


Article

Effects from Converter Slag and Electric Arc Furnace Slag on Chlorophyll a Accumulation of *Nannochloropsis* sp.

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Abstract: The effects of Ca^{2+} in converter slag and electric arc furnace slag leaching solutions on CO_2 content, the effects of CO_2 content in solutions on the accumulation of chlorophyll a of *Nannochloropsis* sp., and the mechanisms were studied. Results showed that addition of 50 mg/L converter slag leaching solution and 400 mg/L electric arc furnace slag leaching solution (lower than 50 vol% concentration) promoted the accumulation of chlorophyll a of *Nannochloropsis* sp., while an increased concentration of 70 vol% inhibited its accumulation. The highest concentration of chlorophyll a was obtained on the addition of 10 vol%. With 10 vol% leaching solutions added, chlorophyll a concentration for converter slag reached 4.2 mg/L, 1.45 times as much as that of pure F/2 medium, and chlorophyll a concentration for electric arc slag reached 3.2 mg/L, 1.10 times as much as that of pure F/2 medium. Under the same addition proportion, the promoting or inhibiting effect from converter slag on the accumulation of chlorophyll a of *Nannochloropsis* sp. was more obvious than that for electric arc furnace slag. The reason for the promotion of chlorophyll a accumulation of *Nannochloropsis* sp. was that an increase of Ca^{2+} in the solutions led to an increase of CO_2 , thereby promoting the photosynthetic rate of microalgae. The study provided new ideas for the green application of solid waste and industrial production of microalgae.

Keywords: leaching solutions; converter slag; electric arc furnace slag; *Nannochloropsis* sp.; chlorophyll a; CO_2 content



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1. Introduction

Slag is a kind of solid waste produced during the process of steelmaking, composed of slagging material, smelting reactant, eroding and shedding furnace body and fettling materials, impurity substances brought in by metal charge, and slagging materials, which are added on purpose to adjust properties of slag [1]. According to steel making technology, slag can be divided into converter slag, electric arc furnace slag (EAF slag), and open hearth furnace slag [2]. According to the world steel statistics in 2021 from the World Steel Association, the world output of crude steel reached 1.878 billion tons in 2020, in which converter crude steel accounted for 73.2% and EAF slag accounted for 26.3%. Producing 1 ton of crude steel would produce 10–15% of slag [3]. Piling up was the main disposal method of slag, which not only occupied a lot of land resources, but also heavy metal elements in slag might dissolve out and enter soil and water bodies because of rainwater leaching, threatening the ecological environment and human health [4]. Currently, the modes of resource utilization of slag mainly include their use in the preparation of steel slag cement [5,6], building materials [7,8], ceramic material [9,10], absorption materials of CO_2 , and so on [11,12]. However, because of problems of large fluctuation, poor activity, poor grindability, and poor stability of slag, the utilization rate of slag in all the countries in the world, especially in developing countries, is still at a low level.

Generally speaking, microalgae refer to a kind of microorganism with microscopic size, rich nutrition, and high photosynthetic utilization degree, widely living in sea and on land [13]. Microalgae, which is easy to cultivate, has features of wide variety, wide distribution, strong adaptability to environment, short growth cycle, and high absorption of N and P [14]. Microalgae, not competing with agriculture for land, could grow on wasteland and beach land, and biomass yield per unit area was several times as much as that of higher plants [15]. Currently, microalgae are mainly used in medical food [16], renewable energy [17], wastewater purification [18], and other areas. The main chemical elements of slag include CaO, SiO₂, MgO, Al₂O₃, FeO, and so on [19]. Microalgae contain several kinds of nutrient elements that are necessary for the growth of microalgae, such as Fe, P, Si, Ca, Mg, and so on. Thus, slag can provide necessary nutrition for the growth of microalgae. Using slag to cultivate microalgae can not only reduce the cost of culturing microalgae but also relieve substantial stacking of slag. According to reports of Nakamura et al. [20], if an appropriate proportion of Fe, Si, P, and N were added into seawater, some types of phytoplankton would grow quite fast. Because of the low cost, sufficient quantity, sufficient mineral concentration, and ionic properties, slag is the best source of this kind of mineral. Table 1 lists the best growth condition of different types of microalgae. Researchers have found that when relatively low concentration of slag or leaching solution of slag was added into an aquatic environment, the kinds of microalgae whose growth could be promoted were *Thalassiosira guillardii* (the best addition concentration of slag was 33 mg/L [21]), *Chaetoceros gracile* (the best addition ratio of leaching solution of slag was 20–40% [22]), *Rhodomonas lens* (the best addition ratio of leaching solution of slag was 20–40% [22]), *Emiliania huxleyi* (the best addition ratio of leaching solution of slag was 20–40% [22]), and *Chlorella* sp. (the best addition concentration of slag 25 mg/L [23] and the best addition ratio of leaching solution of slag was 30% [24]). When a relatively high concentration of slag or leaching solution of slag was added into the aquatic environment, the kinds of microalgae whose growth could be promoted were *Skeletonema costatum* (the best addition concentration of slag was 100 mg/L [25] and the best addition ratio of leaching solution of slag was 40–80% [20,22]), *Thalassiosira allenii* (the best addition ratio of leaching solution of slag was 40–80% [22]), *Isochrysis galbana* (the best addition ratio of leaching solution of slag was 40–80% [22]), *Thalassiosira angulate* (the best addition ratio of leaching solution of slag was 100% [22]) and *Amphidinium carterae* (the best addition ratio of leaching solution of slag was 100% [22]). When an arbitrary concentration of slag or leaching solution of slag was added into the aquatic environment, the kinds of microalgae whose growth could be promoted were *Dunaliella tertiolecta* [22], *Tetraselmis tetrahele* [22], *Synechococcus* sp. [22], *Desmodesmus subspicatus* [26], *Chlorella vulgaris* [26], *Alexandrium tamarense* [25], and *Nitzschia laevis* [27]. Nevertheless, in spite of the research and explorations on the effects of slag on the growth of microalgae, the reason for the influence of steel slag leaching solution on the growth of microalgae was still unclear.

Table 1. The best growth condition of microalgae.

Types of Microalgae	Types of Growth Condition	Best Growth Condition	Reference
<i>Thalassiosira guillardii</i>	Slag	33 mg/L	[21]
<i>Chaetoceros gracile</i>	Leaching Solution of Slag	20 vol%–40 vol%	[22]
<i>Rhodomonas lens</i>	Leaching Solution of Slag	20 vol%–40 vol%	[22]
<i>Emiliania huxleyi</i>	Leaching Solution of Slag	20 vol%–40 vol%	[22]
<i>Chlorella</i> sp.	Slag	25 mg/L	[23]
	Leaching Solution of Slag	30 vol%	[24]
<i>Skeletonema costatum</i>	Slag	100 mg/L	[25]
	Leaching Solution of Slag	40 vol%–80 vol%	[20,22]

Table 1. Cont.

Types of Microalgae	Types of Growth Condition	Best Growth Condition	Reference
<i>Thalassiosira allenii</i>	Leaching Solution of Slag	40–80%	[22]
<i>Isochrysis galbana</i>	Leaching Solution of Slag	40–80%	[22]
<i>Thalassiosira angulate</i>	Leaching Solution of Slag	100%	[22]
<i>Amphidinium carterae</i>	Leaching Solution of Slag	100%	[22]
<i>Dunaliella tertiolecta</i>	Slag or Leaching Solution of Slag	Arbitrary	[22]
<i>Tetraselmis tetrathele</i>	Slag or Leaching Solution of Slag	Arbitrary	[22]
<i>Synechococcus</i> sp.	Slag or Leaching Solution of Slag	Arbitrary	[22]
<i>Desmodesmus subspicatus</i>	Slag or Leaching Solution of Slag	Arbitrary	[26]
<i>Chlorella vulgaris</i>	Slag or Leaching Solution of Slag	Arbitrary	[26]
<i>Alexandrium tamarense</i>	Slag or Leaching Solution of Slag	Arbitrary	[25]
<i>Nitzschia laevis</i>	Slag or Leaching Solution of Slag	Arbitrary	[27]

This research selects converter slag and electric arc furnace slag as representatives, which are commonly used in the process of steelmaking, and aims to study effects and mechanisms of converter slag and EAF slag leaching solutions on the accumulation of chlorophyll a of *Nannochloropsis* sp. In order to compare the effects of accumulation of chlorophyll a under different adding proportions of converter slag and EAF slag leaching solutions and to explore the mechanisms of effects of steel slag leaching solution on the accumulation of chlorophyll a, different proportions of converter slag and EAF slag leaching solutions were added into F/2 medium to continuously culture *Nannochloropsis* sp. Through various characterization means, the main components of steel slag leaching solution, the effects of accumulation of chlorophyll a of *Nannochloropsis* sp., and the mechanisms and effects of steel slag leaching solution on the accumulation of chlorophyll a were analyzed and explained.

2. Materials and Methods

2.1. Materials

Table 2 lists the chemical compositions of converter slag and EAF slag used for this study. Converter slag (dried at 105 °C for 12 h, particle size 106–212 µm) from one steel plant in Tangshan (Hebei, China) was used, and the chemical composition included calcium oxide (46.60 wt%), ferric oxide (31.52 wt%), silicon dioxide (8.25 wt%), phosphorus pentoxide (2.29 wt%), manganese oxide (1.47 wt%), magnesium oxide (4.10 wt%), alumina (1.34 wt%), chromium trioxide (0.94 wt%), vanadium pentoxide (1.75 wt%), and titanium dioxide (0.51 wt%). EAF slag (dried at 105 °C for 12 h, particle size 106–212 µm) from one steel plant in Lishui (Zhejiang, China) was used, and its chemical composition included calcium oxide (27.89 wt%), ferric oxide (2.09 wt%), silicon dioxide (34.57 wt%), phosphorus pentoxide (0.01 wt%), manganese oxide (5.96 wt%), magnesium oxide (4.81 wt%), alumina (3.81 wt%), chromium trioxide (16.95 wt%), vanadium pentoxide (0.26 wt%), and titanium dioxide (2.59 wt%). *Nannochloropsis* sp. (GY-H14 *Nannochloropsis* sp. bacterial strain) from Guangyu Biological Technology Co., Ltd. (Shanghai, China) was used. Table 3 lists the chemical reagents used for this study. Chemical reagents cupric sulfate (≥99%), sodium nitrate (≥99%), calcium hydroxide (≥95%), sodium fluoride (≥98%), potassium bromide (≥99%), anhydrous sodium sulfate (≥99%), anhydrous sodium carbonate (≥99.8%), potassium phosphate dibasic (≥99%), magnesium sulfate (≥99%), ethylenediamine tetra acetic acid disodium salt (≥99%), sodium hydroxide (≥96%), and anhydrous calcium chloride (≥96%) from SHENTAI Chemical Industry Co., Ltd. (Tianjin, China); magnesium chloride hexahydrate (99%) and sodium bicarbonate (99%) from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China); iron chloride hexahydrate (99%), manganese chloride tetrahydrate (99%), cobalt nitrate hexahydrate (99%), calcium chloride dihydrate (≥99.9%), cobalt

chloride hexahydrate (99%), strontium chloride hexahydrate (99.5%), sodium hydrogen phosphate dihydrate (99%), sodium metasilicate nonahydrate (99%), iron citrate (99%), vitamin B1 (98%), vitamin B12 (98%), and D-Biotin (vitamin H) (>98%) from Macklin Biochemical Technology Co., Ltd. (Shanghai, China); sodium chloride ($\geq 99.5\%$), magnesium oxide ($\geq 98\%$), boric acid ($\geq 99.5\%$), and ethanol absolute ($\geq 99.7\%$) from YONGDA Chemical Reagent Co., Ltd. (Tianjin, China); molybdenum trioxide ($\geq 99.5\%$) from Guanfu Fine Chemical Research Institute (Tianjin, China); potassium chloride ($\geq 99.5\%$) from Baishi Chemical Industry Co., Ltd. (Tianjin, China); zinc sulfate heptahydrate ($\geq 99.5\%$) from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China); and hydrochloric acid (36–38%) from Kaixin Chemical Co., Ltd. (Tianjin, China) were selected in this experiment.

Table 2. Chemical compositions of converter slag and EAF slag used for this study, (wt%).

Types of Slag	CaO	Fe ₂ O ₃	SiO ₂	P ₂ O ₅	MnO	MgO	Al ₂ O ₃	Cr ₂ O ₃	V ₂ O ₅	TiO ₂
Converter Slag	46.60	31.52	8.25	2.29	1.47	4.10	1.34	0.94	1.75	0.51
EAF Slag	27.89	2.09	34.57	0.01	5.96	4.81	3.81	16.95	0.26	2.59

Table 3. Chemical reagents used for this study.

Reagents	Purity	Manufacturer	Location
Cupric Sulfate	$\geq 99\%$	SHENTAI Chemical Industry Co., Ltd.	Tianjin, China
Sodium Nitrate	$\geq 99\%$		
Calcium Hydroxide	$\geq 95\%$		
Sodium Fluoride	$\geq 98\%$		
Potassium Bromide	$\geq 99\%$		
Sodium Sulfate Anhydrous	$\geq 99\%$		
Sodium Carbonate Anhydrous	$\geq 99.8\%$		
Potassium Phosphate Dibasic	$\geq 99\%$		
Magnesium Sulfate	$\geq 99\%$		
Ethylenediamine Tetraacetic Acid Disodium Salt	$\geq 99\%$		
Sodium Hydroxide	$\geq 96\%$		
Calcium Chloride Anhydrous	$\geq 96\%$		
Magnesium Chloride Hexahydrate	99%	Aladdin Biochemical Technology Co., Ltd.	Shanghai, China
Sodium Bicarbonate	99%		
Iron Chloride Hexahydrate	99%	Macklin Biochemical Technology Co., Ltd.	Shanghai, China
Manganese Chloride Tetrahydrate	99%		
Cobalt Nitrate Hexahydrate	99%		
Calcium Chloride Dihydrate	$\geq 99.9\%$		
Cobalt Chloride Hexahydrate	99%		
Strontium Chloride Hexahydrate	99.5%		
Sodium Hydrogen Phosphate Dihydrate	99%		
Sodium Metasilicate Nonahydrate	99%		
Iron Citrate	99%		
Vitamin B1	98%		
Vitamin B12	98%		
D-Biotin (Vitamin H)	>98%		
Sodium Chloride	$\geq 99.5\%$	YONGDA Chemical Reagent Co., Ltd.	Tianjin, China
Magnesium Oxide	$\geq 98\%$		
Boric Acid	$\geq 99.5\%$		
Ethanol Absolute	$\geq 99.7\%$		
Molybdenum Trioxide	$\geq 99.5\%$	Guanfu Fine Chemical Research Institute	Tianjin, China
Potassium Chloride	$\geq 99.5\%$	Baishi Chemical Industry Co., Ltd.	Tianjin, China
Zinc Sulfate Heptahydrate	$\geq 99.5\%$	Kemiou Chemical Reagent Co., Ltd.	Tianjin, China
Hydrochloric Acid	36–38%	Kaixin Chemical Co., Ltd.	Tianjin, China

2.2. Preparation of Steel Slag Leaching Solution

One liter of ultrapure water was added into a one-liter conical flask (ultrapure water machine, Exceed-Ad-32, Aike Environmental Protection Equipment Co., Ltd., Chengdu, China). Next, 50 mg converter slag and 400 mg EAF slag were soaked in ultrapure water to form 50 mg/L converter slag solution and 400 mg/L EAF slag solution, respectively. The pH value of the converter slag solution and the EAF slag solution was adjusted to 6 using hydrochloric acid. Slag was continuously oscillated in the solution of adjusted pH value (air bath thermostat, THZ-82A, Jinyi Instrument Technology Co., Ltd., Changzhou, China) and was soaked for 48 h. Solution that was soaked for 48 h was filtered by filter water (0.45 μm aperture) to remove slag particles. Filtered converter slag and EAF slag leaching solutions were used in follow-up experiments.

2.3. Artificial Seawater and F/2 Medium

2.3.1. Artificial Seawater

Table 4 lists the compositions of artificial seawater used for this study. The artificial seawater has the following compositions (g/1 L ultrapure water): 21.1939 NaCl; 3.55 NaSO₄; 0.5993 KCl; 0.2935 NaHCO₃; 0.08627 KBr; 0.02297 H₃BO₃; 0.00275 NaF; 9.5922 MgCl₂·6H₂O; 1.0143 CaCl₂; 0.02186 SrCl₂·6H₂O.

Table 4. Compositions of artificial seawater used for this study, (g/1 L ultrapure water).

Compositions	NaCl	NaSO ₄	KCl	NaHCO ₃	KBr	H ₃ BO ₃	NaF	MgCl ₂ ·6H ₂ O	CaCl ₂	SrCl ₂ ·6H ₂ O
Content	21.1939	3.55	0.5993	0.2935	0.08627	0.02297	0.00275	9.5922	1.0143	0.02186

2.3.2. F/2 Medium

Table 5 lists compositions of F/2 medium used for this study. The F/2 medium has the following compositions (mg/1 L artificial seawater): 75 NaNO₃; 5.62 NaH₂PO₄·2H₂O; 30 Na₂SiO₃·9H₂O; 4.36 Na₂-EDTA; 3.15 FeCl₃·6H₂O; 0.0098 CuSO₄·5H₂O; 0.022 ZnSO₄·7H₂O; 0.01 CoCl₂·6H₂O; 0.018 MnCl₂·4H₂O; 0.1 Vitamin B1; 0.0005 Vitamin B12; 0.0005 Vitamin H.

Table 5. Compositions of F/2 medium used for this study, (mg/1 L artificial seawater).

Compositions	NaNO ₃	NaH ₂ PO ₄ ·2H ₂ O	Na ₂ SiO ₃ ·9H ₂ O	Na ₂ -EDTA	FeCl ₃ ·6H ₂ O	CuSO ₄ ·5H ₂ O	ZnSO ₄ ·7H ₂ O	CoCl ₂ ·6H ₂ O	MnCl ₂ ·4H ₂ O	Vitamin B1	Vitamin B12	Vitamin H
Content	75	5.62	30	4.36	3.15	0.0098	0.022	0.01	0.018	0.1	0.0005	0.0005

2.4. Culturing of *Nannochloropsis* sp.

On a pro rata of 10, 30, 50, and 70 vol%, converter slag and EAF slag leaching solutions were mixed with F/2 medium into 1 L. Then, 5 vol% of *Nannochloropsis* sp. was added and continuously cultured for 15 days in light incubator (GXZ-500, Ningbo Jiangnan Instrument Factory, Ningbo, China) under culture conditions of LD circulation (12 h light/12 h darkness), illumination intensity of 4000 lux and temperature of 23 °C. In this experiment, F/2 medium without adding leaching solution of slag was designated as the control group. All the experiments in this research were carried out under constant light conditions, and the experimental devices are represented in Figure 1 below.

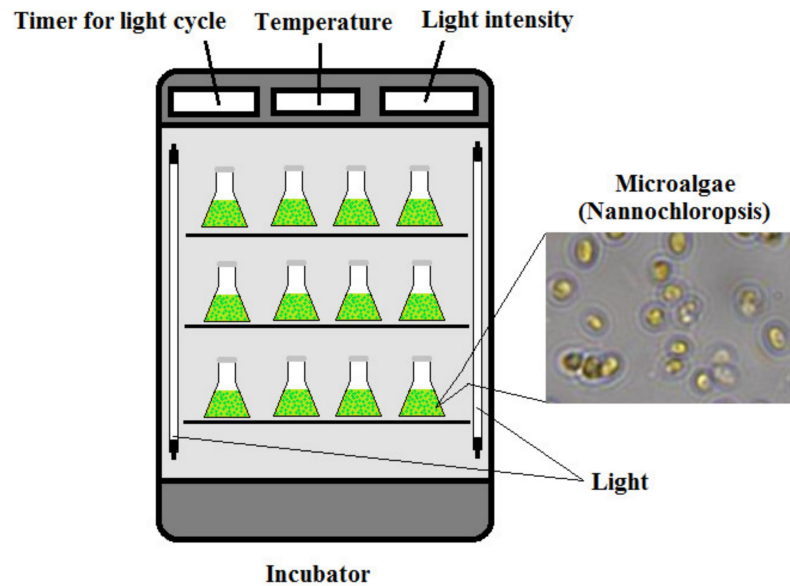


Figure 1. Experimental system used to culture microalgae in this study.

2.5. Measurement of Chlorophyll A Concentration

Twenty milliliters of liquid was taken from each sample that cultured *Nannochloropsis* sp. and a filter membrane (0.45 μm) was used to filter it. After filtration, the filter membrane was taken out and put into a 50 mL liquid storage bottle after being cut into pieces. Next, 3600 μL of 95% ethanol was added to the liquid storage bottle and then 400 μL of distilled water was added. After being mixed evenly, it was heated in an oil bath (constant temperature heating magnetic stirrer, DF-101S, LICHEN-Bx Instrument Technology Co., Ltd., Shanghai, China) for 15 min at 75 $^{\circ}\text{C}$ and cooled naturally to room temperature. When there was no obvious green on the filter membrane, chlorophyll a was considered to have been extracted completely. A Disposable syringe and a 0.22 μm filter membrane were used to transfer the filtrate from the liquid storage bottle to a cuvette. An ultraviolet spectrophotometer (U-T3, Yipu Instrument Manufacturing Co., Ltd., Shanghai, China) was used to measure and record the absorbance under wavelengths of 649, 665, and 750 nm. According to Formula (1), concentration of chlorophyll a was calculated.

$$\text{Chlorophyll a (mg/L)} = 5.24 \times (A_{665} - A_{750}) + 22.4 \times (A_{649} - A_{750}) \quad (1)$$

2.6. Characterization of Steel Slag and Steel Slag Leaching Solution

Chemical compositions and content of converter slag and EAF slag used in this experiment were characterized by X-ray fluorescence spectrum (XRF, ZSX Primus II 03030429, Rigaku Corporation, Tokyo, Japan).

The contents of main elements in converter slag and EAF slag leaching solutions and the contents of Ca^{2+} and Mg^{2+} in F/2 medium containing steel slag leaching solution before and after culturing *Nannochloropsis* sp. were characterized by inductively coupled plasma mass spectrometry (ICP-MS, 7800 type inductively coupled plasma mass spectrometry, Agilent Technologies Inc., Santa Clara, CA, USA).

The pH values of the F/2 medium, converter slag, and EAF slag leaching solutions were characterized by a pH meter (PHS-3C, Yueping Scientific Instrument Co., Ltd., Shanghai, China).

Contents of CO_2 (aq) of the F/2 medium, converter slag, and EAF slag leaching solutions were characterized by a carbon dioxide concentration meter (PXS- CO_2 type, Feng Pro Electronic Technology Co., Ltd., Shanghai, China).

2.7. Effects of Components in Steel Slag Leaching Solution on Concentration of CO₂ (aq)

CO₂ (aq) and HCO₃⁻ in aqueous solution exist in the form of carbonate, as shown in Formulas (2)–(4):



In the above-mentioned forms of carbonate, CO₂ (aq) is necessary for aquatic photosynthetic organisms, including phytoplankton. After quantitative measurement of chemical elements in the steel slag leaching solution, the concentration of CO₂ (aq) was also tested. This experiment did not include algae to remove the effects from algae on the concentration of CO₂ (aq) in the data.

In this research, under acid condition (pH ≤ 4.0), HCO₃⁻ and CO₃²⁻ could be estimated as CO₂ (aq). By adding a solution of adjustable pH value (333 mM citrate and 1.41 M sodium chloride) (pH 1.3), all the forms of carbonate, except CO₂ (aq) were transformed into CO₂ (aq). Then, estimation was finished by using the total amount of carbonate. The concentrations of CO₂ (aq), HCO₃⁻ and CO₃²⁻ were calculated from the total amount of carbonate and pH value according to Henderson–Hasselbalch Formulas (5) and (6):

$$\text{pH} = \text{pK}_1 + \log \left(\frac{[\text{HCO}_3^-]}{[\text{CO}_2(\text{aq})]} \right) \quad (5)$$

$$\text{pH} = \text{pK}_2 + \log \left(\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} \right) \quad (6)$$

Since it was difficult to distinguish CO₂ (aq) from H₂CO₃ (aq) in solution, [H₂CO₃ (aq)] in Formula (3) was expressed as CO₂ (aq) in Formula (5). In the formula, pK₁ = 6.35, pK₂ = 10.33.

2.8. Effects of Concentrations of Ca²⁺ and Mg²⁺ on Concentration of CO₂ (aq)

Briefly, 50, 100, 200, and 400 mg calcium and magnesium oxide were added into 1 L F/2 medium. Concentration of CO₂ (aq) under conditions of different concentrations of Ca²⁺ and Mg²⁺ was test by carbon dioxide concentration meter (PXS-CO₂ type, Feng Pro Electronic Technology Co., Ltd., Shanghai, China).

3. Results and Discussion

3.1. Main Components of Steel Slag Leaching Solution

Since it is difficult to distinguish Fe compounds from Cr compounds in suspended metal solution after alkaline-fusion, the Fe compound and Cr compound in converter slag and EAF slag used in this experiment were called Fe₂O₃ or Cr₂O₃. According to the data in Table 2, the converter slag used in this experiment contained CaO, Fe₂O₃, P₂O₅, and V₂O₅, higher than that in EAF slag. In particular, for CaO, Fe₂O₃, and P₂O₅, the existing quantity in converter slag was much higher than that in EAF slag. Contents of SiO, MnO, Al₂O₃, Cr₂O₃, and TiO₂ in converter slag were lower than that in EAF slag. Especially for SiO₂ and Cr₂O₃, the existing quantity in converter slag was much lower than that in EAF slag. The presence of MgO in converter slag was not significantly different from that in EAF slag.

Table 6 lists the concentrations of main elements in converter slag and EAF slag leaching solutions. Concentration of Total Ca in converter slag and EAF slag leaching solutions were relatively high (5.63 and 2.22 mg/L, respectively), the reason of which was that in these two kinds of leaching solutions, the content of CaO was relatively high, reaching to 46.60 and 27.89 wt%, respectively. Content of Fe₂O₃ in converter slag was also relatively high (31.52 wt%). However, in converter slag leaching solution, the concentration

of Total Fe was very low, only 0.8×10^{-3} mg/L, the reason for which was the different solubility of different elements in water.

Table 6. Concentrations of main elements in converter slag and EAF slag leaching solutions, (mg/L).

Main Elements in Slag	Converter Slag Leaching Solution	EAF Slag Leaching Solution	Waste Synthetical Discharge Standard ^a	Standard of Drinking Water for Life ^b
Total Mg	5.1×10^{-2}	1.8×10^{-1}		4.5×10^2
Total Al	1.2×10^{-2}	3.1×10^{-2}		0.2
Total Ca	5.6	2.2		4.5×10^2
Total Fe	0.8×10^{-3}	0.4×10^{-3}		0.3
Cr (VI)	0.1×10^{-4}	0.7×10^{-4}	0.5	0.5×10^{-1}
Total Mn	0.1×10^{-2}	3.9×10^{-2}	0.1	0.1
Total V	6.8×10^{-2}	0.1×10^{-3}		
Total P	5.1×10^{-2}	0.8×10^{-3}	0.5	

^a Reference Standard: GB 8978-1996. ^b Reference Standard: GB 5749-2006.

In order to discuss the influence of converter slag and EAF slag leaching solutions on the water environment, the concentrations of main elements in converter slag and EAF slag leaching solutions were compared with the emission standard of the national integrated wastewater discharge standard (GB 8978-1996) and the national sanitary standard of drinking water for life (GB 5749-2006). After comparing the concentration of main elements in the leaching solutions of converter slag and electric arc furnace slag in Table 6 with the national integrated wastewater discharge standard (GB 8978-1996) and the national sanitary standard of drinking water for life (GB 5749-2006), it was found that concentrations of main elements in these two leaching solutions were lower than the two standards.

In this research, a steel slag leaching experiment was carried out using pH adjusted ultrapure water, which differs from the natural water in that it contains a variety of substances.

The experimental design might be too simple to elucidate the leaching phenomenon of steel slag in a natural aquatic environment. To prevent steel slag from polluting the natural aquatic environment, additional leaching experiments with natural water are required.

3.2. Effects of Steel Slag Leaching Solution on Accumulation of Chlorophyll A

The content of chlorophyll a, one of the indexes reflexing growth of microalgae, is usually used to measure the growth of microalgae. The increase of chlorophyll a fluorescence value in vivo in microalgae means that growth of microalgae is promoted, while the decrease means it is inhibited [28]. The chlorophyll a method is usually used to measure the biomass of microalgae. There is a significant positive correlation between chlorophyll a and biomass [29]. Thus, biomass increases with the increase of content of chlorophyll a.

Figure 2 shows the relations between concentration of chlorophyll a of *Nannochloropsis* sp. and addition of converter slag leaching solution. Results show that when the addition of converter slag leaching solution was less than 50 vol%, concentrations of chlorophyll a added to converter slag leaching solution were higher than that of control group. Concentration of chlorophyll a decreased with the increase of addition of converter slag leaching solution. Thus, when addition of converter slag leaching solution was less than 50 vol%, accumulation of chlorophyll a of *Nannochloropsis* sp. could be promoted. The promoting effect decreased with the increase of addition of converter slag leaching solution. When the addition of converter slag leaching solution was 10 vol%, the concentration of chlorophyll a reached the highest (4.2 mg/L), 1.45 times as much as that of control group, so when addition of converter slag leaching solution was 10 vol%, the promoting effect was the best. When the addition of converter slag leaching solution was 70 vol%, the concentration of chlorophyll a was lower than that of the control group, so under this addition, accumulation of chlorophyll a of *Nannochloropsis* sp. was inhibited.

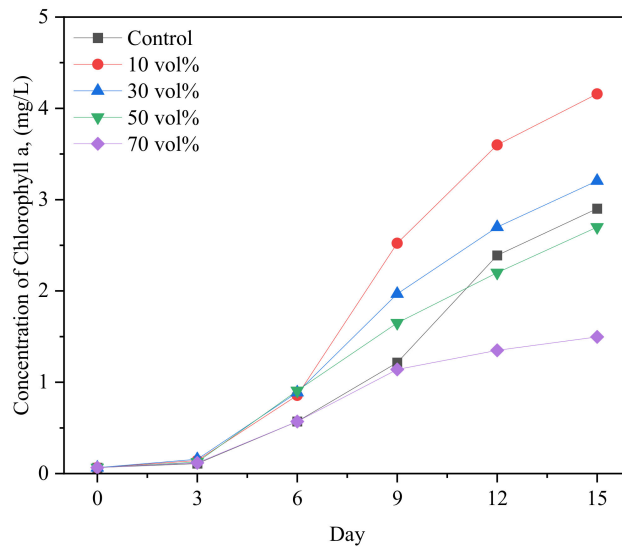


Figure 2. Effects of various additions (vol%) of converter slag leaching solution on concentration of chlorophyll a (mg/L).

Figure 3 shows the relations between the concentration of chlorophyll a of *Nannochloropsis* sp. and the addition of EAF slag leaching solution. Results show that when the addition of EAF slag leaching solution was less than 50 vol%, concentrations of chlorophyll a added to EAF slag leaching solution were higher than that of control group. The concentration of chlorophyll a decreased with the increase of amount of EAF slag leaching solution added. Thus, when the addition of EAF slag leaching solution was less than 50 vol%, accumulation of chlorophyll a of *Nannochloropsis* sp. was promoted. The promoting effect decreased with the increase of addition of EAF slag leaching solution. When addition of EAF slag leaching solution was 10 vol%, the concentration of chlorophyll a reached its highest level (3.2 mg/L), 1.10 times as much as that of control group, so when addition amount of EAF slag leaching solution was 10 vol%, the promoting effect was the best. When the addition amount of EAF slag leaching solution was 70 vol%, the concentration of chlorophyll a was lower than that of control group, so when the addition amount of EAF slag leaching solution was 70 vol%, the accumulation of chlorophyll a of *Nannochloropsis* sp. was inhibited.

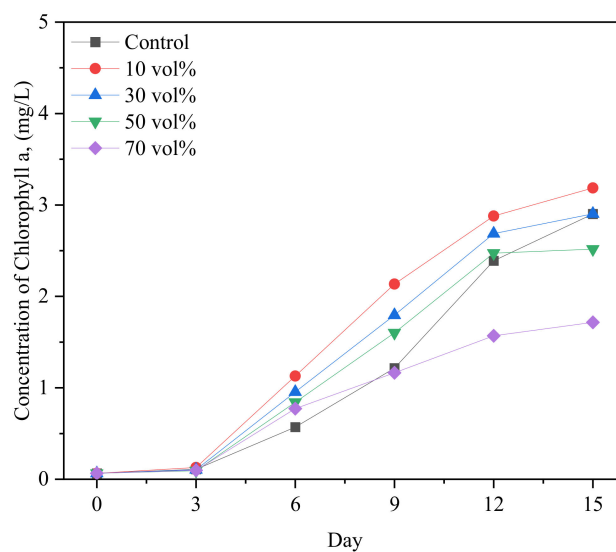


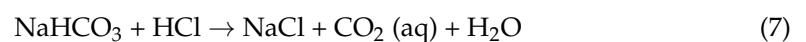
Figure 3. Effects of various additions (vol%) of EAF slag leaching solution on concentration of chlorophyll a (mg/L).

After comprehensively comparing the relationship between the concentration of chlorophyll a of *Nannochloropsis* sp. and the addition of converter slag and EAF slag leaching solutions, it was found that effects of converter slag and EAF slag leaching solutions on accumulation of chlorophyll a of *Nannochloropsis* sp. showed the same trend. When the addition of these two kinds of leaching solutions was less than 50 vol%, the accumulation of chlorophyll a of *Nannochloropsis* sp. was promoted, while when it was 10 vol%, the accumulation reached the highest. When the addition of these two kinds of leaching solution was 70 vol%, the accumulation of chlorophyll a of *Nannochloropsis* sp. was inhibited. Under the same addition proportion, the promoting or inhibiting effect on accumulation of chlorophyll a of *Nannochloropsis* sp. from converter slag leaching solution was more obvious than that for EAF slag leaching solution.

3.3. Effects of Elements in Steel Slag Leaching Solution on Concentration of CO₂ (aq)

Until now, it is still unclear why the accumulation of chlorophyll a in the medium containing steel slag leaching solution is higher than in that without steel slag leaching solution. To a great extent, accumulation of chlorophyll a of terrestrial plant, microalgae, and other photosynthetic organisms relies on photosynthetic efficiency. Photosynthesis can be mainly divided into two metabolic systems, that is, light-dependent reaction (attaining light energy from sunshine) and Calvin cycle (immobilizing CO₂ to synthesize glucose). An increase of CO₂ (aq) concentration can increase the photosynthetic rate, inhibit the respiration rate, promote the accumulation of chlorophyll a, and increase biomass [30]. The increase of CO₂ (aq) promoted microalgae to absorb CO₂ through photosynthesis [31]; however, the concentration of CO₂ (gas) in air determined the concentration of CO₂ (aq) soluble in water. Thus, the concentration of CO₂ (gas) was the key factor driving the rate of photosynthesis [32] not only for terrestrial plants using CO₂ (gas) directly but also for aquatic plankton using dissolved CO₂ (aq) in water. This research studies the concentration of CO₂ in relation to the Calvin cycle.

The mixture configured by NaHCO₃ and HCl was used as reference standard of CO₂ (aq). Emergence of CO₂ (aq) depended on the concentration ratio of these two materials. According to Formula (7), the calculation was performed.



CO₂ (aq) was measured by a PXS-CO₂ type carbon dioxide concentration meter, and the concentration of CO₂ (aq) under all the experimental conditions was tested (Figure 4). The pH value of the medium containing steel slag leaching solution was the same as that of the control group (pH = 7.3). According to the Henderson–Hasselbalch equation, under 10 vol% converter slag and EAF slag leaching solutions condition, the concentration of CO₂ (aq) in the medium containing 10 vol% of converter slag leaching solution was 3.8 mg/L, while it was 3.5 mg/L in the medium containing 10 vol% of EAF slag leaching solution, both higher than that of the control group (3.0 mg/L).

Thus, adding steel slag leaching solution can improve the water environment of microalgae. Compared with the experiment that only used F/2 medium, photosynthesis of microalgae could be further promoted. Additionally, compared with the control group, an increased concentration of CO₂ (aq) by adding steel slag leaching solution could further promote the accumulation of chlorophyll a of *Nannochloropsis* sp. (Figures 2 and 3). Compared with EAF slag leaching solution, converter slag leaching solution could better promote the accumulation of chlorophyll a of *Nannochloropsis* sp., which could also be explained by the different concentrations of CO₂ (aq) between converter slag and EAF slag leaching solutions.

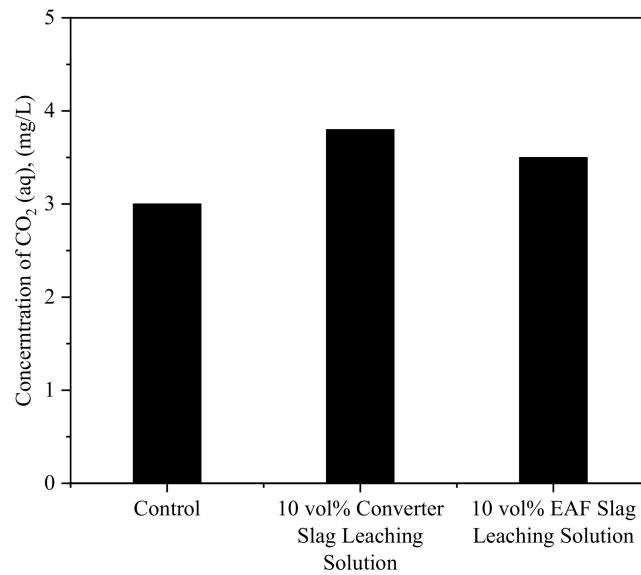
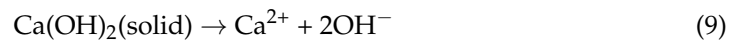
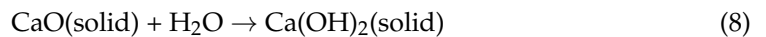


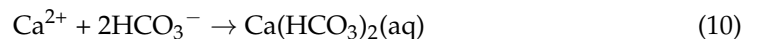
Figure 4. Concentrations of aqueous CO₂ in control, 10 vol% converter slag leaching solution and 10 vol% EAF slag leaching solution.

3.4. Relations between Concentrations of Ca²⁺ and CO₂ (aq) in Steel Slag Leaching Solution

The hardness of water is usually expressed by the contents of Ca²⁺ and Mg²⁺. Ca²⁺ and Mg²⁺ are alkaline-earth metals and easily react with carbonate. Meanwhile, contents of these elements in steel slag leaching solution are higher than that of other elements (Figure 2). Leaching process of CaO in slag can be assumed as the expressions in Formulas (8) and (9).



Although reactions of MgO and CaO could be assumed to be similar, the solubility of hydrated magnesium in water was much lower than that of hydrate calcium [33]. It was assumed that very low content of MgO (whose solubility in water is slightly higher than that of hydrated magnesium) could dissolve in water. Since all the experiments in this research were carried out under the condition of near neutral pH, though MgO could react with acid, reactivity of MgO leaching from slag was not high. As a result, from the perspective of the nature of MgO, MgO would not show high reactivity in the medium. Thus, this research studied the behavior of Ca²⁺ specifically. Figure 5 shows the relations between contents of different forms of carbonate calculated by the Henderson–Hasselbalch equation and pH value. In this research, under an experimental condition of pH = 7.3, among different the forms of carbonate, content of HCO₃[−] was the highest. As a result, Ca²⁺ in leaching solution might react with HCO₃[−], as is shown in Formula (10). According to Formulas (2) and (3), bicarbonate compound might help the solution absorb CO₂ from air.



Ca(HCO₃)₂ could completely ionize, thus in the form of HCO₃[−], Ca(HCO₃)₂ might disturb the chemical equilibrium among different forms of carbonate. The concentration ratio of different forms of carbonate in solution must be constant, an increase in the concentration of HCO₃[−] makes Formula (3) proceed to the left, causing the increase of CO₂ (aq), while the increased CO₂ (aq) might be consumed by microalgae as raw materials for photosynthesis.

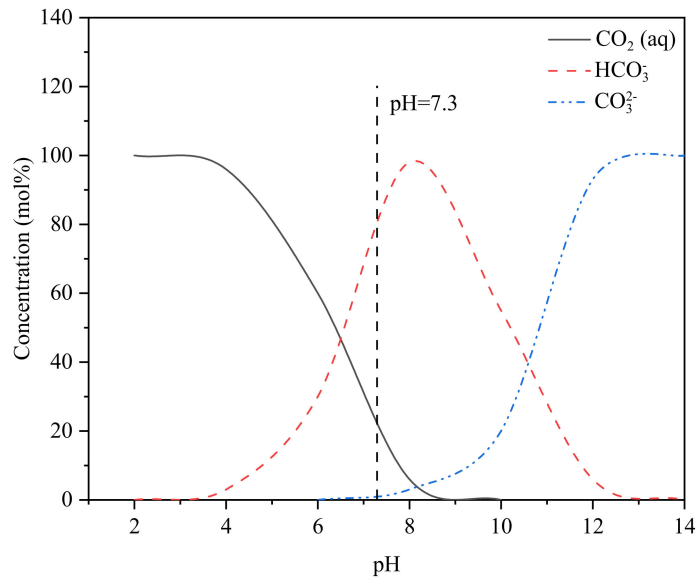


Figure 5. Concentrations of aqueous CO_2 , HCO_3^- , and CO_3^{2-} for each pH (mol%).

In order to test the relations between contents of Ca^{2+} and Mg^{2+} in leaching solution of slag and concentration of CO_2 (aq), 50, 100, 200, and 400 mg/L $\text{Ca}(\text{OH})_2$ and MgO were added into F/2 medium, respectively. Then concentration of CO_2 (aq) in compound was tested. As is shown in Figure 6, the concentration of $\text{Ca}(\text{OH})_2$ was related to the concentration of CO_2 (aq). The concentration of CO_2 (aq) increased with the increase in concentration of $\text{Ca}(\text{OH})_2$. However, since its solubility was relatively low, the concentration of MgO in the test concentration range showed no great effects on the concentration of CO_2 (aq) in the compound.

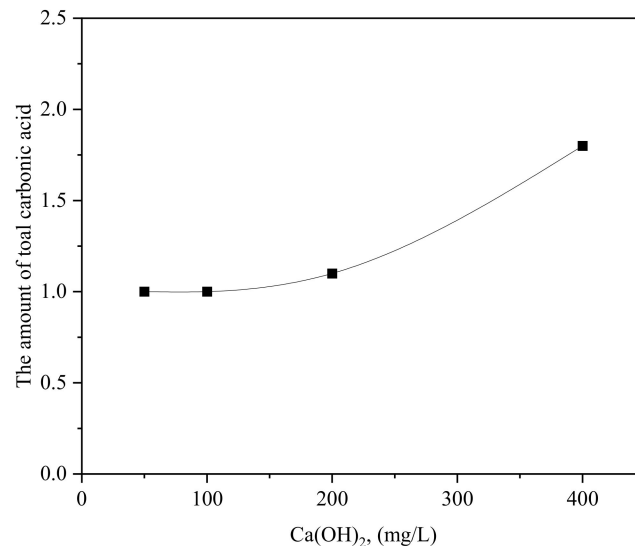


Figure 6. Relation between the amount of total carbonic acid and $\text{Ca}(\text{OH})_2$ content in solution.

With regard to effect of Ca^{2+} on concentration of CO_2 (aq), a part of Ca^{2+} in solution might remain in the medium, so it could not be completely absorbed by *Nannochloropsis* sp. In order to test effect of Ca^{2+} on concentration of CO_2 (aq), the concentrations of Ca^{2+} (Figure 2) in F/2 medium after the addition of converter slag and EAF slag leaching solutions before and after culturing *Nannochloropsis* sp. were tested, and concentrations of Mg^{2+} under the same condition were listed to be used for reference.

In this research, it was found that Ca^{2+} was a factor for promoting the increase of concentration of CO_2 (aq) in steel slag leaching solution. The experiment proved that adding $\text{Ca}(\text{OH})_2$ could increase the concentration of CO_2 (aq) (Figure 6). In the F/2 medium with the addition of converter slag and EAF slag leaching solutions before and after culturing *Nannochloropsis* sp., enough amount of Ca^{2+} and Mg^{2+} existed to react with carbonate (Table 7). Apart from $\text{Ca}(\text{OH})_2$ and MgO , some solutes in the steel slag leaching solution could also react in the F/2 medium to form complex ions and other molecular forms. These hypothetical molecular morphologies composed by second-group elements might be related with the increase of concentration of CO_2 (aq). Further research needs to be carried out to explain the reason due to which the increase of steel slag leaching solution can increase the total content of carbonate and which kinds of molecular forms relate to the increase of concentration of CO_2 (aq).

Table 7. Concentrations of Ca^{2+} and Mg^{2+} in F/2 medium containing steel slag leaching solution before and after culturing *Nannochloropsis* sp.

	Concentration of Ca^{2+} (mg/L)		Concentration of Mg^{2+} (mg/L)	
	Before Culturing	After Culturing	Before Culturing	After Culturing
Converter Slag Leaching Solution	371.14	271.71	1134.56	483.10
EAF Slag Leaching Solution	367.73	254.11	1134.07	471.38

4. Conclusions

This research carried out a leaching test of converter slag and EAF slag leaching solutions. Effects and mechanisms of steel slag leaching solution on *Nannochloropsis* sp. were explored. Conclusion of this research can be summed up in the following points.

- (1) Adding converter slag and EAF slag leaching solutions into the F/2 medium influenced the accumulation of chlorophyll a of *Nannochloropsis* sp. When the addition of these two kinds of leaching solutions was less than 50 vol%, the accumulation of chlorophyll a of *Nannochloropsis* sp. was promoted. While when addition was at 70 vol%, it was inhibited. When the addition of converter slag leaching solution was 10 vol%, accumulation of chlorophyll a reached the highest (4.2 mg/L), 1.45 times as much as that of control group. When 10 vol% of EAF slag leaching solution was added, accumulation of chlorophyll a was 3.2 mg/L, 1.10 times as much as that of the control group. Under the same adding ratio, promoting and inhibitory effects of converter slag leaching solution on accumulation of chlorophyll a were higher than that of EAF slag leaching solution.
- (2) The reason to the increase of chlorophyll a of *Nannochloropsis* sp. was that Ca^{2+} in converter slag and EAF slag leaching solutions increased the content of CO_2 (aq), then the rate of photosynthesis and the growth rate of algae were improved.

Results of this research show that the use of slag not only promotes the growth of aquatic photosynthetic organisms in aquatic environment but it also promotes the absorption of CO_2 through photosynthesis. Additionally, the increased CO_2 (aq) makes secondary contributions to enhance yields of fish and shellfish, because the increased CO_2 (aq) promotes the growth of both microalgae in aquatic ecosystem and that of fish and shellfish feeding on the phytoplankton.

Meanwhile, there are such elements as Fe, P, Ca, and Mg in the slag, which can be used as nutrients to promote the growth and reproduction of microalgae and to improve the accumulation of chlorophyll a and metabolites of microalgae. Thus, the use of slag is beneficial to decrease the cost of the culturing of microalgae.

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