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Removal of metal from acid mine drainage using a hybrid system including a pipes inserted microalgae reactor

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HIGHLIGHTS

- Active treatment and continuous PIMR system are combined for Fe removal from AMD.
- High Fe concentration in AMD is detrimental to microalgae growth and lipid accumulation.
- Pretreated AMD could be used as iron source for microalgae cultivation instead of artificial mineral culture medium.

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ABSTRACT

In this study, the microalgae culture system to combined active treatment system and pipe inserted microalgae reactor (PIMR) was investigated. After pretreated AMD in active treatment system, the effluent load to PIMR in order to *Nephroselmis* sp. KGE 8 culture. In experiment, effect of iron on growth and lipid accumulation in microalgae were inspected. The 2nd pretreatment effluent was economic feasibility of microalgae culture and lipid accumulation. The growth kinetics of the microalgae are modeled using logistic growth model and the model is primarily parameterized from data obtained through an experimental study where PIMR were dosed with BBM, BBM added 10 mg L^{-1} iron and 2nd pretreatment effluent. Moreover, the continuous of microalgae culture in PIMR can be available. Overall, this study indicated that the use of pretreated AMD is a viable method for culture microalgae and lipid accumulation.

1. Introduction

The pollution of surface water by acid mine drainage (AMD) from abandoned mines is a serious problem in South Korea. AMD normally contains high concentrations of Fe, Al, and H_2SO_4 (Cheng et al., 2009). In South Korea, main constituents of AMD from mining sites are Fe, Al, Mn, Ca, Mg, Si, and SO_4^{2-} and from among these high levels of Fe and Al are observed. The contaminated water in a nearby mining site has a red or red-brown color due to the precipitation of Fe. Pollution from these sources may have a detrimental effect on aquatic ecosystem and human.

AMD treatment system are classified into active and passive treatment systems and these treatment system depend on precipitation as the primary method for metal removal from AMD (Das et al., 2009; Johnson and Hallberg, 2005). The most widespread active treatment process uses chemical-neutralizing agents such as limestone, lime, sodium hydroxide, sodium carbonate, and magnesia (Van Hille et al., 1999). These alkaline material is added to AMD for raising the pH, which accelerates the chemical oxidation of metals, resulting in hydroxide and carbonate precipitates. In addition, active treatment systems require treatment facilities such as agitated reactor, precipitate reactor, clarifier, and thickeners. On the other hand, passive treatment systems have advantage of naturally occurring geochemical and biological process to remove AMD treatment with minimal operation and maintenance (Van Hille et al., 1999). Moreover, the limitations of passive treatment system are requirement of wide site for treatment and inappropriate application for treating large amount of AMD (Kalin, 2004). The representatives of passive treatment system are aerobic wetlands, compost reactors/wetlands, permeable reactive barriers, and packed bed iron-oxidation reactors (Johnson, 2006).

Recently, the usage of microalgae has been studied by many researcher and it can be applied to the treating wastewater with

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biosorption and producing biodiesel. Many researcher reported that various species of microalgae could be cultured by nutrient-rich wastewater sources and these produced high lipid yields (Prathiam Devi et al., 2011; Jiang et al., 2011). Furthermore, microalgae has ability of metal removal due to the metal uptake (bioaccumulation) from wastewater streams (Boke and Seda, 2010). Microalgae are more attractive for biodiesel production comparing with conventional oil crops, due to their high photosynthesis efficiency and lipid content (Li et al., 2011). The iron is an important factor for microalgae growth and metabolic activities (Liu et al., 2008). Behrenfeld et al. (2006) reported that iron could promote phytoplankton biomass in oceanic water.

This research investigates the combination of active treatment system using $Ca(OH)_2$ and $Mg(OH)_2$ and biodiesel production system (i.e., PIMR) using microalgae. Iron was used as major target metal for removal using both active treatment system and biodiesel production system. The effects of chemical dosage and reaction time in active treatment system were studied. Moreover, growth and lipid production of microalgae using pretreated AMD including iron were focused on this work. The function of residual iron was investigated to explain enhancing growth rate and lipid production of microalgae under pretreated AMD using active treatment system. In addition, continuous PIMR system for iron removal was also tested.

2. Methods

2.1. Microalgae selection and culture medium

2.1.1. Microalgae selection

The search for microalgae was isolated from a heavy metal-rich environment in an abandoned coal mine located in South Korea. The collected samples were observed microscopically at a magnification factor of 1500× (Fig. A.1, Supplementary data), and 15 new microalgae species were identified (Table A.1, Supplementary data). *Nephroselmis* sp. KGE 8 was selected for this study because of its tolerance to heavy metals; however, its adaptability to heavy metal was not considered in this study.

2.1.2. The components of microalgae culture medium

Bold's basal medium (BBM) was selected to incubate the microalgae. The BBM contains KH_2PO_4 175 mg, $CaCl_2{\cdot}H_2O$ 25 mg, $MgSO_4{\cdot}7H_2O$ 75 mg, $NaNO_3$ 250 mg, K_2HPO_4 75 mg, NaCl 25 mg, H_3BO_3 11.42 mg, Microelement stock solution 1 mL, Solution 1 1 mL, and Solution 2 1 mL L^{-1} as described by Fabregas et al. (2000). The microelement stock solution contains $ZnSO_4{\cdot}7H_2O$ 8.82 g, $MnCl_2{\cdot}4H_2O$ 1.44 g, MoO_3 0.71 g, $CuSO_4{\cdot}5H_2O$ 1.57 g, and $Co(NO_3)_2{\cdot}6H_2O$ 0.49 g L^{-1}. In addition, Solution 1 also contains Na_2EDTA 50 g and KOH 3.1 g L^{-1}, and Solution 2 contains $FeSO_4$ 4.98 g and H_2SO_4 1 mL L^{-1}.

2.2. The measurement of *Nephroselmis* sp. KGE 8 growth according to various concentration of iron (Lab-scale)

Five hundred milliliter culture flasks containing 250 mL BBM were supplemented with increasing iron concentrations from 5 to 70 mg L^{-1} using iron sulfate hydrate. The culture flasks were seeded with 1 mL inoculum from the enrichment culture and incubated at 30 °C and 150 rpm for 1 week in a shaking incubator (IS-971R, JeioTech, Seoul, Korea). A fluorescent light with 53.17 W m^{-2} intensity was employed as the light source for incubation.

2.3. The microalgae culture in PIMR using pretreatment AMD (Pilot-scale)

2.3.1. PIMR and culture conditions

PIMR consisted of an open tank, 2000 mm × 790 mm × 1500 mm, constructed from transparent tempered glass, with 30 acrylic pipes inserted at regular intervals (Fig. A.2, Supplementary data). 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 (58.33 W m^{-2}) were inserted into the pipes. Sunlight (82.38–160.23 W m^{-2}) 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. The temperature in PIMR was 22–27 °C, and amount of aeration for PIMR was 20 L min^{-1}.

2.3.2. Pretreatment of AMD using calcium hydroxide and magnesium hydroxide

The active treatment system is the standard in the AMD treatment industry (Dold et al., 1980). The process consist of 3 steps, aeration, neutralization, precipitation. The principle of neutralization of acid mine drainage lies in the in solubility of heavy metals in alkaline conditions. By controlling pH to a typical set-point of 7, metals are precipitated.

Pretreatment conditions that would allow minerals that may affect microbial growth to remain were determined. For the determination of the comparative effectiveness of alkalinity to reduce iron in AMD, 0.3–0.4 mg L^{-1} of $Ca(OH)_2$ and $Mg(OH)_2$ were spiked to the system at 60 and 90 min.

2.3.3. The culture of microalgae using pretreatment AMD (PIMR, Batch-test)

The 50 mg L^{-1} nitrate and phosphate put in each four pretreatment effluent based on the composition of BBM media. The each batch test was progress during 25 days. The microalgae OD and DCW were 0.1 and 0.04 g L^{-1} was put in PIMR.

2.3.4. The culture of microalgae using pretreatment AMD (PIMR, Continuous-test)

On the basis of preliminary batch results for microalgae growth and efficient removal of metals (Fig. 6), the second type of pretreatment effluent was employed as the influent to supply PIMR. Continuous Fe removal and microalgae cultivation were investigated by supplying the pretreatment effluent and medium with 50 mg L^{-1} nitrate and phosphate based on the composition of BBM media. The flow rate for the second type of pretreatment influent used in PIMR was 47 L h^{-1}. This influent was supplied with 50 mg L^{-1} nitrate and 50 mg L^{-1} phosphate from the supply tank. Retention time was 21.27 h. Before operate continuous PIMR test, the microalgae was incubate until OD and DCW were 3.18 and 1.24 g L^{-1}.

2.4. The analyzed technique

2.4.1. The measurement of microalgae growth and lipid extraction

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). To measured DCW the microalgae in medium was separated by centrifugation at 3000 rpm for 20 min (1580R, Labogene, Korea) and separated microalgae was frozen at −70 °C by deep freezer (DFU-256CE, Operon, Korea). After freeze microalgae, the sample was lyophilized by lyophilizer (FDB-550, Operon, Korea). The calibration curve of cell dry weight versus optical density (Fig. 3) is