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Mechanistically harvesting of *Chlorella vulgaris* and *Rhodotorula glutinis via* modified montmorillonoid

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HIGHLIGHTS

- The harvesting efficiency for *C. vulgaris* and *R. glutinis* with ISMM was evaluated.
- The flocculation efficiency of *R. glutinis* was significantly enhanced.
- *C. vulgaris* and *R. glutinis* were efficiently coagulated by ISMM.

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ABSTRACT

In this study, the flocculation process of *Chlorella vulgaris* and *Rhodotorula glutinis* induced by inorganic salts modified montmorillonoid was conducted. The maximum flocculation efficiency (FE) of 98.50% for *C. vulgaris* and 11.83% for *R. glutinis* were obtained with 4 g/L and 5 g/L flocculant within the dosage scope of 1–5 g/L. The difference of FE was then thermodynamically explained by the extended DLVO theory and the FE of *R. glutinis* was mechanically enhanced to 90.66% with 0.06 g/L cationic polyacrylamide (CPAM) at an optimum pH of 9. After that, aimed to utilize the remainder flocculant capacity, *C. vulgaris* culture was added to the aggregation of *R. glutinis*. Fortunately, the coagulation of *R. glutinis* and *C. Vulgaris* was achieved with 0.05 g/L CPAM and 5 g/L flocculant at pH 9 and the FE reached 90.15% and 91.24%, respectively.

1. Introduction

Biofuels, as one of the most promising resources to solve global energy crisis, are proposed due to their renewable and sustainable properties, and their ability to promote agriculture development. Oleaginous microbes, especially algae and some yeasts, such as *Rhodosporidium kratochvilovae, Cryptococcus curvatus* and *Rhodotorula glutinis* (Patel et al., 2015; Yang et al., 2015; Yen and Chang, 2015), possessing characterizations of high productivity, unrestrained growth environment, and flexible culture patterns are broadly regarded as suitable feedstocks for producing biofuels and other various value-added products (Singh et al., 2014). In generally, biomass harvesting and dewatering are two obstacles of the wholesale biofuels production from oleaginous microbes (Beuckels et al., 2015; Markou et al., 2014). However, the low cell concentration (0.5–5 g/L) of microalgae, small size and stable suspensions due to the negative surface charge of *R. glutinis* and *Chlorella vulgaris* result in a significant cost for the biomass harvesting (Powell and Hill, 2013; Greenwell et al., 2010), which even accounts for 30% of the whole biomass production cost (Horiuchi et al., 2003; Zittelli et al., 2006). Besides, the algae removal is urged to deal with the harmful algal bloom and thus cope with damage to the water system (Sengco and Anderson, 2004). Therefore, an efficient harvesting approach is necessary to reduce the cost of biofuel production as well as satisfy the standard of drinking water sanitary quality (Prochazkova et al., 2013).

Flocculation, as one type of cost effective approaches in harvesting microbes, has attracted much attention and was promptly developed as alternative to the traditional techniques of centrifugation, flotation, membrane filtration and gravity sedimentation which are always energy and time consuming, while hindering large-scale development to a great extent (Milledge and Heaven, 2013; Vandamme et al., 2013; Zhang et al., 2010). Common inorganic and organic flocculants such as $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$, $FeCl_3$ and polyacrylamides, widely used in wastewater treatment and mining, were taken in the lead but not advocated due to economic concepts and safety reasons (Bilanovic et al., 1988). Clay has been reported as a promising candidate due to its characteristics of being inexpensive, effective, nontoxic and environmental friendly

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because the biogeochemical system could prompt the healthy transformation of flocculated microbes in the ecosystem (Sengco and Anderson, 2004). However, the effective surface modification is still urged to improve the flocculation efficiency of those clays with less salinity and solve the secondary contaminant as a result of excessive loading. Therefore, the understanding of flocculation mechanisms is essential to effectively harvest oleaginous yeasts and microalgae.

Flocculation is the process where finely dispersed individual cells destabilize and then form large aggregates by adding various flocculants to the culture medium. The mechanism could be explained as the adsorption of cells to flocculant particles and consists of three stages: surface anchoring, adsorption and bridging (Lartiges et al., 1997). The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory deciphers the second process and determines whether one particle has the potential to adsorb the other. Traditionally, the DLVO theory is applied to determine the stability of colloidal suspensions (whether particles will collide or aggregate) and it involves Lifshitz-van der Waals and electrostatic interactions (Liu et al., 2010). A previous study referred to the extended DLVO (eDLVO) theory with the incorporation of Lewis acid-base interaction and it results in more accurate descriptions of the aforementioned systems compared with the classical DLVO theory (Van Oss, 2006). Subsequently, the DLVO and eDLVO theories have been successfully applied to describe the interaction of cells and solid substrate, such as the adhesion of bacteria to solids (Azeredo et al., 1999), aggregation of cells to particles (Harimawan et al., 2013), and adsorption between two types of cells (Agbakpe et al., 2014). However, little attention has been devoted to apply the eDLVO theory to modify and improve the interactions between particles and thus enhance the flocculation efficiency.

In this study, inorganic salts modified montmorillonoid (ISMM) was utilized as the flocculant. The specific objectives are (1) to thermodynamically explain the different flocculation results of *C. vulgaris* and *R. glutinis* with ISMM *via* eDLVO theory, (2) to enhance the flocculation efficiency of *R. glutinis* mechanically; and (3) to coagulate two oleaginous strains, i.e. *C. vulgaris* and *R. glutinis*, in order to utilize the remainder flocculant capacity.

2. Materials and methods

2.1. Oleaginous strains and culture conditions

The microalga *C. vulgaris* was supplied by the Institute of Hydrobiology and kept at the National Energy R&D Center for Biorefinery, Beijing University of Chemical Technology. The yeast *R. glutinis* (CGMCC No. 2258) was provided by the China National Research Institute of Food and Fermentation Industries and stored in the Beijing University of Chemical Technology.

C. vulgaris was grown in BG-11 medium (Zhao et al., 2012) containing 1.5 g/L $NaNO_3$, 51 mg/L $K_2HPO_4{\cdot}2H_2O$, 20 mg/L Na_2CO_3, 75 mg/L $MgSO_4{\cdot}\mathbf{7}H_2O$, 24 mg/L $CaCl_2$, 6 mg/L citric acid, 6 mg/L ferric ammonium citrate, 1 mg/L EDTA and 1 mL of microelements composed of 2.86 mg/L H_3BO_3, 0.39 mg/L $Na_2MoO_4{\cdot}2H_2O$, 1.81 mg/L $MnCl_2{\cdot}4H_2O$, 0.08 mg/L $CuSO_4{\cdot}5H_2O$, 0.22 mg/L $ZnSO_4{\cdot}7H_2O$, 0.05 mg/L $Co(NO_3)_2{\cdot}6H_2O$ in acidified water. The mediums were autoclaved at 121 °C for 20 min. The cultures were performed in 500 mL Erlenmeyer flasks with 100 mL medium. The fermentation was conducted at 25 °C and illuminated continuously during 10 h a day at a light intensity between 2700 and 3500 lx. The cells at the late exponential phase were used with a cell concentration of 1.12 g dry weight/L and the pH value of medium was 9.0.

R. glutinis was grown in a medium (Xue et al., 2008) containing the following components: glucose 40 g/L, yeast extract powder 1.5 g/L, KH_2PO_4 7 g/L, $(NH_4)_2SO_4$ 2 g/L, $MgSO_4$ 2 g/L, Na_2SO_4 2 g/L. The mediums were autoclaved at 116 °C for 25 min and cultivated in 500 mL Erlenmeyer flasks with 100 mL medium at 30 °C, 180 rpm. The cells were harvested at 96 h (stationary phase) with the final concentration being 16.92 g dry weight/L and the final pH value of the suspension was 3.0.

2.2. Flocculation experiment

2.2.1. Concentration effect of ISMM on FE

ISMM, consisting of C, Al, O, Si, N, Ca, S and Fe elements with the corresponding concentration being 38.71, 22.46, 21.1, 6.31, 4.21, 3.76, 2.97 and 0.18 (atomic wt%) respectively, was used as the flocculant and provided by Beijing University of Chemical Technology. The flocculation was performed with small volume of medium (20 mL) in 50 mL centrifuge tubes. The cell suspension was thoroughly mixed with ISMM by a magnetic stirrer at the speed of 900 rpm for 90 s and then lowered to 200 rpm for 30 s. After that, the mixture was gravitationally settled for 10 min. The dosage of ISMM depended on the desired flocculation efficiency. OD_{600} for *R. glutinis* and OD_{680} for *C. vulgaris* of the supernatant obtained from 80% height of the mixture were determined by triplicated sampling.

2.2.2. pH induced flocculation

The pH of the cell suspension was adjusted by adding 1 N H_2SO_4 or NaOH and the mixture was shaken thoroughly at 1200 rpm for 30 s. Then 0.1 g ISMM was added into the cell suspension and the tubes were stirred at 900 rpm for 3 min and then at 200 rpm for 1 min. After that, the mixture was left standing for 10 min and withdrawn to measure the optical density.

2.2.3. CPAM modified flocculation

CPAM (cationic polyacrylamide), provided by a vendor (Lablead Corp, China), was diluted to 1 g/L in DI water. The CPAM modified *R. glutinis* was obtained by mixing various amounts of CPAM with the cell suspension and stabilizing for 20 min. Then 0.1 g ISMM was added and the flocculation experiment was performed under the condition as described in 2.2.2. CPAM induced flocculation experiment without the flocculant was conducted and determined as a control.

2.2.4. Coagulation of C. vulgaris and R. glutinis

After the optimum concentration of CPAM modified *R. glutinis* was flocculated, the suspension was removed by a pipette and then 20 mL *C. vulgaris* culture was added to different amounts of *R. glutinis* concentrations prepared by flocculating different volumes of *R. glutinis* cultures. The following flocculation experiment conditions were described in 2.2.2. The influence of different volume ratios (1:3, 1:2, 1:1, 2:1, 3:1) of *R. glutinis* to *C. vulgaris* was performed. Besides, the coagulation induced by CPAM and ISMM was conducted as a control. Coagulation of CPAM modified *R. glutinis* with *C. vulgaris* aggregation flocculated by ISMM was performed as a certification.

2.3. Characterization

The size distributions of cells and ISMM were determined by the Dynamic Light Scattering (DLS) technique (Brookhaven Instrument Corp, USA).

The zeta potentials of suspended particles were measured by a zeta potential analyzer (Brookhaven Instrument Corp, USA) with a pH range of 3–11 by adding 1 N NaOH and 1 N H_2SO_4. The 1 mL suspension was pipetted into a cuvette with their respective concentration of 0.20 g/L (*C. vulgaris*), 0.10 g/L (*R. glutinis*) and 0.05 g/L (ISMM).

The contact angles of *C. vulgaris*, *R. glutinis* and ISMM were determined by a sessile drop method (Staicopolus, 1963). The layer