Effects from Fe, P, Ca, Mg, Zn and Cu in Steel Slag on Growth and Metabolite Accumulation of Microalgae: A Review

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Abstract: Steel slag is the solid waste produced by the steelmaking process. At present, there are differences in the treatment and utilization of this waste among countries around the world. The massive accumulation of steel slag not only occupies land, but also the heavy metal elements in steel slag leached by rainwater cause serious pollution to the soil and groundwater, both which threaten the life and survival of the surrounding residents. More and more attention has been paid to the resource utilization of slag because of the gradual promotion of energy saving and emission reduction all over the world. Currently, the fields that utilize slag focus on recycling of steel waste, acting as sinter raw material, dephosphorization of hot metal, road and water conservancy project construction, wastewater treatment material, application of CO₂ capture and flue gas desulfurization or agriculture. Many researchers have carried out research and explorations on the effects of slag on microalgae’s growth and found that slag has enormous potential algal biomasses and huge advantages for promoting microalgae’s growth and the accumulation of metabolites. Under suitable conditions, slag can effectively promote microalgae’s growth and reproduction, as well as promote microalgae’s accumulation of metabolites, especially lipid accumulation. Thus, slag can be used as an ideal nutrient for microalgae. Culturing microalgae with slag can lower the cost and solve the problem of lacking Fe during the process of marine microalgae’s growth. Meanwhile, it can alleviate the phenomenon of the substantial stacking of slag. This study provides new methods for slag’s resource utilization.

Keywords: slag; solid waste utilization; microalgae; biodiesel; energy saving and emission reduction

1. Introduction
Steel slag is the solid waste produced by steelmaking process [1]. According to the data of the World Iron and Steel Association (2020) [2], the world crude steel output reached 1.864 billion tons in 2020. China, Europe, India, Japan and the United States are among the top five regions in the world in crude steel production, accounting for 77.6% of the total. At present, there are differences in the treatment and utilization of steel slag among countries in the world according to their own national conditions. The United States, Germany, Japan and other countries occupy a leading position in the world in the utilization of steel slag. In United States, the utilization rate of steel slag reaches 98%, which is mainly used for steel scrap recovery, sintering and blast furnace reuse, road construction, engineering backfilling and preparation of asphalt concrete aggregate [3,4]. In Germany, through the recycling of steel slag and its application in the cement industry and other fields, about 97% of steel slag is used as aggregate in highway and civil construction projects, which effectively reduces the remaining amount of steel slag [5,6]. According to the statistics of the Japan Iron and Steel Environment Bulletin, the utilization rate of steel slag in Japan is close to 100%. Most of the steel slag in Japan is treated by steam aging, and then used for paving, cement clinker and fertilizer. Among them, the use of steel slag
to repair seafloor water quality is an innovation developed by Japan for improving the marine environment [7,8]. The utilization rate of steel slag in China is lower than that in other developed countries. According to statistics, China’s total crude steel output was 928 million tons in 2019. The new produced steel slag almost reached 200 million tons, compared with the huge discharge of steel slag, meaning its utilization rate is only about 22% [9]. The massive accumulation of steel slag not only occupies land and pollutes the environment, but also the heavy metal elements in steel slag are leached by rainwater to cause serious pollution to the soil and groundwater, which threatens the life and survival of the surrounding residents [10]. With the development of circular economy and the gradual promotion of energy saving and emission reduction all over the world, more and more attention has been paid to the resource utilization of steel slag.

Microalgae refers to the kind of microorganism that is tiny in shape with chlorophyll a in cells and can process photosynthesis [11]. Microalgae, with a variety of kinds, is widely scattered in waters such as oceans and lakes, and has a strong adaptability to environment, a short growth cycle, a high absorption rate of N and P and is easy to cultivate [12]. Microalgae can be produced by wastelands and mudflat lands, leaving much land for agriculture, whose per unit yield is several times more than higher plants [13]. Its various metabolites are rich in such active ingredients as protein, grease and pigment [14]. It has such photosynthetic organs as chloroplast, which can directly convert solar energy into chemical energy. CO_2 sequestration efficiency is high. Algal biomass can be used for the production of energy materials [15]. The mainly factors influencing microalgae’s growth include light, pH value, metal ions, temperature, nutrients, carbon sources and so on. At present, microalgae’s application fields focus on medical food [16], renewable energy [17], wastewater purification [18] and so on. There are many nutrient elements in slag that are necessary for microalgae’s growth, such as Fe, P, Ca, Mg, etc., so slag can provide nutrition for microalgae’s growth. Culturing microalgae by slag can lower the cost and solve the problem of lacking Fe during the process of marine microalgae’s growth. Meanwhile, it can alleviate the phenomenon of the substantial stacking of slag. Culturing microalgae with slag as nutrition provides a new perspective for the industrialization of microalgae and the resource utilization of steel slag.

2. Composition, Feature and Utilization of Slag
2.1. Slag’s Chemical Composition and Mineral Composition

According to method of steelmaking, slag can be divided into converter slag, open hearth furnace slag and electric furnace slag. In accordance with production stage, it can be divided into steelmaking slag, casting slag and splashing slag [19]. Based on basicity value $R = \frac{CaO}{(SiO_2 + P_2O_5)}$, it can be divided into low basicity slag ($R < 1.8$), medium basicity slag ($R = 1.8~2.5$) and high basicity slag ($R > 2.5$) [20]. Due to shape, it can be divided into water quenched granular slag, lump slag and powder slag.

Slag’s chemical compositions mainly include SiO_2, CaO, Fe_2O_3, FeO, Al_2O_3, MgO, MnO and so on [21]. Its mineral compositions include $C_3S$, $Ca_3MgSi_2O_8$, $C_2S$, $C_4AF$, a solid solution containing the Fe compound and P compound, free CaO and so on [22]. Slag’s chemical compositions and corresponding basicity are the main reasons for the difference of mineral composition.

Su et al. [23] believed that the main minerals of low basicity slag included olivine, merwinite and RO phase ($CaO$-$FeO$-$MnO$-$MgO$ solid solution). Calcium hydrogen silicate and tricalcium silicate were the main minerals of medium alkalinity slag, and calcium silicate was the main mineral of high alkalinity slag.

2.2. Features of Slag
2.2.1. Cementitious Property

Slag’s chemical composition and mineral composition are similar to cement clinker, and it is also called overburned cement clinker. During the process of steelmaking, it is difficult for slag to undergo the process of hydration, because slag and liquid steel
experience the melt state together, resulting in coarse grains, C$_2$S and C$_3$S, which are the effective gelling active components in slag. Meanwhile, the existence of trace element oxides, such as P and S, inhibit the process of hydration. Content of RO phase (CaO–FeO–MnO–MgO solid solution) of non-gelling active components is high, taking about 20%–30% of slag mass fraction. Its overall gelling activity is low [24].

2.2.2. Grindability

Slag’s density is between 3.2 g/cm$^3$ and 3.9 g/cm$^3$. From the exterior, it is porous and loose. Since there is a high content of Fe oxides and the existence of metallic iron, it is hard and wear resistant. Meanwhile, mineral compositions of C$_2$S, C$_3$S and RO phase in slag are formed under the high temperature of 1600 °C, and the crystal is coarse and complete with a few defects and is hard to grind. As a consequence, slag’s grindability is low. With the standard that grindability index of standard sand is 1 as baseline, slag’s grindability index is only 0.75 [25].

2.2.3. Stability

Over crystallized f-CaO, f-MgO and RO phase are three main factors influencing slag’s volume stabilization. Hydrations of f-CaO and f-MgO are slow. Volumes of Ca(OH)$_2$ and Mg(OH)$_2$ produced under a water-rich environment expand 1.98–2.48 times, which may cause hidden security problems [26].

2.3. Utilization of Slag

2.3.1. Making Cement and Concrete

Existences of C$_3$S, C$_2$S and C$_4$AF confirm slag’s cementitious property. It is generally considered that slag’s cementitious property increases with the increase of alkalinity. Thus, the slag grained into fine powder can be used for admixture of cement and concrete [27]. Research showed that [28] cement added to 30% micro-powder slag met Turkey’s standard requirements for Portland cement. Huang et al. [29] found that cementitious materials could be produced by the main raw materials of phosphogypsum, steel slag, blast furnace slag and limestone. Wen et al. [30] found that compression strength of concrete added to slag reached 100 MPa and showed excellent resistance to chloride ion penetration. Chen et al. [31] observed that concrete added to grinded electric-arc-furnace slag showed an excellent reduction of water. Qasrawi et al. [32] found that the compression strength of concrete that used slag as a fine aggregate was 1.1–1.3 times higher than ordinary concrete. Papayianni et al. [33] found that high-strength (>70 MPa) concrete could be produced by electric-arc-furnace slag as an aggregate. Slag can also be used as a raw material for producing Portland cement [5] and Belite cement [34].

2.3.2. Road and Water Conservancy Project Construction

Since slag has such features as high strength and durability, it can be processed into an aggregate of high quality that can be on a par with a natural aggregate. Slag is suitable to be used as building material of water conservancy because of its high bulk density, high strength and high wear resistance. In Germany, about 400,000 tons of slag is used as aggregate to stabilize the river bank and riverbed to prevent them from erosion [35]. From 1993 on, the Nippon Slag Association has been devoted to the application of technology research on the utilization of steelmaking slag as foundation reinforcement materials for port construction, and in 2008, they published the “Guide of The Use In Port And Harbor Construction” [36]. The JFE SHOJI Trade Corporation [27] produced an artificial reef by using carbonized slag for seafood and coral cultivation (ocean block).

Slag can be used as aggregates not only for the surface layer of pavement, but also for the bulk base and sub-base, especially on asphalt surface [7]. In Japan and European countries, about 60% of slag is used for road projects, and in England, about 98% of slag is used as aggregates of cement and asphalt pavement.
2.3.3. Applications in Sinter Raw Material, CO\textsubscript{2} Capture and Flue Gas Desulfurization

Slag with more than 50% CaO content can be used as sintering flux and partially replace commercial lime. The addition of slag can improve sinter's quality, reduce fuel consumption produced by the exothermic of Fe and FeO oxidation reaction and reduce the cost of sinter [37]. In America and Germany, the amounts of slag used as sinter raw materials were more than 56% and 24%, respectively [38]. In China, Baoshan Iron and Steel Group (Bao Steel) has been recycling slag used for sintering since 1996. Currently, slag that can be recycled every year has stabilized at 150,000 tons [39].

There is a lot of CaO in slag. Consequently, under mild temperatures and CO\textsubscript{2} pressure conditions, it is possible to store CO\textsubscript{2} in the form of carbonate through the use of slag slurry [40]. Slag is high in CaO, especially free CaO, which can be used for desulphurization. Liu et al. [41] found that desulfurizer that came from the mixing of slag, fly ash and plaster together in definite proportions had the effect of desulphurization. Through theoretical research, Feng et al. [42] testified that it was feasible to condense flue gas by using slag to desulphurize. Ding et al. [43] researched the wet flue gas desulfurization with waste slag and powder slag and found that through reasonable design and proper operation, the rate of wet desulfurization with slag could reach more than 60%. However, this technique is still in the laboratory research phase currently.

2.3.4. Use of Slag in Agriculture

There are necessary elements for crops’ growth, such as Si, Ca and P. During the smelting process, after high-temperature calcination, slag’s solubility increases and is easy to be absorbed by botanies. As a consequence, it is widely used in agriculture. In developed countries such as Germany, America, France, Japan and so on, converter slag is used in producing silicon fertilizer, phosphate fertilizer and trace fertilizer [44]. In China, the first slag fertilizer project invested by Taiyuan Iron and Steel Co., Ltd. (Taiyuan, Shanxi, China) and America Harsco Corporation (Camp Hill, Pennsylvania, USA) began to be constructed in 2011. Practice shows that slag phosphate fertilizer is not only suitable for acid soil and phosphorus-deficient alkaline soil, but also can be used in paddle fields and dry lands, which can achieve good fertilization effects [45]. Since the contents of Ca and Mg are high in slag, it can be used as acid soil modifier, whose alkalinity can make up soil’s acidity [46]. Deng [47] researched the improvement effects of slag on polymetallic-polluting soil. The experiment showed that the slag particles used were fine and contents of heavy metals in crops decreased.

3. Leaching Behavior of Slag

Understanding the dissolution behavior of elements in slag plays an important role in resource utilization of slag. Mombelli et al. [48] found that the ratio of water to slag was a critical factor influencing heavy metal release. Takahashi et al. [49] carried out a leaching test of electric-arc-furnace stainless steel slag and electric-arc-furnace common steel slag. Research findings showed that except for Se in the leaching solution of electric-arc-furnace stainless steel slag, concentrations of all the elements in the leaching solution of the two kinds of slags were lower than Japan’s national discharge standard, environmental quality standard of soil, environmental quality standard of wastewater and drinking water and environmental quality standard of ocean and water pollution. Researchers carried out a leaching test of slag samples from 58 American steel plants and found that all of the leached samples met the safety standards of the American government [50]. As a result, slag can be classified as a kind of harmless byproduct in steel industry and recycled.

4. Influences of Slag on the Growth of Microalgae

There are such elements as Fe, P, Ca and Mg in slag, which can be used as nutrients to promote the growth and reproduction of microalgae and improve the accumulation of the growth and metabolites of microalgae. Thus, the use of slag was beneficial to the decreased cost of the cultivation of microalgae.
4.1. Influences of Fe in Slag on the Growth and Accumulation of Metabolites of Microalgae

There are a lot of iron oxides and iron complexes in slag. Fe in slag is the essential trace element that is needed in the metabolic processes of photosynthesis respiration and an important electron acceptor in microalgae’s photosynthesis. Fe, which is one of the main factors limiting the growth of phytoplankton, plays a critical role in the growth of microalgae. Fe can enhance the ability of microalgae’s nitrogen reduction and fixation, which plays an important role in microalgae’s effective use of C, N and P, as well as in chlorophyll’s biosynthesis and photosynthesis [51].

Proper concentration of iron can promote microalgae’s growth and proliferation. The research findings show that when desulfurization slag was added in the reactor, total iron content of the reactor dramatically increased. Iron concentration increased with the increase of slag concentration. When the amount of slag was over 100 mg/L, pH > 11, Chlorella sp.’s growth was inhibited. When the concentration of 25 mg/L desulfurization slag was added, total iron concentration of the solution was 1.1 mg/L. Compared with the control group of 0.1~0.2 mg/L total iron concentration, concentration of iron increased significantly, and total organic carbon of Chlorella sp. was 56 mg/L. When the concentration of 300 mg/L desulfurization slag was added, total iron concentration of the solution was 4 mg/L and total organic carbon of Chlorella sp. was 41 mg/L, because Fe released by slag and heavy metals of an extremely low concentration stimulated the increase of Chlorella sp.’s biomass, while high-concentration slag would release more heavy metal ions, resulting in the increase of pH, thus inhibiting Chlorella sp.’s growth. Chlorella sp. in the reactor grew through absorbing total iron [52].

In the ocean, especially in the open sea, since land supply and dissolved iron are limited, photosynthesis is limited because of the lack of Fe. To deal with iron stress, slag can be used as marine algae’s iron source, taking use of iron’s quick oxidation and sedimentation to promote marine algae’s growth [53]. Research has found that the iron source released by 20 mg slag was enough for two kinds of marine diatoms, Thalassiosira nordenskioeldii and Thalassiosira oceanica, to grow for 50 days. Fe or Fe$^{2+}$ released by slag and an inorganic iron reagent (FeCl$_3$·6H$_2$O) could effectively promote these two kinds of marine diatoms’ cell proliferation [54].

Slag is different from other inorganic iron reagents, such as Fe powder and ferrous sulfate, which can release dissolved iron ions and colloid Fe (III) and maintain the iron concentration of a solution. Compared with other iron sources, slag has a longer maintaining time. Additionally, slag can also release P and Si into a solution. Including the Fe released, the extended Redfield ratio of P, Si and Fe released by slag into the solution was 1:15:0.0075, which was the average ratio of main nutrients that composed phytoplankton cells [55]. During the process of the use of slag to cultivate Thalassiosira guillardii, it was found that, with slag as the Fe source for the proliferation of Thalassiosira guillardii, the utilization rate of Fe of Thalassiosira guillardii was about 1% of Fe content in slag. With slag as the P source for the proliferation of Thalassiosira guillardii, the utilization rate of P of Thalassiosira guillardii was less than 68% of the P content in slag. When the addition amount of slag was less than 50 mg/L, pH of the solution was not affected [56].

Fe is the essential component for redox reaction in cells and plays an important role in cell respiration, photosynthesis and the process of metalloprotein’s catalytic reaction [57]. Research has shown that Fe plays an important role in micoralgae’s growth and oil accumulation. Xia et al. [58] found that when the concentration of Fe$^{3+}$ was $1 \times 10^{-4}$ mol/L, the growth of Phaeodactylum tricornutum was the fastest and lipid content was the most, reaching up to 47.3%, which was 4.2 times higher than the Phaeodactylum tricornutum cultivated without Fe$^{3+}$. When the concentration of Fe$^{3+}$ was $1 \times 10^{-5}$ mol/L, the growth rate of Isochrysis galbana was the fastest, and the lipid content reached up to 41.9%, which was 2.8 times higher than the Isochrysis galbana cultivated without Fe$^{3+}$. Yeesang et al. [59] found that under the combination conditions of nitrogen deficiency, 82.5 µE/m²s light intensity and 0.74 mM Fe$^{3+}$ concentration, the lipid content of Botryococcus spp. was improved to 35.9%. Zhang et al. [60] studied the effects of Fe on the growth and lipid
accumulation of *Desmodesmus* sp. WC08. The biomass concentration, lipid content and lipid production rate of *Desmodesmus* sp. WC08 were tested when Fe concentration was 0 mmol/L, 0.5 × 10⁻⁵ mmol/L, 1 × 10⁻⁵ mmol/L, 2 × 10⁻⁵ mmol/L, 4 × 10⁻⁵ mmol/L, 6 × 10⁻⁵ mmol/L and 10 × 10⁻⁵ mmol/L. Research findings showed that *Desmodesmus* sp. WC08’s biomass was less affected by the concentration of Fe. When the concentration of Fe was 2 × 10⁻⁵ mmol/L, *Desmodesmus* sp. WC08’s biomass reached the highest value at 2.38 ± 0.21 g/L, which was 3 times as much as without Fe. Under other concentrations of Fe, the accumulation of *Desmodesmus* sp. WC08’s biomass was relatively low. From the perspective of lipid content, Fe concentration had an obvious effect on *Desmodesmus* sp. WC08’s lipid content. The lipid content showed a slowly increasing tendency when Fe concentration was 0~10 × 10⁻⁵ mmol/L. When Fe concentration was 10 × 10⁻⁵ mmol/L, the lipid content reached the highest value (31.99%). Except for the lowest lipid content group (0.5 × 10⁻⁵ mmol/L), it had no significant difference from the lipid contents of other groups. Analysis of the lipid content showed that the Fe concentration had significant influences on the lipid yield of *Desmodesmus* sp. WC08, which meant that the role Fe played in the process of lipid accumulation could not be ignored. In the range of test concentrations, the lipid yield increased with the increase of the Fe concentration. When the concentration was 2 × 10⁻⁵ mmol/L, the lipid yield reached the highest value (0.69 g/L), which was 1.4 times as much as that of blank group. However, compared with the 1 × 10⁻⁵ mmol/L group and the 1 × 10⁻⁴ mmol/L group, there was no significant difference. Consequently, when the Fe concentration was 2 × 10⁻⁵ mmol/L, it was the most helpful for *Desmodesmus* sp. WC08’s accumulation of biomass and lipids.

Different iron sources had different effects on microalgae’s growth. Research compared the influences of three kinds of iron compounds on *Auxenochlorella protothecoides*, that is, ferrous sulfate, ferric ethylenediaminetetraacetic acid (EDTA) and ferric chloride. When the concentration of ferric chloride was 1.15 mM, the content of saturated fatty acids (SFA) of *Auxenochlorella protothecoides* reached the highest value (78.5%). When the concentration of ferrous sulfate was 1.08 mM, content of biomass of *Auxenochlorella protothecoides* reached the highest value (1520 mg/L). Ferric chloride was the most toxic for *Auxenochlorella protothecoides*. When microalgae were cultivated under the condition of 0.2 mM and 14.4 mM ferrous sulfate concentration, as well as 7.19 mM ferric EDTA concentration and 0.07 mM~21.58 mM ferric chloride concentration, biodiesel with a satisfactory quality could be produced [61]. In another research study, researchers studied the influences of three kinds of iron compounds (ferric chloride, ferric EDT and ferric ammonium sulfate) and three kinds of ferrous compounds (ferrous chloride, ferrous EDTA and ferrous ammonium sulfate) on the growth and accumulation of metabolites of *Dunaliella tertiolecta*. It was found that with the increase of Fe concentration, both the growth rate and lipid content of *Dunaliella tertiolecta* had different degrees of increase, and then decreased under a high dose. The optimal cell growth rate could be achieved through the use of ferrous sulfate. Ferrous sulfate was helpful for the accumulation of carbohydrates. When ferric EDTA existed, the lipid content was higher than other iron sources and the carbohydrate content decreased. Ferric EDTA was helpful for the accumulation of microalgae oil, which could be used as the ideal nutrients for microalgae to produce biodiesel [62].

### 4.2. Influences of P in Slag on the Growth and Accumulation of Metabolites of Microalgae

There was a certain amount of P₂O₅ in the slag. P is an essential factor for the primary productivity of aquatic ecosystems, such as oceans and lakes, and is one of the basic nutrients that marine life relies on to grow, as well as being a necessary nutrient source for algae proliferation. P, the substrate and regulator, directly participated in all aspects of photosynthesis in our research, including light energy absorption, formation of assimilatory power, the Calvin cycle, transport of assimilates and playing a regulatory role in the activity of some key enzymes [63]. P, the main component of nucleic acid, proteins and phospholipids, was necessary for chlorophyll synthesis. Compared with nitrogen compounds, the mobility of phosphorus-containing compounds in the natural world was
The lack of P limited the growth rate of most microalgae; when P from the external environment was exhausted, microalgae would consume P reserved by itself until the lowest cell content of P that was suitable for growth was achieved [64]. To further research the reliability that whether slag could be used as nutrient source for marine microalgae and the promoting effects of decarbonization slag and dephosphorization on the growth of *Thalassiosira guillardii*, an experiment of *Thalassiosira guillardii* batch culture using decarbonization slag and dephosphorization slag, without controlling the pH value at the start of the study, was carried out. The research found that, when the slag addition was 33 mg/L, *Thalassiosira guillardii*'s chlorophyll-a fluorescence value increased dramatically. The use of slag boosted the *Thalassiosira guillardii*’s growth obviously. When the slag concentration increased by 100 times (3300 mg/L), *Thalassiosira guillardii*'s chlorophyll-a fluorescence value decreased. *Thalassiosira guillardii*’s growth was inhibited. This was because calcium precipitation in slag decreased the content of phosphate in the culture. Meanwhile, an excessive dose of slag might result in the increase of pH value of the seawater medium, which might greatly reduce the solubility of Fe in slag [65].

P was of great importance for microalgae’s growth. Without enough P, the Calvin cycle efficiency declined and the recycled circulation of NADP+ and NADPH was obstructed. While NADP+ was the terminal electron acceptor of the PSI end of the photosynthetic electron transport chain, when the supply was inefficient, PSI function was sure to decline, which displayed as the decrease of photochemical efficiency of PSI and quantum yield, that is, the Fv/Fm ratio decreased [66–68].

P was the primary limiting factor for *Phaeocystis globosa*’s growth [69]. P was the main factor influencing *Chlorella* sp.’s cell growth [70]. Various algae had different needs for P. *Chrysophyta*’s optimum P moore concentration was 5 µmol/L [71]. *Prorocentrum micans*’ optimum P moore concentration was 0.85 µmol/L [72]. Jiang’s research found that *Heterosigma akashiwo* nearly could not grow under phosphate-free conditions. Under low phosphorus concentration conditions from 0 mg/L to 0.05 mg/L, *Heterosigma akashiwo*’s growth was obviously inhibited. Under the medium phosphorus concentration conditions from 0 mg/L to 1.0 mg/L, its growth rate was directly proportional to concentration of phosphorus. Under the higher phosphorus condition where the concentration reached 5.0 mg/L, the increase of *Heterosigma akashiwo*’s growth rate decelerated [73]. Similar to the lack of N, the lack of P might also influence microalgae’s photosynthesis sometimes. According to Wycoff’s research, after P limitation for 4 days, *Chlamydomonas reinhardtii*’s oxygen evolution rate decreased by 75% [74].

### 4.3. Influences of Ca in Slag on the Growth and Accumulation of Metabolites of Microalgae

There was a lot of Ca in slag and it existed in the form of CaO. After dissolving in seawater, the final content of Ca in slag was from 400 mg/L to 1400 mg/L. Ca was one of the necessary nutrients for microalgae’s growth, which played an important role in the process of microalgae’s metabolism, such as being involved in signal transduction and ion channels, as well as many cell metabolic activities. Ca was the main component of cell membranes. It could improve the contents of chlorophyll and protein. Ca had an important impact on the formation and transformation of carbohydrates [70].

Ge [75] and Cheng et al. [76] found that Ca had a negative impact on the accumulation of *Chlorella* sp.’s biomass. Takahashi et al. [49] studied the influences of electric-arc-furnace slag with two different technologies on *Chlorella* sp. Metallic element was leached from slag by hydrochloride and was filtered by 0.45 µm pore filters to remove slag particles. Thus, a slag leaching solution was attained. After using every type of leaching solution to deal with *Chlorella* sp. for a week, a quantitative detection was conducted through a blood cell counter technique to test *Chlorella* sp.’s growth. When more than 30% of slag leaching solution was added into the culture, *Chlorella* sp.’s growth rate was 150%. There was no slag leaching solution in the control group and *Chlorella* sp.’s growth rate was 100%. On
the contrary, the addition of slag extract into the culture did not directly promote Chlorella sp.’s proliferation. Ca in slag leaching solution increased the concentration of CO$_2$, which promoted Chlorella sp.’s photosynthesis rate and proliferation rate. Zhang et al. [60] studied effects of Ca on the growth and lipid accumulation of Desmodesmus sp. WC08. The biomass concentration, lipid content and lipid production rate of Desmodesmus sp. WC08 were tested when the Ca concentration was 0 mmol/L, 0.05 mmol/L, 0.20 mmol/L, 0.60 mmol/L and 1.00 mmol/L. Research findings showed that when Ca concentration was 1.00 mmol/L, the biomass concentration of Desmodesmus sp. WC08 reached the highest value (2.96 ± 0.16 g/L), which was 1.28 times as much as that of the blank group. When the Ca concentration was 0.20 mmol/L, the lipid content of Desmodesmus sp. WC08 reached the highest value (30.22%), after which the lipid content was 0.05 mmol/L, slightly lower than 30%. When the Ca concentration was 1.00 mmol/L, the lipid production rate of Desmodesmus sp. WC08 reached the highest value (0.87 g/L). Taking costs and the influence of medium precipitation caused by the high concentration of Ca into account, the 0.05 mmol/L Ca concentration was helpful to the accumulation of biomass and lipids of Desmodesmus sp. WC08, under the condition in which the biomass concentration was 2.85 ± 0.25 g/L and the lipid production rate was 0.85 g/L. The results were inconsistent with the research on Chlorella sp. of Ge [75], which might be caused by algae species.

### 4.4. Influences of Mg in Slag on the Growth and Accumulation of Metabolites of Microalgae

There was certain amount of MgO in slag. Mg was one of the necessary nutrients for microalgae’s growth, whose main function was to be used as the chlorophyll’s central atoms, participating in photosynthesis in the processes of light energy and enzymatic action. It also played an important role in protein metabolism [77].

Karemore et al. [78] found that a higher than 10 mg/L Mg$^{2+}$ condition was not helpful for the accumulation of Chlorococcum infudionum’s biomass. Through research on Chlorella protothecoides UTEX 250, Cheng et al. [76] found that, compared with the original medium with added Mg, the culture that removed Mg could increase the lipid content of Chlorella protothecoides UTEX 250 from 4.4% to 9.5%. The research showed that a heavy metal ion was helpful for the increase of some of the microalgae’s lipid content. Under photosynthetic autotrophy conditions, the radish fat content reached the highest value. Under heterotrophic conditions, Mg$^{2+}$ could induce lipid content and lipid production of Monoraphidium sp. FXY-10. When 100 µM Mg$^{2+}$ was added, the lipid content of Monoraphidium sp. FXY-10 reached 59.8% [79]. Polat et al. optimized the culture conditions of Auxenochlorella protothecoides by means of the surface response method. Under the 18.5 mg/L Mg$^{2+}$ and 5.0 g/L NaCl condition, the lipid content reached the highest value [80]. Zhang et al. [60] studied effects of Mg on the growth and lipid accumulation of Desmodesmus sp. WC08. The biomass concentration, chlorophyll content, lipid content and lipid production rate of Desmodesmus sp. WC08 were tested when the Mg concentration was 0 mmol/L, 0.03 mmol/L, 0.09 mmol/L, 0.15 mmol/L and 0.30 mmol/L. Research findings showed that the effects of Mg concentration on biomass concentration of Desmodesmus sp. WC08 were small. When the Mg concentration was 0.09 mmol/L, the biomass concentration of Desmodesmus sp. WC08 reached the highest value (2.44 ± 0.32 g/L), which was 1.21 times as much as that of blank group. When the Mg concentration was 0.30 mmol/L, the chlorophyll content of Desmodesmus sp. WC08 reached the highest value (55 mg/L). Under other Mg concentrations, the chlorophyll contents showed little difference, which meant that photosynthesis of Desmodesmus sp. WC08 was strong when the Mg concentration was relatively high. When the Mg concentration was 0.30 mmol/L, the lipid content of Desmodesmus sp. WC08 reached the highest value (29.40%). When the Mg concentration was 0.09 mmol/L, the lipid production rate of Desmodesmus sp. WC08 reached the highest value (29.40%), 1.2 times as much as that of the blank group, the lipid production rate of which was the lowest. As a consequence, when the Mg concentration was 0.09 mmol/L, it was the most helpful for Desmodesmus sp. WC08’s accumulations of biomass and lipid.
4.5. Influences of Zn in Slag on the Growth and Accumulation of Metabolites of Microalgae

Zn is a kind of heavy metal existing in slag and a necessary microelement for microalgae’s growth. When slag was added to water, the Zn$^{2+}$ concentration in water changed since the Zn$^{2+}$ in slag dissolved out.

The toxicity of zinc is related to the cell membrane, as it may disrupt the calcium uptake necessary for calcium ATPase activity during cell division. Research on Arthrospira platensis [81] showed that after adding slag, Zn$^{2+}$ dissolving out would influence the accumulation of carotenoids. When the Zn$^{2+}$ concentration was lower than 4.0 mg/L, Arthrospira platensis had no obvious influences on carotenoids, but when the Zn$^{2+}$ concentration was higher than 6.0 mg/L, the carotenoid content produced by Arthrospira platensis decreased. When the Zn$^{2+}$ concentration was 1.0 mg/L, the carotenoid content produced by Arthrospira platensis was the highest (2.58 mg/L). When the Zn$^{2+}$ concentration was 4.0 mg/L, the chlorophyll-a content reached the highest value. High zinc conditions severely inhibited the growth of Arthrospira platensis, while under low zinc conditions, the biomass did not change much. An excessive dose of Zn$^{2+}$ might cause the sharp decrease in the content of Octadecenoic acid (C18:1), and even disappearance, while Palmitoleic acid (C16:1) was in contrast to Octadecenoic acid (C18:1). High zinc conditions (4.0 mg/L) might induce the production of Palmitoleic acid (C16:1). Under 8.0 mg/L, the content of Palmitoleic acid (C16:1) reached the highest value. The research found that the content of fatty acid increased with the increase of the Zn$^{2+}$ concentration, which meant that Zn$^{2+}$ was the key element for the accumulation of fatty acid.

When Zn$^{2+}$ concentrations were 0.0 mg/L, 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 4.0 mg/L, 6.0 mg/L and 8.0 mg/L, Coelastrella sp. biomass was in inverse proportion to the Zn$^{2+}$ concentration. When the Zn$^{2+}$ concentration was 0.0 mg/L, Coelastrella sp. biomass reached the highest value (7.79 mg/L/d). When the Zn$^{2+}$ concentration was 8.0 mg/L, Coelastrella sp. biomass was the lowest (3.48 mg/L/d). The protein content and glutathione content (protein/biomass g/g) were 0.207 g/g and 189.9 mg/g, respectively. When the Zn$^{2+}$ concentration was 6.0 mg/L, the maximum value of superoxide dismutase (SOD) was 55.5 U/mg protein. When the Zn$^{2+}$ concentration was 8.0 mg/L, the maximum value of adenosine triphosphate content was 1589 ± 57 µmol/g [82]. Yang et al. [83] studied that the effect of Zn$^{2+}$ on antioxidant enzyme activity of Chlorella vulgaris. The experiment showed SOD activity was positively associated with Zn$^{2+}$ concentration. When the Zn$^{2+}$ concentration was 3.25 mg/L, SOD activity reached the highest amount, but a high concentration would decrease SOD activity. POD activity decreased with the increase of Zn$^{2+}$ concentration. Tripathi and Guar [84] found that, when Scenedesmus sp. was stimulated by a small amount of heavy metal Zn$^{2+}$ (0.005 Mm), the peroxide TBARS content of a kind of lipid in microalgae increased dramatically. Meanwhile, activities of some antioxidant enzymes promoted greatly, such as SOD, APQX, CAT, GR and so on. Li et al. [85] studied the influences of Zn$^{2+}$ on Pavlova viridis antioxidant enzymes and lipid peroxide. The study showed that when the Zn$^{2+}$ concentration was higher than 3.25 mg/L, the TBARS content in microalgae increased obviously, while when the Zn$^{2+}$ concentration was lower than 1.3 mg/L, the CAT content increased gradually with the increase of Zn$^{2+}$ concentration. When the Zn$^{2+}$ concentration was higher than 1.3 mg/L, the CAT content increased rapidly. When Zn$^{2+}$ concentrations were 3.25 mg/L and 6.5 mg/L, CAT contents reached 178.57% and 92.21% of the control samples, respectively. On the contrary, the GPX content decreased gradually with the increase of Zn$^{2+}$ concentrations. SOD was not sensitive to Zn$^{2+}$. Another research study showed that with the increase of Zn$^{2+}$, the content of protein, SOD, glutathione and ATP produced by Coelastrella sp. increased [17].

4.6. Influences of Cu in Slag on the Growth and Accumulation of Metabolites of Microalgae

Similar to Zn, Cu is a kind of heavy metal in slag. Many research studies have shown that slag could help microalgae synthetize glutathione, mercaptan, proline, superoxide dismutase and so on. The reason is that when microalgae was stimulated by Cu$^{2+}$ dissolving from slag, microalgae showed a kind of self-protection mechanism to defend the
oxidative toxicity caused by Cu²⁺ [85,86]. Xia et al. [17] found that, when stimulated by Cu²⁺, Paeolosa viridis’s MDA yield increased obviously. When the Cu²⁺ concentration was 3 mg/L, antioxidants (GSH) and antioxidant enzymes (SOD, CAT and GPX) increased dramatically. Additionally, Rijstenbil et al. [86] also found that a relatively high concentration of Cu²⁺ might cause an obvious increase of SOD in Ditylum brightwellii. Li et al. [87] studied influences of Cu²⁺ on Chlorella protothecoides’s lipid synthesis. It was found that under the stimulation of Cu²⁺, the highest lipid yield was 5.78 g/L.

Liu et al. [88] found that the optimum concentration of Cu²⁺ for the growth of Microcystis aeruginosa was 10⁻⁶ mol/L. When the Cu²⁺ concentration was between 10⁻⁵ mol/L and 10⁻² mol/L, with the increase of Cu²⁺ concentration, the inhibition of Cu²⁺ on the growth of Microcystis aeruginosa increased. Anahi Magdaleno et al. [89] studied effects of Cu²⁺ of different concentrations on three kinds of algae. The research findings showed that different algae showed various sensitivities to the toxicity of the heavy metal, Cu²⁺. The inhibition rate of 200 µg/L Cu²⁺ to Chlorella ellipsoidea, Monoraphidium contortum and Scenedesmus acuminatus was 1%, 54% and 53%, respectively. The EC₅₀ of C. ellipsoidea, M. contortum and S. acuminatus was 489 µg/L, 164 µg/L and 170 µg/L, respectively. Wang et al. [90] studied the effects of Cu²⁺ on the growth and physiological characteristics of Skeletonema costatum and Phaeodactylum tricornutum and found that a relatively high concentration of Cu²⁺ had obvious inhibitory effects on the growth of Skeletonema costatum and Phaeodactylum tricornutum, the EC₅₀ of 72 h of which was (0.546 ± 0.068) and (0.531 ± 0.037) mg/L, respectively. The effects of Cu²⁺ on the activities of SOD and POD of Skeletonema costatum and Phaeodactylum tricornutum displayed as low concentration induction and high concentration inhibition. The content of MDA gradually increased with the increase of Cu²⁺ concentration. Wang et al. [91] found that different concentrations of heavy metal ions (Cu²⁺, Mn²⁺ and Zn²⁺) under different time treatments inhibited the growth of Scenedesmus obliquus, and the ascending sequence of toxicity was Cu²⁺ >Zn²⁺ >Mn²⁺. With the march of the stress time, the chlorophyll fluorescence parameters, PS II maximum light energy conversion efficiency (Fv/Fm), PS II actual light energy conversion efficiency (Y), maximum relative electron transfer efficiency (Re, t, max), half-saturation light intensity (IK) and efficiency of light energy utilization (α) all decreased and the decreasing range increased gradually. Different types of elements in steel slag have different influence on growth and the metabolite accumulation of microalgae. Table 1 shows the influence of different types of elements in steel slag on the growth and metabolite accumulation of microalgae.

Table 1. The influence of steel slag on the growth and metabolite accumulation of microalgae.

| Types of Elements in Steel Slag | Types of Microalgae | Growth Condition | Effects on Growth and Metabolite Accumulation | Reference |
|--------------------------------|---------------------|------------------|-----------------------------------------------|-----------|
| Fe                             | Chlorella sp.       | 25 mg/L          | Promoting Growth                              | [52]      |
|                                | Thalassiosira nordenskioeldii |              | Promoting Growth                              | [54]      |
|                                | Thalassiosira oceanica |       | Promoting Growth                              | [56]      |
|                                | Thalassiosira guillardii |         | Promoting Growth                              |           |
|                                | Isochrysis galbana   | 1 × 10⁻⁴ mol/L   | Promoting Growth and Lipid Accumulation        | [58]      |
|                                | Botryococcus sp.     |                   | Promoting Oil Accumulation                     | [59]      |
|                                | Desmodesmus sp. WC08 | 2 × 10⁻⁵ mmol/L  | Promoting Growth and Oil Accumulation          | [60]      |
|                                | Auxenochlorella protothecoides |       | Promoting Growth                              | [61]      |
|                                | Dunaliella tertiolecta |                 | Promoting Growth and Oil Accumulation          | [62]      |
| Types of Elements in Steel Slag | Types of Microalgae | Growth Condition | Effects on Growth and Metabolite Accumulation | Reference |
|--------------------------------|---------------------|------------------|---------------------------------------------|-----------|
| P                              | Thalassiosira guillardii | 33 mg/L          | Promoting Growth                           | [65]      |
|                                | Phaeocystis globosa   |                  | Influencing Growth                          | [69]      |
|                                | Chlorella sp.         |                  | Influencing Growth                          | [70]      |
|                                | Chrysophyta          | 5 µmol/L         | Promoting Growth                           | [71]      |
|                                | Prorocentrum micans  | 0.85 µmol/L      | Promoting Growth                           | [72]      |
|                                | Heterosigma akashiwo | 0-0.05 mg/L      | Inhibiting Growth                          | [73]      |
|                                |                      | 0-1.0 mg/L       | Promoting Growth                           |           |
|                                |                      | >5.0 mg/L        | Influencing Growth                          |           |
|                                | Chlamydomonas reinhardtii |              | Influencing Growth                          | [74]      |
| Ca                             | Chlorella sp.        | more than 30 vol% of slag leaching solution | Promoting Growth                           | [49]      |
|                                | Desmodesmus sp. WC08 | 0.05 mmol/L      | Promoting Growth and Lipid Accumulation     | [60]      |
|                                | Chlorococcum infusionum | >10 mg/L        | Inhibiting Growth                          | [78]      |
|                                | Chlorella sp.        | Removing         | Promoting Growth and Lipid Accumulation     | [76]      |
| Mg                             | Monoraphidium sp. FXY-10 | 100 µM       | Promoting Accumulation of Lipid Content     | [79]      |
|                                | Auxenochlorella protothecoides | 18.5 mg/L Mg²⁺ and 5.0 g/L NaCl | Promoting Accumulation of Lipid Content     | [80]      |
|                                | Desmodesmus sp. WC08 | 0.09 mmol/L      | Promoting Growth and Oil Accumulation       | [60]      |
| Zn                             | Arthrosira platensis | High Zinc Condition | Inhibiting Growth                         | [81]      |
|                                |                      | 1.0 mg/L         | Promoting Accumulation of Carotenoids       |           |
|                                |                      | 6.0 mg/L         | Promoting Accumulation of SOD               | [82]      |
|                                | Coelastrella sp.     | 8.0 mg/L         | Promoting Accumulation of Adenosine Triphosphate | [17]      |
|                                |                      |                  | Promoting Accumulation of Protein, SOD, Glutathione and ATP |           |
|                                | Chlorella vulgaris   |                  | Promoting Accumulation of SOD               | [83]      |
|                                |                      |                  | Inhibiting Accumulation of POD              |           |
|                                | Scenedesmus sp.      | 0.005 Mm         | Promoting Accumulation of TBARS, SOD, APOX, CAT and GR | [84]      |
|                                |                      | 3.25 mg/L        | Promoting Accumulation of TBARS             |           |
|                                | Pavlova viridis      | 1.3 mg/L         | Promoting Accumulation of CAT               | [85]      |
|                                |                      |                  | Inhibiting Accumulation of GPX              |           |
Table 1. Cont.

| Types of Elements in Steel Slag | Types of Microalgae                        | Growth Condition | Effects on Growth and Metabolite Accumulation | Reference |
|--------------------------------|-------------------------------------------|------------------|---------------------------------------------|-----------|
| Cu                             | *Pavlova viridis*                         | >0.5 mg/L        | Promoting Accumulation of MDA               | [17]      |
|                                |                                           | 3 mg/L           | Promoting Accumulation of GSH, SOD, CAT, GPX |           |
|                                | *Ditylum brightwellii*                    |                  | Promoting Accumulation of SOD               | [86]      |
|                                | *Chlorella protothecoides*                |                  | Promoting Lipid Accumulation                | [87]      |
|                                | *Microcystis aeruginosa*                  |                  | Inhibiting Growth                           | [88]      |
|                                | *Chlorella ellipsoidea*                   |                  | Inhibiting Growth                           | [89]      |
|                                | *Monoraphidium conformum*                |                  | Inhibiting Growth                           |           |
|                                | *Scenedesmus acuminatus*                  |                  | Inhibiting Growth                           |           |
|                                | *Skeletonema costatum*                    | Low Concentration Induction SOD, POD |                                           |           |
|                                | *Phaeodactylum tricornutum*              | Low Concentration Induction SOD, POD |                                           |           |
|                                | *Scenedesmus obliquus*                    |                  | Inhibiting Growth                           | [91]      |

5. Conclusions

As the byproduct in the steelmaking process, there are many nutrient elements in slag that are necessary for microalgae’s growth, such as Fe, P, Ca, Mg and so on. Through research and explorations on the effects of slag on microalgae’s growth, it has been found that slag has enormous potential and huge advantages for promoting microalgae’s growth and accumulation of metabolites. Under suitable conditions, slag can effectively promote microalgae’s growth and reproduction. Culturing microalgae by slag can lower the cost and solve the problem of lacking nutrient elements during the process of microalgae’s growth. It can also alleviate the phenomenon of a large amount of steel slag storage and reduce the environmental pollution caused by steel slag storage. This provides a new method for the utilization of steel slag. Meanwhile, under certain conditions, culturing microalgae by slag can also promote microalgae’s accumulation of metabolites, especially lipid accumulation. Thus, slag can be used as an ideal material for microalgae to produce metabolites.

For the research related to slag’s influences on microalgae’s growth, there is some key information that can hopefully be used as references in future research.

1. Through the laboratory research on slag’s influences on microalgae’s growth, it was found that there are many oxide compositions in slag, which may cause the increase of a solution’s pH value and conversely inhibit microalgae’s growth, which in a marine
environment obviously shows influences of pH decrease. This effect can be verified by a
pilot experiment or even a field experiment.

2. Among the research on slag’s influences on microalgae’s growth, most research
analyzes one certain element in slag. There are few studies on the synergistic effect of
multi-elements in steel slag on the growth of microalgae. It should be further studied
whether the reason why the steel slag promotes the growth of microalgae is the effect of a
single element or the synergistic effect of multiple elements.

3. Among the research on slag’s influences on microalgae’s growth, most research
focuses on slag’s influences on marine microalgae’s growth. In the future, research on
slag’s influences on freshwater microalgae’s growth should be carried out.

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