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Research Paper

Rapid flocculation-sedimentation of microalgae with organosilane-functionalized halloysite

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ABSTRACT

Microalgae is a promising feedstock of biofuel for alternating fossil fuels. The major challenge of microalgal biofuels for commercial applications is in designing an efficient harvesting method with high economic feasibility. In this study, a rapid flocculation-sedimentation harvesting method induced by organosilane-functionalized halloysite flocculant was achieved for *Scenedesmus dimorphus* harvest. The harvesting efficiency was significantly influenced by the pH of microalgal dispersion and the dosage of flocculant. The optimized harvesting condition was pH 3.0 with flocculant dosage of 1.0 $g \cdot g^{-1}$ cell dry mass. Under the optimized harvesting condition, microalgae rapidly reached 93% harvesting efficiency within 0.5 min of settling time, and reached 98% harvesting efficiency within 2 min of settling time. The rapid flocculation was attributed to the charge neutralization of the negatively-charged microalgae cells by the positively-charged organosilane-functionalized halloysite flocculant and to the sweep flocculation by organosilane-functionalized halloysite flocculant. The organosilane-functionalized halloysite flocculant did not affect the lipid extraction of microalgae, and not contaminate the extracted residuals. The organosilane-functionalized halloysite flocculant is of high efficient, cost-effective, and eco-friendly, makes it be of promising application for commercial microalgae harvesting.

1. Introduction

Due to the high content of lipid (up to 80% by mass of the total dry biomass), short growth-cycle (10–50 times faster than terrestrial plants), low land occupation, and low carbon dioxide emission, microalgae has been widely regarded as a promising feedstock for sustainable production of biodiesel, which recognized as ideal substitution for traditional fossil fuels (Chisti, 2007; Demirbas, 2010; Barros et al., 2015; Ummalyma et al., 2017). Usually, the microalgae is in micron size (1–30 μm), of low biomass concentration in culture media (0.5–5 $g \cdot L^{-1}$) and negligible density discrepancy compared with culture media. Moreover, the surface of microalgae cell is negatively charged (−7.5 to −40 mV), leading to a stable algal suspensions. These features of microalgae resulted in great trouble for microalgae harvest, leading to the harvesting and dewatering process consumed much time and energy, approximately account for more than 30% of the total production costs, which significantly impeded the commercialization process of microalgae-based biofuel (Wan et al., 2015).

The cost and energy demand for harvesting microalgae could be significantly reduced if the microalgae could be preconcentrated before dewatering process (Brentner et al., 2011; Vandamme et al., 2013; Ummalyma et al., 2017). Gravity-sedimentation or flocculation-sedimentation are widely used in the preconcentrated process. Gravity-sedimentation is generally recognized as an economical method for separating particles from dilute liquid suspensions, frequently applying in mineral concentration and water treatment (Smith and Davis, 2012). However, the gravity-sedimentation of microalgae is extremely time-consumed because its low density and colloidal stability in suspension resulted in a low sedimentation velocity (e.g. 0.1 $m \cdot day^{-1}$, 0.2 $m \cdot day^{-1}$, and 0.0–0.05 $m \cdot day^{-1}$ for green algae, diatoms, and cyanobacteria, respectively) (Teixeira and Rosa, 2007; Mathimani and Mallick, 2018). Flocculation-sedimentation is commonly employed because the flocculation treatment can increase the effective size of microalgal flocs and decrease the colloidal stability in suspension, leading to the acceleration of the settling rate and promotion of the recovery efficiency (Zheng et al., 2012).

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Flocculation of microalgal can be achieved by chemical flocculation (inorganic metal salts, inorganic polymers, and organic polymers as flocculants), physical flocculation (ultrasound, electro-flocculation, and magnetic separation, etc.), and bio-based flocculation (microbial bio-flocculants-associated bioflocculation, microorganism-associated bio-flocculation, and microalgal cell self-flocculation, etc.) (Vandamme et al., 2013; Wan et al., 2015; Ummalyma et al., 2017). However, these methods possessed their inevitable limitations. For examples, multi-valent inorganic metal salts (e.g. ferric sulphate, ferric chloride, aluminum sulphate and aluminum chloride etc.) were conventional and reliable chemical flocculants, but the toxic nature of metals (e.g. aluminum) often accumulated and interfered with the final applications of the biomass (e.g., food, feed, or fertilizer) or with further processing of the biomass (e.g., lipid extraction and conversion for biofuels) or with the reuse of culture media (Ummalyma et al., 2016; Zhang et al., 2016). The biopolymers (e.g. chitosan, cellulose, cationic starch, poly-γ glutamic acid) are expensive, and the requirement of high dosages made it unavailable for large scale algal biomass production (Laamanen et al., 2016; Mathimani and Mallick, 2018). Moreover, the physical flocculation and bio-based flocculation were established only on lab scales and needed to be confirmed at scale up levels (Barros et al., 2015; Wan et al., 2015; Ummalyma et al., 2017).

The cell surface of microalgae is covered by functional groups (e.g. carboxylic and amine groups). The carboxylic groups are negatively charged above pH 4–5, whereas the amine groups are uncharged at this pH. This results in a net negative surface charge above pH 4–5, leading to the high colloidal stability of microalgae suspensions (Vandamme et al., 2013). Theoretically, the charge neutralization is the key mechanism for microalgae flocculation. Unlike the toxic chemical flocculants and the expensive organic flocculants, clay minerals are eco-friendly and of abundant reserves in nature. Normally, most natural clay mineral particles are negatively charged, which sourced from the permanent charge caused by nonequivalent isomorphic substitution (e.g. Al/Si substitution, Mg/Al substitution) in crystal structure and from the pH dependent charge caused by the exposed surface hydroxyl groups (e.g. SiOH, AlOH) (Bergaya et al., 2006; Liu et al., 2012; Yuan et al., 2015). However, the negatively-charged surface of clay minerals could be regulated as positive charge via physical modification (e.g. surfactant intercalation, chitosan wrapping etc.) or chemical modification (e.g. covalently grafting of organosilane). These features make clay mineral be a potential flocculant for microalgae harvesting.

Halloysite ($Al_2(OH)_4Si_2O_5{\cdot}nH_2O$, n equals 2 or 0) is a naturally occurring dioctahedral 1:1 clay mineral that belongs to the kaolin subgroup. The unit layer of halloysite is composed of an oxygen-sharing tetrahedral SiO_4 sheet and an adjacent octahedral $AlO_2(OH)_4$ sheet. In nature, halloysite shows a dominant tubular morphology, which results from the wrapping of halloysite layers under favorable crystallization conditions and geological occurrences. This wrapping is driven by a mismatch between the larger tetrahedral SiO_4 sheet and the smaller octahedral $AlO_2(OH)_4$ sheet (Joussein et al., 2005; Wei et al., 2019). The lumen surface of halloysite are covered by aluminol (Al-OH) groups, and the external surface of halloysite are covered by siloxane (Si-O-Si) groups (Yuan et al., 2015). This structure results in a positively charged lumen surface and negatively charged external surface. Therefore, halloysite exhibits a negative zeta potential over a pH range of 2.5 to 8.5, which theoretically leads a repulsion force with the negatively charged microalgae. Fortunately, the exposed surface hydroxyl groups are of high chemical reactivity and are available for covalent grafting with many organic compounds (e.g., organosilane, organophosphonic acid etc.) (Tan et al., 2016). The introduction of functional groups onto the surface of halloysite could achieve the orient regulation of the physicochemical property (e.g. change the negatively charged surface into positively charged), which means the organosilane-functionalized halloysite will be a good candidate for microalgae flocculation.

In this work, an organosilane-functionalized halloysite was adopted as flocculant for microalgae harvesting. Attention was focused on the performance and mechanism of halloysite for microalgae flocculation and lipid extraction. This study will provide an efficient and cost-effective method for microalgal harvesting.

2. Experimental

2.1. Microalgae cultivation

The freshwater microalgae *Scenedesmus dimorphus* was grown in a modified BG11 medium (Zhang et al., 2012), and enlarging culture was carried out in indoor 15 L photobioreactors at 25 ± 1 °C under continuous fluorescent illumination with an intensity of 220 μmol·m^{-2}·s^{-1}. Filtered air mixed with 3% (*v*/v) carbon dioxide was continuously supplied at an average rate of 6 L·min^{-1} to provide a carbon source and mix the culture. The pH was maintained at 6.5 ± 0.5 by adjusting the rate of carbon dioxide. Growth of microalgae was terminated at the stationary phase (6 days), and the cell dry mass (DM) was 1.68 g·L^{-1}.

2.2. Organosilane functionalization of halloysite

Raw halloysite sample was collected from Hubei province, China. The sample was purified by hand-pick and sedimentation method and dried overnight at 120 °C. The product was labeled as Hal. Organosilane-functionalized halloysite was prepared following the procedure previously reported (Yuan et al., 2008; Tan et al., 2013; Li et al., 2018). 5 mL of γ-aminopropyltriethoxysilane (APTES) (99%, Aldrich) was dissolved in 200 mL of ethanol, and 10 g of Hal was then added. The dispersion was refluxed at 85 °C for 24 h under constant stirring. The solid phase in the resultant mixture was separated by centrifugation, extensively washed three times with fresh ethanol to remove excess APTES, and then dried overnight at 120 °C. The APTES-modified halloysite was labeled as MHal. The characterization of APTES-modified halloysite has been well established and discussed in our previous work (Tan et al., 2013, 2014; Li et al., 2018).

2.3. Microalgae harvesting

Microalgae harvesting were carried out using a coagulation apparatus (Phipps & Bird, America) with six beakers (500 mL) at 25 ± 1 °C. 400 mL microalgal dispersion was filled into the beaker, and then added a predetermined amount of halloysite samples with varying mass ratio (0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 g·g^{-1} DM of microalgal biomass). The pH of microalgal dispersion was adjusted by 0.1 M HCl or 0.1 M NaOH in the range of 2–11. All samples were rapidly mixed for 1 min at 300 rpm, followed by a slow mixing (50 rpm) for 3 min and natural sedimentation for 15 min. After the sedimentation, an aliquot of the supernatant was withdrawn at a depth of 3 cm below the top of the microalgal dispersion, and the harvesting efficiency (HE) was calculated as Eq. (1):

$$HE = \frac{OD_B - OD_A}{OD_B} \times 100\% \quad (1)$$

OD_B and OD_A are the optical densities of the dispersions before and after harvesting, respectively, measured by an UV-VIS spectrophotometer (DR6000, Hach, USA) at 750 nm. Microalgae harvesting was also conducted via flocculation-sedimentation method (natural halloysite and $AlCl_3$ as flocculant) and natural sedimentation for control experiments. All experiments were performed in duplicates, and the results are expressed as the mean of three independent replicates with error bars representing the standard deviation.

2.4. Lipid extraction

In this study, the microalgal lipid was extracted using a Dionex 350 (Thermo Fisher Scientific, CA, USA) accelerated solvent extraction system with two mixed solvents: solvent A, composed of methanol and

dimethyl sulfoxide (DMSO) with a volume ratio of 9:1, and solvent B, composed of hexane and diethyl ether with a volume ratio of 1:1. The harvested microalgal was dried by a vacuum freeze dryer. Portion of dried microalgae (approximately 45 mg, calculated by microalgal biomass) was loaded into the sample pool, and the lipid was automatically extracted by the pre-set programs. After extraction, a known volume of deionized water (1/3 volume of extracted solution) was added into the extracted solution, followed by a mechanical shake and centrifugation for the delamination, and then the supernatant was collected by a special slender pipette. Finally, the supernatant was firstly concentrated to dry under nitrogen flushing and followed by a freeze-drying process. The total lipid yield (TLY) was calculated as Eq. (2):

$$TLY = \frac{w_l}{w_m} \times 100\% \quad (2)$$

w_l and w_m were the mass of the total extracted lipid and microalgal biomass, respectively.

2.5. Characteration methods

The zeta potentials of the microalgal cell and halloysite samples were measured by a surface potential analyzer (Zetasizer Nano ZS, Malvern, UK) over a pH range of 2–11. SEM micrographs of the harvested microalgal cell (freeze dried) were obtained with a 5-kV FEI-Sirion 200 field emission-scanning electron microscope. Optical micrograph of microalgae and flocculated microalgae were examined under bright optical microscopy with an Olympus microscope (BX53).

3. Results and discussion

3.1. Microalgae harvesting with different flocculant

The harvesting efficiency of microalgae via natural sedimentation and flocculation-sedimentation (Hal, $AlCl_3$, and MHal as flocculant, respectively) was depicted in Fig. 1. In the case of natural sedimentation, microalgae showed a slow settling velocity, corresponding to a low harvesting efficiency, approximately with a 40% harvesting efficiency for 15 min of natural sedimentation (Fig. 1). This poor microalgae harvesting performance was ascribed to that the microalgae dispersion was stable and the microalgae flocs was small (approximately 20 μm × 20 μm) (Fig. 2), which was caused by the low cell density and by the strong electrostatic repulsion between the negatively-charged microalgae cells (approximately −10 mV at pH 3.0) (Fig. 3).

When Hal was used as flocculant, the microalgae harvesting performance was even worse than that in the case of natural sedimentation (Fig. 1). Albeit the halloysite had a relatively heavy density (specific gravity was approximately 2–2.65 $g{\cdot}cm^{-3}$) (www.mindat.org/min-1808.html), the zeta potential of halloysite at pH 3.0 was approximately −40 mV (Fig. 3), leading to a stable halloysite dispersion (Fig. S1). This means that the microalgae cell cannot be harvested through sweep flocculation. In addition, the strongly negatively-charged halloysite was unable to neutralize the negatively-charged microalgae cells, but generated a strong electrostatic repulsion with the negatively-charged microalgae cells and resulted in a more stable dispersion. This means that the microalgae cell cannot be flocculated through charge neutralization. Therefore, these two reasons determined that the natural halloysite was not an effective flocculant, and not suitable for the application of microalgae harvesting.

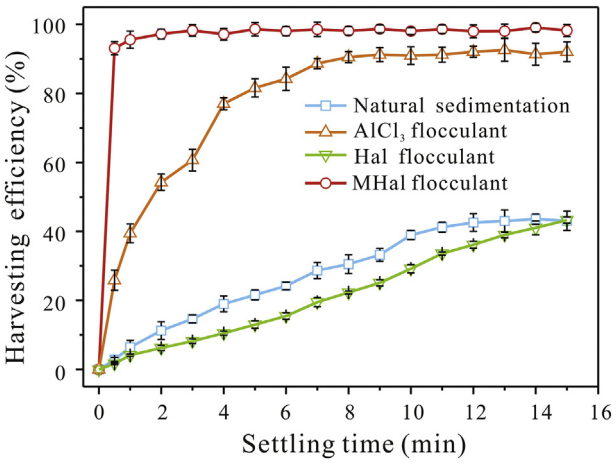

Fig. 1. The dynamic harvesting efficiencies of natural sedimentation (pH 3.0) and flocculation-sedimentation (Hal flocculant, 1 $g{\cdot}g^{-1}$ cell DM, pH 3.0; $AlCl_3$ flocculant, 100 $mg{\cdot}g^{-1}$ cell DM, pH 6.5; MHal flocculant, 1 $g{\cdot}g^{-1}$ cell DM, pH 3.0).

When $AlCl_3$ was used as flocculant, the harvesting performance of microalgae was significantly promoted. The harvesting efficiency was approximately 40% at 1 min, gradually increased with the increase of settling time, and finally reached approximately 90% harvesting efficiency after 10 min settling. This good harvesting performance of $AlCl_3$ was due to the adsorption of positively charged Al species (e.g. $Al(OH)^{2+}$, $Al(OH)_2{}^{+}$, Al^{3+} etc.) (Crittenden et al., 2012) on the surface of microalgae cells resulted in neutralization of the surface charge of microalgae and consequently formation of microalgae flocs, which significantly accelerated the settling velocity and the harvesting process of microalgae. Cui et al. (2014) also used aluminum sulfate as flocculant for the flocculation of *Scenedesmus dimorphus*, and found that the harvesting efficiency of microalgae reached approximately 50% under the optimized flocculation condition (pH 7.5, low ionic strength). Yang et al. (2018) used aluminum sulfate as flocculant for the flocculation of *Scenedesmus acuminatus*, and found that the harvesting efficiency reached approximately 75% for the dosage of 200 $mg{\cdot}g^{-1}$. Rwehumbiza et al. (2012) used aluminum nitrate sulphate as flocculant for the flocculation of *Nannochloropsis salina*, and found that the harvesting efficiency reached 95% within 30 min for a low dosage of flocculant (5.4 $mg{\cdot}L^{-1}$). The discrepancy in harvesting efficiency between these studies and our work was related to the flocculant type, the flocculant dose, the pH of microalgae dispersion, and the type of microalgae etc. Aluminum salts, as traditional flocculants, have been widely used in microalgae flocculation; however, in consideration of its high risk of secondary pollution, it is urgent to develop green and cost-effective alternative flocculant.

When MHal was used as flocculant, surprisingly, microalgae showed a rapid settling velocity (Fig. 2), and the harvesting efficiency explosively reached 93% for 0.5 min of settling time and further increased to 98% for 2 min of settling time (Fig. 1). This outstanding harvesting performance of MHal was contributed to two reasons. (1) APTES modification of halloysite introduced amino groups on the surface (Yuan et al., 2008; Tan et al., 2013, 2014), and changed the zeta potential of halloysite from negative (approximately −40 mV at pH 3.0) to positive (approximately +15 mV at pH 3.0) (Fig. 3). The positively-charged MHal adsorbed onto the surface of microalgae (Fig. 4) and generated electrostatic interaction with microalgae and resulted in charge neutralization of microalgae and the growth of microalgae flocs, which disrupted the stability of microalgae dispersion and facilitated the formation of microalgae flocs for sedimentation (approximately 150 μm × 250 μm and up to 1000 μm × 1500 μm) (Fig. 2, Fig. S2). (2) APTES modification of halloysite decreased the absolute value of the surface charge, meaning the decrease of interaction force (repulsive force) between MHal particles. This issue created a low stability of MHal dispersion (Fig. S1). In addition, the adsorption of MHal onto the surface of microalgae also facilitated the sedimentation of MHal particles, which acted as sweep flocculation for microalgae harvesting. Therefore, microalgae exhibited a rapid flocculation-sedimentation behavior driven by the combined mechanism of charge neutralization and sweep flocculation of the MHal flocculant.

It is noted that the microalgae harvesting condition for MHal

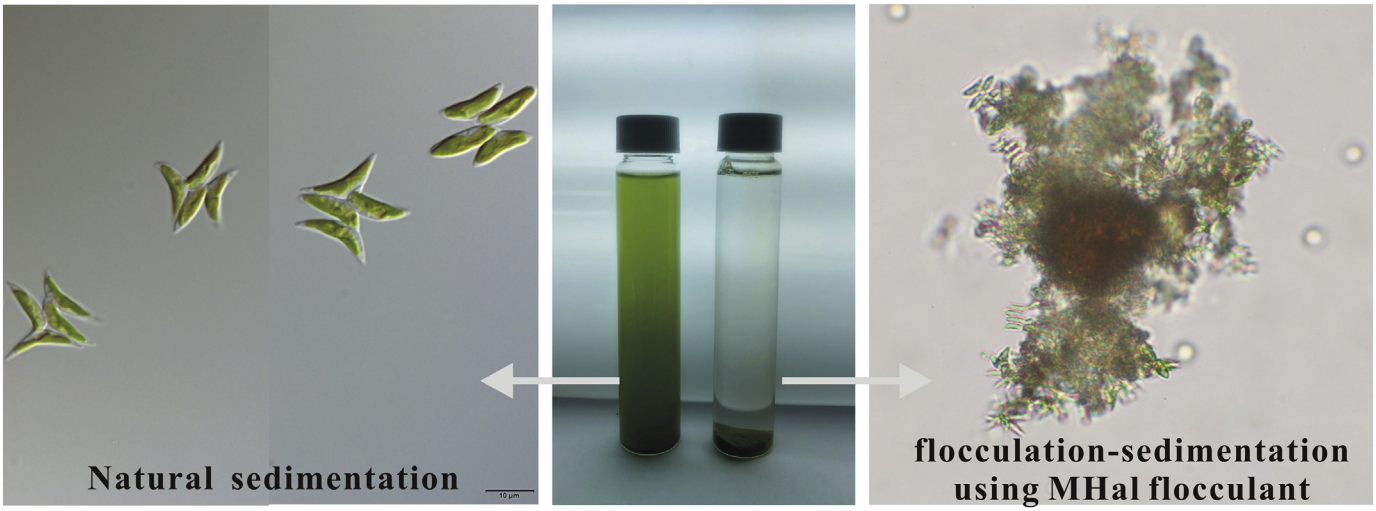

Fig. 2. The optical micrograph of microalgae and flocculated microalgae.

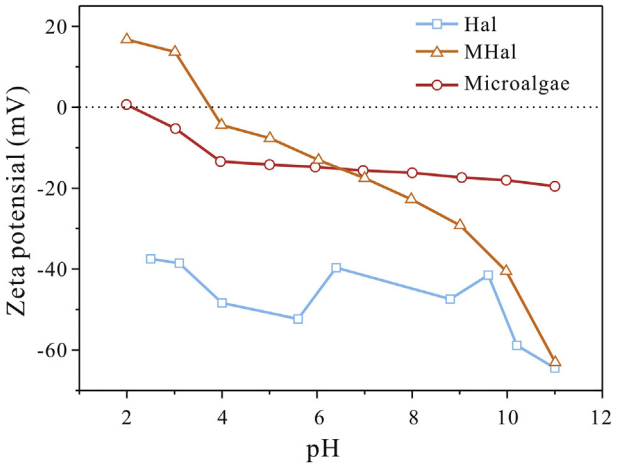

Fig. 3. Zeta potential of MHal, Hal, and microalgae.

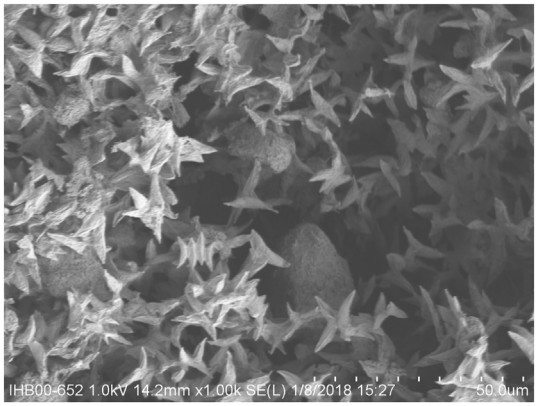

Fig. 4. SEM image of flocculated microalgae.

flocculant (1 $g \cdot g^{-1}$ cell DM, pH 3.0) was much different in compare with that for $AlCl_3$ flocculant (100 $mg \cdot g^{-1}$ cell DM, pH 6.5). It is undeniable that the traditional $AlCl_3$ flocculant is of high efficiency for microalgae harvesting with a small dosage (e.g. 100 $mg \cdot g^{-1}$) and without medium pH adjustment. The dosage of MHal flocculant and medium pH showed significant effect on the harvesting efficiency of microalgae, as discussed in section 3.2. The optimal harvesting condition for MHal flocculant was with a dosage of 1 $g \cdot g^{-1}$ cell DM and with medium pH 3.0. In compare with $AlCl_3$ flocculant, MHal flocculant was much more cost-effective and eco-friendly; moreover, MHal flocculant generated no residual flocculant in culture media, showed no effect on the reuse of culture media; therefore, MHal could be used as alternative flocculant for the traditional chemical flocculant for microalgae harvesting.

3.2. Effect of pH of microalgae dispersion and flocculant dosage on the harvesting efficiency and lipid extraction

The above results showed that MHal was an effective flocculant for microalgae harvesting. In this part, the influence of pH of microalgae dispersion and MHal dosage on the harvesting efficiency were discussed.

In the case of natural sedimentation, the largest harvesting efficiency of microalgae was approximately 40% at pH 2.0, and showed a decreased trend with the increase of pH (Fig. 5), the lowest harvesting efficiency was approximately 6.7%, occurred at pH 6.0, and then harvesting efficiency slightly increased to approximately 10% with the increase of pH. These phenomenon were caused by the variation of zeta potential of microalgae with the increase of pH and by the precipitation of metal ions (e.g. Mg^{2+}, Ca^{2+} etc.) at high pH condition. At pH 2.0, the microalgae had the lowest surface charge (Fig. 3), the microalgae cells had the weakest interaction (repulsive force). Therefore, microalgae had the largest sedimentation efficiency. When pH increased, the zeta

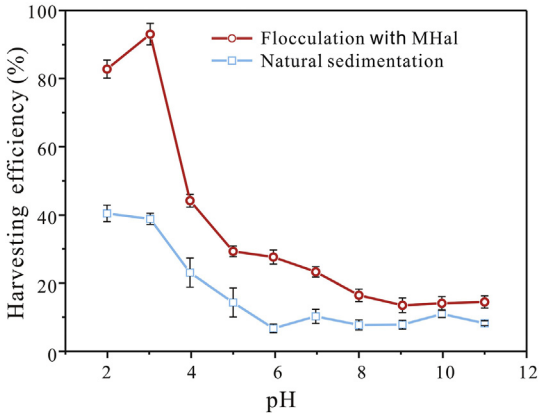

Fig. 5. Effect of the pH on the harvesting efficiency via natural sedimentation and flocculation-sedimentation (MHal flocculant, 1.2 $g \cdot g^{-1}$ cell DM, 15 min of settling time).

potential of microalgae increased, meaning the increase of the interaction between microalgae cells, which resulted in a more stable microalgae dispersion and a lower harvesting efficiency along with the increase of pH. Therefore, the lowest harvesting efficiency occurred at pH 6.0. When pH was higher than 7.0, metal ions (e.g. Mg^{2+}, Ca^{2+} etc.) was precipitated, and resulted in sweep flocculation of microalgae (Zhang et al., 2016), which was benefit for microalgae harvesting. Therefore, under the dual action of negative effect of zeta potential and the positive effect of metal ion precipitation, the harvesting efficiency showed a small extent of fluctuation in the pH range of 7 to 11.

When MHal was used as flocculant for flocculation-sedimentation of microalgae, the initial harvesting efficiency was approximately 82.8% at pH 2.0, and increased to the maximum value (93.0%) at pH 3.0, and then showed a rapid decrease trend with the increase of pH. The possible explanation was as following: (1) at pH 2.0, the zeta potential of microalgae and MHal was +0.6 mV and + 16.7 mV, respectively. Only sweep flocculation exerted for the microalgae harvesting. (2) When pH was 3.0, the zeta potential of microalgae and MHal was −5.3 mV and + 13.7 mV, respectively (Fig. 2). Both charge neutralization and sweep flocculation acted for the microalgae harvesting, resulting the highest harvesting efficiency. (3) When pH was higher than 3.0, both microalgae and MHal possessed negative surface charge, which resulted in repulsive force and showed a negative effect for microalgae harvesting. Therefore, the harvesting efficiency exhibited a decreased trend with the increased pH.

The dosage of MHal flocculant exhibited a positive effect on the harvesting efficiency of microalgae. Without addition of MHal flocculant, the harvesting efficiency was approximately 40%, and it abruptly increased to 74.6% when the dosage was 0.25 $g{\cdot}g^{-1}$ DM, and further increased to 95.7% when the dosage was 1.0 $g{\cdot}g^{-1}$ DM, then it exhibited a small growth extent with the increase of flocculant dosage (Fig. 6). This phenomenon can be explained by collision theory. The average length of *Scenedesmus dimorphus* was about 9.0 μm, and the D_{90} of MHal was 11.85 μm, measured by particle size analyzer (Fig. S3). When MHal flocculant was added into the microalgae dispersion, the similar particle size of MHal particles and microalgae cells was benefit for their collision. Therefore, microalgae flocculated via charge neutralization and sweep flocculation. The larger dosage of MHal flocculant was added, the higher collision probability for flocculant and microalgae occurred, leading to a higher harvesting efficiency. This situation was similar with the collision and attachment between microalgal cells and air bubbles in dissolved air flotation, which can be promoted by increasing the number of bubbles (Zhang et al., 2016).

The microalgae flocs were separated through centrifugation after flocculation-sedimentation or natural sedimentation, then the lipid was extracted after drying process. The total lipid yield for microalgae harvested by natural sedimentation was 37.8%. The theoretical lipid content of *Scenedesmus dimorphus* was about 16%~40% (Warren et al., 2014), indicating the lipid extraction method used in this work was highly efficient. The total lipid yield for microalgae harvested by flocculation-sedimentation with different MHal dosage (0.5, 1.0, 1.5, and 2.0 $g{\cdot}g^{-1}$ DM) was 36.9%, 40.7%, 39.9, and 41.4%, respectively. These results showed that the MHal flocculant did not impede the lipid extraction process, and even facilitated the lipid extraction process through bead-beating mechanism for the cell-disruption (Lee et al., 2010) when MHal flocculant dosage was higher than 1.0 $g{\cdot}g^{-1}$ DM.

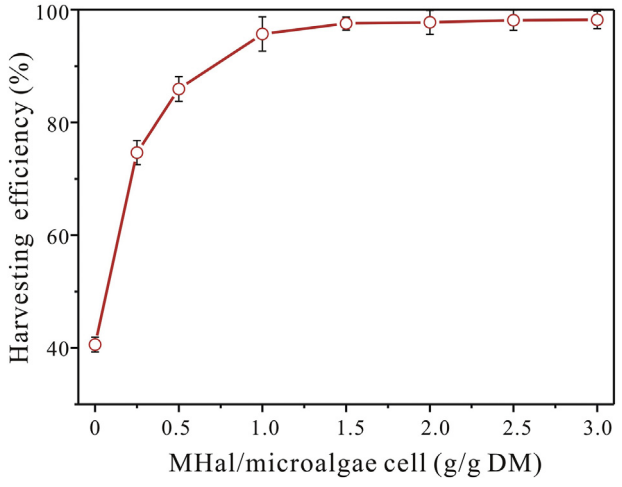

Fig. 6. Effect of MHal dosage on the harvesting efficiency (pH 3.0, 15 min of settling time).

The nutrient content of *Scenedesmus dimorphus* was protein, carbohydrate, and lipid, with a varying DM percentage of 8%~18%, 21%~52%, and 16%~40%, respectively (Warren et al., 2014). After lipid extraction, the residual mainly composed of organics (protein and carbohydrate) and MHal flocculant. The residual organics were good stock for animal feeding stuff, and the MHal flocculant was of high biocompatibility (Vergaro et al., 2010; Shi et al., 2011; Tarasova et al., 2019), and generated no contamination to the residual organics, which means the harvested microalgae could be fully utilized without waste discharge.

The above-mentioned results demonstrated that MHal flocculant possessed some advantages in comparison with other flocculants. (1) High efficiency. The accomplish of 90% harvesting efficiency of microalgae for MHal flocculant only cost 0.5 min, approximately 20 times faster than the $AlCl_3$ flocculant did (10 min). Lee and coworkers (Farooq et al., 2013, 2016; Lee et al., 2013, 2014) synthesized an efficient flocculant, cationic aminoclay, and achieved swift sedimentation of 98% of microalgae within 5 min of settling time through electrostatic interaction between aminoclay and algal cell surface. In addition, Wan et al. (2015) reviewed the harvesting efficiency of different microalgae flocculated by different chemical flocculants (e.g. $Al_2(SO_4)_3$, $FeCl_3$, ammonia, polyacrylamide, chitosan, cationic starch, poly γ-glutamic acid etc.) and found that it would spend 10–120 min for 60%~99% harvesting efficiency, depend on the type of microalgae and chemical flocculant. According to our knowledge, 0.5 min was the fastest record among the already reported flocculation-sedimentation period. (2) No residual flocculant in culture media. Farooq et al. (2013) found there was up to 85% aminoclay flocculant left behind in the supernatant. In addition, for the traditional inorganic chemical flocculants (e.g. $AlCl_3$) and some polymer flocculants (e.g. polyacrylamid), the residual flocculant was inevitably present in culture media. The residual flocculant was of potential risk for secondary pollution of culture media and affected the reuse of culture media. Fortunately, MHal particles were physicochemically stable and not soluble in culture media, it was easy to achieve complete separation from dispersion via centrifugation. The culture media after harvesting microalgae was able to be reused with the feasible pretreatment of the supernatant by pH neutralization and nutrient addition. (3) No biomass contamination and without influence on lipid extraction. Halloysite possessed high biocompatibility (Vergaro et al., 2010; Tarasova et al., 2019), and APTES modification did not affect its biocompatibility (Shi et al., 2011). Therefore, the presence of MHal flocculant in the harvested microalgae did not contaminate the microalgae biomass; in addition, the MHal flocculant did not affect the lipid extraction process of harvested microalgae. However, the metal salts flocculants and some organic flocculants (e.g. polyacrylamid) had been well demonstrated to be harmful for the harvested biomass. (4) Cost-effective and eco-friendly. Halloysite is naturally occurring clay minerals and has large deposits in Australia, United States, China, New Zealand, Mexico, and Brazil (Joussein et al., 2005). Its global supply exceeds thousands of tons per year, its price is about $4 per kg though the purification process could arise the price of raw ores, and this cost is much less expensive in developed countries (Lvov et al., 2008). However, the synthesized inorganic or organic flocculants (e.g. $AlCl_3$, chitosan, aminoclay etc.) are much expensive due to its high dosage, high price, or complicated synthesis procedure. Above-mentioned results

and discussion demonstrated that MHal could be regarded as a green and cost-effective flocculant with high efficiency for microalgae harvesting.

4. Conclusions

Organosilane-functionalized halloysite was found to be a more effective flocculant for flocculation-sedimentation harvest of *Scenedesmus dimorphus* than the traditional inorganic flocculant ($AlCl_3$). Microalgae rapidly reached 93% harvesting efficiency within 0.5 min of settling time, more than 20 times faster than the $AlCl_3$ flocculant. The flocculation mechanism was in combination of charge neutralization and sweep flocculation. The pH of microalgae dispersion and dosage of flocculant affected the flocculation-sedimentation process. The optimized harvesting condition was pH 3.0 with flocculant dosage of 1.0 $g \cdot g^{-1}$ cell dry mass. The organosilane-functionalized halloysite flocculant did not affect the reuse of culture media and the lipid extraction of microalgae; moreover, this flocculant did not contaminate the extracted residuals. This flocculant could achieve rapid, efficient, and cost-effective harvesting microalgae without waste discharge.

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Appendix A. Supplementary data

The stability of halloysite dispersions, the optical micrograph of flocculated microalgae, and the particle size distribution of MHal flocculant. This material is available free of charge via the Internet at http://www.elsevier.com/. Supplementary data to this article can be found online at https://doi.org/10.1016/j.clay.2019.05.005.

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