Harvesting of *Rhodotorula glutinis* via polyaluminium chloride or cationic polyacrylamide using the extended DLVO theory

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Abstract: Polyaluminium chloride (PAC) and cationic polyacrylamide (CPAM) play a crucial role for separating microorganisms from bulk media. However, the mechanism of adsorption between cells and flocculants remain to be further defined to improve the flocculation efficiency (FE) in extreme conditions. This study conducted the flocculation process of *Rhodotorula glutinis* induced by PAC and CPAM, firstly. The result demonstrated that CPAM possessed more efficient harvesting ability for *R. glutinis* compared to PAC. The difference of flocculation capacity was then thermodynamically explained by the extended DLVO (eDLVO) theory, it turned out that the poor harvesting efficiency of PAC was attributed to lacking of binding sites as well as low adsorption force within particles. Based on this, the FE of PAC to *R. glutinis* was mechanically enhanced to 99.84% from 32.89% with 0.2 g/L CPAM

modification at an optimum pH of 9.

Keywords: Flocculation; *Rhodotorula glutinis*; The extended DLVO theory; Polyaluminium chloride; Cationic polyacrylamide

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1. Introduction

Biofuels and Biodiesel, as one type of renewable and sustainable resources, were proposed to alleviate the global energy crisis sharpened due to resource starvation as well as the aggravation of environmental pollution. Oleaginous microorganisms, generally represented by algae and some of yeasts, such as *Chlorella vulgaris*, and *Rhodotorula glutinis*, were regarded as one of the most promising feedstocks to produce biofuels since the properties of low costs, high productivity and accessible culture conditions (Zhao et al. 2008; Dong et al. 2013; Razack et al. 2016). In the wholescale biofuels production from microbes, biomass harvesting is one critical part when taking the cost proportion into consideration. Horiuchi et al. (2003) concluded that the cost of harvesting process could account for up to 30% of the whole production resulting from the small cell size and the stable surface charge (Horiuchi et al. 2003; Powell et al. 2013). Thus, an efficient approach for harvesting oleaginous microbes is urgent to promote efficient biomass harvesting.

Flocculation, broadly regarded as the most promising pretreatment approach, has promptly developed as the optimum candidate of separation techniques (Vandamme et al. 2013), while other traditional techniques, such as centrifugation, gravity sedimentation, flotation, and membrane filtration, was turned out to be time and

energy consuming (Milledge et al. 2013). Therefore, various chemical flocculants have been synthesized and improved to meet the demand of economic concepts and safety standard (Gerde et al. 2014; Gupta et al. 2014). Besides, the cost effective inorganic and organic flocculants, represented by PAC and CPAM, still attracted much attention and was widely utilized in the large-scale biomass harvesting when the problem of secondary contaminant could be ignored (Wang et al. 2015; Zhao et al. 2015). In general, the pleased flocculation efficiency mainly attributes to repeating optimization of conditions or even the surface modification of particles. Therefore, the understanding of flocculation mechanism was essential to improve the flocculation efficiency purposefully.

The interactions, involved in the flocculation process, were generally considered as the combination of electrostatic adsorption, bridging, and netting (Li et al. 2018). Several attempts have been made for explaining the flocculation process. Lartiges et al. (1997) proposed the flocculation process was firstly triggered by the surface anchoring, then stabilized to form pellets by the interactions aforementioned (Lartiges et al. 1997). Bhattacharya et al. developed a mathematical model to describe the algal harvesting process (Bhattacharya et al. 2017). The extended Derjaguin-Landau-Verwey-Overbeek (eDLVO) theory, composed of Lifshitz-van der Waals interaction, electrostatic interactions and Lewis acid-base interaction, was applied to quantitatively determine the adsorption potentials within two particles (Van Oss. 2006). The eDLVO theory have been successfully utilized to explain the interaction energy and attachment of microbial cells and solid substrate, such as the adsorption between extracellular polymeric substance fractions and microbes (Xu et al. 2016), interaction between microalgae and microfiltration membrane (Ahmad et al. 2013),

and adhesion of cells to particles (Agbakpe et al. 2014).

In this study, the soluble flocculants CPAM and PAC were utilized to flocculate *R. glutinis*. The specific objectives are (1) to conduct the flocculation process and compare the FE, (2) to thermodynamically explain the difference of FE via the eDLVO, (3) to enhance the unsatisfied FE of PAC for *R. glutinis* based on the analyses.

2. Materials and Methods

2.1. Oleaginous strains and culture conditions

The yeast of *R. glutinis* (CGMCC No. 2258), used to produce flocculation process, was provided by the China National Research Institute of Food and Fermentation Industries and stored in the Beijing University of Chemical Technology. The cultivation medium (Xue et al. 2008) was made by dissolving 40 g glucose, 1.5 g yeast extract powder, 7 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 2 g $MgSO_4$, 2 g Na_2SO_4 in 1 L deionized water, autoclaved at 116^{o}C for 25 min, and cultivated in 500 mL Erlenmeyer flasks with 100 mL medium at 30^{o}C, 180 r/min. The microorganisms were harvested after 96 h cultivation with the final concentration being 16.92 g dry weight/L.

2.2. Flocculation experiment

2.2.1. Concentration effect of PAC and CPAM on FE

PAC and CPAM, provided by a vendor (Lablead Corp, China), were utilized as the flocculants. The flocculation processes were conducted in 50 mL centrifuge tubes

with 20 mL medium volume. The cell suspension mixed with the flocculants was thoroughly stirred by the magnetic stirrer at the speed of 900 r/min for 90 s and lowered to 200 r/min for 30 s and then left standing for 10 min. OD_{600} for the supension obtained from 80% height of the mixture were determined.

2.2.2. pH induced flocculation

The pH of the cell culture solution was adjusted with 1 mol/L H_2SO_4 and NaOH. Then the desired flocculants were added into the cell suspension and the flocculation processes were performed under conditions as described in 2.2.1.

2.2.3. CPAM modified flocculation

The CPAM solution was made by dissolving 0.1 g CPAM in 100 mL DI water. The CPAM solution was added to cell suspension to obtain the CPAM modified *R. glutinis* cells. Then PAC was added in the mixture and the flocculation experiment was conducted under conditions as described in 2.2.1.

2.3. Characterization

The size distributions of *R. glutinis* were determined by the Dynamic Light Scattering (DLS) technique (Brookhaven Instrument Corp, USA). In this study, the adsorption between cells and the flocculants was assumed as the interaction between cells and the cells coated with flocculants. Thus, the diameters of the soluble flocculants were equal with the average diameter of *R. glutinis*.

The zeta potentials of *R. glutinis* suspension were directly measured by a zeta potential analyzer (Brookhaven Instrument Corp, USA). The zeta potentials of

flocculants were calculated using standard curve that described the relationship between zeta potentials and the dosage.

The contact angles of particles and water, ethylene glycol and α-bromonaphthalene were determined by DSA 100 system (Powereach Inc., China).

2.4. Harvesting efficiency evaluation

The flocculation efficiency (FE) and harvesting efficiency (HE) were used to evaluate the flocculation performance and calculated according to Eq. (1) (Hu et al. 2013) and Eq. (2):

$$FE(\%) = \left[1 - \frac{OD_n}{OD_0}\right] \times 100\% \quad (1)$$

$$HE(\%) = \frac{V_n}{V_0} \times 100\% \quad (2)$$

where OD_0 and OD_n mean OD values measured before and after flocculation process at the optical density of 600 nm; V_n was the volume of aggregated microbes, and V_0 was the initate volume of cell culture.

3. Results and Discussion

3.1. Comparison of CPAM and PAC for harvesting *R. glutinis*

The concentration influences of PAC and CPAM on flocculating results of *R. glutinis* were conducted and the result was shown in **Fig. 1**. As shown in **Fig. 1a**, with CPAM dosage range of 0.1-0.3 g/L, the flocculation efficiency of *R. glutinis* increased while the increment speed decreased. Besides, the maximum FE (99.75%) was

obtained with the concentration of CPAM being 0.3 g/L . Subsequently, FE showed a downward trend with the increment of CPAM. The above relationship between FE and the flocculant dosage was explained by the adsorption process within cells and flocculants. Firstly, the number of cells adsorbed is increasing with the concentration of flocculant increment, so the FE showed an upward trend. However, the flocculant would be added to stabilize the flocculation system again when the dosage of flocculant and the number of cells reached the state of saturated adsorption, and the further addition of the flocculants contributed to the reducing FE. Besides, HE also showed a certain variation with the increment of flocculants. Within the range of 0.1-0.3 g/L, HE increased as the amount of cells adsorbed by the flocculant increased with the flocculation efficiency increased. And the maximum HE (18.67%) was obtained when the dosage of CPAM being 0.3 g/L. Therefore, 0.3 g/L was considered as the optimal concentration of CPAM when flocculating *R. glutinis*.

The effect of PAC concentration on the FE and HE of *R. glutinis* was presented in **Fig. 1b**. The FE showed a steady upward trend at a flocculant dosage scope of 0.4-1.6 g/L, and the maximum FE (32.89%) was obtained when the flocculant dosage being 1.6 g/L. Further addition of PAC did not significantly increase FE compared with 1.6 g/L addition. In addition, HE increased with the addition of PAC, and it reached up to 63.64% when PAC addition being 2.0 g/L. This suggested that additional dosage of PAC, more than 1.6 g/L, contributed little increasement to the FE but resulted in a sharp increment of HE. Thus, the PAC dosage of 1.6 g/L was chosen throughout the whole flocculation experiment of *R. glutinis*.

It was derived that CPAM induced flocculation was more efficient to *R. glutinis* than PAC. The eDLVO theory, utilized to analyze the interaction energies involved in the flocculation process, was applied to explore the difference of the flocculation results mentioned above.

3.2. Explanation of flocculation differences with the eDLVO theory

Table 1 listed the contact angles of the flocculants and cells with water, ethylene glycol and α -bromonaphehalene (surface energy components given in **Table 2**) (Good and Oss 1992). The surface energy components (γ_s^{LW}, γ_s^{+} and γ_s^{-}) of particles (shown in **Table 3**) were calculated according to Eq. (3) (Dann 1970).

The amount of flocculant addition was proportional to the surface area of the cell, and the zeta potential was proportional to the charge of the cell surface. Thus, there existed a linear relationship between the zeta potential and flocculant concentration (shown in **Fig. 2**) and written as :

$$y = 0.00176 + 0.01167x \tag{3}$$

$$y = -0.0424 + 0.2279x \tag{4}$$

where unit of x was mg in Eq.(3), while μg in Eq.(4). The calculated zeta potentials were calculated using optimum concentration based on the abovementioned assumption and the results were summarized in **Table 1**.

In the follow model the cells were assumed as spheres (Zhang, X. et al. 2019) and the flocculants completely covered the surface of the yeast. Therefore, the interactions

between the cells and the flocculants were modeled as particle-particle geometry.

The Lifshitz-van der Waals interaction and Lewis acid-base interaction of cells and the flocculants were derived from Eq.(6) and (7).

$$(1+\cos\theta)\gamma_l = 2¿ \quad (5)$$

$$\Delta G_{mws}^{LW} = -2\left(\sqrt{\gamma_{mv}^{LW}} - \sqrt{\gamma_{lv}^{LW}}\right)\left(\sqrt{\gamma_{sv}^{LW}} - \sqrt{\gamma_{lv}^{LW}}\right) \quad (6)$$

$$\Delta G_{mws}^{AB} = 2¿ \qquad -2¿ \quad (7)$$

In view of their spherical shapes, the Lifshitz-van der Waals interaction and Lewis acid-base interaction separated by distance d can be written as:

$$\Delta G^{LW}(d) = \frac{-Ar}{12d} \quad (8)$$

$$A = -12\pi d_0^2 \Delta G_{mws}^{LW} \quad (9)$$

$$\Delta G^{AB}(d) = \pi r \lambda \Delta G_{mws}^{AB} \exp\left[(d_0 - d)/\lambda\right] \quad (10)$$

where A was the Hamaker constant; λ was the correlation length and d_0 was the closest distance between two particles. The values of λ and d_0 were given being 0.6 nm and 0.157 nm, respectively (Van Oss 2006).

Formula 11 expresses the electrostatic interaction energy separated by distanced (Van Oss et al. 1986):

$$\Delta G^{EL}(d) = \frac{\pi\varepsilon r\left(\zeta_1^2 + \zeta_2^2\right)}{2}\left[\frac{2\zeta_1\zeta_2}{\zeta_1^2 + \zeta_2^2}\ln\frac{1+\exp(-\kappa d)}{1-\exp(-\kappa d)} + \ln\left(1-\exp(-2\kappa d)\right)\right] \quad (11)$$

where ζ and ε were the zeta potential and permittivity of the medium, respectively; κ^{-1} was the double-layer thickness and can be found out as:

$$\kappa^{-1}=\sqrt{\frac{\varepsilon kT}{e^2\sum v_i^2 n_i}} \quad (12)$$

where k was Boltzmann's constant and being 1.38×10^{-23} J/K; e was charge of an electron and being 1.60×10^{-19} C; T was absolute temperature and being 298.15 K; n_i was the number density of different ions in the bulk liquid; v_i was the corresponding valency. In this study, κ^{-1} of *R. glutinis* cultural was calculated to be 1.1862 nm.

The eDLVO theory (Van Oss 2006) proposed that the total Gibbs free energy (ΔG_{TOT}) was consisted of ΔG^{LW}, ΔG^{AB} and ΔG^{EL}. The variation of ΔG_{TOT}, ΔG^{LW}, ΔG^{AB} and ΔG^{EL} versus the separation distance *d* between cells and ISMM were plotted in **Fig. 3**.

It was demonstrated that ΔG_{TOT} between *R. glutinis* and the flocculants showed a primary minimum and totally attractive force due to the negative ΔG^{LW}, ΔG^{AB} and ΔG^{EL}. However, *R. glutinis* showed a preference to CPAM than PAC as ΔG_{TOT} between CPAM and cells was stronger than PAC. As for CPAM, ΔG_{TOT} was negative and presented attract interaction, which was in accordance with the satisfied flocculation results shown in **Fig. 1a**. The negative interaction between PAC and *R. glutinis* indicated that PAC was potential to attractive the yeast, which was inconsistent with the poor flocculation efficiency (shown in **Fig. 1b**). The conflict may attribute to the insufficient binding sites between PAC and cells, thus the

flocculation process was not completely triggered. Besides, the lower zeta potential between cells and PAC contributed to the weaker electrostatic interaction.

3.3. Enhancement of PAC for harvesting *R. glutinis*

The pH induced flocculation of *R. glutinis* cells with PAC was performed because the surface charge of the yeast could be changed in different pH conditions. As apparent in **Fig.4**, PAC showed poor flocculation efficiency of *R. glutinis* (the flocculation efficiencies were 44.52%, 45.2% and 45.86% at pH 3, 5 and 7, respectively) when the pH value was in the range of 3-7. The HE decreased from 61.22% to 51.15%. When the pH was 9, the FE increased to 57.24%, and the corresponding HE was 52.27%. The FE was enhanced with the pH value further increasing, and HE reduced slightly. It was derived that the optimum pH value for PAC flocculating *R. glutinis* was 9, and the FE and HE were 57.24% and 52.27%, respectively.

Based on the results above, the FE could be improved further by enhancing the surface complexing interaction between *R. glutinis* cells and PAC. CPAM was chosen to modify *R. glutinis* cells as it not only adsorbed to cells but also increased the amount of -OH of cells after adsorption. The harvesting results were sharply improved and when flocculated *R. glutinis* modified with different amounts of CPAM *via* PAC (shown in **Table 4**). The FE was as high as 99.84% when the concentration of CPAM and PAC being 0.2 g/L and 0.4 g/L, respectively. And the HE was only 19.34% in this condition. While the optimal FE (32.89%) was obtained when conducting PAC induced flocculation, and the corresponding HE reached up to

63.64%. Thus, it can be concluded that the modification with CPAM can significantly reduce the amount of flocculants and improve the flocculation results.

4. Conclusions

CPAM showed satisfied harvesting efficiency to *R. glutinis* and the optimal FE and HE were 99.75% and 18.67%, respectively. However, PAC was inefficient when utilized to harvest *R. glutinis*, and the optimal FE was 32.89% with the dosage scope being 0.4-2.0 g/L. The eDLVO theory was used to explain the difference between CPAM and PAC, it was concluded that the poor flocculation ability of PAC to *R. glutinis* was attributed to the lacking of binding sites and weak electrostatic interaction. The FE was improved to 57.24% when pH value being 9.0, and the corresponding HE being 52.27%. The flocculation efficiency was further enhanced by CPAM modification, the FE and HE were 99.84 and 19.34% with the dosage of CPAM and PAC being 0.2 g/L and 0.4 g/L, respectively.

Acknowledgments

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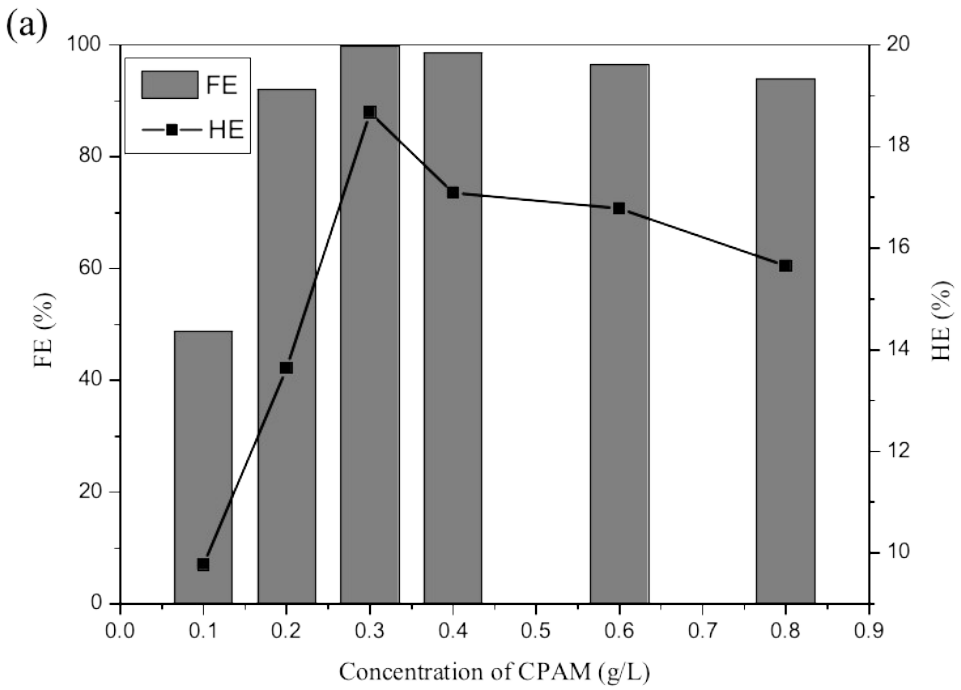
334 **List of figures**


(a)
FE
HE
FE (%)
HE (%)
Concentration of CPAM (g/L)
0
20
40
60
80
100
10
12
14
16
18
20
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
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0.9

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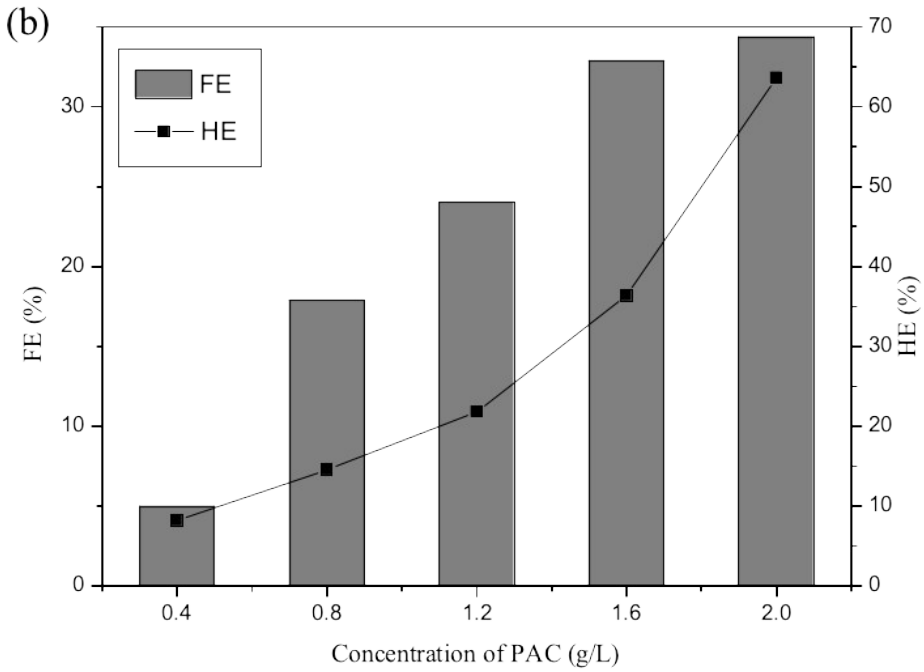

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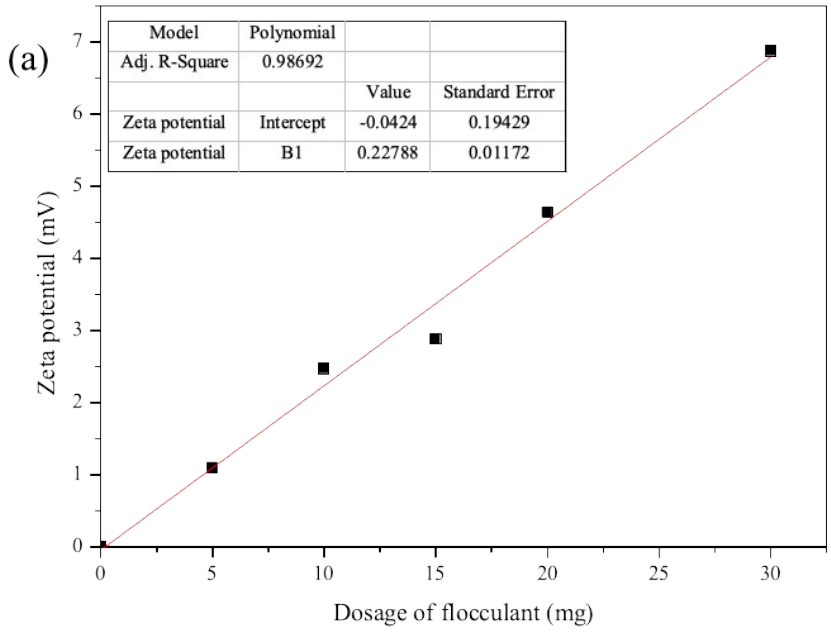
337 **Fig. 1.** Influence of flocculants concentration on FE and HE (a: CPAM and *R. glutinis*; b:

338 PAC coated *R. glutinis*)



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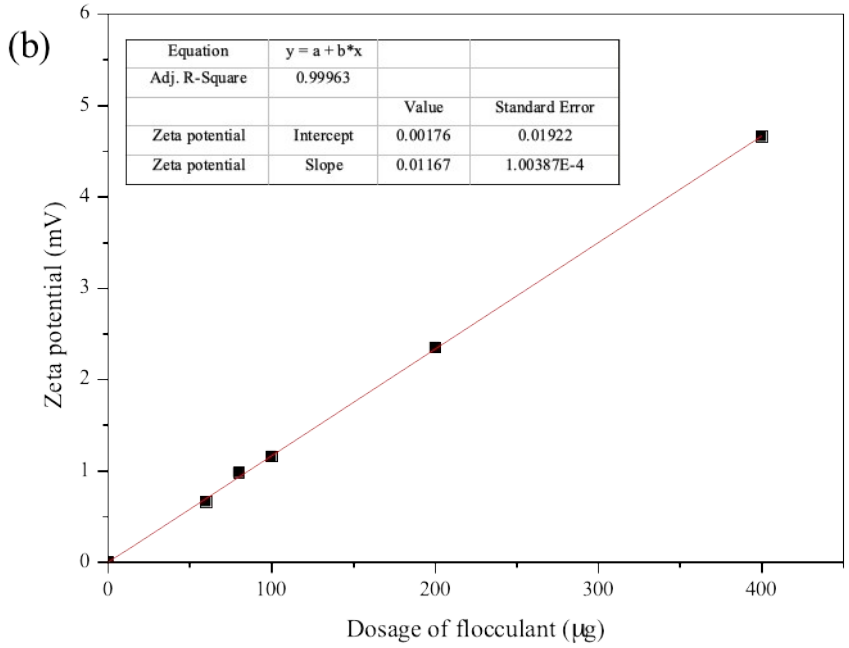

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341 **g. 2.** Influence of concentration on zeta potential of PAC and CPAM in different culture (a: PAC

342 coated *R. glutinis*; b: CPAM coated *R. glutinis*)

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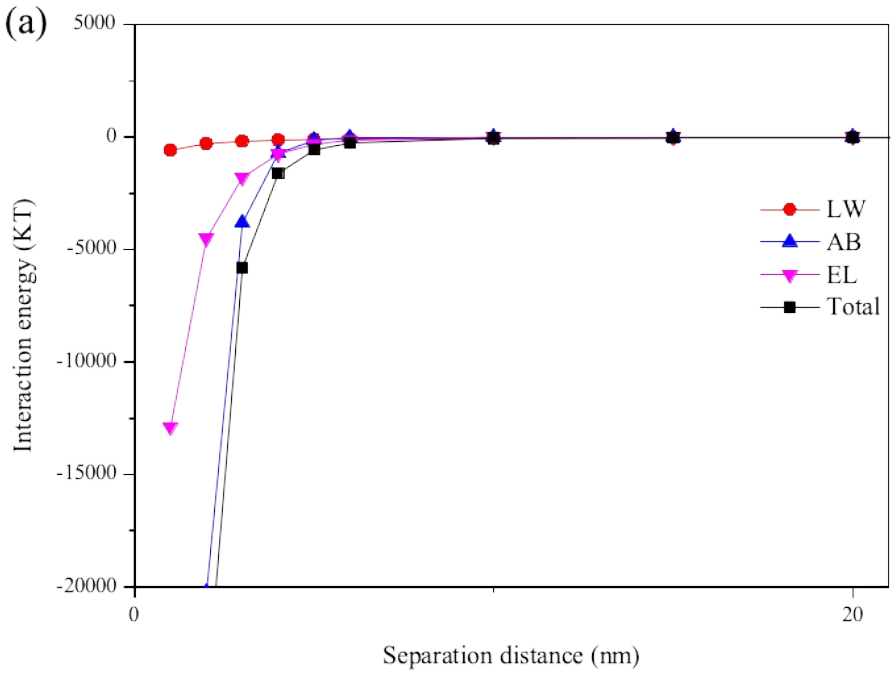

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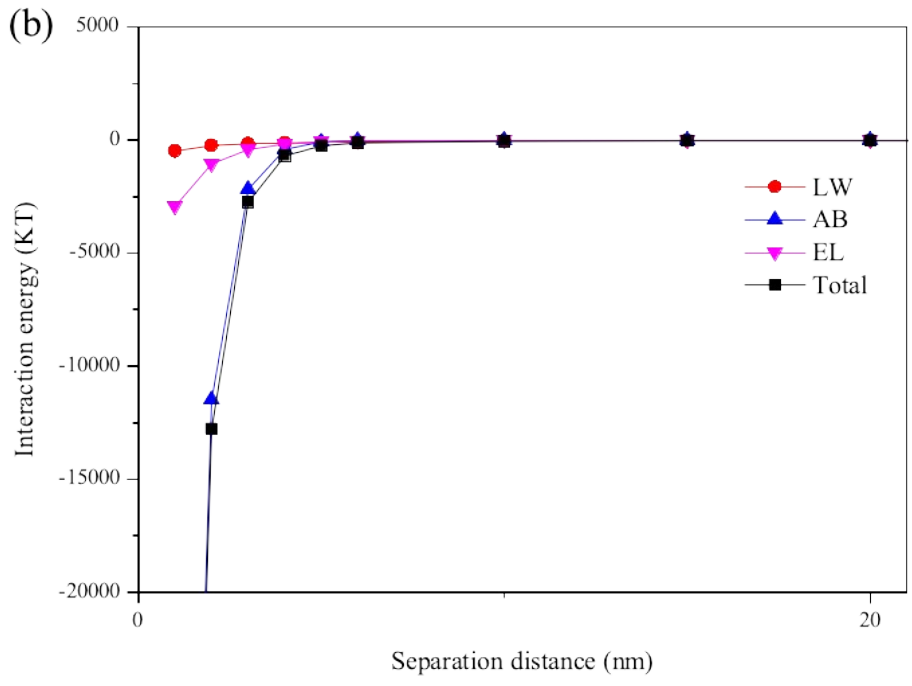

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346 **Fig. 3.** Interaction energies between the flocculants and cells versus the distanced (a:

347 CPAM and *R. glutinis*; b: PAC and *R. glutinis*)

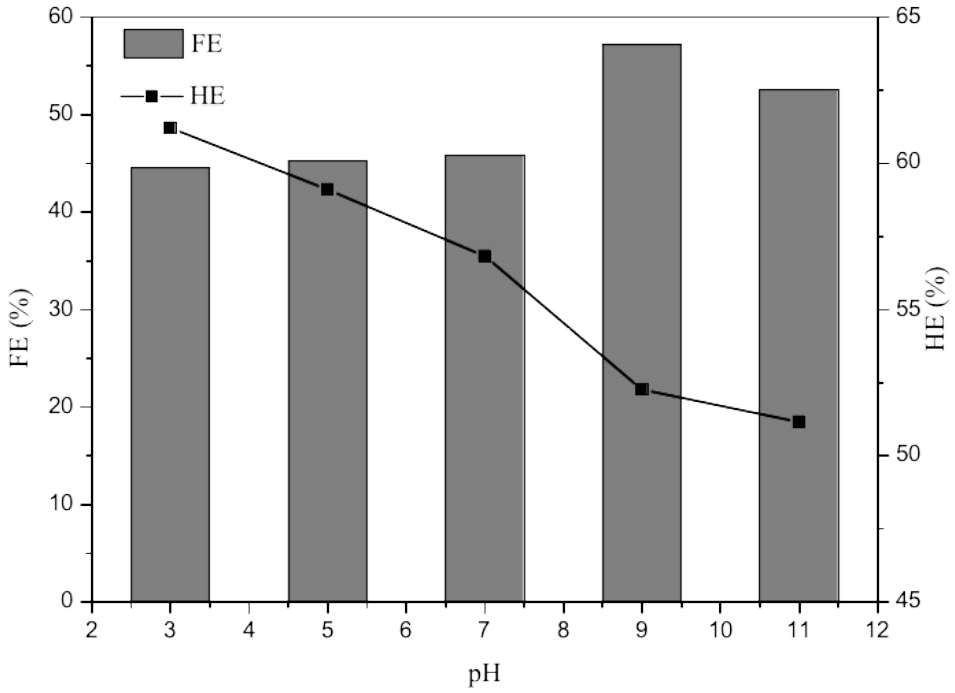

Fig. 4. pH effect of PAC on *R. glutinis* flocculation

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374 **Table 1** Physico-chemical properties of flocculants and *R. glutinis*

Solid	Dm(μm)	Zeta potential(mV)		Contact angle (°)		
				Water	Ethylene glycol	α-bromonaphthalene
CPAM	18.6012	Yeast	75.75	118.38	73.33	40.91
PAC	6.773	Yeast	18.20	68.64	70.48	46

375

376 **Table 2** Surface tension energy components of liquid (mJ/m^2) (Good and Van Oss,

377 1992)

Liquid	γ_l	γ_l^{LW}	γ_l^{AB}	γ_l^+	γ_ℓ
Water	72.8	21.8	51.0	25.5	25.5
Ethylene glycol	48.0	29.0	19.0	1.92	47.0
α-bromonaphehalene	44.4	44.4	0	0	0

378

379 **Table 3** Surface energy components of flocculants and cells (mJ/m^{-2})

Solid	γ_s^{LW}	γ_s^+	γ_s^-
CPAM	34.2225(5.85)	0	0
PAC	31.9225(5.65)	0	21.2521(4.61)
R. glutinis	35.6184(5.97)	0.3703(0.61)	0.0198(0.14)

Table 4

Table 4-1 Influence of CPAM-PAC concentration on FE of *R. glutinis*

PAC \ CPAM	**0.1 g/L**	**0. 2 g/L**	**0.25 g/L**	**0.3 g/L**	**0.4 g/L**
0.4 g/L	50.62	99.84	99.68	99.88	99.57
0.8 g/L	54.68	99.34	99.42	99.89	99.65
1.2 g/L	60.22	99.31	98.28	99.81	99.53
1.6 g/L	63.79	98.58	99.41	99.90	99.81
2.0 g/L	65.76	99.31	99.66	99.93	99.79

Table 4-2 Influence of CPAM-PAC concentration on HE of *R. glutinis*

PAC \ CPAM	**0.2 g/L**	**0. 2 g/L**	**0.25 g/L**	**0.3 g/L**	**0.4 g/L**
0.4 g/L	16.43	19.34	18.51	17.97	18.50
0.8 g/L	23.44	25.23	22.63	20.09	21.14
1.2 g/L	37.44	36.11	32.95	22.17	22.69
1.6 g/L	46.95	42.20	38.72	23.77	24.16
2.0 g/L	69.77	50	43.59	28.89	29.73