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Calcium ions-effect on performance, growth and extracellular nature of microalgal-bacterial symbiosis system treating wastewater

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ABSTRACT

Microalgal-bacterial symbiosis (MABS) system treating wastewater has attracted great concern because of its advantages of carbon dioxide reduction and biomass energy production. However, due to the low density and negative surface charge of microalgae cells, the sedimentation and harvesting performance of microalgae biomass has been one limitation for the application of MABS system on wastewater treatment. This study investigated the performance enhancement of microalgae harvesting and wastewater treatment contributed by calcium ions (i.e., Ca^{2+}) in the MABS system. Results showed that a low Ca^{2+} loading (i.e., 0.1 mM) promoted both COD and nutrients removal, with growth rates of 11.95, 6.53 and 1.21% for COD, TN and TP compared to control, and chlorophyll *a* was increased by 64.15%. Differently, a high Ca^{2+} loading (i.e., 10 mM) caused removal reductions by improving the aggregation of microalgae, with reduction rates of 34.82, 3.50 and 10.30% for COD, NH_4^+-N and TP. Mechanism analysis indicated that redundant Ca^{2+} adsorbed on MABS aggregates and dissolved in wastewater decreased the dispersibility of microalgae cells by electrical neutralization and compressed double electric layer. Moreover, the presence of Ca^{2+} could improve extracellular secretions and promoted flocculation performance, with particle size increasing by 336.22%. The findings of this study may provide some solutions for the enhanced microalgae biomass harvest and nutrients removal from wastewater.

1. Introduction

Developing carbon-neutral operation of wastewater treatment plants has been one of the most important aims all over the world, and the microalgal-bacterial symbiosis (MABS) system has been selected as a promising technology for advanced wastewater treatment because of its advantages for absorbing carbon dioxide, producing oxygen and harvesting microalgae biomass (Ji, 2021; Khan et al., 2021; Jeong and Jang, 2020). Many wastewater treatment technologies based on the MABS system (Ji, 2021; Gao et al., 2021), such as algae-assisted sequencing batch reactor (Tang et al., 2016), algae-assisted sequencing batch biofilm reactor (Tang et al., 2018a, 2018b, 2021), algae-assisted membrane bio-reactor (Sun et al., 2018) and other algae-assisted wastewater treatment system (Su et al., 2016, 2020; Zhang et al., 2020a; Ji et al., 2021; Wang et al., 2021a), have been explored. However, due to the low density and the mutual exclusion caused by the surface charge of microalgae cells, both the settling property of microalgae biomass and the separation effect between microalgae cells and treated water are poor, seriously limiting the promotion and application of MABS system treating wastewater (Acién Fernández et al., 2012; Leong et al., 2021; Olguin, 2012; Tang et al., 2021).

Recently, many efforts have been done to promote the sedimentation performance of microalgae cells in MABS system, as well as the biomass harvesting efficiency (Iasimone et al., 2021; Sun et al., 2019; Kumar et al., 2017). In terms of biological methods, the sedimentation enhancement of microalgae biomass can be realized by the adhesion

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function contributed by extracellular polymeric substances of bacteria (Sun et al., 2019; Oladoja et al., 2020). In terms of physical methods, the negative charge on the surface of microalgae cells is employed to promote directional movements and aggregations of microalgae cells under the action of electric force by introducing external electric fields (Khatib et al., 2021; Kumar et al., 2017). Compared to physical or biological methods, the addition of various chemicals for promoting the sedimentation and aggregation performances of microalgae cells is easier to operate, and has no potential risk for microorganism infection, presenting a great application prospect (Sun et al., 2019; Ogbonna and Nwoba, 2021). Till now, several kinds of chemical flocculants have been studied, especially aluminum and ferric salts (Liang et al., 2019; Vu et al., 2020; Yang et al., 2004, 2020). However, these chemical flocculation processes relies on precipitations (i.e., $Al(OH)_3$ or $Fe(OH)_3$) produced by flocculants (i.e., $AlCl_3$ or $FeCl_3$) to net and bridge microalgae cells, which influences water turbidity (Loganathan et al., 2018). In addition, the color caused by ferric ions also has a significant effect on the chroma of the effluent (Zhou et al., 2016). Thus, it is of great significance to find a more suitable chemical agent.

Considering that the surface of microalgae cells carries negative charge (Zhang et al., 2020b), cationic agent is an alternative to promote the sedimentation performance of microalgae biomass. As well known, calcium is one of the main elements in water body, and commonly presents in the form of divalent cation (i.e., Ca^{2+}) (Borja-Urzola et al., 2020). Different to the high solubility of aluminum salts, most calcium salts are less soluble (Ji et al., 2020; Huynh et al., 2020), leading to them having no prerequisites to form large precipitates. Thus, the calcium salt is more likely to promote flocculation and sedimentation of microalgae cells by the electrical neutralization and compressed double electric layer, rather than the adsorption, bridge and netting contributed by precipitates formation and settling. And the settled microalgae biomass may be much cleaner with less pollution caused by precipitated agent. In addition, calcium ions are colorless and cannot affect the chroma of effluent. These above advantages have the potential to make harvested microalgae biomass away from chemical precipitation pollution caused by aluminium and ferric salts. Moreover, moderate calcium ions can enhance microalgae growth by promoting photosynthesis (Rocha and Vothknecht, 2012), cell division and cell activity (Wang et al., 2011). However, the latest studies mainly focused on the effects of calcium ions on the growth of specific algae species such as *microcystis* sp. (Carvalho et al., 2016), *diatom* sp. (Kröger et al., 1994) and *scenedesmus* sp. (Esakkimuthu et al., 2016), few have been turned attention to the effects of calcium ions on the flocculation and sedimentation of MABS system, including microalgae, bacteria and extracellular secretions (ECS, i.e., or extracellular polymeric substance, EPS), and how Ca^{2+} affects nutrients removal from wastewater in MABS system is needed to be evaluated.

The aim of this study was to investigate the performance of calcium salts (i.e., Ca^{2+}) in the area of wastewater treatment and subsequent biomass separation and harvesting in MABS system. Firstly, the effects of calcium ions on the enhanced nutrients removal from wastewater were studied. Secondly, the growth performance of microalgae and the characteristics of MABS system were analyzed. Finally, a possible mechanism focused on the effects of calcium salts on MABS system was discussed. It is expected that this work can provide some new insights for the enhanced microalgae biomass separation and harvest for MABS system treating wastewater by a kind of relatively eco-friendly and low-pollution agent.

2. Materials and methods

2.1. The properties of influent and inoculum

2.1.1. The compositions of influent

The synthetic wastewater used in this work was the same as domestic wastewater reported in the previous study (Tang et al., 2018b). The details were listed as follows: glucose 200 mg/L, starch 200 mg/L, $NaHCO_3$ 300 mg/L, NH_4Cl 155 mg/L and $K_2HPO_4·3H_2O$ 38 mg/L. In addition, trace elements were also added into influent, with the compositions of HBO_3 2.86 mg/L, $MnCl_2·4H_2O$ 1.86 mg/L, $ZnSO_4$ 0.22 mg/L, $Na_2MoO_4·2H_2O$ 0.39 mg/L, $CuSO_4·5H_2O$ 0.08 mg/L and $Co(NO_3)_2·6H_2O$ 0.05 mg/L. And the theoretical and actual concentrations of NH_4^+-N, TN, PO_4^{3-}-P, TP and COD were 40, 40, 5, 5 and 400 mg/L and 39.59, 42.03, 5.61, 5.65 and 402 mg/L, respectively. The used calcium salts in the present work was calcium chloride ($CaCl_2·H_2O$, analytically pure).

2.1.2. The source and cultivation of inoculum

The inoculum, microalgae and its associated bacteria, was obtained from Moat of Xi'an city, China. In order to avoid the effects of microfauna on the growth of microalgae, the obtained water sample was firstly frozen at −20 °C for a week and then cultured for microalgae enrichment. The light source for microalgae was changed from sunlight to lamp with fixed position, and the illumination intensity were controlled until the chlorophyll *a* (Chl-a) were increased to around 200 μg/L. The algae inoculum was cultivated in BG11 media firstly. With microalgae biomass growing, the synthetic wastewater used in this study was gradually added into the reactor (Tang et al., 2018b).

In order to obviously observe microbial variations in MABS system, microalgae biomass in exponential growth phase was chosen as inoculum. Moreover, considering the response and action time of chemical agent, the whole experiment could be finished in a short time. And the inoculation concentration of volatile suspended solids (VSS) and Chl-a was diluted to around 200 mg/L and 1500 μg/L, respectively, which could ensure a normal growth rate of MABS system for 2–3 days, i.e., in control group.

2.2. Experimental design and operation

The lab-scale experiments were conducted in four sequencing batch reactors (SBRs) with working volume of 1.0 L. The control test (i.e., R_0) was no addition of $CaCl_2•H_2O$. Referencing from the added contents of traditional agent, e.g., $FeCl_3$ were no more than 10 mM (Loganathan et al., 2018; Islami and Assareh, 2020), so the $CaCl_2•H_2O$ was the only source of calcium ions with the theoretical loadings of 0.1, 1, 10 mmol/L for R_1, R_2 and R_3. The inoculum was added into each SBR and diluted by synthetic wastewater with the final volume of 1.0 L. A piece of preservative film was used to cover the top of each SBR to prevent water from evaporating and some holes were made to achieve gas exchange. Magnetic stirring apparatus were employed to realize the homogeneous mixture. The illumination intensity was 4000 lux with light and dark ratio of 12 h: 12 h. The temperature and pH were kept at 23.5 ± 1.5 °C and 7.4 ± 0.2, respectively. And DO in each reactor ranged from 8 to 12 mg/L.

2.3. Analytical methods

2.3.1. Extraction and analysis of ECSs

The heat method was used to extract ECSs (He et al., 2021b; Sheng et al., 2013). And the specific details were listed as follows: 50 mL of mixed liquor sample was centrifuged at 4000 rpm for 5 min and the centrifugated residual was replenished to original volume by 0.05% NaCl solution, then the mixture was treated at 60 °C in water bath for 30 min, the mixture was cooled down to room temperature and centrifugated at 4000 rpm for 15 min. After filtration, the obtained supernatant was total ECSs.

2.3.2. Extraction and measurement of Chl-a

The sample preparation of chlorophyll *a* (Chl-a) relied on mechanical disruption process for Chl-a out and extraction of Chl-a by 90% acetone. And its concentration was measured and calculated by ultraviolet spectrophotometry at four wavelengths: 750, 663, 645 and 630 nm. The details can be found in the previous study (Tang et al., 2016).

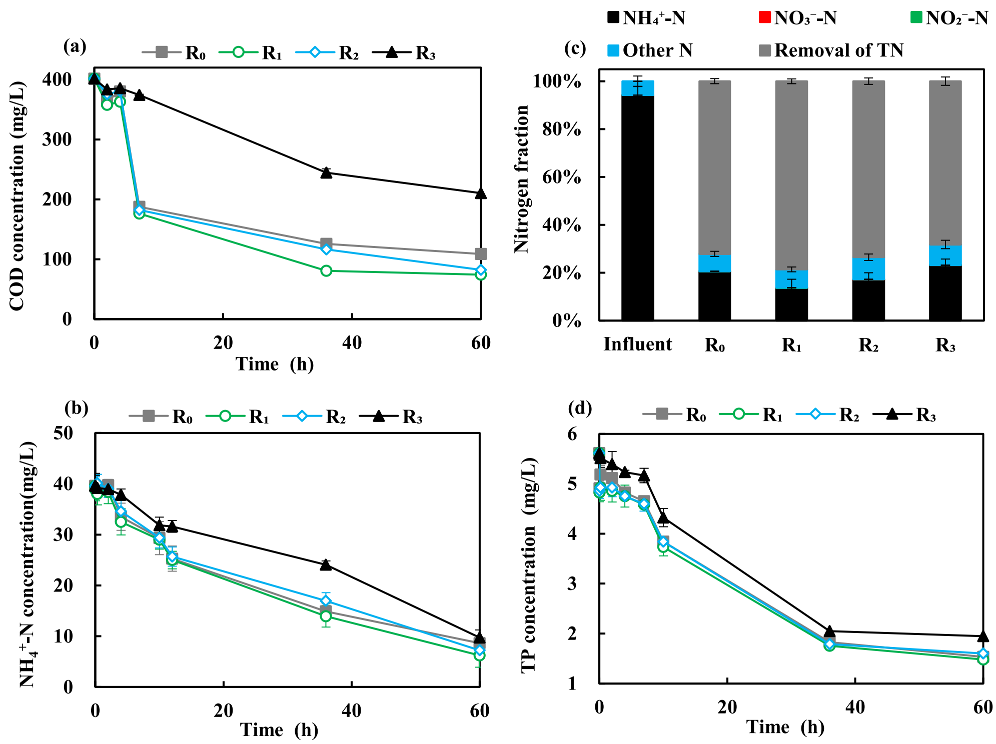

Fig. 1. Removal performance of contaminants at different Ca^{2+} loadings. (a) COD, (b) NH_4^+-N, (c) Nitrogen balance, and (d) TP.

2.3.3. Characteristics analysis of MABS aggregates

The morphology of MABS aggregates was observed by a light microscope (Nikon 50i, Japan). The particle size distribution of MABS aggregates was analyzed by a sync laser granularity analyzer (Microtrac, England) and the zeta potential was detected by a zeta potential analyzer (ZS90, Malvern, England). Samples for the determination of zeta potential was the mixture of supernatant, i.e., after centrifugation, and a few drops of MABS aggregates, i.e., suspended form.

2.3.4. Chemical analysis

The measurements of COD, TN, NH_4^+-N, NO_3^--N, NO_2^--N and TP were conducted by Standard Methods (APHA, 2005). The concentration of polysaccharide (PS) was determined by phenol-sulphuric acid method (DuBois et al., 1956), and the protein (PN) was measured by a protein kit (He et al., 2021a). The content of Ca^{2+} was measured by an atomic absorption spectrometry (ICP-1100, Thermo, USA). Each sample above was parallelly observed and determined for three times. The difference analysis of data between control group (R_0) and experimental group (R_1-R_3) was conducted by *t*-test.

3. Results and discussion

3.1. Performance response of MABS system to different Ca^{2+} loadings

3.1.1. COD removal

Fig. 1 presents performance responses of MABS system to different Ca^{2+} loadings. As shown in Fig. 1(a), the concentrations of COD in R_0 decreased from 402 to 109 mg/L with the removal efficiency of 72.89%. More decreases were obtained in R_1 and R_2 with effluent COD concentrations of 74 and 82 mg/L, the corresponding removal efficiencies increased to 81.60% and 79.60%, which increased by 11.95% and 9.21% compared to R_0. However, a further increase of Ca^{2+} loading led to a decrease of COD removal, e.g., the final COD in R_3 was 211 mg/L, with the removal efficiency of 47.51%, which was 34.82% lower than that in R_0. These results suggested that either a low or medium Ca^{2+} loading (i.e., 0.1 and 1 mM) exhibited a promoted effect on COD removal in MABS system, whereas a high Ca^{2+} loading (i.e., 10 mM) could do an adverse effect. Previous studies have proved that microalgae generally utilize inorganic carbon sources, such as CO_2 and HCO_3^-, in routine conditions with free solar irradiance (Moreira and Pires, 2016). Therefore, the removal of COD may be mainly attributed to the biodegradation of bacteria around microalgae, and the different removal responses of COD to Ca^{2+} loadings may be related to the impacts of Ca^{2+} on bacteria inhibition.

3.1.2. Nutrients removal

The removal performance of NH_4^+-N under different Ca^{2+} loadings is shown in Fig. 1(b). The NH_4^+-N concentration in R_0 decreased from 39.60 to 8.64 mg/L with removal efficiency of 78.16%, and it further reduced to 6.22 and 7.23 mg/L in R_1 and R_2 with removal efficiencies of 84.30% and 81.74%. In contrast, the concentration of NH_4^+-N in R_3 showed an increase compared to R_0, with removal efficiency reduction of 3.50%. Similar with COD removal, both low and medium Ca^{2+} loadings (i.e., 0.1 and 1 mM) could promote NH_4^+-N removal, whereas a high Ca^{2+} loading (i.e., 10 mM) caused a reduction. In addition, in order to clarify the removal of TN and the transformation of nitrogen-containing compounds affected by Ca^{2+}, the nitrogen balance in each reactor was analyzed and shown in Fig. 1(c). The removal amount of TN in R_0 was 30.32 mg/L, and the corresponding removal efficiency was 72.15%. Similar to the change trends of NH_4^+-N, the removal efficiencies of TN in R_1 and R_2 were also higher than that of R_0, and the highest removal efficiency of TN was observed in R_1, with the value of 76.86%, which was 6.53% higher than that of R_0 ($p < 0.05$). Inversely, the TN removal in R_3 was the lowest, with the removal efficiency of 68.24%. The contents of both NO_3^--N and NO_2^--N in each reactor were all lower than 0.02 mg/L. As the contents of DO in all reactors were ranged from 8 to 12 mg/L, there were no suitable conditions for the occurrence of denitrification process. In addition, the denitrification process under aerobic condition is a rare occurrence in wastewater treatment process (Cantera et al., 2021; Sayara et al., 2021; Wang et al., 2021b), so the low abundance and activity of ammonia-oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) resulted in a lower

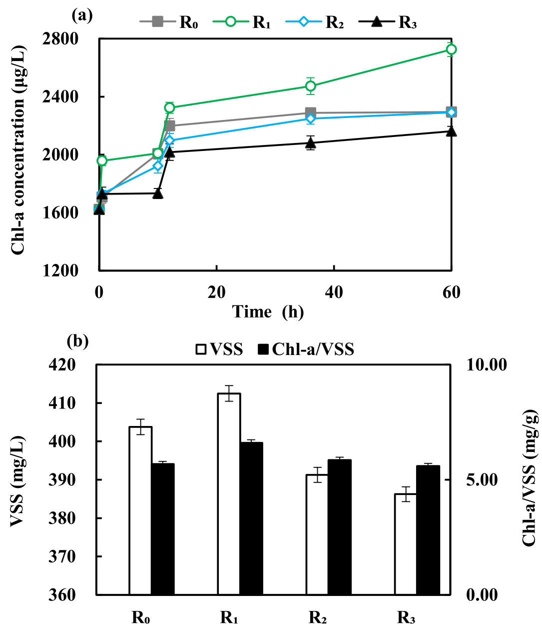

Fig. 2. Microbe growth in MABS system at different Ca^{2+} loadings. (a) Chl-a, (b) VSS and Chl-a/VSS.

NO_3^--N and NO_2^--N production. As the pH value, i.e., neutral, was suitable for nitrifying bacteria (Cho et al., 2014), the activity of nitrifying bacteria was more likely to be significantly inhibited by sunlight because of the destruction for electron transfer from cytochrome *c* to nitrite reductase (Barak et al., 1998; Kaplan et al., 2000). Thus, the removal of NH_4^+-N or TN was mainly due to the assimilation of microorganism and its associated bacteria, in MABS system.

Fig. 1(d) shows the change trends of TP affected by Ca^{2+}. It can be seen that the TP concentration in R_0 decreased from 5.62 to 1.54 mg/L, and the corresponding removal efficiency was 72.69%. Different from the removal of COD and NH_4^+-N, the removal effects and trends of TP in R_1 and R_2 were closed to that of R_0, but the highest TP removal efficiency was still observed in R_1, with the value of 73.57% ($p < 0.05$). And consistent with COD and NH_4^+-N, the lowest TP removal was still obtained in R_3, with removal efficiency of 65.20%.

Based on the above results, it can be found that the performance responses of both COD and nutrients removal from wastewater in MABS system to Ca^{2+} loadings were almost consistent. The growth and assimilation (or uptake) of microalgae was the main removal pathway for nutrients, including TN and TP (Rout et al., 2021), whereas COD removal was mainly achieved through the degradation of bacteria (Lee and Lei, 2019). Therefore, the different Ca^{2+} loadings in MABS system were likely to affect the growth rate of microorganisms and then do effects on pollutants removal from wastewater.

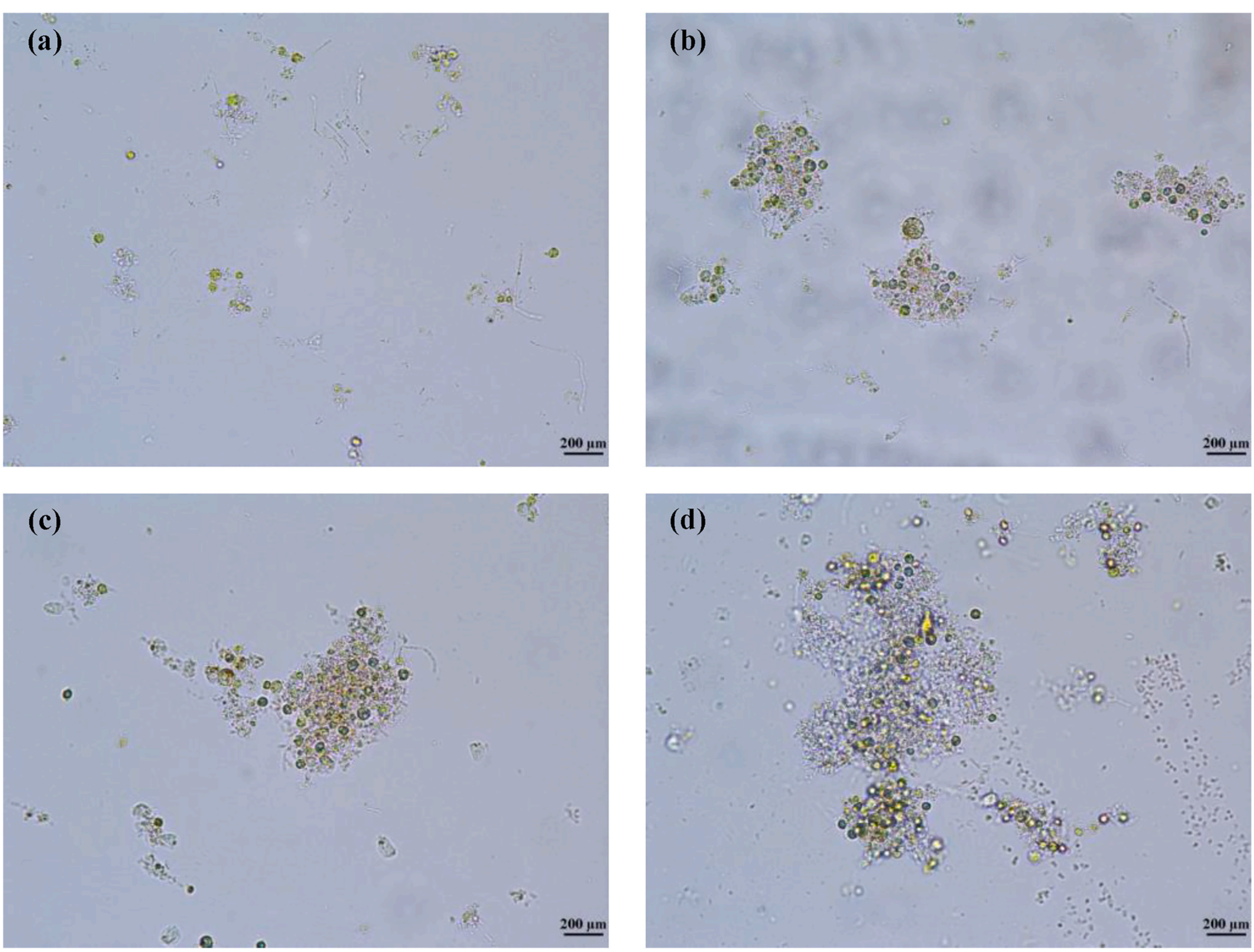

Fig. 3. Morphology features of MABS system in each reactor. (a) R0, (b) R1, (c) R2 and (d) R3.

3.2. The changes of biomass affected by different Ca^{2+} loadings in MABS system

In this study, the changes of Chl-a were used to evaluate the effects of Ca^{2+} loadings on the growth of microalgae, and the changes of VSS were employed to investigate biomass growth situation in MABS system. As shown in Fig. 2(a), Chl-a concentration in R_0 increased from 1622 to 2294 μg/L, with an increase rate of 41.45%. A further increase of Chl-a was observed in R_1, with an increase rate of 68.04%, whereas the increase rate of Chl-a concentration in R_3 was reduced compared to R_0, with the value of 33.23%. Interestingly, there was no significant difference in Chl-a concentration increase between R_2 and R_0 ($P = 0.93 > 0.05$). These results proved that the growth of microalgae could also be affected significantly by the content of Ca^{2+}. A low loading of Ca^{2+} (i.e., 0.1 mM) could promote the growth of microalgae, and a high loading (i.e., 10 mM) showed inhibition effects on microalgae growth, which was consistent with the changes of nutrients removal effects, and these results corroborated the different nutrients removal performances related to Ca^{2+} loading in MABS system.

Fig. 2(b) shows the changes of VSS and Chl-a/VSS in each reactor. The VSS values were 403.75, 412.50, 391.25 and 386.25 mg/L, with the Chl-a/VSS values of 5.68, 6.61, 5.86 and 5.60 mg/g for R_0, R_1, R_2 and R_3, respectively. VSS reflected the amount of the whole biomass related to microorganism growth and secreted organic substances, and its variation trend was the same as that of Chl-a. The ratios between microalgae growth and whole biomass (i.e., Chl-a/VSS) in R_1 and R_2 were a little higher than that in R_0 and R_3. These results indicated that a high Ca^{2+} loading (i.e., R_3) inhibited the growth of both microalgae and bacteria, and a low Ca^{2+} loading mainly promoted the growth of microalgae, or promoted more in growth of microalgae rather than bacteria. Previous studies have reported that a moderate Ca^{2+} loading can promote microalgae for cell wall synthesis and photosynthesis (Gökçe, 2021), which was also responsible for microalgae growth improvement at a low Ca^{2+} loading in present study. Moreover, as the exclusive O_2 producer in MABS system, microalgae controlled both the growth and metabolism rate of other microorganisms, mainly aerobic bacteria. Thus, it suggested that the growth inhibition of microalgae at a high Ca^{2+} loading could also indirectly influence the growth rate of bacteria through controlling O_2 supply. The indirect influence of Ca^{2+} on bacteria was consistent with the previous research, in which, the TN removal effect of activated sludge system was inhibited because of larger flocs formation rather than bacterial activity inhibition at a high Ca^{2+} loading, i.e., around 10.25 mM (Zhou et al., 2021).

3.3. Characteristics of MABS aggregates affected by different Ca^{2+} loadings

3.3.1. Morphological structure of MABS aggregates

Cell dispersibility, one of key factors for microalgae to take in nutrients and receive luminous energy, is of great significance on the growth of microalgae (Wu et al., 2020). To evaluate impacts of Ca^{2+} loadings on cell dispersibility of microalgae, the structure characteristics and properties of MABS aggregates were observed (Fig. 3). With Ca^{2+} loading increasing, the agglomeration property of microalgae cells gradually became obvious, and larger flocs were formed by the dispersed microalgae cells and bacteria. When the dispersed microalgae cells formed agminated aggregates, it was difficult for microalgae cells to effectively receive enough light energy because of the shading and blocking contributed by aggregates, so the agglomeration of microalgae cells was not conducive to microalgae growth. In addition, the microalgae cells also presented mild agglomeration at low Ca^{2+} loading. The possible reason was that both the formation of microalgae cell wall and the aggregation of microalgae cells were influenced by the presence of Ca^{2+}, and then this combined action was presented as the promoted effect on microalgae growth.

The effects of Ca^{2+} on morphology of MABS aggregates was qualitatively analyzed by microscopic examination method. In order to quantitatively evaluate the changes of MABS aggregations, the particle size distribution of MABS aggregates in each reactor was measured (Fig. 4). There existed two peaks in each reactor, one was the range of 1–10 μm, and the other was over 50 μm. The first peak was mainly distributed at 5.5 μm, representing relative content of dispersive microalgae cells (Quijano et al., 2017). With the increased addition of Ca^{2+}, the relative content of dispersive microalgae cells decreased from 4.12% to 1.39%. The particle size for the second peak in each reactor gradually increased from 74 to 322.8 μm, suggesting that more and more dispersive microalgae cells gathered to aggerates and further aggregated to flocs with larger size.

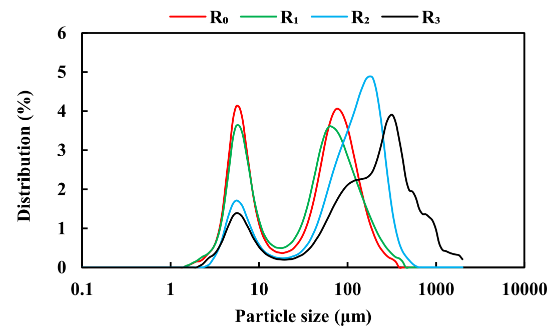

Fig. 4. Particle size distribution of MABS system in each reactor.

Table 1
Zeta potential and Ca^{2+} distribution in each reactor.

		R_0	R_1	R_2	R_3
Zeta potential (mV)		−20.70	−19.60	−13.70	−11.50
Ca^{2+} concentration (mM)	Theoretical value in influent	/	0.10	1.00	10.00
	Measured value in influent	/	0.10	1.08	10.27
	Effluent	/	0.01	0.72	8.73
	MABS aggerates	/	0.09	0.36	1.54
Absorption rate of Ca^{2+}(%)		/	95.276	32.685	15.038

3.3.2. Surface charge variation in MABS system

The aggregation property of microalgae cells in MABS system was presented by qualitative and quantitative methods, which indeed proved the surface properties and structure changes of MABS system. Microorganism uptake and utilization for Ca^{2+} into cell was relatively less than its added content, and the residual Ca^{2+} was more likely to affect both microalgae and its surrounding bacteria by cell surface and ECSs. Previous studies have been proved that the microbe aggregation was mainly affected by the surface charge (Yuheng et al., 2011) and extracellular secretions (Sun et al., 2019), which played important roles in keeping physical-biological property of microbe aggregation. A reduction of negative surface charge on microalgae cells could result in decrease of both repulsive force and distance among microalgae cells, and more ECS production could improve the adhesive force between microalgae cells and other particles, such as bacteria. The above two jointly formation processes promoted microbe aggregation or flocculation.

In order to confirm the key influencing factors on MABS aggregation, the changes of surface charge and ECS in MABS system were analyzed (Table 1). With the increase of Ca^{2+} loadings, the corresponding zeta potentials (i.e., absolute value) were decreased, indicating a destabilization trend of dispersoid-microalgae cells (Samari-Kermani et al., 2021; Pei and Zhang, 2021; Chen et al., 2021). As shown in Table 1, the contents of Ca^{2+} in both effluent and MABS aggregates increased with the increased Ca^{2+} loadings, indicating that electrical neutralization and compressed double electrical layer were the main mechanisms for

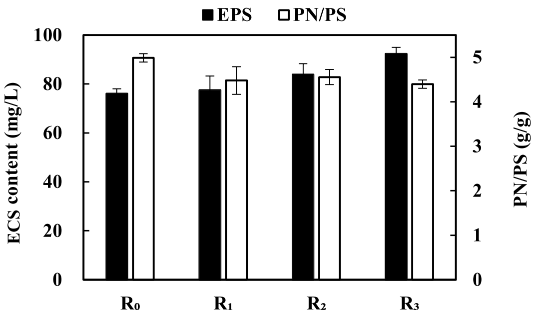

Fig. 5. Effects of Ca^{2+} on extracellular secretions in MABS system.

aggregation effect. Specifically, the microalgae cell surface carried negative charge and tended to adsorb Ca^{2+}, then surface charges of microalgae cells were decreased, presenting as the decreased zeta potential. Finally, the repulsive force was weakened. Meanwhile, from R_0 to R_3, the increase of Ca^{2+} content in solution improved the cation concentration, which compressed and thinned diffusion layer of colloid particles (i.e., microalgae cells) to be closed to each other. Moreover, the Ca^{2+} solubility in each reactor was relatively stable, proved by the similar values between theoretical and measured concentrations of Ca^{2+}. This result meant there was almost no calcium precipitate formed during the experiment period, suggesting that the other two mechanisms, including bridge and netting effects, for aggregation and flocculation did not occur.

3.3.3. Extracellular secretions in MABS system

The changes of ECSs affected by different Ca^{2+} loadings are shown in Fig. 5. The total amounts of ECS were 72.44, 74.96, 80.69 and 88.82 mg/L with the added Ca^{2+} increasing from 0 to 10 mmol/L. Combining the corresponding VSS (Fig. 2(b)), the ECS contents were 179.42, 181.72, 206.24 and 230.00 mg/g VSS. Moreover, the ratios between PNs and PSs showed opposite trends. Similar results have been reported, Ca^{2+} could promote the production of PS to improve the formation of aerobic granular sludge (Nancharaiah and Reddy, 2018), also, Ca^{2+} could increase the binding site of PN and PS secreted by bacteria (Liu et al., 2015) or fungi (Zamalloa et al., 2017). Therefore, both content and viscosity of ECSs were promoted to improve and regulate the shape of flocs in MABS system. The viscosity of ECSs, a kind of macro phenomenon of polymer, was corresponded to molecular migration at a microscopic level. The larger the molecular weight, the more processes were needed to move the barycenter, and then the slower the migration of polymer. That is, the viscosity of ECS was closely positive to its molecular weight. It has been proved that ECS produced by microalgae was less and has smaller molecular weight than that of bacteria (Zhao and Evans, 2021), it's more likely that ECS was produced by bacteria firstly and then adsorbed by microalgae cells. Thus, Ca^{2+} influenced the contents of ECS produced by bacteria.

3.4. Possible mechanisms for biomass aggregation and growth affected by Ca^{2+}

Based on the above results, the possible mechanisms for biomass aggregation and growth affected by Ca^{2+} in MABS system were stated (Fig. 6). The addition of Ca^{2+} into MABS system not only affected the growth and aggregation of microalgae cells, but also did effects on COD and nutrients removal. Firstly, microalgae cells could actively take in Ca^{2+} for their cell wall synthesis and chlorophyll formation to improve their growth based on Chl-a content at a low Ca^{2+} loading (Fig. 2). Secondly, on one hand, the negative surface charge of microalgae cell was neutralized by Ca^{2+} adsorbed on its adsorbed layer, and on the other hand, residual Ca^{2+} in solution compressed its diffusion layer. The above two processes contributed to the reduction of the repulsion force between cells (Table 1). Thirdly, Ca^{2+} improved the PS secretion of ECSs produced by bacteria (Fig. 5) and promoted its adhesive power for microalgae cells (Fig. 4). In the interactions of those two manners, microalgae cells tended to aggregate to lager flocs with Ca^{2+} content increasing (i.e., 10 mM). However, the illumination aera of each cell surface became lower because of shading and blocking contributed by other microorganisms and ECSs, directly leading to a decreased growth rate of microalgae, and then the growth of aerobic bacteria was inhibited indirectly because of oxygen production rate reduction. Correspondingly, both COD and nutrients removal efficiency were worse at a high Ca^{2+} loading (Fig. 1). The findings of this study may provide some solutions for enhancing microalgae biomass harvesting and nutrients removal from wastewater by an eco-friendly and low-pollution agent.

4. Conclusion

This work studied the role of Ca^{2+} on the enhanced microalgae biomass harvesting and wastewater treatment efficiency in MABS system. A low Ca^{2+} loading (i.e., 0.1 mM) promoted COD and nutrients removal through enhancing microalgae growth with the Chl-a increase

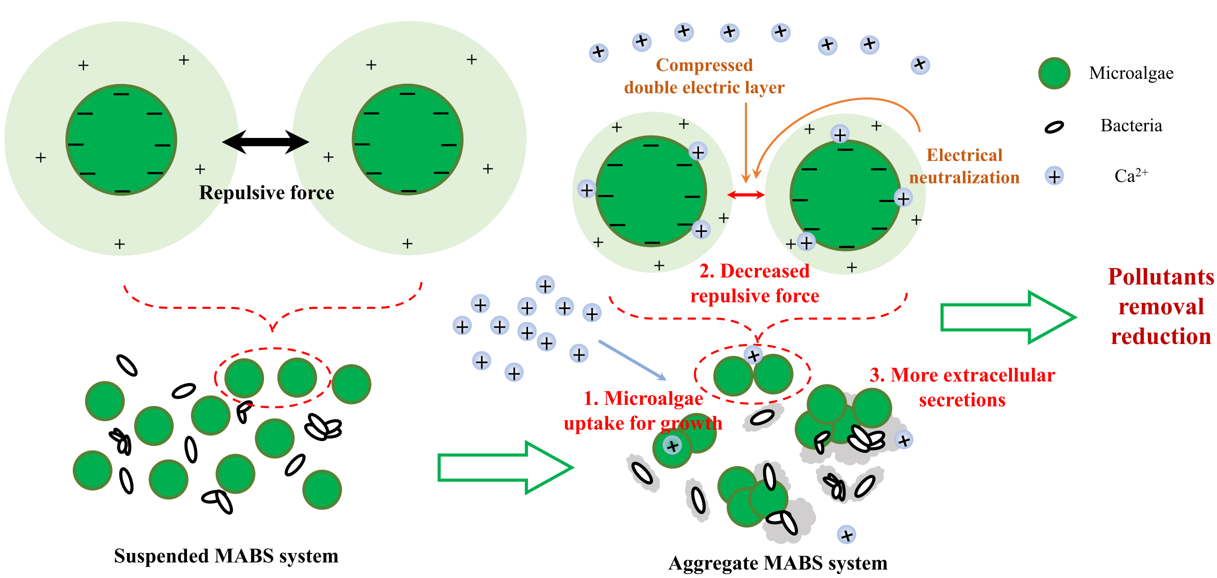

Fig. 6. Mechanisms for cell aggregation and growth affected by Ca^{2+} in MABS system.

rate of 64.15%, whereas a high Ca^{2+} loading (i.e., 10 mM) did an adverse effect on pollutants removal through motivating microbe aggregation with particle size increasing from 74 to 322.8 μm, and the illumination performance for microalgae was shaded in MABS system. Partial electrical neutralization of surface charge contributed by adsorbed Ca^{2+}, compressed double electric layer contributed by dissolved Ca^{2+}, and the promotion of ECS affected by Ca^{2+} were the main mechanism for the formation of MABS aggregates, suggesting that the performance and status of the MABS system could be affected by Ca^{2+}, and greatly related to the Ca^{2+} loading. The findings of this work may provide some new solutions for developing wastewater treatment technology based on MABS system to achieve the enhanced microalgae biomass harvesting and pollutants removal.

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Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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