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Algae as an electron donor promoting sulfate reduction for the bioremediation of acid rock drainage

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Abstract

This study assessed bioremediation of acid rock drainage (ARD) in permeable reactive barriers (PRB) using algae, *Chlorella sorokiniana*, as the sole electron donor (e-donor) for sulfate reducing bacteria (SRB). Lipid extracted algae (LEA), residues of biodiesel production, were compared with whole cell algae (WCA) as an e-donor to promote SRB activity. Inoculated columns containing anaerobic granular sludge were fed a basal-medium containing H_2SO_4 and Cu^{2+} . SO_4^{2-} , S^{2-} , Cu^{2+} and pH were monitored throughout the experiment of 123 d. Cu recovered in the column packing at the end of the experiment was evaluated using sequential extraction. Both WCA and LEA promoted 80% of sulfate removal ($12.7 \text{ mg SO}_4^{2-} \text{ d}^{-1}$) enabling near complete Cu removal (>99.5 %) and alkalinity generation raising the effluent pH to 6.5. No noteworthy sulfate reduction, alkalinity formation and Cu^{2+} removal were observed in the endogenous control. In algae amended-columns, Cu^{2+} was precipitated with biogenic H_2S produced by sulfate reduction. Confirmation of the resulting CuS was evidenced by sequential extraction and X-ray diffraction. LEA and WCA provided similar levels of e- donor based on the COD balance. The results demonstrate an innovative passive remediation system using residual algae biomass from the biodiesel industry in PRBs.

Keywords

heavy metal; acid drainage; algae waste; biodiesel; permeable reactive barrier

1. INTRODUCTION

Acid rock drainage (ARD) is produced by the contact of sulfide mineral residues of hard rock mining with moisture and air. The ARD is characterized by low pH and dissolved heavy metals. Low pH which ranges from 2 to 6 is due to the oxidation of sulfides, and iron generation of protons and sulfuric acid [1]. Examples of the composition of ARD from different mining locations are shown in Table S1 in the supplementary information (SI). The acidity extracts and dissolves heavy metals (HM) such as Cd, Pb and Cu. Heavy metals can reach surface waters and may accumulate to a toxic levels causing severe impact on aquatic organisms [2].

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Acidity and heavy metals impact human health and the environment. In humans, exposure to high doses of Cu can cause headaches, dizziness, nausea, and diarrhea [3]. Cu is well known for its toxicity to aquatic life; acute Cu toxicity at lethal concentrations (LC_{50}) in the range of 5 to 50 mg L⁻¹ has been observed in several fish species [4]. Cu is toxic to green algae grown in fresh water at pH of 5.7 to 6.5; cell division is affected at concentrations as low as 1 µg L⁻¹, and Cu is 20-fold more toxic than uranium at the same concentrations [5]. Certain heavy metals such as Pb and Cd are toxic to humans. Pb is known to cause neurotoxicity and affect the cardiovascular system and kidneys [6]. Cd causes acute toxicity in liver, lungs and kidneys and is also a known carcinogen [7].

The main approaches to the remediation of ARD utilize alkali to precipitate heavy metals and neutralize acidity or to use the activity of sulfate-reducing bacteria (SRB) to form biogenic sulfide and precipitate heavy metals. Typically, either NaOH or limestone is used to promote the chemical precipitation of heavy metals. Metal hydroxides precipitate metals due to an increase in pH, and metal carbonates precipitate using the soluble CO_3^{2-} from limestone [8]. HM precipitation with biogenic sulfide from SRB could be an economically attractive passive ARD treatment.

SRB are a group of anaerobic organisms that use organic compounds or molecular H₂ as electron donor (e-donor) to reduce sulfate (an external electron acceptor) to sulfide, also known as dissimilatory sulfate reduction [9–11]; as illustrated in Eq. (1). SRB reduce sulfate to sulfide, while the organic matter, the e-donor, is oxidized to CO₂ and acidity is simultaneously consumed [12].

The formation of HM-sulfides (MeS) results from a chemical reaction between hydrogen sulfide produced by SRB and the soluble heavy metal cation (Me²⁺), Eq. (2).

A comparison of solubility constants (K_{sp}) of hydroxide, carbonate and sulfide metals is shown in Table S2 in the SI. In this table, the K_{sp} values indicate the lower solubility of MeS compared to metal hydroxides and carbonates [13]. Biogenic sulfide (H₂S) generated during sulfate reduction is an excellent ligand of heavy metals and is extensively used for remediation of HMs such as Cu, Pb, Zn and Cd in acidic effluents.

E-donating compounds enable sulfate reduction to convert sulfate to sulfide. For the remediation of ARD, slow release electron donors such as lignocellulosic materials and polysaccharide complex materials are generally preferred to enable passive treatment low maintenance systems. Cellulosic wastes, cow manure, municipal compost and wood chips have been tested as e-donor for SRB [12, 14–16]. The lower the content of lignin, the higher the biodegradability and capacity for developing bacterial activity [17]. Lignocellulosic

materials are slow release e-donors, which are desirable in a long-lasting system, such as a permeable reactive barrier (PRB).

A PRB is capable of removing contaminants in a passive treatment below-ground where a wall is installed to intercept an ARD plume, producing a clean effluent. PRB are filled with pea-gravel and sand, mixed with reactive media. The PRB's sustainability is greatly impacted by the selection of reactive media. The goal in biological PRBs is to use an organic carbon source with a life span exceeding 5 years. In HM remediation, the reactive materials vary from zeolite, limestone, zero-valent iron and local compost materials [18–20].

Residual algae from the biofuels industry may provide large quantities of biomass in the future. Microalgae can play the dual role of treating wastewater and generation of biomass for biodiesel production [21]. With current technology there is an excellent economic outlook for algae based biodiesel production [22]. Two microalgal strains have emerged as production candidates from recent US Department of Energy research: *Chlorella sorokiniana*. (DOE 1412) and *Nannochloropsis gaditana* (CCMP-1775) [23]. *Chlorella sorokiniana*-1412, a genetically modified microalga has been widely tested for its biodiesel production potential. Lipid extracted algae (LEA) is the residual material left after lipid extraction from whole cell algae (WCA).

The composition of algae cells lends itself as a slow release e-donor. LEA is expected to be a massive waste product [24–26] that could be used as an e-donor for bioremediation applications such as the treatment of heavy metals in ARD by sulfate reduction. The effectiveness of algal biomass as both carbon source and energy to drive the process of sulfide generation by SRB has not been studied except in two cases evaluating extracellular polymeric substances (EPS) from algae, mainly composed by exopolysaccharides, as an e-donor for sulfate reduction [27, 28]. Due to the high content of complex cell wall polysaccharides in algae, LEA is expected to degrade slowly and last a long time in PRB as a slow-release e-donor, which is conducive to low cost and low maintenance.

In this study, *Chlorella sorokiniana*-1412 is utilized as the e-donor. The objective of this study is to compare WCA with LEA and determine if algae biomass residues could serve as an e-donor to support SRB in ARD remediation.

2. Materials and Methods

This study uses WCA and LEA as an e-donor for SRB to remediate ARD. Columns were packed with glass beads and inoculated with sludge having sulfate reducing activity to mimic PRBs. The experiment compares an endogenous column (with no electron donor added) with columns containing WCA or LEA. The experiment evaluates whether the algae substrate is able to reduce sulfate to biogenic sulfides, enhancing Cu removal and increasing ARD pH.

2.1 Algae growth conditions

Chlorella sorokiniana-1412 was cultivated on a BG-11 medium in a photo bioreactor [29]. The chemical composition of WCA and LEA is illustrated in Table 1.

2.2 Anaerobic inoculum

Sulfate reducing anaerobic granular sludge was obtained from a containerboard mill wastewater treatment plant, (RockTenn, Syracuse, NY). It had a volatile suspended solids (VSS) content of $0.094 \text{ g VSS g}^{-1}$ (wet weight of pellet after centrifugation). When unused, the sludge was stored anaerobically at 4°C .

2.3 Chemicals

The main chemicals used were $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 98+%, CAS 7758-998 (Sigma-Aldrich, St. Louis, MO) to add a heavy metal to the synthetic ARD medium. H_2SO_4 , 98% V/V; 18M, CAS 7664-93-9 from Fisher Scientific (Fair Lawn, NJ) combined with cupric sulfate to prepare $250 \text{ mg L}^{-1} \text{ SO}_4^{2-}$ and lower the pH to 4. NaOH, 97%, CAS 1310-73-2, from Fisher Scientific (Fair Lawn, NJ) was used to adjust the ARD medium to pH 4.

2.4 Chemical Oxygen Demand content

The chemical oxygen demand (COD) content of *Chlorella sorokiniana*-1412 was quantified using standard methods [30], with $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ at 150°C per 2 h and measured at 600 nm. The COD (mg COD/mg dwt algae) was: 1.43 for WCA and 1.32 for LEA. Table 1 shows the algae composition.

2.5 Lipid extraction from microalgae biomass

Lipids were extracted using 7.5 g-dry algae biomass with 1.43 g COD/g-dwt algae in a mixture of methanol and chloroform (3:1, v/v) and microwaved at 80°C [31]. The remaining solids were washed with water 5 times and dried in a 160 mL bottle with nitrogen at 3 psi over 24h. The total lipids extracted amounted to 9.4 %. The algal residue remaining is described as LEA, COD value of 1.32 g COD/g-dwt algae.

2.6 Media for bioreactors

The basal mineral medium used for sulfate reducing experimentation contained (in mg L^{-1}): NH_4Cl (30); K_2HPO_4 (200); KH_2PO_4 (300); $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (20), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (80), and 1mL per L of trace element solution [32]. Cu was added as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (50), gradually increased from 10 to 50 mg L^{-1} , and additional sulfate (SO_4^{2-}) was added as H_2SO_4 to reach (250). The pH was adjusted by addition of NaOH.

2.7 PRB columns

This study was designed for a SRB process using 250 mg L^{-1} of SO_4^{2-} as the electron acceptor and algae as the electron donor, with no other exogenous source of e-donor. Fig. 1 illustrates three glass columns (0.07 L^{-1}). The same amounts of granular sludge, equivalent to 12 g VSS L^{-1} reactor, and 2mm diameter glass beads were used to pack the columns and increase permeability. The endogenous column did not contain any algae. The WCA column was filled with intact *Chlorella* and the LEA column was filled with *Chlorella* after lipid extraction. The mass composition of the packing and the algae COD content are described in Table 2.

All columns were operated in continuous parallel mode at $(24 \pm 1^\circ\text{C})$ with the same source of influent (synthetic ARD). The influent was kept at $(24 \pm 1^\circ\text{C})$ and then pumped upward using a peristaltic pump. The feed medium was pumped at a flow rate of $0.064 \text{ L}\cdot\text{day}^{-1}$ or an empty bed hydraulic retention time (HRT) of 1.1 day, lasting 123 days. The sulfate and acidity of the synthetic ARD medium (basal medium) were constant, while the Cu content was gradually increased at different periods (Table 3).

The influent sulfate concentration was always 2.6 mM of SO_4^{2-} (supplied as sulfuric acid). The pH of the medium was kept at 4.0 by partially neutralizing the sulfuric acid with NaOH. Cu was added after the first 26 days, at three intervals. As illustrated in Table 3, the first period (a) had no Cu addition; this period was followed by periods (b–d): 10, 30 and $50 \text{ mg L}^{-1} \text{ Cu}^{2+}$, and the last period (e) had no Cu, to test sulfate reducing activity and the absence of toxicity due to H_2S .

2.8 Analysis

Influent and effluent were analyzed to determine pH, S^{2-} , SO_4^{2-} and Cu^{2+} . Sulfide was analyzed colorimetrically by the methylene blue method [33]. Sulfate was measured by ion chromatography with suppressed conductivity using a Dionex AS11-HC4 column (Dionex, Sunnyvale, CA) and a conductivity detector. To avoid Sulfate formation from sulfide oxidation in sample handling and storage, the liquid samples were acidified, followed by stripping H_2S with $\text{N}_2\text{-CO}_2$, as recommended by Hughes [34]. Cu was determined by inductively coupled plasma-optical emission spectrometry (2100 Optima ICP-OES, PerkinElmer, Waltham, MA). Wavelength used for Cu^{2+} determination was 327.3 nm at detection limit range of 0.1 to $1.0 \mu\text{g L}^{-1}$.

At the end of the experiment, the packing of each column was extracted, glass beads were removed and the remainder was homogenized. To compare the amount of Cu retained in the columns against the Cu recovered from the columns, sequential extractions were performed using water, 1 M HCl and $3:1 \text{ HNO}_3\text{-HCl}$ (15.7 and 12 M , respectively) sequentially to extract the Cu retained in the packing. A single sample was collected from each column and the precipitates were characterized by X-ray diffractometer (XRD), from (PANalytical X'Pert Pro, Netherlands). To estimate the algae washout, a daily sample was analyzed by the previously described COD method. The cumulative volume and the dilution factor were used to calculate the total COD lost by algae washout. The COD was quantified using the whole solution of the algae amended columns and corrected by the endogenous column.

The total sulfide (TS) associated with the liquid and gas was calculated by Eq. (3). Effluent of 0.5 mL was analyzed for dissolved sulfide (DS), the sum of $\text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$, by the methylene blue method. The concentration of undissociated sulfide $[\text{H}_2\text{S}]$ was calculated based on the DS, accounting for pH and using the first dissociation constant ($\text{pK}_{a1} = 6.98$).

$$\text{TS} = \text{DS}(1 + \alpha_0 \text{HF}) \quad (3)$$

Where:

TS = total sulfide in system (gas & liquid) per liter liquid

DS = dissolved sulfide (measured with methylene blue method)

α_0 = fraction H_2S of DS

H = dimensionless H constant C_g/C_{aq} (0.4)

F = vol headspace/vol liquid (stripping factor)

$\alpha_0 = 1/(10^{(pH-pKa)} + 1)$

pKa = dissociation constant of H_2S (6.98 at 25°C)

Sulfide in the mineral fraction was estimated by the decrease in Cu. This calculation used a molar ratio of $Cu/S = 1.0$ with the formation of CuS as the only precipitate.

Methane production was measured by volume, using a 1.0 L empty gas sampling bag Tedlar-SCV (Sigma-Aldrich, MO) connected to the upper part of each column. The amount of methane was significant only during the first three weeks; after that period the gas production declined to zero. In calculating methane produced, it was assumed the gas was composed 70% of methane [35].

The initial algae COD of 7g at time zero (COD_{t0}) accounted for 100% of the COD in both algae-amended columns. At the end of this study, the accounting of COD considered four fractions making up the total COD: cumulative H_2S -COD (COD in all forms of sulfide), algae washout-COD, CH_4 -COD and the COD remaining in reactor, which was calculated as follows:

$$COD_{remaining} = COD_{t0} - (H_2S-COD + washout-COD + CH_4-COD).$$

The sulfur balance was calculated considering: 1) soluble H_2S -S (measured + stripped), 2) SO_4^{2-} -S in the effluent and 3) CuS -S due to the sulfur precipitated and adsorbed in the column in the periods when Cu was added.

3. Results

3.1 Sulfur

Both WCA and LEA contributed to sulfide formation in the effluent of the laboratory PRBs (Figure 2A). In the case of WCA, the highest effluent sulfide concentration, 50 mg L^{-1} , was observed in the latter half of period (b). In the case of LEA, the maximum sulfide concentration of 55 mg L^{-1} occurred at the end of period (e). Consistently, throughout reactor operation, the algae amended columns produced orders of magnitude higher effluent sulfide concentrations compared to the endogenous column. In fact, the endogenous column produced only very low levels of sulfide (maximally 5 mg L^{-1}) from d 5–40. Thereafter, there was no longer detectable sulfide in the effluent of the endogenous column.

Further evidence of sulfate reduction is supported by the removal of sulfate in the algae amended columns (Figure 2B). The highest sulfate removal occurred from the latter part of period (b) to the end of period (d) in the WCA column or from period (c) through (e) in the

LEA column, corresponding to 60–80% sulfate removal. There was little to no sulfate removal in the endogenous column.

Cu did not appear to impact sulfate reduction. Period (d), which had the highest Cu concentration, also had the highest sulfate removal (Figure 2B) and thus Cu inhibition was not witnessed. Sulfide levels dropped in periods (c) and (d) when Cu concentrations increased (Figure 2A), but the drop was due to copper sulfide precipitates (Figure 3) and not to toxicity.

In the beginning of period (e), a noteworthy difference in behavior was observed between the WCA column and the LEA column. Both the sulfate removal and the sulfide production increased at faster pace in the LEA column. This indicates a greater release of e-donor from LEA as compared to WCA in the final period as the experiment was ending. Alternatively, the sudden removal of Cu at the start of period (e) caused sulfide inhibition of SRB, and the LEA column acclimated to the toxicity faster. These observations taken together indicate that both algae e-donor sources were quite suitable as e-donor for sulfate reduction and there was no evidence of Cu inhibition.

Sulfur balances during the system operation are shown for the algae containing column in Figures 3A and 3B. The figures clearly show that sulfate was converted to aqueous sulfide (with a minor fraction of stripped S) and copper sulfide minerals. The balances are excellent on any given day; the sum of sulfur species was very similar to the inlet S quantity. The balance in the endogenous column primarily demonstrated that the effluent was sulfate-S equal to the input sulfate-S (Figure S3 in SI), what one would expect if no reaction had taken place.

3.2 pH

Figure 4 shows the pH evolution of the PRBs' effluent. The algae amended columns had effluent pH values near neutral, ranging from 6.0 to 6.5. This contrasts starkly with the pH values in the endogenous columns, i.e., approximately 4.0, from period (b) onwards. The pH values were correlated with sulfate reduction activity. This activity was consistently high in algae-amended columns. The endogenous column, however, had low activity from the start of reactor operation until d 40, at which time activity decreased to zero and concomitantly as the pH dropped to 4.0.

3.3 Copper

The influent and effluent Cu concentrations are shown in Figure 5. Figure 5A is zoomed out to show the influent and effluent concentrations ranging from 0 to 50 mg L⁻¹. Figure 5B is zoomed in to the concentration range from 0 to 0.5 mg L⁻¹, in order to appreciate differences at very low effluent concentrations. The endogenous column reached Cu breakthrough towards the end of period (b). Thereafter, only partial or no Cu retention was observed. At the end of period (d), 50 mg L⁻¹ of Cu were discharged with the effluent and this was equal to the influent. This behavior is consistent with lack of sulfate reduction. Conversely, Cu was effectively retained in the algae-amended columns. Cu was retained in the reactor throughout the experiment as shown in Figure 5A. The Cu removal percentages were higher than 99.5 and 99.7 for the WCA- and LEA columns, respectively. The effluent

Cu concentrations from these columns are shown in Figure 5B. The effluent Cu concentrations ranged from 50 to 400 $\mu\text{g L}^{-1}$ in the WCA column and from non-detect to 450 $\mu\text{g L}^{-1}$ in the LEA column.

Cu was precipitated as CuS as evidenced by XRD and sequential extraction. The XRD evidence is presented in Figure 6. The figure shows clear evidence of CuS crystals that was only observable in the solids from the two algae-amended columns. No such evidence was found in the endogenous column.

The cumulative Cu balance is presented in Table 4. Firstly, the table shows that there was approximately 4× more Cu retention in the algae-amended columns than the endogenous column. Secondly, the table shows that the retained Cu speciation was mostly in the form of sulfides because most of the Cu could only be extracted by a strong oxidative acid HNO_3 -HCl (3:1), expected to be required for sulfides. The HNO_3 -HCl was not necessary in the endogenous column because all the Cu was extracted with water and 1M HCl (indicating that CuS were not present in that column consistent with the XRD data). Thirdly, the table shows a good balance of Cu extracted versus cumulative Cu that was retained in all columns (as measured comparing inlet – outlet Cu). In the algae columns, the recovery of Cu extracted ranged from 95 – 98 % of that retained.

3.4 COD

A cumulative COD balance is provided for the columns containing algae in Figure 7. In period (a) (the first 26 d of operation), biogas production provoked washout of the biomass that accounted for the loss of 40.7 and 26.7 % of the algae COD. The COD metabolized during the study corresponded to only 9.5 and 9.2% of the added algae-COD, respectively, to produce sulfide. And to a lesser extent (only 3.5% and 1.3% of COD) of the WCA- and LEA-COD, was converted into methane COD.

4. Discussion

4.1 Main Findings

Both WCA and LEA algae biomass served as an e-donor for sulfate reduction. Sulfide formed from this reaction precipitated Cu in the form of copper sulfides as evidenced by XRD and sequential extraction. Sulfate reduction was active in the algae amended columns even as the experiment ended on d 123. By comparison, the endogenous reactor exhibited low sulfate reduction activity during the first 40 d of the experiment and then activity ceased. Thus, algae could serve as a long-term e-donating substrate. Active sulfate reduction was linked to highly efficient Cu removal and an increase in ARD pH.

4.2 Algae as Electron Donor in Anaerobic Conditions

This study provides direct evidence that WCA and LEA can serve as e-donors to drive sulfate reduction. Previously, microbially driven sulfate reduction in a constructed wetland was hypothesized to be linked to the degradation of algal biomass from two genera, *Scenedesmus* and *Carteria*, naturally occurring algae in that wetland [27]. Additionally, extracellular polysaccharides produced by a mixed culture of algae in a high rate pond were

considered to be responsible for sulfate removal [28]. Studies of a wastewater ponding system containing heavy metals [36] demonstrated that a combination of algae growing in the pond and co-disposed organic wastes served as a carbon source for sulfate reduction, enabling Cu removal with an efficiency of 80% (from 500 mg L⁻¹). This study provides for the first time direct evidence that WCA and LEA can be utilized as e-donor for sulfate reduction.

In addition to algae, the use of phototrophic cyanobacterial biomass for sulfate reduction has also been reported. Sulfate reduction by a mixed culture utilized 31% COD from *Spirulina* sp. biomass as a sole carbon source [37]. The *Spirulina* cell wall composition has a high glucan content similar to other cyanobacteria [38].

The cell walls of *Chlorella*, are rigid and more resistant to biodegradation because of a matrix containing glucosamine, the dominant cell wall polymer, combined with hemicellulose with glucose and mannose [39, 40]. Cellulosic and hemicellulosic material as e-donor for SRB has been demonstrated in several studies. A significant sulfate reduction rate of 12.3 mg L⁻¹ d⁻¹ was observed in one study [41], for bioremediation of acid mine drainage (AMD) at pH 2.0, using rice straw as e-donor corresponding to a Cu removal efficiency of 98% (inlet 1.5 mg L⁻¹ Cu). In another study, grass cellulose was used as the carbon and energy source for microorganisms in rumen fluid, enabling sulfate removal efficiency of 86% [42].

While little is known about algae as a donor by sulfate reducing consortia, it is well established that algae can serve as an electron donor for methane production. In massive algae cultures, anaerobic digestion is a necessary step to make microalgal biodiesel sustainable [43]. The COD conversion of algae biomass to methane under anaerobic digestion of *Chlorella sorokiniana* and *Chlorella vulgaris* ranged from 40 – 73 % [44]. In a different study, around 50% of the biomass of *Chlorella vulgaris* was converted to methane [45]. Methane yield of *Chlorella vulgaris* ranged from 189 to 450 mL CH₄ g⁻¹ VS, but anaerobic digestion can also provide nutrients from LEA [46], which is essential to make biodiesel production sustainable.

Sonicated and thermally treated algae provided higher methane yields, compared to WCA. The lipid extraction method, characterized by type of solvent and temperature, is by itself a pretreatment that increases methane production of LEA [47]. Thermal hydrolysis of *Nannochloropsis* at 170°C supported methane productivity enhancement of 40% for the WCA and 15% for the LEA [48]. Another study utilizing ultrasound at 128.9 MJ/kg and thermal hydrolysis at 80 °C increased methane production from *Scenedesmus* biomass by 2-fold and 1.6-fold, respectively, compared with untreated biomass [49]. The increase was attributed to cell wall disruption and COD solubilization.

4.3 Copper and Sulfide Toxicity to SRB and Methanogens

In this study, copper and sulfide had no apparent inhibitory effect on sulfate reducing activity. The 50% Cu inhibiting concentration (IC₅₀) to acetoclastic and hydrogenotrophic sulfate reducers was 32.3 mg L⁻¹ and >200 mg L⁻¹ respectively. In the same study the Cu IC₅₀ for acetoclastic and hydrogenotrophic methanogens was reported as 20.7 and 8.9 mg

L⁻¹, respectively [50]. A greater Cu IC₅₀ to sulfate reducers and methanogens has been reported 1136 and 130 mg L⁻¹ [51]. The observed IC₅₀ values of Cu for hydrogenotrophic SRB were higher than the concentration used in this study of 50 mg L⁻¹, thus our findings are consistent with the lack of toxicity expected at this concentration.

Undissociated sulfide (H₂S) is the main toxic form of sulfide, as only the neutral form permeates the cell membrane [52]. In toxicity studies, on average, IC₅₀ of undissociated H₂S, at pH of 6.8, of acetoclastic and hydrogenotrophic sulfate reducers was 272 and 299 mg L⁻¹, respectively, while to acetoclastic and hydrogenotrophic methanogens was 97 and 136 mg L⁻¹ respectively [53]. In a similar study sulfide IC₅₀, at pH of 6.8, of acetoclastic and hydrogenotrophic sulfate reducers was 270 and 380 mg L⁻¹ undissociated H₂S, respectively, while the acetoclastic and hydrogenotrophic methanogens was 160 and 220 mg L⁻¹ of undissociated H₂S, respectively [54]. In our study the effluent pH typically ranged from 6.4–6.7. In the worst case scenario this corresponds to 62.5 mg L⁻¹ of undissociated H₂S (at pH=6.5), which is much lower than the IC₅₀ reported in literature for hydrogenotrophic sulfate reducers.

4.4 Implications of Algae Use as e-donor for AMD Remediation in PRB

The utilization of LEA may have remarkable advantages as a slow release e-donor to remediate ARD in PRBs. Additionally; algae can sustain PRBs for years producing benefits to the environment by removing HMs and acidity from ARD. LEA utilization could also add profitability to the biodiesel industry. The main challenge in using algae as a reactive material in PRBs, however, is how to reduce the washout of the suspended algae that we suspect was exacerbated by biogas production in the initial period. Eventually SRB will outcompete methanogens under prolonged sulfate-reduction and low pH conditions in ARD plumes.

5. Conclusions

- WCA and LEA were both shown to be effective e-donor to drive sulfate reduction of ARD, enabling the precipitation and removal of Cu in lab-scale PRB.
- Efficient sulfate reduction was maintained during the experimental period of 4 months, using a low pH synthetic ARD influent. Sufficient algae-COD remained to sustain the operation for another 20 months
- The Cu removal efficiency was greater than 99.5% in the WCA and LEA columns.
- Due to metal sulfide precipitation, the SRB community was not inhibited at 50 mg L⁻¹ of influent Cu.
- The precipitate retained in the columns was composed mostly of insoluble copper sulfide formed from the biogenic sulfide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A descriptive diagram of the three up-flow packed bed columns used to test the algae biomass as electron donor for the SRB process. A) Endogenous column, B) WCA column and LEA column.

Figure 2.

Panel A: Total sulfide in the effluent (sulfide measured + stripped): Endogenous column (○), WCA column (■) and LEA column (△). Concentration of Cu²⁺ was 0, 10, 30, 50 and 0 (mg L⁻¹) added to the synthetic basal medium during periods a, b, c, d and e respectively. The maximum H₂S concentration stripped (at pH 6.5) was 0.23 mg L⁻¹-effluent during week 3. This corresponds to 5.1% of total H₂S. Sulfide stripped was corrected only the first 40 days of active gas production. Panel B: Sulfate removal (%) Calculated based in the percent of (SO₄²⁻ in minus SO₄²⁻ out) / SO₄²⁻ × 100.

Figure 3. Sulfur balance in WCA (panel A) and LEA (panel B) columns: sulfide aqueous + stripped (■), sulfur in CuS (□), sulfate effluent (■).

Figure 4.
Effluent pH: Endogenous column (O), WCA column (■) and LEA column (△). The horizontal line represents the constant influent pH 4.

Figure 5.

Effluent and influent copper concentration: Panel A: View of data in the range from 0 to 60 mg L⁻¹ Panel B: Close up view of effluent data in the range of 0 to 0.6 mg L⁻¹. Endogenous column (○), WCA column (■) and LEA column (△). The influent concentration of Cu²⁺ Was 0, 10, 30, 50 and 0 (mg L⁻¹) added to the synthetic basal medium during period. a, b, c, d and e respectively. The dash horizontal line represents the influent Cu²⁺ concentration in each of the periods.

Figure 6. XRD spectrum of the column packing at the end of the column experiment. Endogenous column (1), WCA column (2) and LEA column (3). Copper sulfide (Covellite) used as the reference.

Figure 7.
Algae-COD balance in WCA and LEA columns: Remaining algae (■), S²⁻-COD (□), CH₄-COD (■) and Wash out algae (▨).

Table 1Chemical composition of *Chlorella sorokiniana* before and after lipid extraction.

Composition (Dwt)	WCA	LEA
Total solids (g TS/g wet wt)	0.20	1.0 [†]
Volatile solids (g VS/dwt)	0.96	0.94
Total nitrogen (% dwt)	5.5	5.6
Total phosphorous (% dwt)	0.57	0.53
Total carbon (% dwt)	51.0	48.4
Total lipids (% dwt)	9.4	ND

[†]LEA is dry powder.

Table 2

Composition of packed-bed columns.

Component	PRB Columns [*]			
	DBD ^{**}	Endogenous	WCA [‡]	LEA [†]
	g/cm ³	-----g dwt/column-----		
Glass beads(2 mm)	1.5	32	17.0	17.0
Sludge (Rock Tenn) [‡]	1.0	0.94	0.94	0.94
Whole Cell Algae (WCA) [*]	0.42	0	4.9	0
Lipid Extracted Algae (LEA) [†]	0.41	0	0	5.3

^{*} Packing volume (0.07 L).^{**} DBD = dry weight bulk density.[‡]WCA (1.43 mg COD/mg dwt algae)[†]LEA (1.32 mg COD/mg dwt algae)[‡] sludge inoculum of 9 g wet sludge (0.094 g VSS g⁻¹ wet sludge).

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Table 3

Periods of column operation as defined by influent Cu²⁺ concentrations.

Period:	a	b	c	d	e
Time(d)	0 – 26	27 – 59	60 – 74	75 – 100	101 – 123
Influent Cu ²⁺ (mg L ⁻¹)	0	10	30	50	0

Sequential extraction of total copper from the homogenized column packing at the end of the experiment by means of: water, 1M HCl and 1:3 ratio (v/v) of concentrated HNO₃:HCl.

Table 4

Column	Sequential Extraction of Cu (mg)				% Cu Recovered [†]
	(water)	(1M HCl)	(HNO ₃ :HCl) [‡]	Total Extracted	
Endogenous	0.3	33.1	0.2	33.6	36.0
WCA	0.0	28.6	92.3	120.9	127.6
LEA	1.5	24.1	98.0	123.6	126.0

[‡]HNO₃-HCl, 3:1(v/v); 15.7M and 12M respectively.

[†]Cumulative copper removal calculated from difference between flux in and flux out.

[‡]% Recovery = (Cu extracted from packing material/cumulative Cu retained)* 100.