₃ ⁻ (58.3%)— NO ₂ ⁻ (92%)—TP (79.5%)—PO ₄ ³⁻ (67.7%)	(Gupta et al., 2016)
Gravel Biochar	Synthetic wastewater	COD (93.4%)—TN (94.9%)—NH ₄ ⁺ (99.4%)	(Liang et al., 2020)
Gravel Biochar	Synthetic wastewater	COD (99.84%)—NH ₄ ⁺ (92.00%)—TP (88.63%)	(Liao et al., 2022)
Clay-ceromite Biochar	Domestic wastewater	COD (75.5%)—TP (76.2%)—TN (59.2%)— NH ₄ ⁺ (62.5%)	(Ji et al., 2020)
Gravel Biochar (sludge)	Synthetic wastewater	COD (90.99%)—NO ₂ ⁻ (99.50%)—NH ₄ ⁺ (99.59%) —TN (90.94%)—TP (51.59%)	(Zheng et al., 2022)

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Sand Biochar Gravel	Wastewater swine	COD (77.18%) – NH ₄ ⁺ (96.54%) – TN (40.12%)	(Feng et al., 2021)
Sand Biochar	Synthetic stormwater	TSS (71.1%) – TOC (29.3%) – NH ₄ ⁺ (13.5%) – TN (11.7%) – TP (8%) – <i>E.coli</i> (87.1%)	(Lun, L. Chen, 2018)
Sand Biochar Gravel	Domestic wastewater	COD (91.80%) – NH ₄ ⁺ (50.05%) – TN (49.90%)	(Zhou et al., 2018)
Soil Quartz sand Zeolite Biochar Cobblestones	Synthetic wastewater	TN (62.98%) – NH ₄ ⁺ (93.93%) – NO ₂ ⁻ (93.28%) – COD (86.64%)	(Yuan et al., 2020)
Sand Biochar Gravel	Synthetic wastewater	COD (93.21%) – NH ₄ ⁺ (98.30%) – TN (72.22%) – TP (53.32%)	(Li et al., 2019)
Gravel Biochar (cattail)	Synthetic wastewater	COD (77.41%) – NO ₂ ⁻ (84.72%) – NH ₄ ⁺ (96.12%) – TN (80.73%) – TP (43.95%)	(Zheng et al., 2022)
Gravel Biochar Sand	Synthetic wastewater	COD (89.1%) – TN (90.2%) – NH ₄ ⁺ (81%)	(Ajibade et al., 2020)
Biochar Zeolite Gravel	Synthetic wastewater	COD (99.9%) – NH ₃ ⁻ (99.9%) – Phenols (99.9) – Pb (99.9%) – Mn (99.9%)	(Abedi and Mojiri, 2019)
Biochar Sand Gravel Rocks	Domestic wastewater	COD (96.8%) – NO ₂ ⁻ (57.85%) – TN (68.02%) – NH ₄ ⁺ (88.16%) – PO ₄ ³⁻ (75.26%) – SO ₄ ²⁻ (80.50)	(Chand et al., 2021)
Biochar (corn cobs) Gravel	Industrial wastewater	COD (59%) – BOD ₅ (75%) – TN (37%) – NH ₄ ⁺ (76%) – PO ₄ ³⁻ (71%)	(Kizito et al., 2017)
Biochar (wood) Gravel	Industrial wastewater	COD (72%) – BOD ₅ (83%) – TN (47%) – NH ₄ ⁺ (83%) – PO ₄ ³⁻ (85%)	(Kizito et al., 2017)
quartz sand	Tailwater	NO ₂ ⁻ (95.30%) – TN (86.68%) – NH ₄ ⁺ (86.33%) –	(Jia et al.,

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Soil Fe-Biochar		NO ₂ ⁻ (79.35 %) – COD (63.36 %)	2020)
Biochar LECA	Municipal wastewater	TN (20.0 %) – TP (22.5 %)	(Kasak et al., 2018)

1
2 The biochar can be used at various stages of the wastewater treatment process to increase treatment
3 capacity and recover value-added by-products. The adsorption, buffering, and immobilization mechanism of
4 microbial cells may influence the use of biochar in the wastewater treatment system. For example, properly
5 modified biochar could effectively adsorb nutrients such as phosphorus and nitrogen from treated effluent,
6 allowing it to be used for soil rehabilitation as a nutrient-enriched material. In addition, biochar could help
7 develop activated sludge's treatment and settling capacity by adsorbing inhibitors and hazardous chemicals or
8 providing a surface for microbial immobilization when used in the treatment process. The introduction of
9 biochar to the biological system can also help increase the soil amendment capabilities of biosolids, extend the
10 value chain, and provide other economic benefits as interest in its use in soil applications increases (Mumme et
11 al., 2014). The following sections discuss biochar's role in removing various contaminants from wastewater.

12 **3.4.1. Removal of organic pollutants**

13 Numerous studies have been conducted in recent years to test the effectiveness of biochar in
14 removing various organic substances from water, such as antibiotics, drugs, agrochemicals, polycyclic aromatic
15 hydrocarbons (PAHs), cationic aromatic dyes, and volatile organic compounds (VOCs) (see **Table 2**) (Adeel et
16 al., 2016; Mondal et al., 2016).

17 **4.1.3.4.1.1. Removal of conventional pollutants**

18 Organic pollutants are another important type of pollutant in the aquatic environment, the biochar has
19 shown a high removal efficiency towards this kind of pollutants. Based on the literature, the Numerous studies
20 have been conducted in recent years to test the effectiveness of biochar in removing various organic substances
21 from water, such as antibiotics, drugs, agrochemicals, polycyclic aromatic hydrocarbons (PAHs), cationic
22 aromatic dyes, and volatile organic compounds (VOCs) (Adeel et al., 2016; Mondal et al., 2016). Generally,
23 biochar prepared at a higher pyrolysis temperature will improve non-polar organic compounds' removal
24 efficiencies due to higher microporosity and surface area (Mohamed et al., 2016; Mohanty et al., 2013). On the
25 other hand, the biochar prepared at a temperature below 500 °C comprises a higher amount of hydrogen and
26 oxygen-containing functional groups, so it is more likely to have a high affinity for polar organic molecules
27 (Suliman et al., 2016). For example, biochar derived from rice husk and pyrolyzed soybeans at 600-700 °C
28 facilitates the removal of trichloromethylene (VOC) and non-polar carbofuran (pesticide) from contaminated
29 water (Suliman et al., 2016). In addition, at T >700 °C, red gum wood chips and chicken litter-derived biochar
30 efficiently removed pyrimethanil and diesopropylatrazine (fungicide/pesticide), whereas the same biochar at T

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1 <500 °C proved ineffective (Chen and Chen, 2009; Yu et al., 2010). And for the removal of polar insecticides
2 and herbicides such as norflurazon, 1-naphthol and fluridone was performed using biochar produced at <300 °C,
3 as a result of the pollutant's interaction with the biochar's functional groups (Li et al., 2016; Sun et al., 2011). On
4 the other hand, the biochar with more O and H functional groups (<400 °C) showed higher sorption of aromatic
5 cationic dyes such as methyl-blue and methyl-violet. Still, the process strongly depended on pH (Adeel et al.,
6 2016; Teixid et al., 2011). In addition, the polar antibiotic sulfamethazine (SMZ) exhibits pH-dependent
7 interactions when sorbed to softwood/hardwood-derived biochars (pyrolyzed at 300-700 °C) (Mohan et al.,
8 2014). Therefore, it can be considered an important parameter for biochar interactions and polar organic
9 contaminant removal.

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10 Generally, organic matter from wastewater may be removed by filtration, adsorption, hydrolysis,
11 chemical reduction or oxidation by microbial degradation, etc. (Vymazal and Tereza, 2015). The degradation by
12 the microbiota attached to the substrates is responsible for the elimination of organic matter in aqueous solutions
13 (Faulwetter et al., 2009). Conventional organic compounds such as chemical oxygen demand (COD) and
14 biological oxygen demand (BOD₅) can be removed effectively due to the coupling role of anaerobic and aerobic
15 degradation in CW systems (Saeed and Sun, 2017; Zhao et al., 2020). Thus, the integration of biochar into CWs
16 plays an important role in COD removal, even though organic matter can be leached from biochar (Zhou et al.,
17 2019). However, Several studies have shown that biochar amendment promotes COD removal in CWs (Deng et
18 al., 2019; ~~F~~-Guo et al., 2020). This result can be explained ~~because biochar has a~~ by the good adsorption capacity
19 of biochar toward organic molecules and provides a heterogeneous surface with very high porosity for oxygen
20 filling and habitation by various organic degradation microbes. Moreover, biochar can promote plant growth,
21 releasing additional oxygen into CW substrates for aerobic COD decomposition. A recent finding by some
22 researchers ~~is~~ show that the introduction of biochar into CWs can reduce the quantity of microbial extracellular
23 polymeric substances (EPS) accumulated in the wastewater matrix and induce ~~their~~ metabolization of heavy
24 molecular weight EPS metabolites into lower molecular weight compounds because biochar increases the
25 metabolic and abundance activities of heterotrophic bacteria, thus reflecting organic decomposition, which is
26 conducive to mitigating the clogging of wastewater treatment substrate.

27 3.4.1.2. Emerging pollutants

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28 Emerging hazardous organic pollutants that can be contained in stormwater, livestock wastes,
29 agricultural waters, and industrial wastewaters, etc., such as dyes, pesticides, herbicides, endocrine disruptors
30 (e.g., phthalic acid esters, polycyclic aromatic hydrocarbons, and bisphenol A), and antibiotics (Table 2), pose
31 serious long-term threats to ecosystems and public health, even at minute concentrations (Vymazal and Tereza,
32 2015). Hydrophobic effects, electrostatic attraction, conjugation of aromatic-donors and cationic-acceptors, pore
33 filling, and hydrogen bonding are all processes that biochar can use to adsorb these contaminants (Xiang et al.,
34 2020; Zhang et al., 2019). Most importantly, biochar possesses catalytic and redox-reactive activities, allowing it
35 to accept/donate electrons or promote generate ROS and electrical conduction, thus accelerating the abiotic
36 decomposition of adsorbed organic pollutants (Devi and Saroha, 2015; Zhang et al., 2019). In addition, biochar
37 substrates may stimulate the reproduction and development of microbes involved in decomposing organic

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1 pollutants. However, this augmentation role of biochar has only been studied profoundly so far (Yan et al., 2017;
2 You et al., 2020). The mechanisms involved depend mainly on biochar properties, operating conditions and
3 contaminants. Due to the exceptional ability of biochar to adsorb bisphenol A, Lu and Chen. (2018) found that
4 the integrating biochar into CWs improved the elimination of bisphenol A from stormwater and increased the
5 life of CW systems. integration of biochar to CWs improved the elimination of bisphenol A from stormwater and
6 increased the life of CW systems and that According to the same authors, the bbiochar prepared at 700 °C
7 performed significantly better than biochar prepared at 300 and 500 °C. In addition, the biochar substrate
8 supported the increase of functional microbes and served as an excellent biofilm carriers to indirectly enhance
9 the decomposition of bisphenol A. Improved plant growth in CWs also facilitates the removal of organic
10 pollutants of organic pollutants removal (Lun, L. Chen, 2018). Tang et al. (2016) used plant-derived biochar that
11 was planted in a *Cyperus alternifolius* constructed wetland CW and then modified with Fe(NO₃)₃ solution to
12 achieve higher removal efficiencies (>99%) and rate-constant rates for four pesticides in wastewater than the
13 non-biochar control (64 - 99%) (Tang et al., 2016). The cause is that biochar adsorbs the pesticides and promotes
14 their microbial decomposition. The use of biochar derived from fruit pits in zeolite-based CWs significantly
15 increased antibiotic removal rates (sulfamethazine and ciprofloxacin) while also decreasing the production of
16 sulfonamide and quinolone resistance genes, which was attributed to the biochar's ability to facilitate antibiotic
17 biodegradation and adsorption (Yuan et al., 2020). Biochar is a good attachment medium for microbes that
18 degrade organic matter. For example, Mahmood et al. (2015) used corn-derived biochar manufactured at 400 °C
19 as a biofilm support for *Pseudomonas putida* cells to adsorb and reduce dyes and Cr (VI) in a continuous flow
20 bioreactor for the efficient treatment of tannery wastewater containing azo dyes, aniline and Cr (VI).

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21 Other organic compounds, such as pharmaceuticals and pesticides, are considered emerging
22 contaminants because of their effects on human health, and have been detected in municipal wastewater
23 treatment plants (Firouzsafari et al., 2019); (Shi et al., 2021). Wastewater from the pharmaceutical industry
24 contains pharmaceutical intermediates used in production (Karunanayake et al., 2017). antibiotics and active
25 ingredients such as hormones (Rashid et al., 2021). However, pesticides are found in industrial wastewater
26 through pesticide production (Baehmann-Pinto et al., 2018). washing of commercial containers used to store or
27 transport pesticides (Zapata et al., 2010). and agri-food industries (Lopes et al., 2020). The biochar as adsorbent
28 promote the degrade antibiotic and antibiotic resistance genes (ARGs) from wastewater, and dissolved organic
29 carbon release in CWs indicated that water and alkaline media portray the optimum conditions for SMX
30 and ARGs removal, this shows the feasibility of using biochar for regulated sulfamethoxazole (SMX), removal
31 and ARG accumulation (Ajibade et al., 2021). However, the study of (Feng et al., (2021b) showed the relation
32 between ARGs removal and dissolved organic matter (DOM). They, noted that the photosensitized DOM is
33 responsible for producing reactive intermediates to remove, ARGs. Hence incorporating biochar under forced
34 aeration into CWs could remove ARGs up to 99.3% and DOM 72% effectively from swine wastewater. (Abas
35 et al., (2022) confirmed that the integration of biochar substrate has an effect in improving Chlorantraniliprole
36 (CAP) removal. CAP mass removal was very high in biochar (99%). The biochar also enhance the efficiency of
37 the treatment pharmaceuticals and personal care products (PPCPs) form wastewater. the presence of the
38 colonization of arbuscular mycorrhizal fungi (AMF) in CWs enhanced the best removal performance for PPCPs

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1 in biochar added systems (more than 99.99%). These results can be attributed to the higher adsorption capacity
2 of PPCPs of biochar, due to its large surface area and porous structures of biochar substrate, which could also
3 promote the development and growth of microbes and the adsorption of PPCPs, thus enhancing its
4 biodegradation (B-Hu et al., 2022; Y-Hu et al., 2022).

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5 Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic compounds (Gaurav et al., 2021),
6 with at least two aromatic rings (Kang et al., 2019). They include compounds such as phenanthrene, naphthalene,
7 anthracene, pyrene, fluorine and benzo(a)fluoranthene (Jain et al., 2020; Kong et al., 2021). Several studies have
8 used biochar as an adsorbent substrate to remove this pollutant, because biochar may provide a reproduction
9 habitat for microbes and enhance the microbial community to improve denitrification and PAHs removal
10 performance (Cao et al., 2021). Furthermore, the biochar was also tested to remove benzo(a)fluoranthene (BbFA), a
11 typical PAH in CWs, and has shown higher BbFA with its removal efficiency exceeding 99%, which could be
12 attributed to enhanced PAH biodegradation (Z-Guo et al., 2020). In the same way (Kang et al., (2023), was
13 studying removal efficiency of representative PAH, benzo(a)fluoranthene (BbFA), using biochar modified by iron
14 as a supplement to the CW substrate. They reached to increase the performance of BbFA removal by 20.4 %,
15 because the biochar may increase dissolved organic carbon content, particularly low-aromaticity, which
16 contributed to PAH degradation by microorganisms. In addition, the presence of functional groups on the
17 biochar surface may improve the electron interactions between microorganisms and PAHs.

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18 4.2.3.4.2. Removal of inorganic pollutants

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19 Inorganic contaminants in wastewater include compounds such as nitrite (NO₂⁻), ammonium (NH₄⁺),
20 nitrate (NO₃⁻), hydrogen sulfide (H₂S), phosphorus (PO₄³⁻) and heavy metals (Cu, Cr, Cd, Pb, Fe, Hg, Zn and As
21 ions) (Table 2) that pose a dangerous risk to human health and the environment (CAO et al., 2009; CAO
22 et al., 2009). Generally, biochar produced at low pyrolysis temperature (about 500°C) is used to remove
23 inorganic contaminants, produced at low pyrolysis temperature (about 500°C). The nature of biochar sorption is
24 influenced by the morphological structure and chemical composition (Abdelhafez and Li, 2016).

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25 4.2.3.4.2.1. Nitrogen removal

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26 Multiple pathways are used to remove nitrogen from wastewater in CW, substrate adsorption, ammonia
27 volatilization, plant uptake and microbial processes. Multiple pathways remove nitrogen from wastewater plant
28 uptake, substrate adsorption, ammonia volatilization, and microbial processes (Saeed and Sun, 2017). Classical
29 microbial nitrification, followed by denitrification, and finally converting N to N₂O or N₂, is considered the most
30 common mechanism (Jia et al., 2020b; Vymazal, 2011). However, the insufficient ability of sand, and gravel to
31 adsorb nitrogen and provide habitable microsites for denitrifying microorganisms remains a major challenge in
32 conventional CW systems filled with gravel, ceramite, or sand (Kizito et al., 2017; Yang et al., 2018), although
33 ceramite gives better results than gravel or sand which are widely used (Vohla et al., 2011). In addition, low
34 dissolved oxygen (DO) due to inadequate reoxygenation may limit nitrification in flooded streams, and/or
35 denitrification can be limited by electron donors deficient for nitrate reduction (Lu et al., 2020; Vymazal, 2011).

1 Therefore, several solutions are being investigated to improve nitrogen removal from wastewater, including
2 introducing substrates with high nitrogen removal capacity (Jia et al., 2020b; Shen et al., 2018).

3 Cation exchange can keep cations in biochars with a high surface charge density. Consequently, the
4 internal porosity, high biochar surface, and presence of polar and non-polar sites on the biochar surface promote
5 nitrifier growth and nutrient adsorption and simpler and easier atmospheric aeration and oxygen replenishment at
6 the bottom of the CW matrix. As well as, the addition of the biochar substrate can increase the rate of
7 nitrification, resulting in a great improvement in total nitrogen (TN) and NH_4^+ removal in CW (Kizito et al.,
8 2017; Rozari et al., 2018; Zhou et al., 2019). However, the leaching of dissolved organic matter (DOM) can be
9 done with the help of biochar, which is mainly based on humic acid, which allows it to temporarily trap the
10 influent DOM in the pores as a carbon source to stimulate denitrification after desorption (Li et al., 2018a; Zhou
11 et al., 2019). Denitrifier proliferation may also be enhanced, resulting in nitrate denitrification for low C/N
12 effluents (Zhou et al., 2019). On the other hand, biochar acts as a chemically redox-active material with
13 electroactive functional groups on its surface (e.g. phenols and quinones), which promotes the biochemical
14 transfer of the material into wastewater (Yuan et al., 2018; Zhang et al., 2019). According to Wu et al. (2018),
15 biochar derived from cattail stalks prepared at 300°C can increase the electron conversion efficiency between the
16 metabolism of carbon and nitrate reduction by modulating the electron shuttle mechanism and increasing the
17 activities of denitrifying enzymes, which can increase the rate of denitrification in wastewater, in contrast,
18 biochar made at 800 °C inhibits these mechanisms. As a result, many studies have reported that biochar addition
19 to domestic, swine, anaerobic, and secondary wastewater effluents improved nitrogen removal efficiency (by
20 more than 20% on average). Removal efficiency increased proportionally with biochar dosage, although the
21 performance improvement depended on biochar loading and preparation conditions, wastewater properties, and
22 wastewater operating conditions. Biochar substrates in settling ponds showed better nitrogen removal than
23 conventional gravel or sand and some functional fillers, such as zeolite and ceramite (Ji et al., 2020; Yuan et al.,
24 2020).

25 4.2.2.3.4.2.2. Phosphorus removal

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26 Phosphorus compounds (P) in wastewater may be eliminated by a variety of processes, including
27 substrate precipitation, adsorption, plant uptake, and microbial uptake into wastewater, with substrate retention
28 generally being the most widely used process (Kumar and Dutta, 2019; Saeed and Sun, 2017). Elements such as
29 Fe, Ca, Mg, and Al in CW fillers can bind phosphorus stably; therefore, materials rich in these elements (Fe, Ca,
30 Al, Mg) are preferable as CW substrates enable phosphorus removal efficiently and also increase the lifetime of
31 CW systems. Conventional CW substrates consisting of sand or gravel can only effectively remove total
32 phosphorus (TP)-P from wastewater for a short time (Chang et al., 2016; Shi et al., 2017). In some studies,
33 biochar-based filters (CWs) were found to have higher phosphorus removal efficiencies than control systems
34 filled with zeolite or gravel. Still, the improved impact for Phosphorus compoundsP removal was much lower
35 than for N removal. The biochar substrates could trap more phosphorus from wastewater than gravel, especially
36 from wastewater with a high phosphorus concentration (e.g., anaerobic digestion effluent) (Kizito et al., 2017).
37 In addition, the incorporation of biochar into CWs can enhance plant growth and the proliferation of Phosphorus

1 ~~compounds~~ ~~P~~-accumulating microorganisms (PAOs), thereby improving biotic ~~Phosphorus~~ ~~P~~-removal pathways
2 (Ji et al., 2020; Shi et al., 2017). However, this ameliorative effect cannot be easily maintained. The chemical
3 properties of biochar and wastewater, especially the biochar's surface charge, are important factors in removing
4 anionic phosphates (Wichern et al., 2018). However, other studies have shown that adding biochar to gravel-
5 filled CW did not improve phosphorus removal (Zhou et al., 2019). Mixed biochar and sand substrates are even
6 less efficient than sand alone in phosphorus removal (Rozari et al., 2016). These results can be explained
7 because biochar has a negative surface charge and a low affinity for phosphate. Other negatively charged
8 molecules in the wastewater (organic matter) can compete with phosphate for exchange sites in biochar (Rozari
9 et al., 2016). Biochar substrates made from ~~/Fe/Al/Ca~~-rich feedstocks, such as crab shells, can improve P's
10 recovery/removal capacity from wastewater (Dai et al., 2017). Biochar can be modified with metal salts (iron,
11 magnesium, and aluminum compounds) to make metallic biochar before filling (Wang., 2019; Zheng et al.,
12 2019), or combined with other fillers with high ~~Phosphorus compounds~~ ~~P~~-adsorption efficiency (crab shells) to
13 prepare biochar (Shi et al., 2017; Yang et al., 2018). There is still a need for further research and relevant
14 applications in phosphorus removal using biochar substrates.

15 ~~4.2.3.3.4.2.3.~~ ~~Metals~~ ~~Removal of metals~~

16 Heavy metals are generally non-biodegradable and are found in large quantities in rainwater, mining
17 effluents, and ~~some~~ industrial wastes. Biochar with a unique pore structure, a high percentage of organic carbon,
18 and many functional groups have a high chance of interacting with heavy metals in several ways (Oliveira et al.,
19 2017). Heavy metals are absorbed by biochar mainly through complexation and ion exchange between heavy
20 metal ions and functional groups of biochar (e.g., COOH, OH, R-OH) (Hsu et al., 2009; Lu et al., 2011).
21 Additionally, the coordination of metal ions with π -electrons (C=C) of biochar (Yu et al., 2010) and the
22 formation of metal precipitates with inorganic constituents (Ippolito et al., 2012; Lu et al., 2011) ~~could play a~~
23 ~~role in the P removal by biochar~~. Adsorption through the biochar matrix is affected by its chemical properties,
24 which are affected by feedstock type, pyrolysis temperature, application rate, pH, and other factors. For example,
25 copper (Cu²⁺) had a high affinity for OH- and COOH- groups in hardwood and crop biochars, which varied with
26 pH and feedstock type (Lima et al., 2010). Similarly, biochars derived from soybean straw, guayule shrub,
27 hermaphrodite sida, and wheat straw effectively removed Ni²⁺, Cu²⁺, Zn²⁺, and Cd²⁺ (Lu et al., 2017). The higher
28 biochar efficiency was attributed to the high O and C contents, polarity index and high O/C molar ratio, which
29 were regulated mainly by pH (Bogusz et al., 2015; Peng et al., 2016). In addition, the removal of mercury (Hg²⁺)
30 was effectively performed using alkaline biochar prepared from both manure and various agricultural residues
31 (corn stover, soybean straw, cocoa husks, switchgrass, and corn stover). Due to its high sulfur content (SH and
32 sulfate groups), biochar produced from cocoa hulls and animal manure was particularly effective in removing
33 Hg²⁺, precipitating up to 90% of the Hg²⁺ as HgCl₂ or Hg(OH)₂, mainly by co-precipitation with the anions (O,
34 S, Cl) in the biochar (Baltreinaite, 2015; Mohamed et al., 2016). Similarly, the biochar dosage affected the
35 removal of heavy metals such as Cd²⁺, Zn²⁺, Pb²⁺ and Cu²⁺. Thus, the removal efficiency was higher with rising
36 biochar loading in the aqueous system, due to the increase in surface area and pH (Laird et al., 2010; Xu et al.,
37 2013).

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1 Dissolved heavy metals in wastewater, such as hydroxides and sulfides, can be removed mainly by
2 precipitation, adsorption from the abiotic substrate, and microbial reduction of sulfates for hydroxides and
3 sulfides precipitation (Kosolapov et al., 2004). Adding biochar can help gravel ponds improve metal holding
4 capacity by increasing abiotic pathways. Under ideal conditions, a study was conducted in a gravel-filled pond to
5 remove just 58% Mn and 51.6% Pb from synthetic industrial wastewater. In comparison, adding biochar and
6 zeolite increased the removal efficiency of both metals up to 99.9%. These results can be explained because both
7 metals have high adsorption capacities toward biochar and zeolite (Abedi and Mojiri, 2019). In addition, the
8 inorganic components of the biochar impart an alkaline nature to the biochar, allowing it to raise the pH value of
9 acidic mine wastewater and subsequently reduce the metal ions solubility by inducing the formation of metal
10 hydroxide precipitates (Gwenzi et al., 2017). Biochar substrates can be modified before amendment with
11 heteroatoms and oxidizing agents, acids, or anionic moieties (e.g., HSO₃, OH, S₂, etc.) to enhance the metal
12 retention capacity of CWs (Wang et al., 2019).

13 **4.3.3.4.2.4. Pathogens Removal of pathogens**

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14 The removal of pathogens from wastewater is essential for protecting human health. Removal was
15 accomplished by filtration, predation, adsorption, oxidation, and inactivation by exposure-several regulatory
16 standards for pathogens in wastewater effluent for reuse (Wu et al., 2016). The high porosity of biochar, high
17 specific surface area, numerous pores with a wide range of sizes, hydrophobicity and organic leaching may make
18 biochar more suitable for removing microbial contaminants than gravel or sand. However, there has been
19 relatively little research on removing pathogens from wastewater using biochar-enhanced CWs. According to
20 Mohanty et al. (2014) and Lau et al. (2017), the introduction of biochar into sand-based biofilters (FBs)
21 significantly increased the presence of *Escherichia coli* in stormwater. In addition, it decreased the
22 remobilization of sequestered nuisance bacteria during intermittent influx and highlighted the high potential of
23 using biochar substrate in CWs for wastewater disinfection. Furthermore, biochar with volatile content and
24 polarity had a higher removal efficiency for *E. coli* (Mohanty et al., 2014). This improvement effect may be
25 explained by the fact that biochar can produce antimicrobials that significantly adsorb viruses and bacteria
26 mainly using hydrophobic interactions and reduce the driving forces that detach pathogens.

27 On the other hand, another recent study by Kaetzl et al. (2019) found that CWs filled with rice husk-
28 derived biochar can remove bacteriophages and fecal indicator bacteria (FIB) from pretreated municipal
29 wastewater much better or as much as CWs filled with sand or original rice husk (Kaetzl et al., 2019). The ability
30 of biochar to remove pathogens varies with preparation conditions and feedstock (Mohanty et al., 2014).
31 Modifying biochar with H₂SO₄ increases the surface area of biochar prepared from wood, reflecting a significant
32 improvement in *E. coli* elimination in bioretention systems and reducing remobilization during drainage and
33 intermittent flow (Lau et al., 2017). Even though biochar-based filters show high FIB removal efficiency
34 comparable to sand-based filters (Wichern et al., 2018), biochar remains an attractive feedstock in CW systems
35 for pathogen removal due to its economic production and performance, using locally available biological waste,
36 and can be reused as a soil amendment.

5-4. Mechanisms and factors influencing the pollutants adsorption on biochar

Pollutant adsorption mechanisms on biochar

The heterogeneity of the biochar surface allows a variety of sorption processes to occur. The chemical characteristics of the adsorbent surface and the nature of the contaminants determine the adsorption mechanism (Rosales et al., 2017). The three main adsorption mechanisms, according to Pignatello (Pignatello., 2011), are the precipitation mechanism, in which the adsorbent forms layers on the adsorbent surface, and the physical mechanism, in which the adsorbate (e.g., pollutants) is deposited on the adsorbent surface (e.g., biochar), and the pore-filling mechanism, in which the adsorbate (e.g., pollutants) condenses in the adsorbent pores (e.g., biochar). The adsorption process of organic pollutants is generally carried out by electrostatic attraction, complex adsorption, electron-acceptor- donor interaction, pore filling, hydrophobic interactions and hydrogen bonding (see Fig. 4) (Pignatello., 2011). For example, the sorption of organic contaminants by the biochar surface via the pore filling process is influenced by the total volume of the mesopores and micropores; so that the penetration of the pollutant into the internal structure of the biochar is all the more favored when its ionic radius is small, which reflects an increase in the biochar adsorption efficiency (Ahmad et al., 2014; Rosales et al., 2017). Soluble pollutants may attach to the alkaline surface of the hydrophobic biochar using their hydrophobic functional group or be precipitated. Due to the dissociation of oxygen-containing functional groups on the biochar surface, the biochar is generally negatively charged, causing an electrostatic attraction between the positively charged molecules and biochar (Ahmad et al., 2014; Qambrani et al., 2017).

The biochar produced at high temperatures lost its functional group-containing hydrogen and oxygen, making it more aromatic and less polar and, consequently, less suitable for removing polar organic pollutants. However, the electrostatic repulsion between the biochar and the negatively charged anionic organic molecules could favor the production of hydrogen bonds, leading to adsorption. On the other hand, if there is no hydrogen interaction, non-polar pollutants are more likely to penetrate hydrophobic areas (Ahmad et al., 2014). On the other hand, many mechanisms can be involved in removing inorganic pollutants such as heavy metals, such as ion exchange and complexation, surface precipitation under alkaline circumstances, and anionic and cationic electrostatic attraction (Fig. 4). Similarly, Lu et al. (2011) examined the relative contributions of different Pb adsorption mechanisms on sludge-derived biochar. They arrived at the following mechanisms: (i) co-precipitation and complexation with mineral oxides and organic matter in the biochar, (ii) electrostatic complexation due to the exchange of the metal with cations (sodium and potassium) present in the biochar, (iii) surface precipitation as lead silicate- phosphate ($5\text{PbO}\cdot\text{P}_2\text{O}_5\cdot\text{SiO}_2$), and (iv) surface complexation with free carboxyl^{-s} and mineral oxides in the biochar.

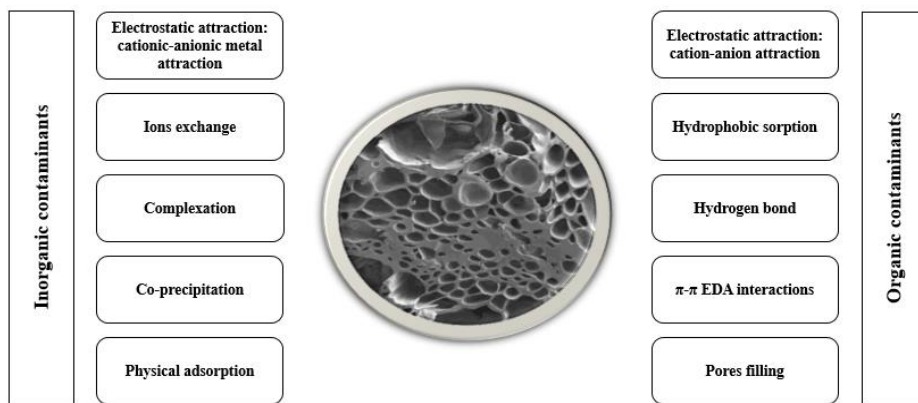


Fig. 2: Mechanisms for biochar's elimination of organic and inorganic contaminants.

The variation in these removal mechanisms and the physicochemical properties of biochar greatly implicates its suitability and efficacy for the remediation of the targeted pollutants. Several factors such as biochar characteristics, dosage of biochar, solution pH and temperature of the medium greatly influence the biochar's overall adsorption capacity by modifying the removal mechanisms involved in the remediation of specific pollutants aqueous systems (Abbas et al., 2018; Ambaye et al., 2021).

6. Factors influencing the pollutants adsorption on biochar:

6.1.4.1. Characteristics of biochar

The volume of micropores in an adsorbent controls its ability to absorb an adsorbate (Lowell, 2004; Zabaniotou et al., 2008). Pores of different sizes are found in adsorbent materials, and classified into macropores, micropores, and mesopores based on the width of the opening (Mosher, 2011). The experimental conditions strongly influence the distribution and size of the pores during the preparation of the biochar, and especially the pyrolysis temperature has the greatest influence (Zhou et al., 2010). The micropores are the most abundant in the biochar structure and would be responsible for their high adsorption capacity and surface area. Zabaniotou et al. (2008) reported that biochar prepared at a high pyrolysis temperature contains a very high volume of micropores that varies between 50%-78% of the total pores. The sorption rate of the biochar is controlled by the size of the adsorbate, such that larger particles can cause blockage or exclusion of sorption sites. In comparison, smaller particles increase the van der Waal force of penetration of the adsorbate into the adsorbent and decrease the mass transfer limitation (Daifullah and Girgis, 1998). It also depends on the surface functional groups' levels and types (Qambrani et al., 2017). The carbonization process, the feedstock's chemical composition, and the carbonization temperature all influence the distribution of surface functional groups (Ahmad et al., 2012). Gascó et al. (2018) compared the properties of hydrochar and biochar produced from pig manure using HTC and pyrolysis.

1 The results showed that when the pyrolysis temperature is high, the broad peak around 3400 cm⁻¹,
2 corresponds to the -OH stretching vibration in the hydroxyl and carboxyl groups and becomes less visible for
3 biochars compared to the feedstock. Due to the decarboxylation and dehydration reactions during the HTC
4 process, the HTC hydrochars revealed broadband at 3400 cm⁻¹ with less intensity than the feedstock. Several
5 scientists agreed that a high aromatic structure characterizes biochar prepared at a high temperature of around
6 600 °C. On the other hand, hydrochar prepared using the HTC method at a temperature between 200 and 240 °C
7 for 2 h favors biochar with more aliphatic structures. According to Qambrani et al. (2017), the functional groups
8 (-CH₂, O-H, C=O, C=C and -CH₃) of biochar have changed due to the pyrolytic conditions, which promote the
9 hydrophobic interactions of biochar. The hydrophobic character of biochar is determined by the amount of
10 oxygen and nitrogen-containing functional groups; the lower the nitrogen and oxygen-containing functional
11 groups in the biochar, the higher hydrophobic the biochar (Moreno-castilla, 2004). Hence, the presence of
12 oxygen-containing functional groups on the hydrophilic biochar surface facilitates water to penetrate through
13 hydrogen bonds, resulting in competition between the adsorbate and water on the available sites of the biochar
14 surface. Hydrophobic biochars are expected to contribute to insoluble adsorbate adsorption, while hydrophilic
15 biochars are considered less effective due to water sorption. Adsorbates that are less soluble or insoluble are
16 most likely to be absorbed into the biochar pores in aqueous solutions (Li et al., 2002).

17 6.2.4.2. Dosage of the adsorbent

18 The adsorbent dosage significantly impacts the sorbent-sorbate balance of an adsorption system. Hence,
19 using ~~of~~ a high adsorbent dosage increases the removal efficiency of inorganic and organic contaminants due to
20 the availability of a larger number of sorption sites (Chen, 2013; Chen et al., 2011). On the other hand, the
21 application of a dosage rate that is too high leads to a reduction of the adsorption capacity of the biochar and
22 consequently, an overlapping of the adsorption layers will be produced, which protects the accessible active sites
23 on the sorbent surface (Kizito et al., 2015; Linville et al., 2017). Therefore, the adsorbent dosing must be well
24 optimized to achieve high elimination capacity and make the process cost-effective.

25 6.3.4.3. pH of the solution

26 The pH of the solution is a crucial factor that controls the adsorption process by influencing the
27 ionization degree and charge of the adsorbate, the adsorbent surface charge and the speciation (Kılıc et al.,
28 2013). The competition between protons and cationic pollutants decreases as the pH of the solution is above the
29 point of zero charges, and a negative charge appears on the adsorbent surface as a result of the deprotonation of
30 carboxylic groups and phenolic on the surface. Basic functional groups, such as amines, are protonated and
31 positively charged at low pH.~~At low pH, basic functional groups, such as amines, are protonated and positively~~
32 ~~charged,~~ improving anions' adsorption (Kumar et al., 2011). This means that deprotonation of the functional
33 groups and the pH of the medium influences the biochar adsorption behavior~~the biochar adsorption behavior is~~
34 ~~influenced by the deprotonation of the functional groups and the pH of the medium.~~ Kizito et al. (2015) and Hu
35 et al. (2019) studied the effect of pH on the adsorption capacity of biochar towards ammonium (NH₄⁺). They

1 showed that the adsorption capacity of NH_4^+ increased with the initial solution pH between 4 and 8 and then
2 decreased when the pH was above 9.

3 **6.4.4.4. Temperature of the medium**

4 The medium temperature in which the biochar is applied impacts its adsorption capacity. Most studies
5 showed that adsorption efficiency increased with temperature, confirming that the adsorption process is
6 endothermic. The study by Enaime et al. (2017) indicated that the indigo carmine sorption on potassium
7 hydroxide (KOH) activated biochar rises with temperature due to the endothermic nature of the sorption process.
8 The increase in temperature leads to an increase in the mobility of the dye molecule and the possibility of an
9 increase in the adsorbent porosity. This can be explained by the swelling effect of the adsorbent internal structure
10 when the temperature increases, allowing more dye to penetrate further. Another study, Kizito et al. (2015)
11 found that increasing the temperature above 300 °C to 450 °C is beneficial for maximum removal efficiency.

12 **7.5. Advantages and limitations of biochar as a CW substrate**

13 The use of biochar as a substrate in CWs solves the problem of environmental pollution (Table 34).
14 Due to ~~their~~ low-cost, availability of the raw materials, and the high commercial potential of biochar, the
15 preparation of biochar has ~~been~~ developed rapidly in recent years (Lili et al., 2017). Due to its adsorption
16 capacity and porous structure, biochar is commonly used as a slow-release fertilizer filler (Xu and Lu, 2019).
17 However, biochar is rarely used in water treatment due to its high cost, high ash content, and difficulty in ash
18 removal (Kasak et al., 2018). Theoretically, biochar may considerably enhance the purification of wastewater
19 (Deng et al., 2019), as an additional carbon source for CWs (Kasak et al., 2018), and their surface allows the
20 adsorption of various pollutants.

21 Furthermore, biochar may improve the activity of the microorganisms in CWs (Tang et al., 2017).
22 Therefore, biochar could improve the degradation of high molecular weight compounds ~~metabolized to~~ low
23 molecular weight compounds in CW (Deng et al., 2019). The biochar's main objective is to increase the
24 adsorption efficiency of the substrate and provide the carbon source to enhance the denitrification
25 efficiency. However, the application of the CW substrate is easy to generate a blockage due to the low
26 structural strength of the biochar and the ease of generating a powder (Saeed et al., 2019).

27

28 *Table 43: Limitations and advantages of biochar as a CW substrate.*

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Advantages

Reference

Disadvantages

Reference

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- Sustainable and abundant resources, cheap and more oxygen groups present in biochar improves pollutants adsorption.	(Houben et al., 2013)	- Elimination pollutants efficiency is undetermined and heavy metals retain in soil.	(Houben et al., 2013)
- Effective medium for capturing pollutants from wastewater which can connect to the soil and result in an alteration -Reduce greenhouse gas emissions	(Yaashikaa et al., 2020)	- <u>High cost, high ash content, and difficulty in ash removal</u> - Raise weed growth by 200% during lentil culture after using of biochar at a rate of 15 t/ha.	(Kasak et al., 2018)(Khorram et al., 2018)
- Improve the activity of microorganisms in CWs	(Tang et al., 2017)	- <u>Easy to generate a blockage and the ease of generating a powder</u> - High cost, high ash content, and difficulty in ash removal	(Saeed et al., 2019)(Kasak et al., 2018)
- Provide reactive sites for microbes	(Li et al., 2019)	- <u>Easy to generate a blockage and the ease of generating a powder</u>	(Saeed et al., 2019)
- Adsorb NO ₃ -N, NH ₄ ⁺ and PO ₄ ³⁻ - Remove suspended solids, BOD ₅ , metals and coliforms	(Gao et al., 2018)	<u>Substance release (e.g. N, P, salt, alkaline)</u>	(L. L. Zhuang et al., 2022)
- Improve the retention of water	(Ahmad et al., 2014)		

1

2 **8.6. Conclusion and perspectives**

3 The present review highlighted the constructed wetlands (CWs) a natural system that are largely
4 investigated for different kind of wastewater (urban, industrial, mixture) treatment throw physical (porosity of
5 substrate), chemical (adsorption, precipitation and biological processes (biodegradation, nitrification
6 denitrifications), under vertical or horizontal flow regime. The constructed wetland has proven good
7 performances for the elimination of organic matter (99 %), nutrients especially phosphates (88 %) and nitrogen
8 (96 %). However, constructed wetlands still very limited on removing recalcitrant or emergent pollutant such as
9 heavy metals, pesticides, drugs, PAHs, volatile organic compounds (VOCs) etc., According to previous
10 literature, removal capacity of CW depends on the type of macro-phytic plant and the substrate of the bed.
11 According to the analyzed references, different plants can be used in CW. Nevertheless, phragmites australis and
12 Around donax have been the most applied that are considered as the most resistant or high organic load and
13 present the capacity to oxygenate the substrate and enhance the hydraulic conductivity in the filter. The substrate
14 plays also an important role in constructed wetland depuration efficiency that could reach NH₄⁺-N (40.23%),
15 NO₃-N (48.94 %), TN (52%), and COD (35%) when sand or gravel substrate are used. Any improvement of
16 the CW efficiency must be performed via the integration of a good substrate in the filter. Among several

1 materials generally tested as substrate for CW such as zeolite, pozzolan, charcoal, and biochar is gaining big
2 interest recently, due to its promising characteristics as an optimal adsorbent having the ability to remove not
3 only conventional pollutants but owing to good removal performances for even emergent ones that are very toxic
4 and recalcitrant. Furthermore, biochar could bring carbon to the substrate and have a great impact on the
5 pollutants biodegradation by giving a good niche of functional group of microorganisms. The removal
6 percentage could reach COD (99 %), TP (88 %), NH₄⁺ (96 %), Abamectin (99 %), TSS (71 %), Total coliforms
7 (70 %), TN (40 %), and ARGs (99 %).

8 Theses interesting characteristics of the biochar are obviously dependent on the processes used to
9 prepare the material, and the conditions of the preparation including conditions of thermal conversion and the
10 kind of feedstock used. Based on the literature review, it was found that the optimum pyrolysis temperature must
11 be around 400 and 600 °C, with a possibility to have an oriented prepared biochar depending of the targeted
12 pollutants basing on the temperature. Furthermore, feedstock must have some specific characteristics to give a
13 good quality of the biochar that depends of the feedstock richness in carbon (c) and low quantity of mineral
14 matter. The large pore volume and high specific surface area reaching 200 m²/g, thus allowing to effectively
15 remove pollutants and pathogens from wastewater. The biochar quality is affected by the conditions involved in
16 preparing biochars (e.g., pyrolysis temperature, heating rate and carbonization time).

17 Several factors alter the removal efficiency of pollutants in CWs, such as substrate chemical and
18 physical properties, hydraulic retention time, the oxygenation conditions, and redox conditions. In addition,
19 configuration where the biochar is implemented as interlayer between two inert layers (sand, gravel, zeolite) has
20 been reported as optimal design for CW integrating biochar to avoid clogging of the filtration system or biochar
21 flotation.

22 Overall, the use of biochar in horizontal flow CW is still limited, and a few papers discussed this aspect.
23 Similarly, there is only limited information on the removal of emerging organics, and pathogens from
24 wastewaters by biochar CWs, that mean the involved mechanisms and potential capability of biochar CWs in the
25 removal of these pollutants should be further explored and elucidated. Moreover, it is undeniable that biochar
26 offers various economic and environmental benefits and advantages, and its effectiveness in removing various
27 contaminants at the laboratory scale has been widely reported. However, more in situ experiments should be
28 conducted to test the effectiveness of biochar using real effluents and to examine the actual effect of biochar on
29 the environment before its large-scale application. Furthermore, the biochar stability after many use cycles and
30 its regeneration should be further studied. The economic and environmental advantages of biochar preparation,
31 combined with the improved physicochemical properties of the material, make its application more feasible for
32 wastewater treatment. Although, in addition, biochar improves the removal of various pollutants from
33 wastewater in CWs, this improvement effect is dependent on several parameters. Therefore, this study
34 systematically presents an overview of the different raw materials and conditions used for the production of
35 biochar constituting the CWs substrate, its characteristics, the role of macrophytes and categories of plants used,
36 the location of biochar in the substrate, its dimensions, and the effectiveness of biochar in removing various
37 pollutants from wastewater. Overall, it is undeniable that biochar offers various economic and environmental

1 ~~benefits and advantages, and its effectiveness in removing various contaminants at the laboratory scale has been~~
2 ~~widely reported. However, more in situ experiments should be conducted to test the effectiveness of biochar~~
3 ~~using real effluents and to examine the actual effect of biochar on the environment before its large scale~~
4 ~~application. Furthermore, the biochar stability after many use cycles and its regeneration should be further~~
5 ~~studied.~~

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7
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