Environ. Sci. Technol. **2007**, *41,* 4620−4625

DLVO Approach to the Flocculability of a Photosynthetic H_2-Producing Bacterium, *Rhodopseudomonas acidophila*

XIAO-MENG LIU, GUO-PING SHENG, AND HAN-QING YU*

School of Chemistry, University of Science & Technology of China, Hefei, 230026 China

The DLVO theory was used to explore the flocculation characteristics of a H_2-producing photosynthetic bacterium, *Rhodopseudomonas acidophila.* The relationship between the surface characteristics of this strain and its flocculability was evaluated. Its flocculability was governed by both electrolyte concentration and pH, and the appropriate electrolyte concentration and pH were found to be 0.1 M NaCl solution and pH 7.0, respectively. In addition, the extracellular polymeric substances produced by *R. acidophila* were observed to have a significant effect on its flocculation. The effective Hamaker constant between *R. acidophila* and water was only 2.27×10^{-23} J, suggesting that the contribution of van der Waals interaction energy to the total interaction energy could be neglected. As a result, the bacterial particles could not overcome the total energy barrier to flocculate effectively. Otherwise, because the repulsive total interfacial free energy between the bacterial cells and water was positive, the cell particles of *R. acidophila* repelled each other, resulting in a great stability of the cell suspensions.

Introduction

H_2 is considered as a viable alternative fuel and "energy carrier" of the future. Due to an increasing need for H_2 energy, development of cost-effective and efficient H_2 production technologies has gained significant attention in recent years (*1*, *2*). Photosynthetic bacteria (PSB) are regarded as one of the most promising cultures for biological H_2 production because of their high H_2 conversion yield (*3*, *4*). To achieve a rapid and stable H_2 production, PSB are expected to be cultivated in a photobioreactor to produce H_2 continuously. However, because PSB flocculate poorly (*5*), cells cannot be efficiently separated from supernatant and flow away with effluents, resulting in a low PSB cell concentration and a low H_2 production rate in H_2-producing reactors. To solve this problem, it is essential to explore and accordingly improve the flocculation characteristics of PSB.

Bacterial flocculation is highly related to the bacterial surface characteristics, inert electrolyte concentration (*6*), and extracellular polymeric substances (EPS) (*7*). A large amount of research work has been conducted regarding the flocculation capacity of pure or mixed cultures (*8*, *9*), such as *Rhodopseudomonas sp.* (*3*, *5*, *7*), activated sludge (*10*−*12*) and biofilm (*13*). However, the relationship between bacterial surface characteristics and flocculation is not clear yet and the reason for the poor flocculability of PSB is not elucidated.

The DLVO theory (*14*), named after Derjaguin, Landau, Verwey, and Overbeek, was first proposed to describe the stability of colloidal suspensions in the field of colloid chemistry, and has been widely used in different fields (*15*−*17*). The pioneering work by Marshall et al. (*18*) suggested that bacterial sorption to surfaces involves an initial reversible sorption step, followed by an irreversible adsorption process. They also claimed that the effect of electrolyte concentration on the initial reversible step could be elucidated by DLVO theory (*18*). Since then, the DLVO theory has been applied as both qualitative and quantitative models to explain microbial adhesion (*19*−*23*). van Loosdrecht et al. (*21*) estimated the energy of adhesion per cell. Zehnder et al. (*22*, *23*) also quantified the polymer interactions from the deviation of bacterial adhesion from the DLVO-based expectations.

In our previous studies on *Rhodopseudomonas acidophila* (*3*, *5*, *7*), the production of EPS by *R. acidophila* under various conditions was investigated, and the relationship among the EPS contents, components, and the bacterial flocculation was also evaluated. EPS and their contents and composition have a significant effect on the bacterial surface characteristics and flocculability. However, the reasons for the poor flocculability of *R. acidophila* are not clear yet. Therefore, in this work the DLVO theory was adopted to analyze the stability of cell suspension and flocculation of *R. acidophila*. In addition, the relationship between the bacterial surface characteristics and flocculation was explored. It would be useful to understand the reasons for the poor flocculability of PSB and to elucidate the relationship between their surface characteristics and flocculation. With such information, strategies to improve the flocculability of PSB for biological H_2 production might be identified.

Materials and Methods

Photosynthetic Bacterium and Chemical Analysis. *R. acidophila* used in this work was obtained from the East Sea Fisheries Research Institute, Shanghai, China. This strain is able to utilize acetate, propionate, and butyrate as substrates to produce H_2 (*3*). This strain was anaerobically grown in a medium at pH 7.0. The composition of this medium can be found in our previous paper (*3*). The strains were grown in 500-mL rubber-stoppered vials at 30 °C and 3000 lux for 70−80 h. Anaerobic conditions were created by purging with pure argon.

All chemicals used in this work were of analytical grade. Bacterial growth was described by measuring the dry cell weight. After 70-h cultivation, the bacterial cells were collected by centrifugation. Because of the poor flocculability of *R. acidophila*, a centrifugation time as long as 10 min and a centrifugation speed as high as 12000 rpm were selected for the efficient separation of cells from their solution. This method was proven to be effective for separating the cells and rarely causing the removal of EPS from the surface of *R. acidophila* in a previous study (*3*). After being harvested by centrifugation, the PSB cells were washed twice with 0.1 M NaCl solution. Thereafter, the cell pellets were re-suspended in NaCl solutions with different NaCl concentrations for analysis. After that, this PSB suspension was used for the electrokinetic potentials (e.g., ζ potential) measurement (NanoZS, Malvern Co., UK). The optical density of this suspension was also measured as absorbance at 650 nm using a spectrophotometer (UV751GD, Analytical Instrument Co., Shanghai, China) in all experiments.

* Corresponding author fax: +86 551 3601592; e-mail: hqyu@ustc.edu.cn.

10.1021/es070107n CCC: $37.00
Published on Web 05/31/2007

The DLVO Theory. The total energy of interaction (W_{TOT}) between two spherical particles can be obtained by summation of the electric double layer (W_R) and van der Waals energies (W_A):

$$W_{TOT} = W_A + W_R \quad (1)$$

where

$$W_A = -\frac{A_{BLB}R}{12H} \quad (2)$$

$$W_R = 2\pi\epsilon R\psi_s^2 \ln(1 + \exp(-\kappa H)) \quad (3)$$

where A_{BLB} is the effective Hamaker constant, which is measured by a contact angle approach. The A_{BLB} value is 2.27 $\times 10^{-23}$ J in this work. H is the separation distance between the cells. R is the cell radius, and the cells are assumed to be spherical with a diameter of 2 μm. Ψ_s and κ represent the stern potential and inverse of the Debye length, respectively, which are related to the electric double layer interaction W_R.

In the DLVO theory, W_R in eq 3 is an approximately exponential function of the distance between the particles with a range of the order of the thickness of the double layer (i.e., κ^{-1}), and W_A in eq 2 decreases as an inverse power of the distance between the particles. Hence, the van der Waals attraction will predominate at small interparticle distances, whereas the double-layer repulsion may predominate at intermediate distances. In general, if a large energy barrier prevents the close contact of particles to the surface, the flocculation and deposition is highly prevented and the system should be kept stable.

Contact Angle and Surface Characteristic Evaluation. The interfacial tension and surface free energy of *R. acidophila* cells were estimated using a contact angle approach (JC2000A, Powereach Co., Shanghai, China). Homogeneous cellular layers were prepared by collecting bacterial cells on 0.45-μm acetate cellulose membranes, which were washed twice with double distilled water and then placed on 1% agar plate. Prior to the measurement, the membranes were mounted on glass slides and air-dried for 10 min, and the advancing contact angles were directly measured using the sessile drop technique with a drop of the liquids water, 1-bromonaphthalene, and formamide. All contact angle values were based on arithmetic means of at least ten independent measurements.

The total surface tension (γ_i) for a surface i can be separated into two components, i.e., an apolar Van der Waals surface tension component γ_i^{LW}, and a polar or acid-based surface tension component γ_i^{AB}. Hence, the total surface tension of bacterium and liquid in this work can be respectively expressed as following:

$$\gamma_B = \gamma_B^{LW} + \gamma_B^{AB} \quad (4)$$

$$\gamma_L = \gamma_L^{LW} + \gamma_L^{AB} \quad (5)$$

The surface tension component and parameters of *R. acidophila* were calculated with eq 6 (*20*):

$$(1 + \cos\theta)\gamma_L = 2((\gamma_B^{LW}\gamma_L^{LW})^{1/2} + (\gamma_B^+\gamma_L^-)^{1/2} + (\gamma_B^-\gamma_L^+)^{1/2}) \quad (6)$$

where θ is the contact angle between the bacterial surface and the drop liquid. γ^+ and γ^- are the electron-acceptor (Lewis base) and electron donor (Lewis base) parameters, respectively. $\gamma^{AB} = 2\sqrt{\gamma^+\gamma^-}$.

The surface tension component and parameters for *R. acidophila* and drop liquids used in this work are given in Table 1. From the data above, the total interfacial tensions between the bacterial surface and water can be calculated with eqs 7−9 (*20*, *24*):

TABLE 1. Surface Tension of *R. acidophila*, Total Interfacial Tension, and Total Interfacial Free Energy between *R. acidophila* and Water and Their Components (mJ m^{-2})

R. acidophila	γ_B	γ_B^{LW}	γ_B^{AB}
	38.9	20.8	18.2
R. acidophila-water	γ_{BL}	γ_{BL}^{LW}	γ_{BL}^{AB}
	−9.3	0.012	−9.31
R. acidophila-water	ΔG_{adh}	ΔG_{BL}^{LW}	ΔG_{BL}^{AB}
	18.6	−0.024	18.62

$$\gamma_{BL} = \gamma_{BL}^{LW} + \gamma_{BL}^{AB} \quad (7)$$

where

$$\gamma_{BL}^{LW} = (\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}})^2 \quad (8)$$

$$\gamma_{BL}^{AB} = 2(\sqrt{\gamma_B^+\gamma_B^-} + \sqrt{\gamma_L^+\gamma_L^-} - \sqrt{\gamma_B^+\gamma_L^-} - \sqrt{\gamma_B^-\gamma_L^+}) \quad (9)$$

With these equations, the interfacial free energy (ΔG_{adh}) between the bacterial particles and waters could be calculated from eq 10 (*20*):

$$\Delta G_{adh} = -2\gamma_{BL} = -2(\gamma_{BL}^{LW} + \gamma_{BL}^{AB}) \quad (10)$$

The parameters related to the surface characteristics of *R. acidophila* and water are listed in Table 1.

Hamaker Constant (*A*). The Hamaker constant is an important parameter for determining the apolar free energy of interactions between two molecules or particles in vacuum or in condensed media. The Hamaker constant A_{ii} of a homogeneous material (i) in the condensed state, either liquid or solid, can be estimated using eq 11:

$$A_{ii} = 24\pi l_0^2\gamma_i^{LW} \quad (11)$$

where l_0 is the minimum equilibrium distance (≈0.157 nm) (*16*), and γ_i^{LW} can be calculated from eq 6.

In the case of two molecules or particles of material (i) across water (w), the effective Hamaker constant (A_{iwi}) can be calculated from eq 12 (*25*):

$$A_{iwi} = (\sqrt{A_{ii}} - \sqrt{A_{ww}})^2 \quad (12)$$

Thus, through introduction of eq 11 to eq 12, the effective Hamaker constant between bacterial cells and water (A_{BLB}) used in this work can be calculated as follows:

$$A_{BLB} = (\sqrt{A_{BB}} - \sqrt{A_{LL}})^2 = 24\pi l_0^2(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}})^2 \quad (13)$$

Flocculation Tests. In this work an index, F, was used to describe the flocculability of *R. acidophila* cells under given conditions. *R. acidophila* was harvested by centrifugation at 12 000 rpm for 10 min and washed twice with 0.1 M NaCl solution. The cell pellets were then re-suspended in NaCl solution with various concentrations and the solution optical density was measured at 650 nm (A_0). Thereafter, the PSB suspensions were centrifuged at 1000 rpm for 2 min, and the supernatant optical density was measured again at 650 nm (A_t). Thus, the F value can be calculated using the following equation:

$$F\% = \left(1 - \frac{A_t}{A_0}\right) \times 100 \quad (14)$$

Results

ζ Potentials and Flocculability of *R. acidophila* at Various Ionic Strengths. The ζ potential measurements of *R. acidophila* in 0.001−5.0 M NaCl solutions are shown in Figure 1a. Because of the effect of double layer compression, the negativity of the ζ potential of *R. acidophila* decreased with the increasing electrolyte concentration. With an increase in NaCl concentration from 0.001 to a nearly saturated 5.0 M, the ζ potential decreased from −36.5 mV to a minimum (negative) of −5.3 mV.

Figure 1b shows the flocculability of *R. acidophila* at various ionic strengths. Usually, a lower *F* value indicates a more stable cell suspension, i.e., the poorer flocculability of cells. The *F* value increased from 9.8 to 18.6 with an increase in NaCl concentration from 0.001 to 0.1 M, indicating that a high solution ionic strength could improve the flocculability of *R. acidophila* cells. At 0.1 M NaCl solution, the *F* value peaked of 18.6, but decreased with a further increase in NaCl concentration. These results are in good agreement with the predictions of the DLVO theory.

As shown in Figure 1a and b, both ζ potential and ionic strength are the main factors affecting the stability of cell suspensions. These two factors are both related with the parameter κ^{-1}, which is defined as the Debye length or as the "thickness" of the diffuse double layer (*14*). The κ value can be calculated with the following equation:

$$\kappa = \left(\frac{4e^2 N_A I}{\epsilon kT}\right)^{1/2} \quad (15)$$

From this equation, it could be found that the addition of NaCl increased the ionic strength and κ value (Figure 1a), which caused the compression of the diffuse double layer and finally resulted in a corresponding decrease in ζ potential. This is also consistent with the experimental results shown in Figure 1a.

Flocculability of *R. acidophila* in $CaCl_2$ Solutions. The effect of divalent cations, e.g., Ca^{2+}, on the flocculability of *R. acidophila* was evaluated. The concentrations of NaCl and $CaCl_2$ solutions were adjusted to identical ionic strengths. Figure 1b shows that the *F* values of *R. acidophila* suspension in $CaCl_2$ solutions were higher than those in NaCl solutions at the same ionic strengths, indicating that *R. acidophila* suspension in $CaCl_2$ solutions had a better flocculability. In both $CaCl_2$ and NaCl solutions, the *F* value increased with an increase in ionic strength from 0.001 to 0.1 M. With a further increase in NaCl concentrations, the *F* value decreased. However, in the $CaCl_2$ solutions the *F* value continuously increased to 31.6 until the ionic strength reached 1 M, but decreased later. In the high salt area (imaginary line area in Figure 1b), the bacterial flocculability became worse in both solutions and the *F* value also decreased sharply.

ζ Potentials and Flocculability of *R. acidophila* at Various pHs. The effect of pH value on the ζ potential and flocculability of *R. acidophila* cells is shown in Figure 2. The natural pH of the suspension was approximately 7.0. With the addition of 0.01 M NaOH or 0.01 M HCl, the pH values of the suspension were adjusted to 3.0−11.0. The negativity of the ζ potential of *R. acidophila* cells re-suspended in 0.1 and 0.01 M NaCl solutions increased with the increasing suspension pH value from 3.0 to 8.0 (Figure 2a). At pH 8.0, the ζ potential increased to a maximum (absolute value) for the two electrolyte concentration suspensions. With a pH increase from 8.0 to 11.0, the negativity of the ζ potential slightly decreased.

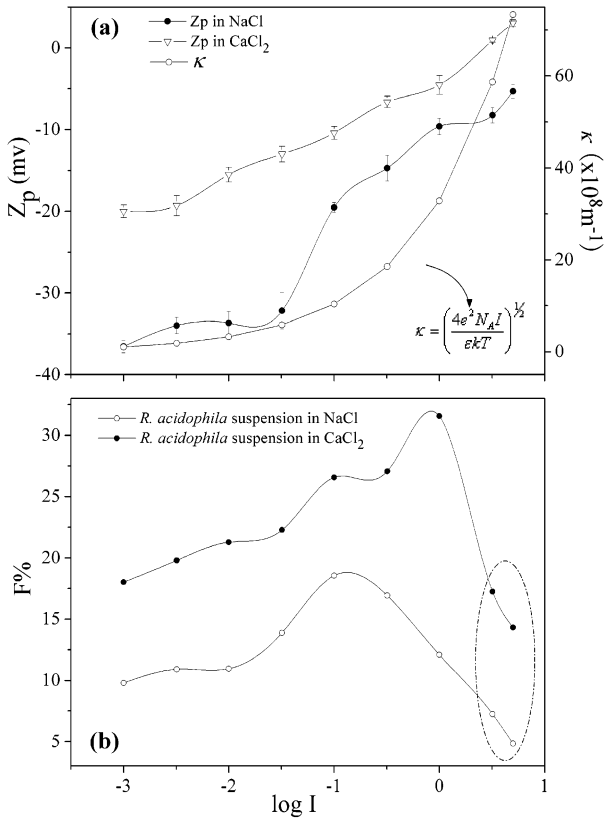

FIGURE 1. Effect of ionic strength on (a) ζ potential and *k*; and (b) flocculability of *R. acidophila* suspensions.

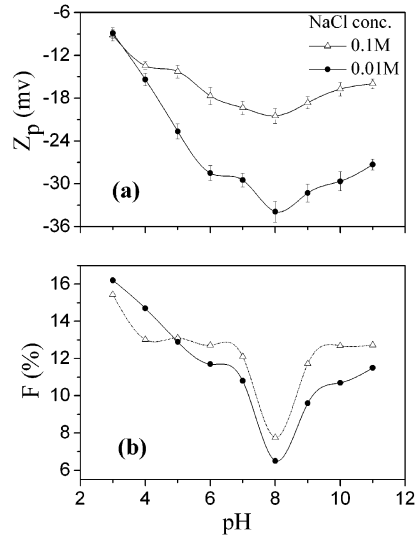

FIGURE 2. Effect of pH on (a) ζ potential; and (b) flocculability of *R. acidophila* suspensions at various NaCl concentrations.

The flocculability of *R. acidophila* in 0.01 or 0.1 M NaCl solutions at various pHs is illustrated in Figure 2b. Similar to Figure 2a, the *F* value decreased with an increase in pH from 3.0 to 8.0 and reached a minimum of 7.8 and 6.5 in 0.1 and 0.01 M NaCl solutions at pH 8.0, respectively, suggesting the lowest flocculability of *R. acidophila* at pH 8.0. Thereafter, the *F* value increased with an increase in pH from 8.0 to 11.0. As an interpretation by the classical DLVO theory, the electrostatic repulsive energy increases with increasing ζ potential, accordingly the *F* value decreases. As pH was increased from 7.0 to 8.0, the flocculability of *R. acidophila* decreased significantly for the suspensions in 0.1 and 0.01 M NaCl solutions. This might be related to the pH shift from

TABLE 2. Change of Characteristics of *R. acidophila* in the EPS Extraction Process

item	before EPS extraction	after EPS extraction
F%	22.4	7.0
ζ potential[a] (mV)	−24.4 ± 1.0	−20.5 ± 0.9
contact angle (deg)		
water	39.2 ± 1.7	38.8 ± 2.0
1-bromonaphthalene	63.0 ± 1.2	68.0 ± 2.0
formamide	38.4 ± 1.3	42.4 ± 1.5
effective Hamaker constant (J)	5.6×10^{-23}	1.5×10^{-23}
ΔG_{adh} (mJ m^{-2})	17.8	21.7

[a] ζ potential in 0.1 M NaCl

neutrality to alkalinity for the suspensions of *R. acidophila*.

Characteristic Change of *R. acidophila* in EPS Extraction Process. The EPS extraction experiment was conducted to evaluate the relationship between EPS and bacterial flocculability. The alterations of *R. acidophila* characteristics in the EPS extraction process are summarized in Table 2. The *F* value of *R. acidophila* suspensions decreased from 22.4 to 7.0, indicating that the bacterial flocculability decreased significantly after the EPS extraction. The contact angles of *R. acidophila* measured before and after the EPS extraction are also given in Table 2 to show the changes of bacterial surface characteristics.

Discussion

The poor flocculability of *R. acidophila* might be highly related to its inherent surface characteristics. Analysis of the experimental results with the DLVO theory indicates that two reasons seemed to be responsible for the poor flocculability of *R. acidophila*. Because of the small value of effective Hamaker constant, the contribution of W_A could be neglected, which caused a significant increase in energy barrier. On the other hand, due to the positive interfacial free energy ΔG_{adh}, which reflects the interaction between the bacterial particles and waters, the cell particles of *R. acidophila* repelled each other, resulting in a great stability of the cell suspensions.

Influence of Ionic Strength and pH on the Flocculability of *R. acidophila*. The influence of inert electrolyte concentration, NaCl, on the total potential energy of interactions of two bacterial particles is illustrated in Figure 3. With the addition of electrolyte at 0.001−0.1 M, the thickness of the diffuse double layer (i.e., κ^{-1}) decreased. As a result, the ζ potential also decreased, and the electrostatic repulsive energy had a lower contribution to the total interaction energy. The energy barrier of the suspensions dropped from 1981 to 533 *kT* with an increase in ionic strength. A higher energy barrier means a more stable suspension. Hence, the flocculability of *R. acidophila* increased with the increasing electrolyte concentration. Although the flocculation behavior of *R. acidophila* is well described by the DLVO theory in the electrolyte range of 0 to about 0.1 M, beyond this concentration, however, an increase in ionic strength did not increase the flocculability of *R. acidophila*. At a higher ionic strength, the bacterial flocculability decreased. This is contradictory to the predictions of the DLVO theory, which suggests that at a higher ionic strength the flocculation should become better. As the NaCl concentration was increased to 0.32 M, even to nearly saturated 5 M, κ^{-1} decreased to 0.54 and 0.14 nm, respectively. Therefore, the double layer interaction had a lower contribution at a higher ionic strength. Other reasons at high salt concentrations, such as dehydration causing "hydrophobization" and "salting out" (*25*), rather than those described by the DLVO theory, influenced the bacterial flocculation in this case. Moreover, electrolyte at a high level might cause some ion exchanges within the EPS matrix of the bacterial cells. This may also reduce the bacterial flocculability. These effects could change the cell structure and alter the interfacial tensions between the interacting phases (*26*). Such a change could have an effect on the energetics of attachment and might finally cause a change of the flocculability. Similar results at high salt concentrations have been reported (*26*).

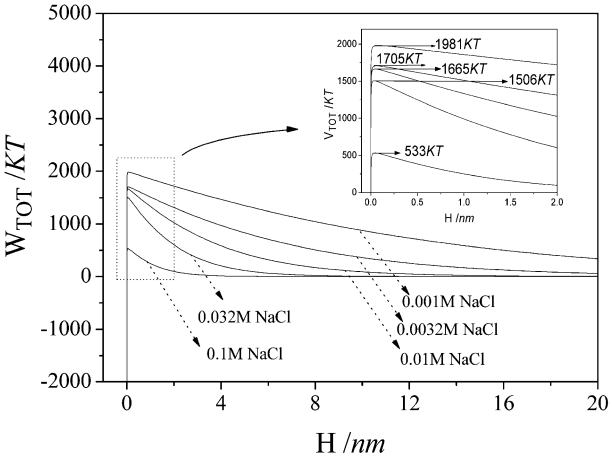

FIGURE 3. Total interaction energy profiles as a function of interparticle distance at various ionic strengths.

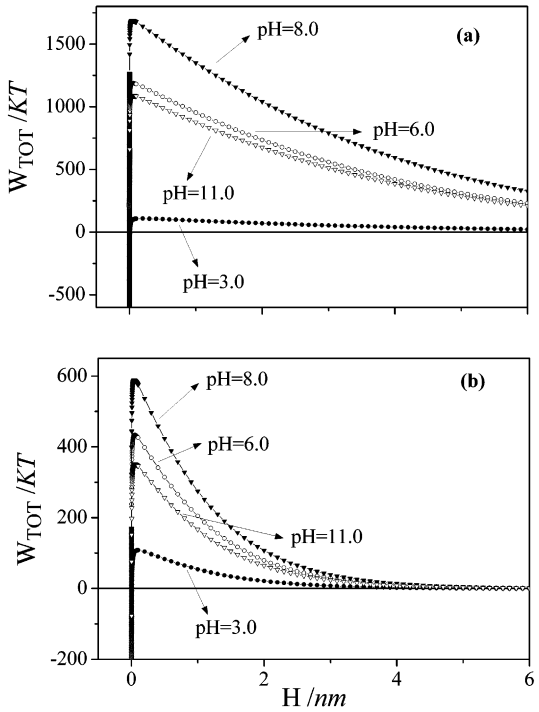

FIGURE 4. Plots of free energy versus interparticle distances of *R. acidophila* at various pH values re-suspended in (a) 0.01 M NaCl solution; and (b) 0.1 M NaCl solution.

Figure 4 illustrates the total interaction energy profiles of *R. acidophila* suspended in NaCl solutions at various pH values. With an increase in pH from 3.0 to 8.0, the energy barrier increased with the increasing ζ potential. Thus, the suspension at pH 8.0 was stable and its flocculability was low. At pH 3.0, the energy barrier decreased to approximately 100 *kT*, and the *R. acidophila* suspension under this condition could flocculate more readily. However, the bacterium could not grow well under such high acidic or alkalic (pH = 11.0) conditions (*8*). Hence, its cultivation at pH 7.0 might be more reasonable.

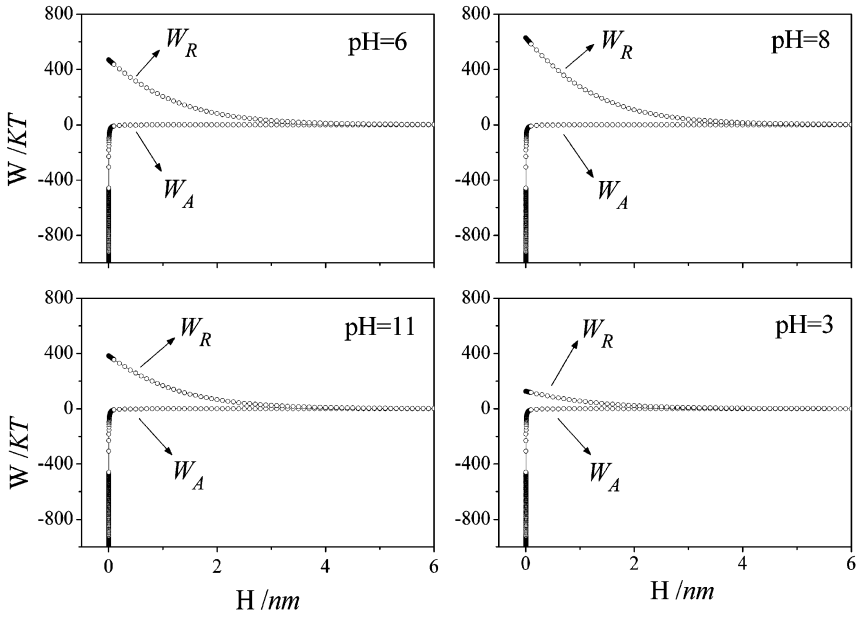

FIGURE 5. Plots of W_A and W_R contribution to the total interaction free energy of *R. acidophila* suspeneded in 0.1 M NaCl solutions at different pH values.

Influence of W_A on the Flocculability of *R. acidophila*. W_A and W_R values of the *R. acidophila* suspension at 0.1 M NaCl solution as a function of interparticle distance at pH 3.0, 6.0, 8.0, and 11.0 are shown in Figure 5. The contribution of W_A to the total energy and the energy barrier was identical for the four pH values. Because of the different ζ potentials at various pHs, the contribution of W_R was also changeable. Such a difference might be responsible for the various flocculabilities of *R. acidophila* at different pH values.

At 0.1 M NaCl concentration, the relatively better flocculability of *R. acidophila* suspension was observed. However, the height of the energy barrier was nearly 550 *kT*. At such a high energy barrier, the bacterial particles could not overcome the barrier to flocculate. Hence, the suspension of *R. acidophila* was still stable. The van der Waals attraction (W_A) decreased substantially to nearly nil with the increasing interparticle distance, thus the contribution of W_A to the total energy was very insignificant. The main factor governing W_A is the effective Hamaker constant (A_{BLB}), as shown in eq 13. The apolar Van der Waals surface tension component of *R. acidophila* (γ_B^{LW}), which governed the value of effective Hamaker constant (eq 13), was 20.8 mJ m^{-2}. This value was close to the apolar Van der Waals surface tension component of water (γ_L^{LW}, 21.8 mJ m^{-2}). Thus, the effective Hamaker constant between *R. acidophila* and water was so small that the contribution of W_A could be neglected.

Contribution of Interfacial Free Energy to the Flocculability of *R. acidophila*. The calculated interfacial free energy (ΔG_{adh}) between the bacterial particles and water also suggests that the suspension of *R. acidophila* was very stable. The first term of ΔG_{adh}($\Delta G_{BL}^{LW} = -2\gamma_{BL}^{LW}$) is usually negative, indicating that the van der Waals force is responsible for attraction. The contribution of the second AB force ($\Delta G_{BL}^{AB} = -2\ \gamma_{BL}^{AB}$) to the interfacial interaction is variable. They could be either repulsive or attractive, depending upon the surface thermodynamic parameters of the dispersed surface, *i* (i.e., bacterial particles), and of the dispersing medium (i.e., water). The value of the attractive LW forces was -0.02 mJ m^{-2} and the repulsive AB interfacial free energy was 18.6 mJ m^{-2} (Table 1). The repulsive interfacial free energy predominated the suspension media of *R. acidophila*. As a result, the cell particles repelled each other and the suspension was kept stable for a long period. This might be the main reason behind the poor flocculability of *R. acidophila*.

Influence of Ca^{2+} and EPS on the Flocculability of *R. acidophila*. Addition of the Ca^{2+} to the *R. acidophila* suspensions can improve its flocculability (Figure 1a and b). At the same level of ionic strength, the bacterial flocculation abilities in $CaCl_2$ solutions were higher than those in NaCl solutions. This might be attributed to two reasons. First, the negativity of the ζ potential in $CaCl_2$ solutions was lower than that in NaCl solutions. This implies that the Ca^{2+} was bound to the microbial cells to reduce their surface negative charges. Accordingly, the energy barrier of bacterial suspension in $CaCl_2$ solutions was lower than that in NaCl solutions (Figure 1a), resulting in a better flocculability of *R. acidophila* in $CaCl_2$ solutions. Second, divalent ions, e.g., Ca^{2+}, are important for the cross-linking of the charged compounds in EPS. The interaction force between Ca^{2+} and EPS could enhance the bacterial flocculability. Unlike in NaCl solutions, the flocculability of *R. acidophila* in $CaCl_2$ solutions increased until the ionic strength reached 1 M. This might be attributed to the competition between the two interactions. As a result, the force between Ca^{2+} and polymers in EPS may weaken the "salting out" effect, and the flocculability is increased at relatively higher ionic strengths.

EPS are located at the cell surface. The stability of microbial aggregates is also regulated by other interactions, such as ion bridging and polymer entanglement, in addition to the van der Waals attraction, electrostatic repulsion, and hydrophobicity interaction (*27*). The main components in EPS of *R. acidophila*, i.e., proteins, carbohydrates, and nucleic acids, have a high content of negatively charged groups (*7*). This work shows that the addition of $CaCl_2$ could improve its flocculability, suggesting the importance of the ionic bridging interaction between the EPS and divalent metals (e.g., Ca^{2+}). In addition to the ionic bridging interaction, the polymer entanglement interaction also played an important role in microbial flocculation, as evidenced by the significant decrease in bacterial flocculability after EPS extraction using EDTA method. Moreover, the EPS content and the ratio of proteins/carbohydrates had an effect on the bacterial surface energy, and accordingly the change of bacterial surface characteristics. This resulted in a change in the bacterial adhesion energy, corresponding to a decrease in the effective Hamaker constant and an increase in the positive interfacial free energy (ΔG_{adh}). All of these might be responsible for the worse flocculability of *R. acidophila* after the EPS extraction.

With the DLVO theory and experimental results about the surface characteristics of *R. acidophila*, interesting information could be acquired to explain the poor flocculability of a H_2-producing PSB, *R. acidophila*. It can also provide useful information for cultivating a strain with good flocculation and designing an efficient phototrophic H_2-producing reactor. Through manipulating the content and component of bacterial EPS, the surface characteristics of *R. acidophila* could be changed and its flocculability might be improved (*7*). In addition, the cultivation pH, temperature, and inert electrolyte may also be manipulated to enhance the flocculability of PSB.

Acknowledgments

We thank the Natural Science Foundation of China (NSFC) (Grants 20577048 and 50625825), and the NSFC-RGC Joint Project (50418009) for the partial support of this work.

Supporting Information Available

The ζ potential measurement method, EPS extraction procedures, and the measured EPS contents of *R. acidophila*. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Oh, S. E.; van Ginkel, S.; Logan, B. E. The relative effectiveness of pH control and heat treatment for enhancing biohydrogen gas production. *Environ. Sci. Technol.* **2003**, *37*, 5186−5190.
(2) Park, W.; Hyun, S. H.; Oh, S. E.; Logan, B. E.; Kim, I. S. Removal of headspace CO_2 increases biological hydrogen production. *Environ. Sci. Technol.* **2005**, *39*, 4416−4420.
(3) Sheng, G. P.; Yu, H. Q.; Yu, Z. Extraction of the extracellular polymeric substances from a photosynthetic bacterium *Rhodopseudomonas acidophila*. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 125−130.
(4) Barbosa, M. J.; Rocha, J. M. S.; Tramper, J.; Wijffels, R. H. Acetate as a carbon source for hydrogen production by photosynthetic bacteria. *J. Biotechnol.* **2001**, *85*, 25−33.
(5) Sheng, G. P.; Yu, H. Q.; Yue, Z. B. Production of extracellular polymeric substances from *Rhodopseudomonas acidophila* in the presence of toxic substances. *Appl. Microbiol. Biotechnol.* **2005**, *69*, 216−222.
(6) Lance, J. C.; Gerba, C. P. Effect of ionic composition of suspending solution on virus adsorption by a soil column. *Appl. Environ. Microbiol.* **1983**, *47*, 484−488.
(7) Sheng, G. P.; Yu, H. Q. Relationship between the extracellular polymeric substances and surface characteristics of *Rhodopseudomonas acidophila*. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 126−131.
(8) Chang, Y. I.; Chang, P. K. The role of hydration force on the stability of the suspension of *Saccharomyces cerevisiae*-application of the extended DLVO theory. *Colloids Surf., A* **2002**, *211*, 67−77.
(9) Dengis, P. B.; Nelissen, L. R.; Rouxhet, P. G. Mechanisms of yeast flocculation: comparison of top and bottom-fermenting strains. *Appl. Environ. Microbiol.* **1994**, *61*, 718−728.
(10) Bura, R.; Cheung, M.; Liao, B.; Finlayson, J.; Lee, B. C.; Droppo, I. G.; Leppard, G. G.; Liss, S. N. Composition of extracellular polymeric substances in the activated sludge floc matrix. *Water Sci. Technol.* **1998**, *37*, 325−333.
(11) Sheng, G. P.; Yu, H. Q. Chemical-equilibrium-based model for describing the strength of sludge: Taking hydrogen-producing sludge as an example. *Environ. Sci. Technol.* **2006**, *40*, 1280−1285.
(12) Wilen, B. M.; Jin, B.; Lant, P. The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water Res.* **2003**, *37*, 2127−2139.
(13) Jahn, A.; Nielsen, P. H. Extraction of extracellular polymeric substances (EPS) from biofilms using a cation exchange resin. *Water Sci. Technol.* **1996**, *32*, 157−164.
(14) Shaw, D. J. *Introduction to Colloid & Surface Chemistry*, 4th ed.; Butterworth Heinemann: Boston, 1992.
(15) Chen, K. L.; Mylon, S. E.; Elimelech, M. Aggregation kinetics of alginate-coated hematite nanoparticles in monovalent and divalent electrolytes. *Environ. Sci. Technol.* **2006**, *40*, 1516−1523.
(16) Wu, W. J.; Nancollas, G. H. Application of the extended DLVO theory-the stability of alatrofloxacin mesylate solutions. *Colloids Surf., B* **1999**, *14*, 57−66.
(17) Redman, J. A.; Walker, S. L.; Elimelech, M. Bacterial adhesion and transport in porous media: Role of the secondary energy minimum. *Environ. Sci. Technol.* **2004**, *38*, 1777−1785.
(18) Marshall, K. C.; Stout, R.; Mitchell, R. Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* **1971**, *68*, 337−348.
(19) Van Oss, C. J. Hydrophobicity of biosurfaces origin, quantitative determination and interaction energies. *Colloids Surf., B* **1995**, *5*, 91−110.
(20) Van Oss, C. J. *Interfacial Forces in Aqueous Media*; Marcel Dekker: New York, 1994.
(21) Van Loosdrecht, M. C. M.; Lyklema, J.; Norde, W.; Zehnder, A. J. B. Bacterial adhesion: a physicochemical approach. *Microbiol. Ecol.* **1989**, *17*, 1−15.
(22) Schafer, A.; Harms, H.; Zehnder, A. J. B. Bacterial accumulation at the air−water interface. *Environ. Sci. Technol.* **1998**, *32*, 3704−3712.
(23) Jucker, B. A.; Zehnder, A. J. B.; Harms, H. Quantification of polymer interactions in bacterial adhesion. *Environ. Sci. Technol.* **1998**, *32*, 2909−2915.
(24) Van Oss, C. J.; Chaudhury, M. K.; Good, R. J. Interfacial Lifshitz−van der Waals and polar interactions in macroscopic systems. *Chem. Rev.* **1988**, *88*, 927−941.
(25) Brown, D. G.; Jaffe, P. R. Effects of nonionic surfactants on the cell surface hydrophobicity and apparent hamaker constant of a *Sphingomonas sp. Environ. Sci. Technol.* **2006**, *40*, 195−201.
(26) Hermansson, M. The DLVO theory in microbial adhesion. *Colloids Surf., B* **1999**, *14*, 105−119.
(27) Mozes, N.; Leonard, A. J.; Rouxhet, P. G. On the relations between the elemental surface composition of yeasts and bacteria and their charge and hydrophobicity. *Biochim. Biophys. Acta-Biomembr.* **1988**, *945*, 324−334.

Received for review January 15, 2007. Revised manuscript received April 19, 2007. Accepted April 30, 2007.

ES070107N