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Research paper

Harvesting microalgal biomass using magnesium coagulation-dissolved air flotation

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ABSTRACT

Coagulation with magnesium was found to be more effective for harvesting microalgae *Chlorella zofingiensis* with dissolved air flotation (DAF) than the use of Fe^{3+}, Al^{3+} or chitosan, and the required coagulant dosage was in the order Mg^{2+} < chitosan < Al^{3+} < Fe^{3+}. The Mg^{2+} dosage required depended on the growth phases and culture medium characteristics. An early exponential culture required the highest Mg^{2+} dosage (226 mg g^{-1}), while a late stationary culture required the lowest dosage (36 mg g^{-1}). HPO_4^{2-} and CO_3^{2-} in the culture medium competed with the microalgal cells for Mg^{2+} and increased the Mg^{2+} dosage necessary. No Mg^{2+} addition was required to harvest the freshwater microalgae *Scenedesmus dimorphus* grown in a pond with tap water with a high Mg^{2+} concentration or the marine microalgae *Nannochloropsis* sp. The critical coagulation pH ranged between 10.8 and 11.8, with a lower pH requirement at a higher Mg^{2+} concentration. Magnesium hydroxide precipitated with the harvested biomass; however, over 99.5% of the precipitated Mg^{2+} was recovered by washing the biomass with 0.1 M HCl. Microalgal harvesting with Mg^{2+} did not introduce extrinsic coagulant; thus, neither the biomass nor the medium was contaminated.

1. Introduction

Harvesting algal biomass with coagulation/sedimentation or coagulation/dissolved air flotation (DAF) has been considered a more cost-effective method for biofuel production than other harvesting methods such as filtration and centrifugation [1–4]. The selection of an appropriate coagulant is critical for low-cost and effective microalgal harvesting. Fe^{3+} and Al^{3+}, although widely used in algae containing water and wastewater treatment, are problematic because they reduce the quality of the microalgal biomass for food, feed and fertilizer and increase the complexity of lipid extraction and conversion for biofuels. The residual Fe^{3+} and Al^{3+} in DAF subnatant may affect microalgal growth [5,6] and cause problems with recycling the culture medium.

Microalgae can be self-flocculated (autoflocculation) with a pH increase [7–9] due to the chemical precipitation of calcium and/or magnesium salts at high pH [10]. Sukenik and Shelef [11] suggested that autoflocculation was caused by the precipitation of calcium phosphate but could be accurately simulated by the addition of a base. Vandamme et al. [8] stated the unique importance of magnesium (≥0.15 mM) for high pH-induced coagulation in the presence of a low phosphate concentration. As the pH increases, positively charged magnesium precipitates the microalgal cells via charge neutralization and sweep flocculation. It has been reported that the Mg^{2+} in fresh culture medium may not be sufficient and that additional Mg^{2+} is required for coagulation [9].

Magnesium coagulation followed by gravity sedimentation or flotation has been used for harvesting both marine and freshwater microalgae [12,13]. However, the interaction between Mg^{2+} and microalgal cells and the effects of HPO_4^{2-}, CO_3^{2-} are still not well understood. Compared with sedimentation, DAF uses small air bubbles (approximately 60 μm) to float the microalgal flocs, resulting in a higher processing rate and a higher solid content in the harvested biomass [14].

In this study, Mg^{2+} coagulation-DAF harvesting was evaluated with *Chlorella zofingiensis* and further tested with different microalgal strains. The harvesting efficiency and the metal concentration in the harvested biomass and subnatant were compared with Al^{3+}, Fe^{3+} and chitosan coagulation-DAF harvesting. The potential for recycling the culture medium and the coagulant was also

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investigated. The objective of this work was the development of an efficient Mg^{2+}-based coagulation method that closes the loop with respect to coagulant and water resources by elucidating 1) the mechanism of Mg^{2+} coagulation for the DAF harvesting of microalgal biomass and 2) the influence of Mg^{2+} coagulation on the quality of the harvested biomass and DAF subnatant.

2. Materials and methods

2.1. Microalgal culture

The freshwater microalgae *Chlorella zofingiensis* and *Scenedesmus dimorphus* and the marine microalgae *Nannochloropsis* sp. were cultured in modified BG11 (Table 1) and modified f/2 medium (Table 1) with a low initial NO_3^--N of 32 mg L^{-1}, respectively. Na^+, K^+, Ca^{2+}, Mg^{2+} were the primary cations in both media, and the primary anions were NO_3^-, HPO_4^{2-} and CO_3^{2-} for the modified BG11 and Cl^- and NO_3^- for the modified f/2 medium. Cultures were maintained in indoor 15 L panel reactors with a light path of 5 cm and continuous fluorescent illumination. The average light intensity on the reactor surface was 220 μmol m^{-2} s^{-1}. To study the effects of the growth phase, *C. zofingiensis* was harvested at 2, 4, and 8 days, and the culture dry weights were 1.0, 1.5 and 1.9 g L^{-1} with the microalgal cells in the early exponential, late exponential, and late stationary phase, respectively, as identified by cell number [15]. The organic matters in the recycled medium were quantified using a total organic carbon (TOC) analyzer, and further characterized using Fluorescence Excitation-Emission Matrix (FEEM). To triger the Mg^{2+} coagulation-DAF harvesting by pH increasing for the cultures which has a high Mg^{2+} concentration, a *Scenedesmus dimorphus* culture grown in an outdoor 1500 L open pond with 128 mg L^{-1} NO_3^--N and a daily maximum light intensity of 2000 μmol m^{-2} s^{-1} was also evaluated. Tap water (28.2 ± 3.8 mg Mg^{2+} L^{-1} and 85.2 ± 8.6 mg Ca^{2+} L^{-1}) was used for open pond inoculation and for refilling to compensate for evaporation.

2.2. Microalgal harvesting

Mg^{2+} coagulation-DAF harvesting was evaluated using a DAF jar tester (DBT6, EC Engineering, Canada). For cultures in which the Mg^{2+} concentration was insufficient, Mg^{2+} was added to the culture with rapid mixing ($G = 200\ s^{-1}$). A pre-determined amount of 1 M NaOH was then added to reach the target pH. Slow mixing was maintained for 10 min for flocculation ($G = 30\ s^{-1}$). Flotation was initiated by the injection of supersaturated water after flocculation, and mixing was stopped immediately after bubble injection. After 10 min, 50 mL DAF subnatant was taken for dry weight (g L^{-1}) measurement. The harvesting efficiency was calculated on the basis of the dry weight of the microalgal biomass in the subnatant and in the original medium.

The change in DAF-harvesting efficiency with the change in the Mg^{2+} concentration can be described by the dose-effect model or logistic function shown in the following equation:

$$\eta = A_1 + \frac{A_2 - A_1}{1 + \exp(A_0 - B \cdot X(\eta))}$$

where η is the DAF-harvesting efficiency, $X(\eta)$ is the Mg^{2+} dosage (mg/g) at an efficiency η, and A_1, A_2, A_0 and B are regression parameters obtained using a least-squares method.

To explore the effects of major ions in the freshwater culture medium on the Mg^{2+} coagulation-DAF harvesting, late exponential *C. zofingiensis* was collected by centrifugation at 9000*g* for 10 min. The microalgal pellet was resuspended in deionized water. Different concentrations of Ca^{2+} and Mg^{2+} were added to the microalgal-deionized water suspension to obtain 0.22 mM HPO_4^{2-} and 0.19 mM CO_3^{2-} values, respectively, and then the pH was adjusted to 11.5 for DAF harvesting. The effects of 0.2 mM Ca^{2+} and 0.002 mM Na_2EDTA on Mg^{2+} coagulation-DAF harvesting were further studied. The concentrations of HPO_4^{2-}, CO_3^{2-} and Na_2EDTA were the same as those in the BG11 medium, while the Ca^{2+} concentration was maintained at 80 mg L^{-1}, which was 10 times higher than that in the BG11 medium (8.0 mg L^{-1}).

Chitosan (Sigma-Aldrich 417963-100G, practical grade), Al^{3+} as aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$), and Fe^{3+} as ferric sulfate ($Fe_2(SO_4)_3$) coagulation were compared with Mg^{2+} for the harvesting of late-exponential *Chlorella zofingiensis*. The pH was adjusted to an optimal value of 7.0 ± 0.2, 6.2 ± 0.2, and 8.0 ± 0.2 for chitosan, aluminum, and iron, respectively, with 1 M NaOH [16]. Coagulant dosages were normalized by microalgal biomass dry weight and reported as mg coagulant ion g^{-1} dry biomass.

To explore the mechanisms of the effectiveness of Mg^{2+} coagulation, the zeta potential of the microalgal surface was measured. A microalgae pellet collected by centrifugation was resuspended in a 0.01 M $NaNO_3$ solution to maintain an ionic strength of 0.01 M and eliminate the effects of the medium on the microalgal surface. The zeta potential of the microalgal cells was measured using a ZetaPlus Analyzer (DLS, Brookhaven Instrument Corporation).

2.3. Measurement of the metal concentration in the DAF subnatant and harvested biomass

DAF-harvested biomass and subnatant were analyzed for metal concentration. After DAF harvesting of late-exponential *C. zofingiensis* with 100 mg Mg^{2+} g^{-1}, 220 mg Al^{3+} g^{-1}, 400 mg Fe^{3+} g^{-1} and 140 mg chitosan g^{-1}, 0.6 g (dry equivalent) of harvested biomass and 50 mL subnatant were collected. Triplicate harvesting experiments were conducted and three biomass and medium samples were taken for metal analysis. After filtration through a 0.7 μm glass fiber filter, the Ca^{2+}, Mg^{2+}, Al^{3+} and Fe^{3+} concentrations (mg L^{-1}) in the DAF subnatant were measured using ICP-OES (Thermo iCAP6300).

The DAF-harvested biomass was centrifuged to remove the residual culture medium. Approximately 0.2*g* (dry equivalent) of the centrifuged biomass was freeze dried for metal analysis. Another

Table 1
Original composition and concentrations of cations and anions in freshwater culture medium modified BG11 and marine culture medium modified f/2.

		BG11		f/2	
		mg L^{-1}	mM	mg L^{-1}	mM
Cations	Na^+	459	19.97	10994	478.02
	K^+	17.16	0.44	399.75	10.25
	NH_4^+	0.54	0.03	0.84	0.05
	Mg^{2+}	7.2	0.30	1332.5	55.52
	Ca^{2+}	8.0	0.20	430.5	10.76
	Fe^{3+}	1.02	0.14	1.31	0.02
	Mn^{2+}	0.495	0.0090	0.099	0.0018
	Zn^{2+}	0.05	0.0008	0.0104	0.0002
	Cu^{2+}	0.019	0.0003	0.005	0.0001
	Co^{2+}	0.010	0.0002	0.006	0.0001
Anions	NO_3^-[a]	141.7	2.29	141.7	2.29
	Cl^-	7.74	0.22	19731	555
	$H_2PO_4^{2-}$	NA	NA	3.42	0.04
	SO_4^{2-}	2.98	0.03	4.69	0.00
	CO_3^{2-}	11.3	0.19	NA	NA
	MoO_4^{2-}	0.26	0.00	0.01	0.00
	HPO_4^{2-}	21.12	0.22	NA	NA
	SiO_3^{2-}	NA	NA	8.06	0.11
	BO_3^{3-}	2.71	0.05	NA	NA
	$C_6H_5O_7^{3-}$	10.11	0.06	NA	NA
	EDTA	0.58	0.002	6.79	0.02

[a] NO_3^-–N concentrations were reduced to 32 mg L^{-1} for nitrogen starvation.