Metal remediation of acid mine drainage using a hybrid system of microalgae reactor

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Abstract: Acid mine drainage(AMD) contains high concentrations of heavy metals and has become a serious environmental problem. A pipes inserted microalgae reactor(PIMR) was constructed to cultivate microalgae and purify AMD. The effects of metal concentration, pH and sulfate after pretreatment on the removal of iron and microalgae growth were investigated. Batch studies showed that PIMR and microalgae can adsorb iron with an uptake of 63.21 ± 9.8 mg/L iron. Microalgae growth was measured by optical density (OD) and dry cell weight (DCW); OD and DCW were 3.96 and 1.54g/L respectively. Continuous studies also proved that PIMR can be used for metal remediation and microalgae cultivation.

Keywords: Pipes inserted microalgae reactor, Acid mine drainage, Microalgae, Metal removal

1. INTRODUCTION

Acid mine drainage (AMD) is one of the major sources of heavy metals. AMD occurs when sulfide minerals, such as pyrrhotite (FeS) and chalcocite (Cu_2S), are exposed to air and water. Pyrite (FeS_2) is one of the most common sulfide mineral that reacts in the presence of water and oxygen to yield sulfuric acid and iron [1].

Microalgae are photosynthesis microbial cell factories that convert carbon dioxide to potential biofuels, foods supplements, animal feed, and high-value bioactive materials. Microorganisms have been used for bioremediation of pollutants under in-situ and ex-situ conditions. Furthermore, microalgae are able to remove a xenobiotic by a biosorption process using living or dead nitrogen fixing biofertilizers [2-5].

This study investigated the feasibility of using AMD as a source of minerals for microalgae cultivation. We also investigated the microalgae's effectiveness in terms of heavy metal removal. For this purpose, a two- step process was developed. In the first step, AMD is neutralized by chemical materials, and iron in AMD is precipitated. However, iron colloids exist in the effluent. This may result in the effluent being a reddish color, which is sometimes considered to be an aesthetic problem [6]. In the second step, a pipes inserted microalgae reactor (PIMR) is introduced to remove iron colloids and cultivate microalgae by supplying minerals simultaneously.

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2. Materials and Methods

2.1. Microalga strain and growth media

The microalga selected for this study, *Nephroselmis sp*. KGE 8 was isolated from a heavy metal-rich environment in an abandoned coal mine located in South Korea. This microalga was selected because of its tolerance to heavy metals; however, its adaptability to heavy metal was not considered in this study. Bold's basal medium (BBM) was selected to incubate the microalgae [7]. The components of BBM are shown in Table 1.

Table 1. The components of BBM media for incubate microalgae

BBM media components	
KH_2PO_4	175 mg/L
$CaCl_2*H_2O$	25 mg/L
$MgSO_4*7H_2O$	75 mg/L
$NaNO_3$	250 mg/L
K_2HPO_4	75 mg/L
NaCl	25 mg/L
H_3BO_3	11.42 mg/L
Microelement stock solution	1 ml
Solution 1	1 ml
Solution 2	1 ml

Microelement stock solution components	
$ZnSO_4*7H_2O$	8.82 g/L
$MnCl_2*4H_2O$	1.44 g/L
MoO_3	0.71 g/L
$CuSO_4*5H_2O$	1.57 g/L
$Co(NO_3)_2*6H_2O$	0.49 g/L

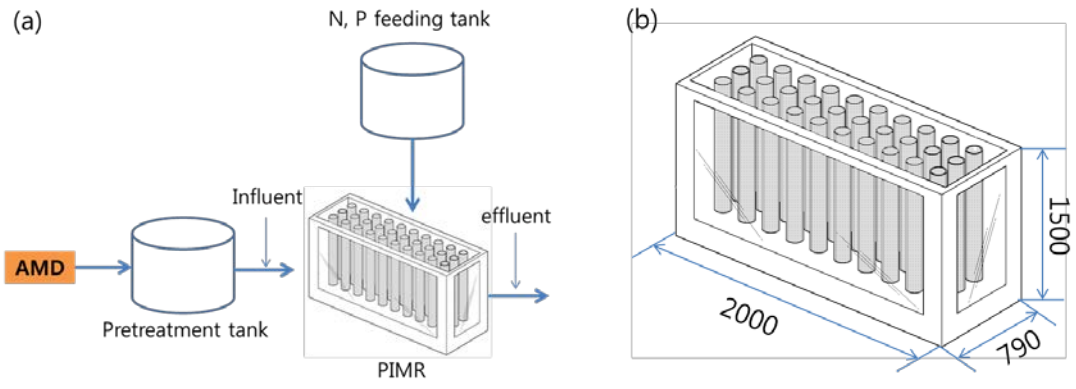
Solution 1	
Na_2EDTA	50 g/L
KOH	3.1 g/L

Solution 2	
$FeSO_4$	4.98 g/L
H_2SO_4	1 ml/L

2.2. PIMR and culture conditions

PIMR consisted of an open tank, 2000mm × 600mm × 1100mm, constructed from transparent tempered glass, with 30 acrylic pipes inserted at regular intervals (Fig. 1). The pipes could deliver light evenly although the incubated microalgae stuck to the reactor and interfered with light distribution. The light sources were sunlight and LED sticks. The LED sticks (147 μmol/m^2/s) were inserted into the pipes. Sunlight provided light from 10:00 to 16:00. The LED sticks were used from 16:00 to 10:00. The microalgae reactor without pipes could contain 1 ton of water with one input pipe for air flow to agitate the microalgae and provide carbon dioxide.

Figure. 1 : Schematic diagram of the pipes inserted microalgae reactor (a) and the size of PIMR (b)



2.3. Chemical treatment for AMD pretreatment

The selected abandoned coal mine, the Yeong-Dong mine, is located 200km from Seoul, along the northeastern border of the Tae-back Mountain. AMD was contaminated with 217.8 mg/L Fe and 5.7 mg/l Mn.

Calcium hydroxide ($Ca(OH)_2$) and magnesium hydroxide ($Mg(OH)_2$) were used to pretreat AMD. Depending on amount of $Ca(OH)_2$ and $Mg(OH)_2$ required and the reaction time, effectiveness of pretreatment were determined. For pretreatment of 1000 L AMD, air was injected into the reactor at a rate of 100 L/min. When the chemical reaction was complete, the treated AMD was transferred to a settling tank and the supernatant was stored.

2.4. Microalgae growth measurement.

Optical density (OD) and dry cell weight (DCW) were used to measure microalgae growth. OD was measured at 680 nm using a spectrophotometer (HS-3300; Humas, Daejeon, Korea). Microalgae growth by DCW was determined by: biomass productivity (*P),* as expressed in Eq. (1).

$$P = M_b - M_{b0}/T - T_0 \quad (5)$$

where M_b and M_{b0} are microalgae biomass at time T and starting time T_0 respectively

2.5. Analysis of various parameters.

To measure metal (Fe, and Mn) concentration, samples were collected and digested using organic matter and added sulfuric acid. The samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES 730; Varian Inc. Palo Alto, CA, USA).

To investigate the effect of anions in AMD, the sample was diluted 1/10, 1/100 and analyzed for sulfate (SO_4) by ion chromatography (850 Professional IC; Metrohm, Herisau, Switzerland). The phenanthroline method was employed to quantify ferrous iron [8]. All experiments were performed in duplicate and results were expressed as the mean value.

3. Results and Discussion

3.1. Batch test of *Nephroselmis* sp. KGE 8 in PIMR

Microalgae cultivation was performed in PIMR to examine the effects of initial concentrations of cations and anions in the pretreated AMD. Four types of pretreated AMD (Table 2) were used initially as culture media at lab scale. (For PIMR equal light energy and cell concentrations were initially provided to the cultures, which were cultivated for 25 days.) Time courses of cell growth and biomass productivity relative to the type of pretreatment influent are shown in [9]. For cell concentration in the second type of pretreatment influent, OD and DCW were 3.96 and 1.54g/L, respectively. In the control tanks, where microalgae were incubated in BBM media, the respective measurements were lower, i.e., OD was 3.54 and DCW was 1.38g/L. Biomass productivity (P) for the second type of pretreatment influent increased with increasing Fe concentration until 8.52 ±2.4.

The maximum biomass productivity (P) was 0.0602 g/L/day. These results indicate that Fe in AMD could promote biomass productivity. However, for the second and fourth types of pretreatment influents, higher Fe concentrations could inhibit microalgae growth.

Table 2. The change of AMD characteristics after pretreatment by Calcium hydroxide ($Ca(OH)_2$) and Magnesium hydroxide ($Mg(OH)_2$) [9]

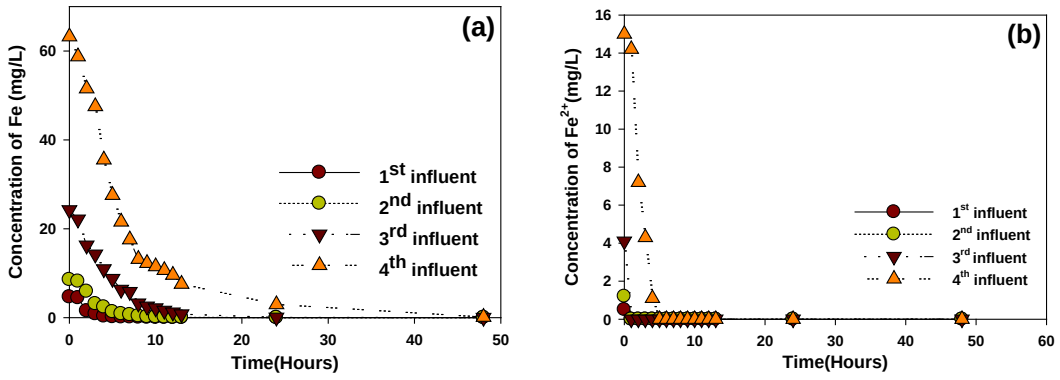
	Initial AMD	1st pretreatment	2nd pretreatment	3rd pretreatment	4th pretreatment
Fe	237.8 ±12.5 mg L^{-1}	4.64 ± 0.5 mg L^{-1}	8.52 ± 2.4 mg L^{-1}	24.21 ± 2.7 mg L^{-1}	20.51 ± 9.8 mg L^{-1}
Fe^{2+}	187.3 ± 8.6 mg L^{-1}	0.5 ± 0.1 mg L^{-1}	1.2± 0.2 mg L^{-1}	4.1 ± 0.9 mg L^{-1}	3.2 ± 1.4 mg L^{-1}
Mn	5.7 ± 1.5 mg L^{-1}	3.9 ± 0.7 mg L^{-1}	4.8 ± 0.5 mg L^{-1}	5.3 ± 0.6 mg L^{-1}	5.4 ± 0.5 mg L^{-1}
SO_4	320.4 ± 24.3 mg L^{-1}	214.6 ± 19.6 mg L^{-1}	252.9 ± 16.2 mg L^{-1}	294.2 ± 17.1 mg L^{-1}	311.7 ± 14.3 mg L^{-1}
NO_3	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}
PO_4	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}
T-N	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}
T-P	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}	< 0.1 mg L^{-1}
pH	3.7	7.1	6.2	6.0	5.7

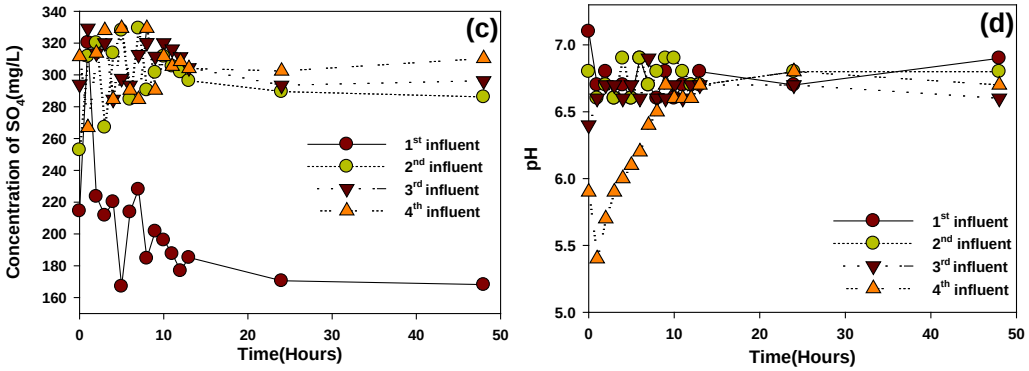
3.2. The removal of Fe in PIMR

Figure 2a, 2b, 2c shows that variation in total Fe, Fe (II), and SO_4 concentration in effluent. Fe could be removed through various processes that included (i) absorption of microalgae for cell growth [10], (ii) biosorption of metal ions on microalgae [11], and (iii) precipitation of metals inside the biological reactor [12]. A direct relationship was observed between the initial Fe concentration and the amount of Fe taken up; the higher the initial Fe concentration, the larger the amount of Fe taken up. The maximum Fe loading capacity of Nephroselmis sp. KGE 8 was found to be 59.92 mg/g for the fourth type of pretreatment wastewater (Fig. 2a). However, the pH in PIMR ranged from 5.4–7.1 (Fig. 2d) and precipitation could occur [13]. Therefore, it is significant that the PIMR system can remove both the insoluble Fe that remains in suspension and Fe precipitates in a sludge system.

The ferrous iron was removed immediately until 4.1±0.9 mg/L in the third pretreatment influent because ferrous iron oxidation occurred because of air input to PIMR. For the fourth pretreatment influent, ferrous iron was removed at 5 h reaction time in PIMR. Although the sulfate concentration varied during the reaction time, the Fig. 2c indicates that except for the first type of influent, sulfate concentration did not change appreciably, and 20% of the sulfate was removed when the first type of influent was used in PIMR. Some previous studies have reported the importance of sulfate reduction processes to AMD remediation [14]. In particular, it has been reported that dissimilatory sulfate reduction is an important mechanism in AMD purification [15].

Figure 2. Variation of influent (t=0) and sample in PIMR. (a) Concentration of total iron, (b) Concentration of ferrous (Fe^{2+}). (c) Concentration of Sulfate, (d) pH





3.3. Continuous operation of PIMR

On the basis of preliminary batch results for microalgae growth and efficient removal of metals (Fig.2), we employed the second type of pretreatment effluent as the influent to supply PIMR. Continuous Fe removal and microalgae cultivation were investigated by supplying the pretreatment influent and medium with 50 mg/L nitrate and phosphate based on the composition of BBM media. The flow rate for the second type of pretreatment influent used in PIMR was 47L/h. This influent was supplied with 50 mg/L nitrate and 50 mg/L phosphate from the supply tank. Retention time was 21.27hours.

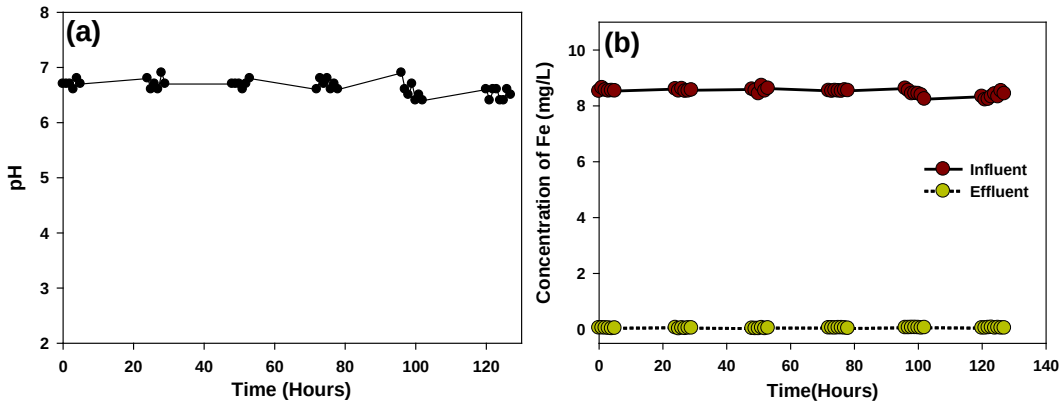
3.4. Variation in pH and removal of mineral.

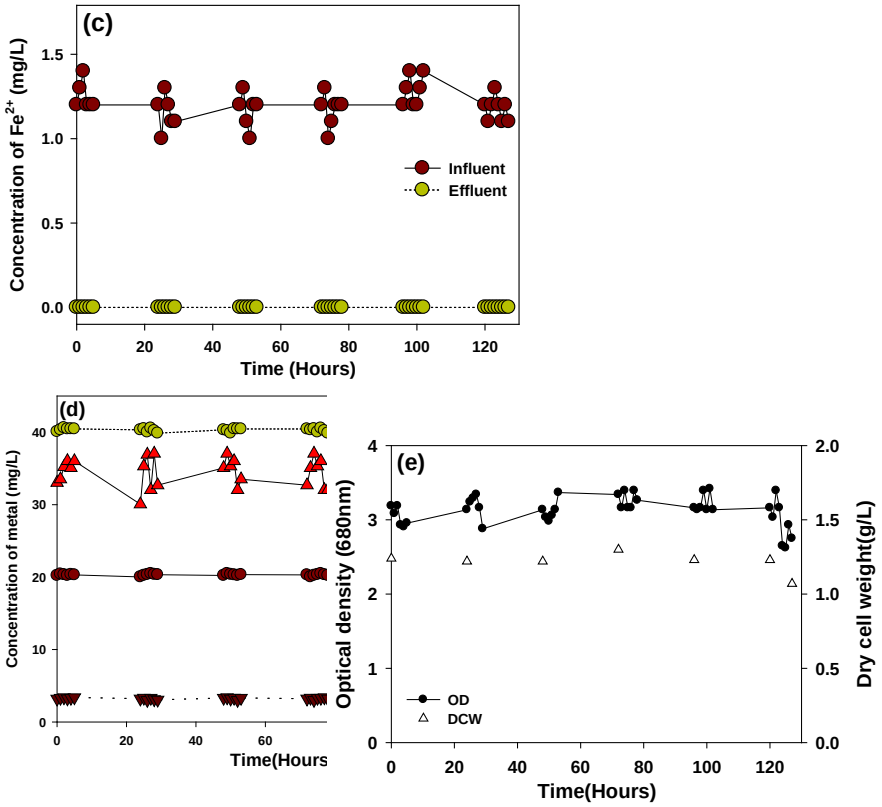
The results of the pH profile are graphically illustrated in Fig. 3a. The results indicate that the pH in PIMR was maintained from 6.4 to 6.8 over the lasting 120 hours. This pH condition could be optimal for microalgae growth and Fe removal.

Figure 3b and 3c shows the influent and effluent concentrations for both total Fe and ferrous iron. Fe concentrations in the influent were 8.21 - 8.64 mg/L and in the effluent were < 0.1 mg/L. Iron oxide particles were observed in the influent but not in the effluent.

There was 1.4 - 1.0 mg/L of ferrous iron in the influent. Ferrous iron was removed from the effluent. Ca, Mg, and Mn concentrations were 20.02 - 20.48 mg/L, 39.86 - 40.47 mg/L, and 3.02 - 3.38 mg/L, respectively (Fig. 3d). Although Ca, Mg, and Mn were taken up by the microalgae, their concentrations did not change because Ca and Mg were used for pretreatment and Mn occurs in AMD and accumulated in PIMR. Sulfate concentration was 267.13 - 329.42 mg/L and unlike the results for the batch test, did not change significantly.

Figure 3. pH profile (a) on effluent and removal of iron (b), ferrous (c) and calcium (Ca), magnesium (Mg), manganese (Mn) and sulfate (d) and variation of optical density (OD) and dry cell weight (DCW) (e) during continuous PIMR operating in 127 hours





3.5. Cell growth in continuous cultivation.

The time courses of biomass concentration for continuous cultivation are shown in Fig. 3e. In the batch test, OD and DCW for *Nephroselmis* sp. KGE 8 cultivation were 3.96 and 1.54g/L respectively. In the continuous cultivation mode, microalgae growth was maintained at OD 2.64 - 3.41 and DCW 1.07 - 1.30g/L for 25 days. OD decreased because few microalgae can persist in the effluent. However, the cells can be maintained during continuous growth. This study demonstrated that compared with the batch test, the continuous growth mode can achieve proximity harvest cell.

4. Conclusions

A PIMR containing pretreatment system including Ca and Mg, was developed and employed for microalgae-mediated heavy metal remediation. It reduced the initial high Fe and Mn concentrations released from AMD and supplied PIMR. The hybrid system was combined with a pretreatment system, and PIMR enhanced heavy metal reduction in AMD. It was economical in improving bioremediation and enhancing microalgae production.

A PIMR system was developed and operated for microalgae cultivation. The pipes in PIMR allowed effective light penetration and distribution. Moreover, PIMR could be used efficiently for Fe removal from AMD and microalgae cultivation. Batch studies showed that PIMR and microalgae can adsorb Fe with an uptake of 63.21 ±9.8 mg/L. Continuous studies also proved that PIMR can be used for metal remediation and microalgae cultivation.

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