

Regeneration of copper-loaded pine bark biochar using simultaneous bio-sulfide precipitation of copper

Misbah Bashir, Chander Mohan and Ajit P. Annachhatre *

Indian Institute of Technology, Mandi, Himachal Pradesh, 175005, India

*Corresponding author. E-mail: ajit@iitmandi.ac.in

 APA, 0000-0001-6212-4154

ABSTRACT

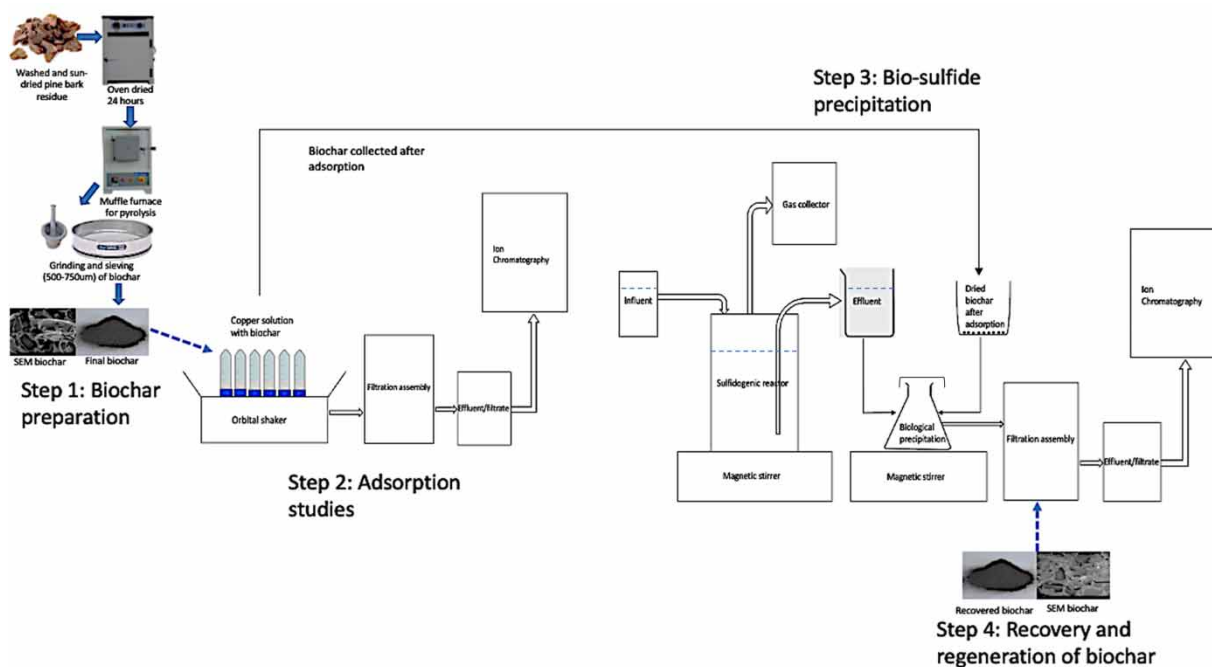
This research investigated adsorption of copper from aqueous solution onto the pine bark biochar, removal of adsorbed copper by bio-sulfide precipitation, and simultaneous regeneration of pine bark biochar adsorbent. A sulfidogenic reactor was established and operated under anaerobic conditions. During the sulfidogenic phase, COD:SO₄²⁻ was gradually increased from 24:1 to 4:1. Use of sulfide-rich effluent from bio-sulfide reactor at neutral pH yielded above 99% copper removal from the aqueous solution. In the experiment's second stage, pine bark biochar was prepared through slow pyrolysis at 650 °C from pine bark residue that had a carbon content of 81% and a surface area of 368 m²/g. This biochar was then used in subsequent experiments. Initially, copper was adsorbed onto the biochar under neutral pH at contact time of 6 h. Maximum biochar adsorption capacity of 106 mg/g of copper was obtained. Finally, biochar was regenerated by precipitating the adsorbed copper as copper sulfide using sulfide-rich effluent from the sulfidogenic reactor. Complete recovery of adsorbed copper from biochar as copper sulfide precipitates were obtained was also confirmed by EDX-SEM analysis of biochar and precipitates. Regenerated biochar could be reused as an adsorbent in the subsequent adsorption cycle.

Key words: adsorption, biochar regeneration, bio-sulfide precipitation, continuously stirred tank reactor, copper-loaded biochar, loading rate

HIGHLIGHTS

- Pine bark biochar produced through slow pyrolysis at 650 °C successfully adsorbed copper from an aqueous solution.
- Biochar adsorption capacity of 106 mg/g of copper was obtained.
- Simultaneous removal of adsorbed copper and regeneration of biochar were accomplished through bio-sulfide precipitation.
- The regenerated biochar could be reused in the successive adsorption cycle.

GRAPHICAL ABSTRACT



INTRODUCTION

Urban as well as industrial activities have contributed significantly to environmental pollution and need to be addressed on an urgent basis due to their serious environmental impacts (Deniz 2017; Sulyman *et al.* 2017). Several industrial processes discharge effluents containing heavy metals that are hazardous in nature, causing severe health effects (Bashir *et al.* 2020). Several treatment processes are used for the removal of heavy metals from industrial effluents such as ion exchange, oxidation, reduction, reverse osmosis, electrodialysis, and ultrafiltration (Qasem *et al.* 2021). Table 1 presents the advantages and disadvantages of various heavy metal removal technologies.

Chemical precipitation is a widely used technology employed for the treatment of heavy metal removal from wastewater due to its simple operation. In this process, heavy metal contaminants are separated from an aqueous solution by simple hydroxide or sulfide precipitation followed by sedimentation and filtration (US EPA, 2000). Figure 1 shows the heavy metal solubility as a function of pH with respect to metal hydroxide as well as metal sulfide (EPA 625/8-80-003; Prokkola *et al.* 2020). Metal hydroxide precipitation is a simple and economical process that can be achieved by calcium or sodium hydroxide (Saravanan *et al.* 2021). But this process generates a lot of low-density sludge which is difficult to settle and dewater. On the contrary, using metal sulfide precipitation with the help of sodium sulfide or calcium sulfide provides an economical and easy method due to its several advantages such as the smaller quantity of sludge generation, wide pH range for precipitation, and a higher degree of metal removal due to lower dissolved heavy metal concentration in the treated effluent (Zainuddin *et al.* 2019).

Recent studies have shown that using metal sulfide precipitation can also be achieved biologically (biological sulfide precipitation) using sulfate-reducing bacteria (SRB) by hydrogenotrophic sulfate reduction and acetotrophic sulfate reduction as shown in Equations (1) and (2). Biological sulfide precipitation of heavy metal (Equation (3)) is a widely used technique in which sulfide-rich effluent from a sulfidogenic reactor is used for metal sulfide precipitation (Wang *et al.* 2012; Tyagi *et al.* 2020; Estay *et al.* 2021) (Glombitza *et al.* 2015) (Ozuolmez *et al.* 2015).



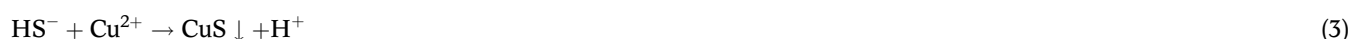
$$\Delta G = -48.1 \text{ kJ (mol acetate)}^{-1}$$



Table 1 | Advantages and disadvantages of heavy metal removal technologies

Heavy metal removal technology	Advantages	Disadvantages	References
Chemical precipitation	<ul style="list-style-type: none"> - Simple operation - Economical - Highly efficient 	<ul style="list-style-type: none"> - High sludge generation 	Matlock <i>et al.</i> (2002)
Ion exchange	<ul style="list-style-type: none"> - Economical - Wide range of heavy metals can be removed 	<ul style="list-style-type: none"> - Disposal of the adsorbent - Highly pH sensitive 	Ariffin <i>et al.</i> (2017); Barakat (2011)
Oxidation	<ul style="list-style-type: none"> - Rapid and efficient process for heavy metal removal 	<ul style="list-style-type: none"> - Not economical due to high energy cost - Unnecessary by products formation 	Ariffin <i>et al.</i> (2017)
Reduction	<ul style="list-style-type: none"> - Easily controlled for treating metal wastewater 	<ul style="list-style-type: none"> - Not economical 	Chen <i>et al.</i> (2013)
Reverse osmosis	<ul style="list-style-type: none"> - Promising in removing heavy metal from wastewater 	<ul style="list-style-type: none"> - High maintenance - High quality feed is required 	Ipek (2005)
Electrodialysis	<ul style="list-style-type: none"> - Possibility of metal recovery - Efficient in metal removal especially in acidic medium 	<ul style="list-style-type: none"> - High maintenance - Waste generation - Precipitation in the setup 	Juve <i>et al.</i> (2022)
Ultrafiltration	<ul style="list-style-type: none"> - Hybridization of ultrafiltration with other removal techniques provides highly efficient treatment 	<ul style="list-style-type: none"> - Efficiency for heavy metal removal is low - Not economical 	Yaqub & Lee (2018)

$$\Delta G = -262.06 \text{ kJ (mol hydrogen)}^{-1}$$



where ΔG = Gibbs Free Energy.

Pinus roxburghii is an evergreen deciduous plant found in the Himalayan region which produces abundant forest residue such as pine bark, needle, and cone (Tyagi *et al.* 2022). The pine forest residue accumulates on the forest soil which leads to several adverse environmental impacts such as inhibition of groundwater recharge and forest fires which leads to deterioration in air quality (Bashir *et al.* 2022). Biochar can be produced from pine forest residues through slow pyrolysis at a temperature of 400–650 °C in the absence or limited supply of oxygen (Bashir *et al.* 2022). The properties of biochar and other pyrolysis products are presented in Table 2. Biochar is reported to have superior adsorption properties mainly due to its large surface area, charged surface, and the existence of several functional groups (Ali *et al.* 2021; Bashir *et al.* 2022). Researchers have reported biochar having a microporous structure with a pore size/diameter ranging from 0.004 to 150 μm . Moreover, biochar having a surface area of up to 600 m^2/g has also been reported (Leng *et al.* 2021). As a result, biochar has been successfully used as an adsorbent for the removal of heavy metals such as copper, lead, and arsenic from the aqueous phase (Bashir *et al.* 2022). It has been reported that pine bark biochar produced under those conditions of pyrolysis has a copper adsorption capacity of 60 mg/g which is significantly higher than the copper adsorption capacity of pine bark residue, which is 3.6 mg/g (Bashir *et al.* 2022; Tyagi *et al.* 2022).

It has been found that chemical modification of biochar using ammonium sulfate, hydrochloric acid, and nitric acid improves its adsorption capacity mainly due to the addition of certain functional groups like carboxylic, carbonyl, lactonic, and phenolic groups (Chen *et al.* 2019). Chemical modification of the biochar also enhances the surface area which helps in increasing the adsorption capacity of the biochar (Wang *et al.* 2020). Hailegnaw *et al.* (2021) have reported a superior effect

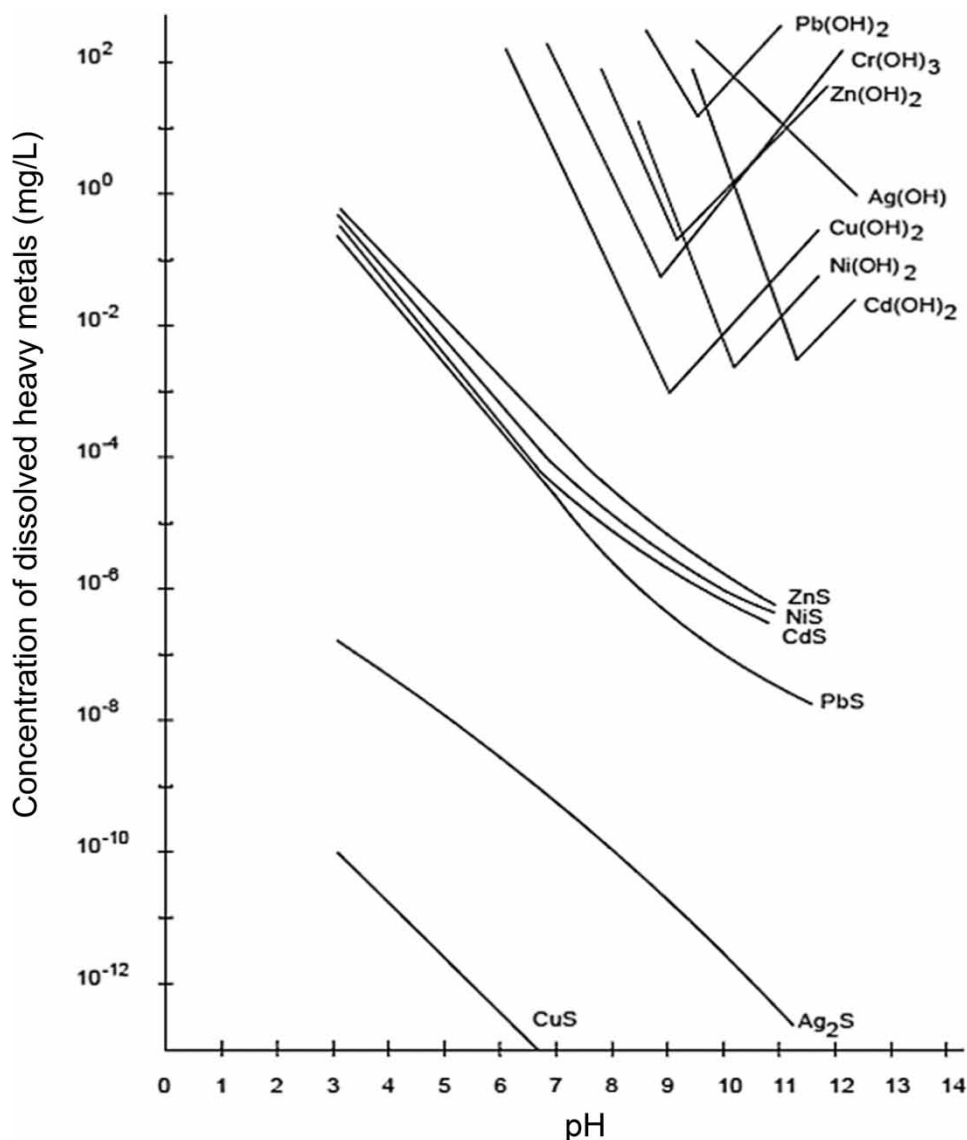


Figure 1 | Metal hydroxide and metal sulfide solubility vs. pH (Prokkola *et al.* 2020).

for the uptake of phosphorus and potassium by ammonium sulfate-modified biochar. Many researchers have also shown that biochar modified by HCl, HNO₃, H₂SO₄, and CaCO₃ improved heavy metal adsorption capacity as well as removal efficiency (Li *et al.* 2016; Wu *et al.* 2018; Sonu *et al.* 2020; Wang *et al.* 2020).

The standard practice of biochar regeneration is by desorption using acidic desorbing agents such as 0.1 M HCl, 0.1 M H₂SO₄, or 0.1 M HNO₃ (Liu *et al.* 2020; Bashir *et al.* 2022). Desorbed heavy metal is then removed from the aqueous solution through hydroxide precipitation. As a result, two separate processes in series are employed, namely, desorption followed by precipitation for the complete removal of adsorbed heavy metal. However, in this research, the feasibility study is conducted for simultaneous desorption and sulfide precipitation of an adsorbed heavy metal from pine bark biochar. Removal of adsorbed heavy metal is accomplished in a single-step process through desorption followed by simultaneous heavy metal sulfide precipitation. Sulfide-rich effluent from the biological sulfate reduction process is used as a desorbing agent for the regeneration of biochar as well as for simultaneous sulfide precipitation of copper in a single-step process. The regenerated pine bark biochar can be used in the next cycle for the adoption of heavy metal.

Table 2 | Properties of biochar, syn-gas, and bio-oil at 600–700 °C

Properties		Biochar	Bio-oil	Syn-gas	Reference
Elemental composition (%)	C	80–82	Lignocellulosic biomass under slow pyrolysis	NA	Mohan <i>et al.</i> (2006); Bashir <i>et al.</i> (2022); Imam & Capareda (2012)
	H	2–2.4			
	N	1.2–1.3			
	O	14.1–18.8			
Gaseous composition (%)	CO	NA	NA	30–60	Ciferno & Marano (2002)
	CO ₂			5–15	
	CH ₄			0–5	
	H ₂			25–30	
Surface characteristics	Surface area (m ² /g)	36–600	Lignocellulosic biomass under slow pyrolysis	NA	Bashir <i>et al.</i> (2022); Jia <i>et al.</i> (2018)
	Pore size (nm)	0.004–150			
	Pore Volume (cm ³ /g)	0.009–1.66			
Physical properties	Colour	Black	Brown, dark red, or black	NA	Brewer <i>et al.</i> (2009); Xu <i>et al.</i> (2011); Yargicoglu <i>et al.</i> (2015); Banks & Bridgwater (2016); Bashir <i>et al.</i> (2022); Zhang <i>et al.</i> (2007); Gupta & Demirbas (2010); Ghenai (2010)
	Viscosity (cP)	NA	40–100	10	
	Density (kg/m ³)	1,240–3,380	1.1–1.3	0.95	
	Specific gravity	0.59–1.65	1.2	NR	
	pH	7.3–9.13	2–3	NA	
	Heating value (MJ/kg)		15–22	10.4–27.8	
Yield (%)		25–30	37–40	26–30	Imam & Capareda (2012); Bashir <i>et al.</i> (2022); Moreira <i>et al.</i> (2017)
Applications		<ul style="list-style-type: none"> – Organic or inorganic contaminant removal from aqueous solution – Soil conditioner – Carbon capture and storage – Solid fuels 	<ul style="list-style-type: none"> – Fuels in boilers, engines and turbines for heat and power generation – By upgrading bio-oil, it can be converted to transportation biofuels 	<ul style="list-style-type: none"> – Used for the production of other liquid fuels like methanol and diesel fuel via catalytic synthesis – Clean alternative to fossil fuels in generating electricity – Wide range of chemical production 	Bashir <i>et al.</i> (2022); Speight (2019); Pattiya (2018)

METHODOLOGY

Seed sludge

Cow dung and digested sludge from the sewage treatment plant of IIT Mandi were used for seeding the continuously stirred tank reactor (CSTR) (Malik *et al.* 2020).

CSTR operation

Figure 2 presents the schematic of the CSTR employed in this research (Chander *et al.* 2020). A CSTR reactor having 5 L of volume (effective volume 4.75 L) was used for the continuous operation. The reactor's content was continuously mixed using a magnetic stirrer. The gas from the methanogenic reactor was collected using the liquid displacement method. As shown in Table 3, the CSTR was operated in three phases, namely the start-up phase, the methanogenic phase, and the sulfidogenic phase.

Chemical precipitation

Sulfide precipitation of copper was carried out using solutions of 95 mg/L of cupric nitrate and 95 mg/L of sodium sulfide. These solutions were mixed at a metal to sulfide ratios (M:S) of 1:1, 1:2, and 1:3 at pH of 2–3 and at a contact time of 30 min (Tyagi *et al.* 2020).

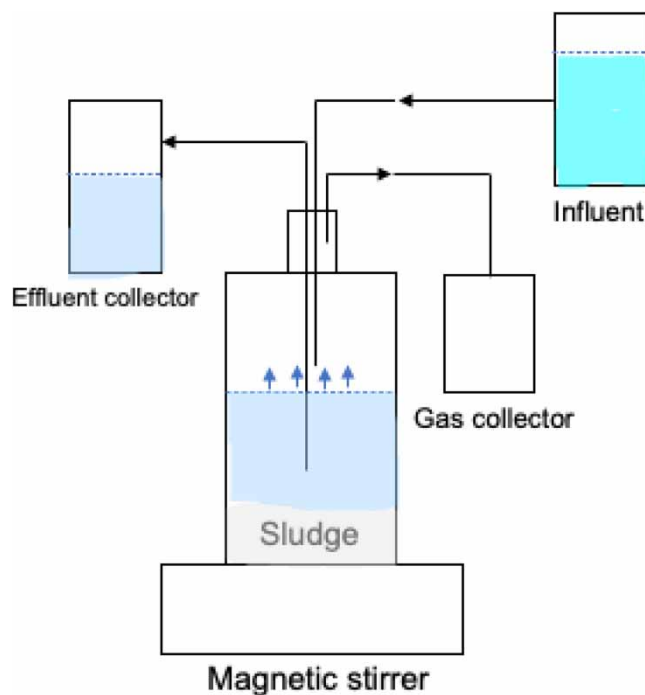


Figure 2 | Schematic of the CSTR.

Table 3 | CSTR operation up to 143 days

Sr. No.	Reactor phase	Wastewater	Nutrients	COD (mg/L)	COD:N:P	COD:SO ₄ ²⁻
1	Start-up phase	Jaggery (1–43 days)	NA	5,000–7,000	NA	24:1
2	Methanogenic phase	Jaggery (44–108 days)	Salts of N and P	6,500–7,500	200:5:1	24:1
3	Sulfidogenic phase	Jaggery and sulfate (109 days onwards)	Salts of N and P	6,500–7,500	200:5:1	24:1–4:1

Bio-sulfide precipitation

Figure 3 presents the schematic of the bio-sulfide precipitation process in which the sulfide-rich effluent of the sulfidogenic reactor was mixed with a cupric nitrate solution of 95 mg/L. In order to obtain optimized parameters like M:S, pH, and contact time, these parameters were individually varied while keeping others constant as per the data presented in Table 4.

Biochar preparation

Pine bark biochar was produced through slow pyrolysis (under a limited supply of oxygen) of a pine bark forest residue in a muffle furnace using a crucible. The pyrolysis was carried out at the optimum temperature of 650 °C with a heating rate of 10 °C/min for the period of 1 h. The crucible containing pine bark biochar was then cooled in a desiccator at room temperature. Biochar was then crushed and sieved to obtain a particle size in the range of 500–710 µm which was subsequently used in adsorption and bio-sulfide precipitation experiments (Bashir *et al.* 2022).

Bio-sulfide precipitation with biochar

As shown in Figure 4, sulfide precipitation was carried out with sulfide-rich effluent and pine bark biochar at a solid-to-liquid ratio (S:L) of 1:2. The optimized parameters (M:S, pH, and contact time) obtained from earlier experiments were used for bio-sulfide precipitation with pine bark biochar.

Adsorption studies

Batch adsorption studies using pine bark biochar were carried out as per the procedure outlined elsewhere (Bashir *et al.* 2022). The experiments were performed in a 15-mL falcon tube, continuously agitated by an orbital shaker at 200 rpm. As

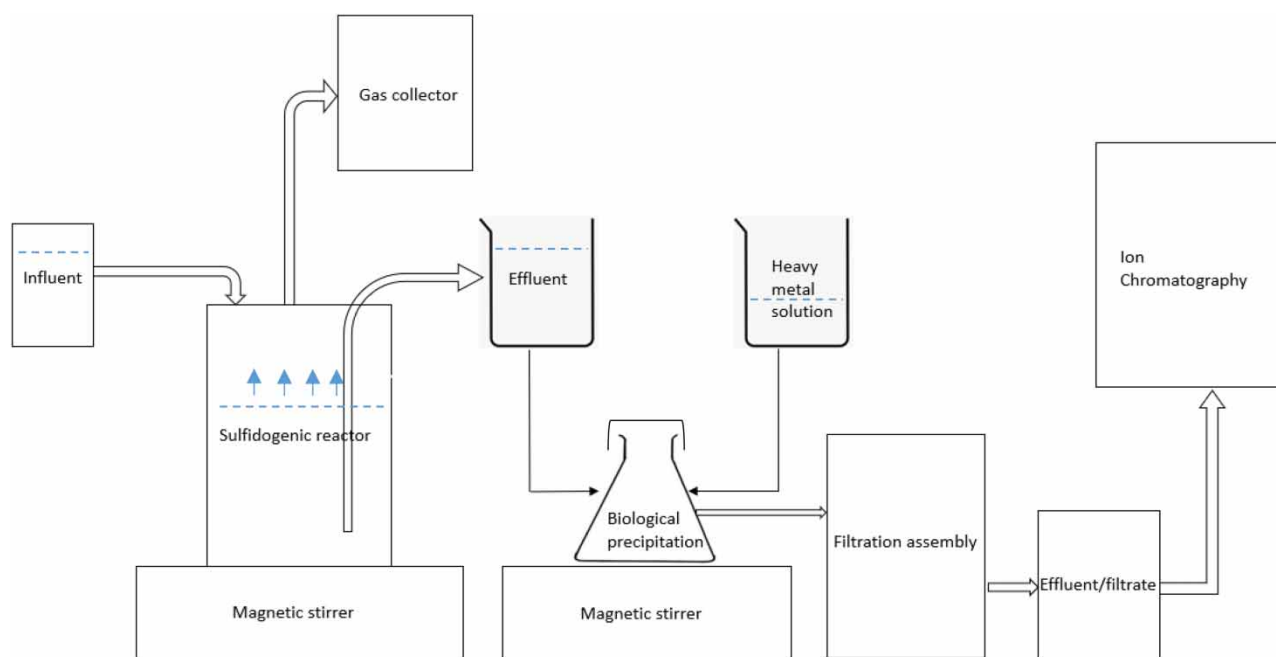


Figure 3 | Schematic of bio-sulfide precipitation for copper removal.

Table 4 | Optimization parameters for bio-sulfide precipitation

Experiment no.	M:S	Contact time (min)	pH
1	1:1–1:3	10	7
2	1:3	10–90	7
3	1:3	10	2–8

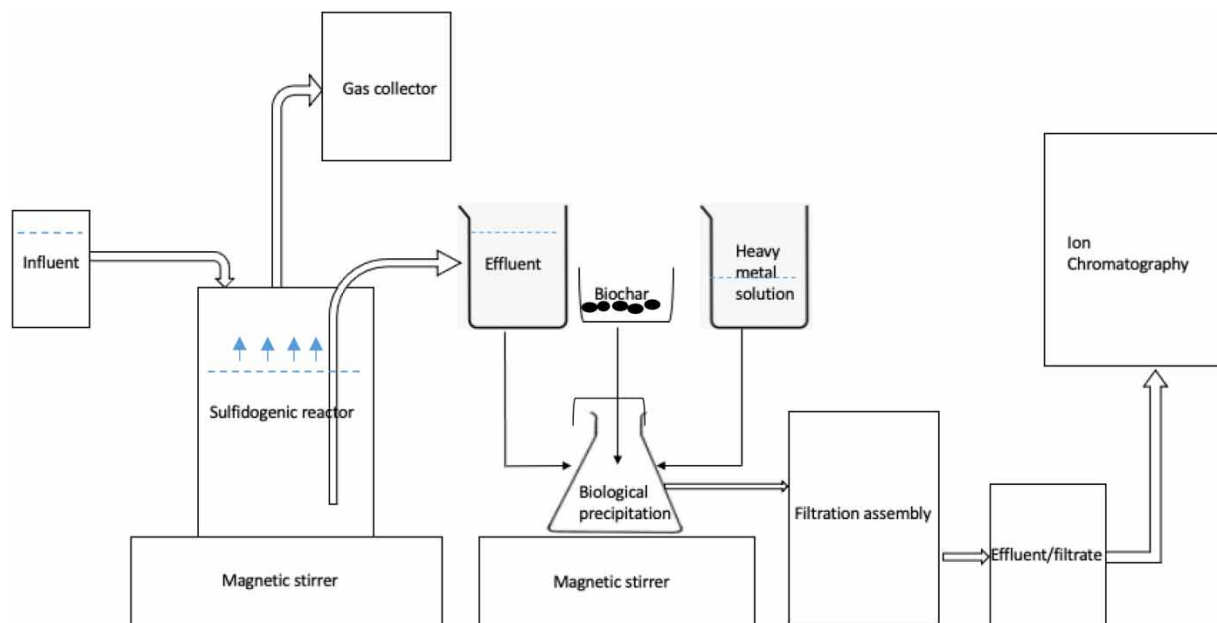


Figure 4 | Schematic of bio-sulfide precipitation with pine bark biochar for copper removal.

per the findings, the optimum conditions for the adsorption of copper using pine bark biochar was at pH 7 under room temperature. The contact time was varied between 3 and 16 h to check its adsorption capacity and removal efficiency at S:L of 1:2 and initial copper concentration of 95 mg/L under a neutral pH.

Bio-sulfide precipitation of adsorbed copper from copper-loaded biochar

As presented in Figure 5, sulfide-rich effluent of the sulfidogenic reactor was mixed with copper-loaded pine bark biochar. This experiment was divided into two stages. In the first stage, adsorption experiments using pine bark biochar were carried out. In the second stage, the copper-loaded biochar generated after adsorption (as shown in Figure 5) was used as a source of copper for metal sulfide precipitation. Continuous stirring using a magnetic stirrer was provided along with a contact time of 10 min and the pH of the effluent was neutral. The biochar was regenerated by simultaneous precipitation of adsorbed copper as copper sulfide using sulfide-rich effluent from a sulfidogenic reactor.

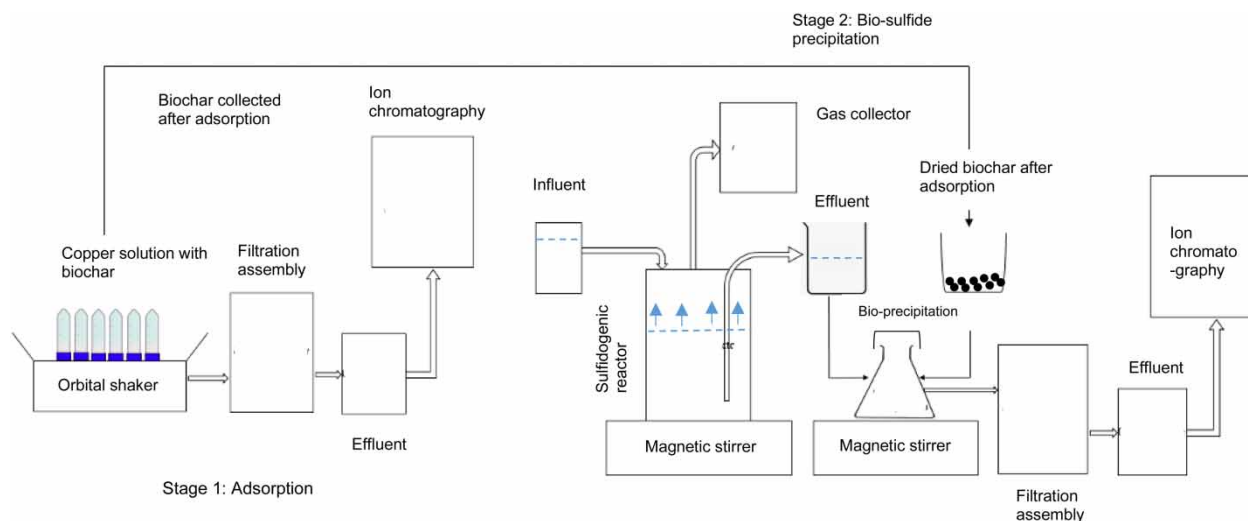


Figure 5 | Schematic of bio-sulfide precipitation of copper using heavy metal-loaded biochar.

Statistical analysis

All the batch experiments were performed three times while each sample in individual experiments was replicated three times. Regression (to determine the character and strength of the relationship between a dependent variable with respect to other independent variables), standard deviation or SD (measures the dispersion of the dataset with respect to its mean value), and error bars (T-shaped bar on a particular graph that helps in visualizing the data that depicts uncertainty in the data set) were also calculated with the help of MS-Excel software.

Total sample size = $m \times n$

where m indicates the number of batches and n indicates the replicates in each sample.

Analytical experiments

Elemental analysis up to 1- μ m depth and surface morphology of pine bark biochar and precipitates for semi-quantitative analysis were carried out using EDX-SEM (Nova Nano SEM-450). Gold coating of the samples up to 10 nm was carried out before analysis and approximately 1 mg of each sample was used for EDX-SEM analysis. Copper deduction (sample size of 5 mL) was carried out using ion chromatography (930 Compact IC Flex). Influent and effluent streams from the CSTR were analyzed for pH, VFA, COD, and sulfate. The pH was measured using a pH meter Deluxe model-101. Sulfate was measured using UV-spectrophotometer (HACH, model no. DR6000). The VFA, COD, and sulfate were analyzed as per the Standard Method (APHA 2017).

RESULTS AND DISCUSSION

Performance of the CSTR

The CSTR was operated in three phases, namely the start-up phase, the methanogenic phase, and the sulfidogenic phase. The start-up of the reactor (Phase 1) was operated for 43 days during which a loading rate of 0.18 kg-COD/(m³d) was recorded as shown in Figure 6(a). The methanogenic stage (Phase 2) was operated for up to 108 days. COD removal of 85% and gas production of 0.8 L/day were recorded during this stage (Figure 6(d) and 6(f)).

On the other hand, the reactor was operated in the sulfidogenic phase (Phase-3) from 109 days to 143 days by gradually increasing the sulfate content from 290 to 1,800 mg/L. By the end of the sulfidogenic phase, 66% of sulfate removal was achieved as shown in Figure 7. The negative sulfate removal is due to the fact that the sulfidogenic reactor was not fully matured in the initial stage. Since the sulfidogenic population was not fully established, the accumulated sulfate in the reactor escaped into the effluent. Therefore, in the initial stage, the effluent had more sulfate than the influent. This resulted in negative sulfate removal efficiency. Similar results have been reported where the accumulation of sulfate has taken place during the methanogenic stage of the reactor (Ozuolmez *et al.* 2020).

Chemical sulfide precipitation of copper

Chemical sulfide precipitation was carried out at a pH of 2–3 and a contact time of 30 min since these parameters were found to be optimum (Tyagi *et al.* 2020). Table 5 presents the results of chemical sulfide precipitation of copper at varying M:S and contact time of 30 min and pH of 2–3. M:S of 1:3 was found optimum with the resulting copper removal of 95%.

Bio-sulfide precipitation of copper

Effect of M:S

Table 6 presents the results of bio-sulfide precipitation of copper using sulfide-rich effluent from a sulfidogenic reactor. During these experiments, M:S varied between 1:1 and 1:3 when a contact time of 10 min, pH of 7.0, and initial copper concentration of 95 mg/L was maintained. The highest copper removal efficiency was obtained at M:S of 1:3 with a removal efficiency of 88%. As a result, an M:S of 1:3 was maintained in subsequent experiments. Similar observations are reported elsewhere (Deng *et al.* 2019).

Effect of contact time

During this set of experiments, M:S of 1:3, pH of 7.0, and initial copper concentration of 95 mg/L were maintained while contact time varied from 10 to 90 min. As shown by the data in Table 7, the sulfide precipitation primarily occurred in the first 10 min which remained at a higher level until the contact time of 90 min. As reported by other researchers and

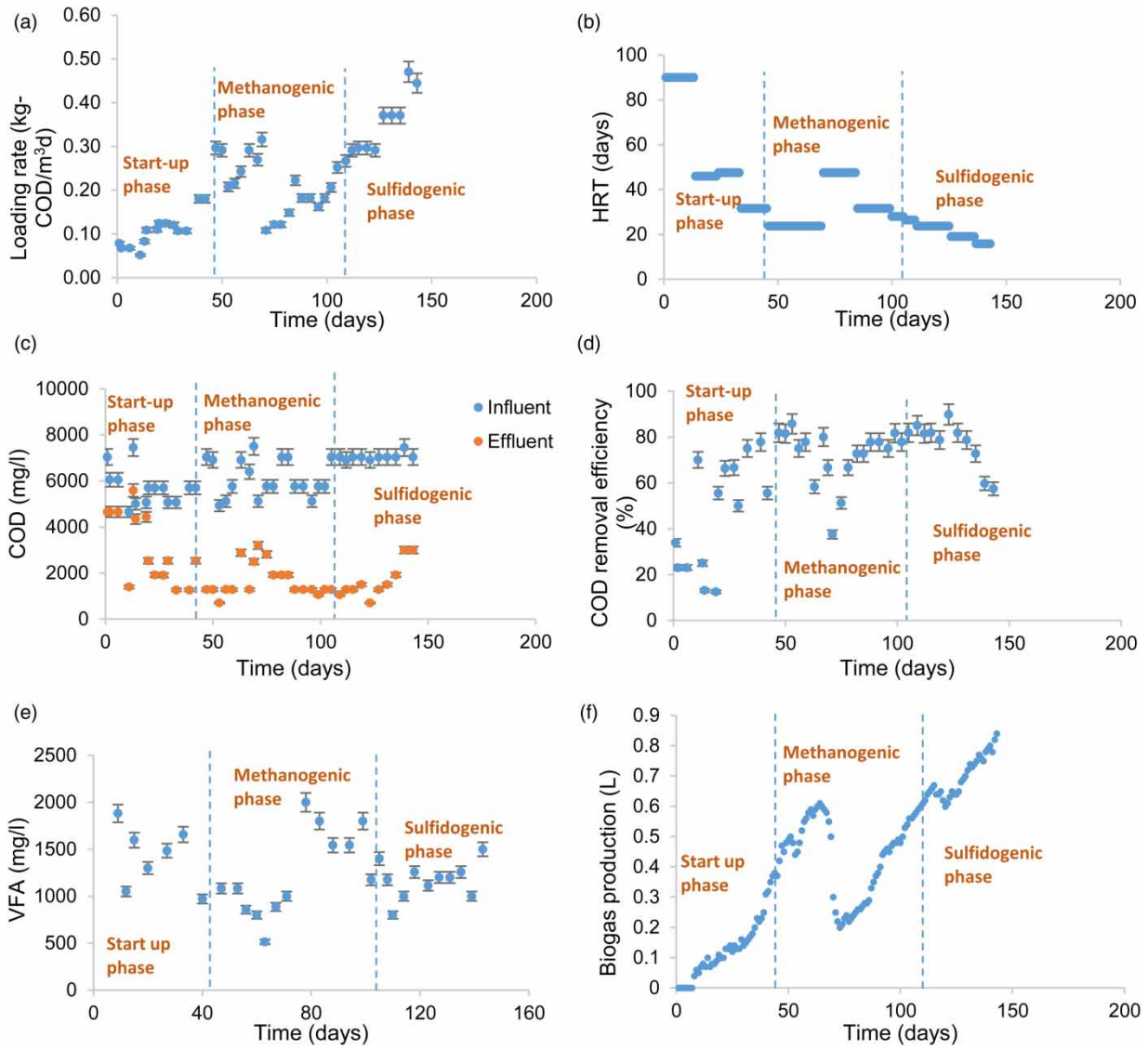


Figure 6 | Overall performance of the CSTR.

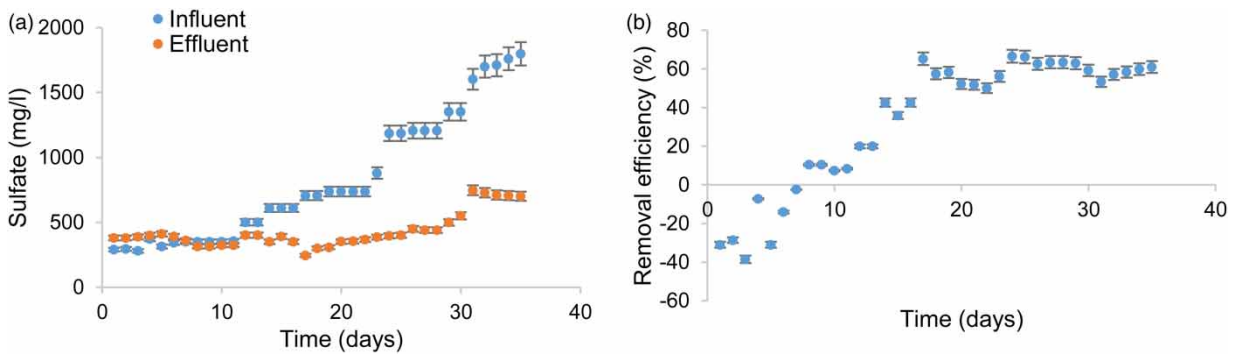


Figure 7 | Performance of the CSTR during a sulfidogenic phase.

Table 5 | Chemical sulfide precipitation at varying M:S for copper removal

S. No.	Contact time (min)	pH	M:S	Initial copper conc. (mg/L)	Final copper conc. (mg/L)	Removal efficiency (%)
1	30	2–3	1:1	95	11.2 ± 0.2	88–89
2	30	2–3	1:2	95	5.9 ± 0.4	93–94
3	30	2–3	1:3	95	4.7 ± 0.1	94–95

Table 6 | Bio-sulfide precipitation for copper removal at varying M:S (COD:SO₄²⁻ = 10:1)

S. No.	Ratio (M:S)	Contact time (min)	Initial conc. (mg/L)	Final conc. (mg/L)	Removal efficiency (%)
1	1:1	10	95	39.0 ± 0.3	58–59
2	1:1.25	10	95	38.0 ± 0.1	59–60
3	1:1.5	10	95	26.4 ± 0.3	71–72
4	1:2	10	95	20.0 ± 0.4	78–79
5	1:3	10	95	11.3 ± 0.2	87–88

Table 7 | Bio-sulfide precipitation for copper removal at varying contact time (COD:SO₄²⁻ = 8:1)

S. No.	Contact time (min)	Initial concentration (mg/L)	Final concentration (mg/L)	Removal efficiency (%)
1	10	95	4.4 ± 0.2	95–96
2	20	95	11.2 ± 0.3	87–88
3	30	95	10.3 ± 0.2	88–89
4	60	95	6.6 ± 0.1	92–93
5	90	95	4.4 ± 0.4	95–96

also shown in [Figure 1](#), the solubility of copper during sulfide precipitation is significantly low, hence higher removal efficiency is recorded ([Lewis 2010](#); [Deng et al. 2019](#)).

Effect of pH

[Table 8](#) presents the effect of varying pH (2–8) on bio-sulfide precipitation at a contact time of 10 min, initial copper concentration of 95 mg/L, and M:S of 1:3. As shown by the data in [Table 8](#), the removal efficiency always remained higher than 98% for all pH values between 2 and 8. Similar observations were reported elsewhere ([Nielsen et al. 2008](#)).

Adsorption studies

The adsorption studies were carried out as per the procedure presented elsewhere ([Bashir et al. 2022](#)). The details of adsorption studies are provided in [Table 9](#). Adsorption studies were carried out to check the removal efficiency and adsorption

Table 8 | Bio-sulfide precipitation for copper removal at varying pH (COD:SO₄²⁻ = 5:1)

S. No.	pH	Initial concentration (mg/L)	Final concentration (mg/L)	Removal efficiency (%)
1	2–3	95	1.2 ± 0.4	98–99
2	3–4	95	1.2 ± 0.1	98–99
3	4–5	95	0.9 ± 0.2	98–99
4	5–6	95	0.8 ± 0.1	99–99.5
5	6–7	95	0.6 ± 0.2	99–99.5
6	7–8	95	0.7 ± 0.3	99–99.5

Table 9 | Pine bark biochar adsorption studies

Initial conc. (mg/L)	Contact time (h)	Final conc. (mg/L)	Removal efficiency (%)	Adsorption capacity (mg/g)
95	3	49.6 ± 0.2	48–49	90.8
95	6	45.4 ± 0.2	52–53	99.2
95	10	45.0 ± 0.4	52–53	100
95	16	42.0 ± 0.1	55–56	106

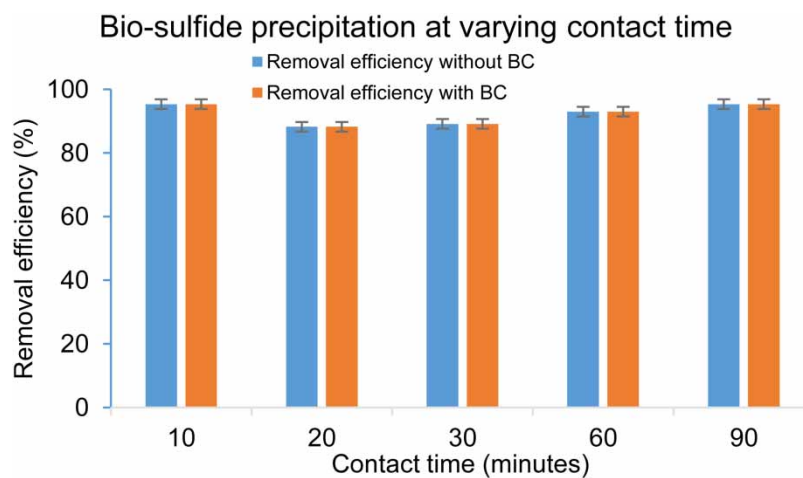
capacity of pine bark biochar so that the copper-loaded biochar can be regenerated by treating it with sulfide-rich effluent from a sulfidogenic reactor. Adsorption capacity at various contact times was calculated using 0.5 g/L of pine bark biochar with an initial copper solution of 95 mg/L and neutral pH. It was observed that after 6 h of contact time, adsorption capacity did not show much variation. The maximum adsorption capacity observed was 106 mg/g at a contact time of 16 h. However, considering that 99.2 mg/g of adsorption capacity was achieved in the first 6 h, a contact time of 6 h was considered sufficient in subsequent experiments.

Bio-sulfide precipitation of copper with unloaded and copper-loaded biochar

Experiment with unloaded pine bark biochar

An attempt was made to check the effect of pine bark biochar addition on bio-sulfide precipitation of copper. As shown in Figure 8, there was no appreciable difference observed in bio-sulfide precipitation of copper with and without the unloaded biochar. When the reactor got fully stabilized at COD:SO₄²⁻ of 4:1, the biochar enhanced the copper removal efficiency and promoted the complete removal of copper from the aqueous solution (Hardyanti *et al.* 2018). Although this improvement was insignificant, the idea was to evaluate the role of pine bark biochar during sulfide precipitation.

Table 10 presents the effect of pine bark biochar's particle size on bio-sulfide precipitation of copper at a pH of 7.0, initial copper concentration of 95 mg/L, and contact time of 10 min. It was observed that the pine bark biochar particle size had no

**Figure 8** | Comparison of bio-sulfide precipitation with and without unloaded biochar.**Table 10** | The effect of biochar's particle size on bio-sulfide precipitation

S. No.	Contact time (min)	Biochar size (µm)	Initial concentration (mg/L)	Final concentration (mg/L)	Removal efficiency (%)
1	10	100–500	95	2.9 ± 0.1	96–97
2	10	500–700	95	2.4 ± 0.4	97–98
3	10	700–1,000	95	2.5 ± 0.1	97–98

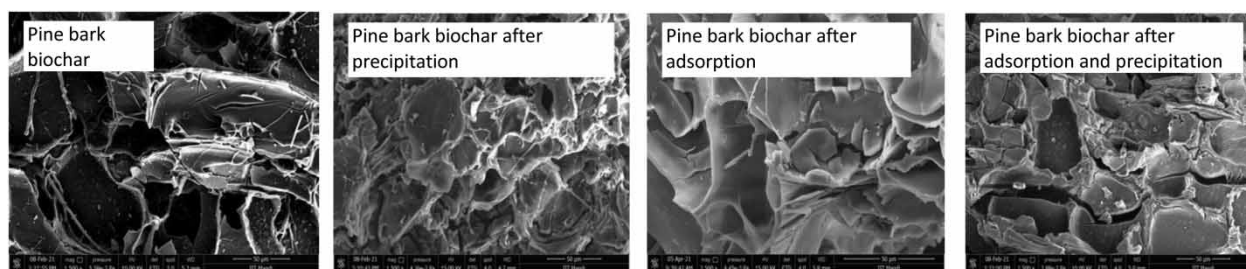


Figure 9 | SEM images of pine bark biochar (PB-BC) before and after adsorption as well as precipitation.

Table 11 | Comparison of different copper precipitation techniques

S. No.	Type of precipitation	Initial conc. (mg/L)	pH	Contact time (min)	Final conc. (mg/L)	Removal efficiency (%)
1	Chemical sulfide	95	2–3	30	4.78	95
2	Bio-sulfide	95	7–8	10	0.4	99.5
3	Bio-sulfide with unloaded biochar	95	7–8	10	0	100
4	Bio-sulfide with copper-loaded biochar	95	7–8	10	No copper deducted	NA

significant effect on copper removal efficiency since copper removal efficiency of about 97% was observed for the entire range of biochar particle size of 100–1,000 μm .

Experiment with copper-loaded pine bark biochar

Copper-loaded pine bark biochar was used to carry out bio-sulfide precipitation of adsorbed copper with the help of sulfide-rich effluent from a sulfidogenic reactor. A contact time of 10 min and pH of 7 was maintained. SEM images (Figure 9) of pine bark biochar before and after bio-sulfide precipitation clearly confirmed that there was no adsorbed copper remaining on the surface of pine bark biochar after bio-sulfide precipitation. The EDX results (as presented in the supplementary file) also suggested that there was no adsorbed copper remaining on the surface of pine bark biochar after bio-sulfide precipitation. The copper sulfide precipitates were filtered using filtration assembly and the filtrate was analyzed for residual copper by ion chromatography. The results from ion chromatography confirmed that there was no copper in the filtrate which confirmed that all the adsorbed copper was desorbed from the surface of biochar and precipitated as copper sulfide.

Comparison of all types of precipitation

Table 11 presents the comparison data of all types of precipitation for copper removal when the reactor was fully converted into the sulfidogenic phase at COD:SO₄²⁻ of 4:1. It was observed that under the sulfidogenic phase (COD:SO₄²⁻ of 4:1), 99% of copper removal was achieved. It can be seen from the table that bio-sulfide precipitation with copper-loaded biochar regenerated pine bark biochar as no copper ion concentration was deducted on the biochar's surface (verified by EDX-SEM) and in the effluent.

CONCLUSIONS

The main objective of the study was to remove copper from an aqueous solution by adsorption onto pine bark biochar produced by slow pyrolysis of the residue at 650 °C followed by simultaneous removal and precipitation of adsorbed copper from biochar using bio-sulfide precipitation, thereby regenerating the biochar. The anaerobic methanogenic CSTR was established and converted into a sulfidogenic phase by decreasing COD:SO₄²⁻ from 24:1 to 4:1. Sulfide-rich effluent from CSTR at COD:SO₄²⁻ of 4:1 was able to remove more than 99% of copper from the aqueous solution. Pine bark biochar when used as an adsorbent for copper removal from the aqueous solution yielded a biochar adsorption capacity of 106 mg/g at S:L of 1:2. Simultaneous removal of adsorbed copper and regeneration of pine bark biochar was accomplished through bio-sulfide precipitation of copper using a sulfide-rich effluent from the fully stabilized sulfidogenic reactor as confirmed by EDX-SEM analysis.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Ali, M., Tariq, M., Sun, Y., Huang, J., Gu, X., Ullah, S., Nawaz, M. A., Zhou, Z., Shan, A., Danish, D. & Lyu, S. 2021 **Unveiling the catalytic ability of carbonaceous materials in fenton-like reaction by controlled release cao₂ nanoparticles for trichloroethylene degradation.** *Journal of Hazardous Materials* **416**, 125935. ISSN 0304-3894. <https://doi.org/10.1016/j.jhazmat.2021.125935>.
- Ariffin, N., Abdullah, M. M. A. B., Zainol, M. R. R. M. A., Murshed, M. F., Zain, H., Faris, M. A. & Bayuaji, R. 2017 **Review on adsorption of heavy metal in wastewater by using geopolymer.** *MATEC Web of Conferences* **97** (1), 01023. doi:10.1051/mateconf/20179701023.
- Banks, S. W. & Bridgwater, A. V. 2016 **Catalytic fast pyrolysis for improved liquid quality.** In: *Handbook of Biofuels. Production (Second Edition)*. Woodhead Publishing, pp. 391–429. ISBN 9780081004555. <https://doi.org/10.1016/B978-0-08-100455-5.00014-X>.
- Barakat, M. A. 2011 **New trends in removing heavy metals from industrial wastewater.** *Arabian Journal of Chemistry* **4** (4), 361–377. ISSN 1878-5352. <https://doi.org/10.1016/j.arabjc.2010.07.019>.
- Bashir, M., Mohan, C. & Annachhatre, A. P. 2020 **Adsorption of copper from aqueous solution onto agricultural adsorbents: kinetics and isotherm studies.** *Materials Today: Proceedings* **28** (Part 3), 1279–2030.
- Bashir, M., Mohan, C., Tyagi, S. & Annachhatre, A. P. 2022 **Copper removal from aqueous solution using chemical precipitation and adsorption by himalayan pine forest residue as biochar.** *Water Science and Technology* **86** (3), 530–554. <https://doi.org/10.2166/wst.2022.222>.
- Brewer, C. E., Schmidt-Rohr, K., Satrio, J. A. & Brown, R. C. 2009 **Characterization of biochar from fast pyrolysis and gasification systems.** *Environmental Progress & Sustainable Energy* **28** (3), 386–396.
- Chander, M., Bashir, M. & Annachhatre, A. P. 2020 **Community based biogas plant utilizing food waste and cow dung.** *Materials Today Proceedings* **28** (3), 1910–1915. <https://doi.org/10.1016/j.matpr.2020.05.312>.
- Chen, X., Huang, G. & Wang, J. 2013 **Electrochemical reduction/Oxidation in the treatment of heavy metal wastewater.** *Journal of Metallurgical Engineering* **2** (4).
- Chen, X., Chen, G., Chen, L., Chen, Y., Lehmann, J., McBride, M. B. & Hay, A. G. 2019 **Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution.** *Bioresource Technology* **102** (19), 8877–8884.
- Ciferno, J. & Marano, J. 2002 **Benchmarking biomass gasification technologies for fuels, chemicals and hydrogen production.** *National Energy Technology Laboratory* **1**, 19.
- Deng, Z., Oraby, E. A. & Eksteen, J. J. 2019 **The sulfide precipitation behaviour of Cu and Au from their aqueous alkaline glycinate and cyanide complexes.** *Separation and Purification Technology* **218**, 181–190.
- Deniz, T. 2017 **Adsorption of Cu(II) on three adsorbents, Fe₃O₄/Ni/NixB nanocomposite, carob (Ceratonia siliqua), and grape seeds: a comparative study.** *Turk. J. Chem.* **41**, 760–772.
- Estay, H., Barros, L. & Troncoso, E. 2021 **Metal sulfide precipitation: recent breakthroughs and future outlooks.** *Minerals* **11**, 1385.
- Ghenai, C. 2010 **Combustion of syngas fuel in Gas turbine can combustor.** *Advances in Mechanical Engineering*. doi:10.1155/2010/342357.
- Glombitza, C., Jaussi, M., Røy, H., Seidenkrantz, M. S., Lomstein, B. A. & Jørgensen, B. B. 2015 **Formate, acetate, and propionate as substrates for sulfate reduction in sub-Arctic sediments of southwest Greenland.** *Front. Microbiol.* **6**, 846. doi:10.3389/fmicb.2015.00846.
- Gupta, R. & Demirbas, A. 2010 **Bio-Oil from Biomass Pyrolysis.** In: *Gasoline, Diesel, and Ethanol Biofuels From Grasses and Plants*. Cambridge University Press, pp. 140–157 doi:10.1017/CBO9780511779152.010.
- Hailegnaw, N. S., Mercl, F. & Kulhánek, M. 2021 **Co-application of high temperature biochar with 3,4-dimethylpyrazole-phosphate treated ammonium sulphate improves nitrogen use efficiency in maize.** *Scientific Reports* **11**, 5711.
- Hardyanti, N., Utomo, S., Oktaviana, A., Serafina, K. & Junaidi, J. 2018 **Effect of COD/SO₄²⁻ supply ratio variations of sulfate-reducing bacteria of sulphood raise in acid mine drainage.** *E3S Web of Conferences* **73**, 05009.
- Imam, T. & Capareda, S. 2012 **Characterization of bio-oil, syn-gas and biochar from switchgrass pyrolysis at various temperatures.** *Journal of Analytical and Applied Pyrolysis* **93**, 170–177. doi:10.1016/j.jaap.2011.11.010.
- Ipek, U. 2005 **Removal of Ni(II) and Zn(II) from an aqueous solution by reverse osmosis.** **174** (2), 161–169. doi:10.1016/j.desal.2004.09.009.
- Jia, Y., Shi, S., Liu, J., Su, S., Liang, Q., Zeng, X. & Li, T. 2018 **Study of the effect of pyrolysis temperature on the Cd²⁺ adsorption characteristics of biochar.** *Appl. Sci.* **2018** (8), 1019. doi:10.3390/app8071019.
- Juve, J. M. A., Christensen, F. M. S., Wang, Y. & Wei, Z. 2022 **Electrodialysis for metal removal and recovery: a review.** *Chemical Engineering Journal* **435** (Part 2), 134857. ISSN 1385-8947. <https://doi.org/10.1016/j.cej.2022.134857>.
- Leng, L., Xiong, Q., Yang, L., Li, H., Zhou, Y., Zhang, W., Jiang, S., Li, H. & Huang, H. 2021 **An overview on engineering the surface area and porosity of biochar.** *Science of The Total Environment* **763**, 144204.
- Lewis, A. 2010 **Review of metal sulfide precipitation.** *Hydrometallurgy* **104** (2), 222–234. doi:10.1016/j.hydromet.2010.06.010.

- Li, K., Jiang, Y., Wang, X., Bai, D., Li, H. & Zheng, Z. 2016 Effect of nitric acid modification on the lead(II) adsorption of mesoporous biochars with different mesopore size distributions. *Clean Techn Environ Policy* **18**, 797–805. <https://doi.org/10.1007/s10098-015-1056-0>
- Liu, C., Wang, W., Wu, R., Liu, Y., Lin, X., Kan, H. & Zheng, Y. 2020 Preparation of acid- and alkali-modified biochar for removal of methylene blue pigment. *ACS Omega* **5** (48), 30906–30922. <https://doi.org/10.1021/acsomega.0c03688>.
- Malik, W., Mohan, C. & Annachhatre, A. P. 2020 Community based biogas plant utilizing food waste and cow dung. *Materials Today: Proceedings* **28**, 1910–1915.
- Matlock, M. M., Howerton, B. S. & Atwood, D. A. 2002 Chemical precipitation of heavy metals from acid mine drainage. *Water Research* **6** (19), 4757–4764. ISSN 0043-1354. [https://doi.org/10.1016/S0043-1354\(02\)00149-5](https://doi.org/10.1016/S0043-1354(02)00149-5).
- Mohan, D., Pittman Jr., C. U. & Steele, P. H. 2006 Pyrolysis of wood/biomass for bio-oil: a critical review. *Energy Fuels* **20** (3), 848–889.
- Moreira, R., Orsini, R. R., Vaz, J. M., Penteado, C. & Spinace, E. V. 2017 Production of biochar, bio-oil and synthesis gas from cashewNut shell by slow pyrolysis. *Waste Biomass Valor* **8**, 217–224. doi:10.1007/s12649-016-9569-2.
- Nielsen, A. H., Hvitved-Jacobsen, T. & Vollertsen, J. 2008 Effects of pH and iron concentrations on sulfide precipitation in wastewater collection systems. *Water Environment Research* **80** (4), 380–384.
- Ozuolmez, D., Hyunsoo, N., Mark, L., Kjeldsen, K. U., Jørgensen, B. B. & Caroline, C. M. 2015 Methanogenic archaea and sulfate reducing bacteria co-cultured on acetate: teamwork or coexistence? *Frontiers in Microbiology* **6**. doi:10.3389/fmicb.2015.00492.
- Ozuolmez, D., Stams, A. J. M. & Plugge, C. M. 2020 Propionate converting anaerobic microbial communities enriched from distinct biogeochemical zones of Aarhus Bay, Denmark under sulfidogenic and methanogenic conditions. *Microorganisms* **8**, 394. <https://doi.org/10.3390/microorganisms8030394>.
- Pattiya, A. 2018 *Fast Pyrolysis. Direct Thermochemical Liquefaction for Energy Applications*. Woodhead Publishing, pp. 3–28. ISBN 9780081010297. <https://doi.org/10.1016/B978-0-08-101029-7.00001-1>.
- Prokkola, H., Nurmesniemi, E. T. & Lassi, U. 2020 Removal of metals by sulphide precipitation using Na₂S and HS – solution. *ChemEngineering* **4**, 51.
- Qasem, N. A. A., Mohammed, R. H. & Lawal, D. U. 2021 Removal of heavy metal ions from wastewater: a comprehensive and critical review. *Clean Water* **4**, 36.
- Saravanan, A., Kumar, P. S., Jeevanantham, S., Karishma, S., Tajsabreen, B., Yaashikaa, P. R. & Reshma, B. 2021 Effective water/wastewater treatment methodologies for toxic pollutants removal: processes and applications towards sustainable development. *Chemosphere* **280**, 130595.
- Sonu, K., Sogani, M., Syed, Z., Dongre, A. & Sharma, G. 2020 Effect of corncob derived biochar on microbial electroremediation of dye wastewater and bioenergy generation. *Chemistry Select* doi:10.1002/slct.202002652.
- Speight, J. G. 2019 *Unconventional gas. Natural Gas (Second Edition)*. Gulf Professional Publishing, pp. 59–98, ISBN 9780128095706. <https://doi.org/10.1016/B978-0-12-809570-6.00003-5>.
- Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation 2017 In: *Standard Methods for the Examination of Water and Wastewater* (Lipps, W. C., Baxter, T. E. & Braun-Howland, E. eds.). APHA Press, Washington DC.
- Sulyman, M., Namiesnik, J. & Gierak, A. 2017 Low-cost adsorbents derived from agricultural By-products/Wastes for enhancing contaminant uptakes from wastewater: a review. *Pol. J. Environ. Stud.* **26** (2), 479–510.
- Tyagi, S., Malik, W. & Annachhatre, A. P. 2020 Heavy metal precipitation from sulfide produced from anaerobic sulfidogenic reactor. *Materials Today: Proceedings* **32**, 936–942.
- Tyagi, S., Bashir, M., Mohan, C. & Annachhatre, A. P. 2022 Characterization of pine residues from himalayan region and its use as heavy metal adsorbent. *Water, Air and Soil Pollution*. doi:10.1007/s11270-022-05624-7.
- US Environmental Protection Agency (US EPA). 2000 Wastewater technology fact sheet chemical precipitation. 832-F-00-018. US EPA, Washington, DC.
- Wang, H., Chen, F., Mu, S., Zhang, D., Pan, X., Lee, D. & Chang, J. 2012 Removal of antimony [Sb(V)] from Sb mine drainage: biological sulfate reduction and sulfide oxidation-precipitation. *Bioresour. Technol.* **146**, 799–802.
- Wang, L., Chen, L., Tsang, D. C. W., Guo, B., Yang, J., Shen, Z., Hou, D., Ok, Y. S. & Poon, C. S. 2020 Biochar as green additives in cement-based composites with carbon dioxide curing. *Journal of Cleaner Production* **258**, 120678. ISSN 0959-6526. <https://doi.org/10.1016/j.jclepro.2020.120678>.
- Wu, J., Huang, D., Liu, X., Meng, J., Tang, C. & Xu, J. 2018 Remediation of As(III) and Cd(II) co-contamination and its mechanism in aqueous systems by a novel calcium-based magnetic biochar. *Journal of Hazardous Materials* **348**, 10–19. ISSN 0304-3894. <https://doi.org/10.1016/j.jhazmat.2018.01.011>.
- Xu, Y., Hu, X., Li, W. & Shi, Y. 2011 Preparation and Characterization of Bio-oil from Biomass. *Progress in Biomass and Bioenergy Production*. IntechOpen. <https://doi.org/10.5772/16466>.
- Yaqub, M. & Lee, S. H. 2018 Heavy metals removal from aqueous solution through micellar enhanced ultrafiltration: a review. *Environmental Engineering Research. Korean Society of Environmental Engineering*. <https://doi.org/10.4491/eer.2018.249>.
- Yargicoglu, E. N., Sadasivam, B. Y., Reddy, K. R. & Spokas, K. 2015 Physical and chemical characterization of waste wood derived biochars. *Waste Management* **36**, 256–268. <http://dx.doi.org/10.1016/j.wasman.2014.10.029>.

- Zainuddin, N. A., Mamat, T. A. R., Maarof, H. I., Puasa, S. W. & Yatim, S. R. M. 2019 Removal of Nickel, Zinc and Copper from Plating Process Industrial Raw Effluent Via Hydroxide Precipitation Versus Sulphide Precipitation. *IOP Conf. Series: Materials Science and Engineering* **551**, 012122.
- Zhang, Q., Chang, J., Wang, T. & Xu, Y. 2007 [Review of biomass pyrolysis oil properties and upgrading research](#). *Energy Conversion and Management* **48** (1), 87–92. doi:10.1016/j.enconman.2006.05.010.

First received 11 May 2022; accepted in revised form 26 March 2023. Available online 8 April 2023