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Research paper

Adsorption of bacteria onto layered double hydroxide particles to form biogranule-like aggregates

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ABSTRACT

Biogranulation is a key process in biological treatment of wastewater, which forms biogranules from the agglutination of bacteria and suspended solid in wastewater. In this paper, the adsorption behaviors of bacteria onto layered double hydroxides (LDH) are investigated, and the biogranulation mechanism between bacteria and LDH particles is elucidated. It is found that $NiFeCO_3$-LDH is able to absorb 45–55 mg/g of *Bacillus subtilis* under various conditions. The adsorption follows the first-order Lagergren model. Scanning electron microscope (SEM) images support the formation of bacteria-LDH aggregates. Mechanism study shows that electrostatic interaction between LDH and bacteria should be the most important for the adsorption of bacteria onto LDH. Furthermore, one bacterium adhering to a few LDH particles is suggested, which maximizes the interactions between bacteria and LDH particles. The present results provide a possible means for biological treatment of wastewater through bacterial granulation with LDHs.

1. Introduction

Biological wastewater treatment is nowadays a widely accepted and well-established technology that has been extensively used in the treatment of biodegradable wastewater prior to discharge. One of the most important factors ensuring the success of biological treatment is to introduce enough biomass and suspended solid into the system (Tay and Show, 2006). The use of biogranules is advantageous for such a purpose due to their regular, dense, and strong structures with good settling properties (Hulshoff Pol et al., 2004). As observed by Young and McCarty in 1979, the substitution of biogranules for activated sludge enables high biomass retention and withstands high-strength wastewater and shock loadings (Liu and Tay, 2004; Yu, 2006).

The process of formation of well-defined discrete biogranules, namely biogranulation is achieved by agglutinating suspended solids with bacteria (Hulshoff Pol et al., 2004). Often, a relatively long start-up period required for the development of biogranules is problematic in wastewater treatment applications (Vlyssides et al., 2008). One solution is to introduce nuclei or bio-carriers with high specific surface areas and specific gravity, similar to granular sludge or spherical shape, for microbial adhesion, which is key for the origin of biogranulation due to bacteria-to-bacteria or bacteria-to-solid surface interactions (Hulshoff Pol et al., 2004; Vlyssides et al., 2008; Wana et al., 2009). Several research groups have used inert particles such as foam, zeolite, water-absorbing polymer (WAP), granular activated carbon (GAC) and cationic polymer to promote the granulation (Wang et al., 2004; Yoda et al., 1989; Yu, 2011). However, the employment of materials featuring positively charged surface to enhance the granulation is rare, which are believed to be ideal nuclei for microbial adhesion (You et al., 2003). This is because the surface of bacteria usually carries a lot of negative charges under physiological conditions, arising from the weak acidic and basic functional groups of the polypeptides in bacteria (e.g., carboxylic acid and amine moieties) (Jeong-Ann Park et al., 2010; Jin et al., 2007).

One prominent type of nucleus bearing positively charged surface is derived from layered double hydroxides (LDHs) (Ma et al., 2006). LDHs are a group of anionic clay materials with a unique layered structure (Rives and Angeles Ulibarri, 1999). The general formula can be expressed as $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}(A^{m-})_{x/m}\cdot nH_2O$, where M^{2+} and M^{3+} are divalent and trivalent cations, respectively, in octahedral positions, and A^{m-} is an anion located between the inter layers to balance the positive charges in the hydroxide layers (Auerbach et al., 2004; Duan and Evans, 2006; Forano et al., 2006; Wang and O'Hare, 2012; Zhang et al., 2008). LDHs can be easily synthesized by coprecipitating divalent and trivalent metal salts in a basic solution under controlled conditions. The sizes of LDH crystals can be in a range of 50–100 nm (Xu and Lu, 2006), providing a relatively large surface area (10–200 m^2/g). Carbonate/chloride LDH particles in aqueous suspensions possess a positive zeta potential up to 40–50 mV (Jin et al., 2007). It is also well known that LDHs have a high anion exchange capacity (2–5 mmol/g) (Miyata, 1983), showing an increasing interest in the application of potential sorbents and drug delivery (Jin et al., 2007; Liu et al., 2011). These properties enable LDHs to be promising nuclei that would accelerate granulation via adhering bacteria.

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On the other side, it is necessary to add some metal elements as nutrient for biotreatment of wastewater with simple composition (Morgan and Watkinson, 1992). As it is well known, Fe, Ni, Co and Cu are the most common trace metal elements for bacteria (Shen et al., 1993). Herein Fe and Ni were firstly selected for components of LDH to investigate the interaction of LDH and bacteria. The released Fe and Ni (the ratio can be greatly adjusted according to the demand of bacteria) in the biotreatment reactor could be the possible nutrient elements.

Although adsorption of viruses and bacteria onto LDHs has been reported (Jeong-Ann Park et al., 2010; Jin et al., 2007; Rivera-Utrilla et al., 2012; You et al., 2003), the search for new types of LDHs is still widely sought. In particular, the adsorption mechanism of the specific bacteria/virus onto LDHs still needs to be elucidated.

To these ends, we herein report: (1) the examination of the feasibility of using $NiFeCO_3$-LDH as the adsorbent for bacterium *Bacillus subtilis* to form bacteria-LDH aggregates; (2) the investigation of adsorption behaviors of bacterium *B. subtilis* over $NiFeCO_3$-LDH under various conditions; and (3) revealing the adhesion mechanisms of *B. subtilis* on $NiFeCO_3$-LDH. Our results demonstrate that $NiFeCO_3$-LDH could be used to enhance biogranulation during the biological treatment of wastewater.

2. Materials and methods

2.1. Bacteria growth

Growth procedures for *B. subtilis* were similar to those described earlier by Rong et al. (2008) In brief, the colony of *B. subtilis* was initially inoculated into 10 mL of Broth Bouillon and shaken with aeration at 150 rpm at 35 °C for 8 h. Three mL of the preculture was then transferred to 150 mL of the same medium and cultivated for 14 h. *B. subtilis* bacteria were harvested by centrifuging the suspension at 4000 rpm for 15 min, followed by three-time wash with sterilized distilled–deionized (DDI) water, and then resuspended in DDI water to obtain the bacterial suspension. The bacterium concentration of aqueous solution was determined by measuring the optical absorbance (OD410) of the suspension, which was then dried (105 °C) and weighed. Dry weights were plotted vs. measured absorbance to produce the standard curve that was linear and used in all subsequent experiments.

2.2. Preparation of LDH

$Ni/FeCO_3$ LDH with a Ni/Fe molar ratio of 3 was chosen in this study. A co-precipitation method was used to prepare $NiFeCO_3$-LDH similar to that described by Saiah (Saiah et al., 2008). The samples were obtained by simultaneously drop wise adding one solution containing 1.5 mol $Ni(NO_3)_2 \cdot 6H_2O$ and 0.5 mol $Fe(NO_3)_3 \cdot 9H_2O$ in 250 ml of distilled water. Another solution (250 mL) containing NaOH (4 M) and Na_2CO_3 (0.25 M) was placed into a beaker containing 100 mL of deionized water at a constant $pH = 11.0 \pm 0.5$ under vigorous stirring at room temperature. The suspension obtained was stirred mechanically for 60 min at room temperature. The suspension was then aged at 85 °C for 4 days to full crystallization. After the aging treatment, the precipitation product was collected by filtration and washed several times with distilled water to remove excess soluble ions, until the filtrate pH was close to 7.0. The collected precipitate was dried in an oven at 60 °C for 12 h and then passed through a 200-mesh sieve for subsequent use. The chemical formula of the $NiFeCO_3$-LDH was approximately $Ni_{0.77}Fe_{0.23}(CO_3)_{0.115}(OH)_2 \cdot 0.64H_2O$, as determined through element analyses.

2.3. Adhesion of B. subtilis onto $NiFeCO_3$-LDH

For the adsorption equilibrium of *B. subtilis* over $NiFeCO_3$-LDH, the LDH (3, 5 or 10 mg) was added to 50 mL Erlenmeyer flask containing 6 mL of bacterial suspension (10–150 mg/L). The adsorption was performed in a shaker with a shaking speed of 150 rpm at 25 ± 1 °C for 180 min. For the adsorption kinetics of $NiFeCO_3$-LDH, 6 mL of different bacterial suspensions (30, 45 and 60 mg/L) and 5 mg of $NiFeCO_3$-LDH were added to 50 mL Erlenmeyer flask. Control test was performed in DDI water with 5 mg of LDH under the same conditions, compared with the bacterial suspension (45 mg/L) without LDH addition.

After the injection of 1 mL of 60% (w/w) sucrose solution (to obtain the required density gradient using density gradient centrifugation method) (Dubnau and Davidoff-Abelson, 1971), the suspension was centrifuged at 1000 rpm for 5 min in order to separate the unattached bacteria in the supernatant. The bacterial concentration was then measured directly by spectrophotometry. The percentage of adsorbed bacteria was calculated by subtracting the amount of unabsorbed bacteria from the initial amount.

Adsorption was also conducted at various temperatures (15, 20, 25, 30, 35 and 40 °C) in which 5 mg of $NiFeCO_3$-LDH and 6 mL of bacterial suspension (pH 7.0) containing 45 mg/L of *B. subtilis* were employed. The similar experiment was also carried out in a range of pH from 4.0 to 11.0 (adjusted by 0.01 M NaOH or 0.01 M HCl solution) and in the presence of 0–100 mM of KCl and 0–100 mM of $MgSO_4$ at 25 ± 1 °C.

2.4. Collection of bacteria-LDH aggregate

After the adsorption equilibrium of *B. subtilis* over $NiFeCO_3$-LDH (5 mg of $NiFeCO_3$-LDH and 6 mL of bacterial suspension at pH 7.0, 25 ± 1 °C), the bacteria-LDH aggregates were collected by centrifugation, followed by washing three times with sterilized distilled–deionized water. The solid sample was obtained after freeze-drying in Free Zone (Labconco Corporation) for 24 h. $NiFeCO_3$-LDH and *B. subtilis* bacteria were also collected in the same way.

2.5. Characterization

The morphology of samples was imaged in a scanning electron microscope (SEM, JSM-6700F) combined energy dispersive X-ray (EDX) analyzer at a voltage of 15 keV. Before SEM imaging, samples were coated with Pt for 20 min in JFC-1600 sputter coater (JEOL, Japan). The overlapped peaks of P and Pt in the energy spectrums of *B. subtilis* and *B. subtilis*-$NiFeCO_3$ LDH were separated by fitting the curve with Gaussian peak functions using Origin Pro 8.0 (Originlab Corporation, U.S.A).

XRD patterns were collected in DLMAX-2550 with Cu–K radiation (at a scan speed of 6°/min) over a range of 4–70°. Identification of the crystalline phases was conducted by comparison with the JCPDS files.

Fourier transform infrared spectra (FTIR) were recorded in a Nicolet FT-IR 380 instrument (Thermo Scientific Co., Ltd, U.S.A) with KBr press disk technique by scanning in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

3. Results and discussion

3.1. Adhesion of B. subtilis onto LDH

The SEM images show that *B. subtilis* with a rod-like shape has a length of approximately 2–3 μm (Fig. 1b), which is adsorbed on bulk LDH (Fig. 1c). The adhesion of bacteria to LDH particles is also evidenced by the fact that the turbid *B. subtilis* suspension (Fig. 1d(B)) becomes a transparent solution (Fig. 1d(C)) after addition of LDH. The resulting bacteria-LDH aggregates are larger and more compact (Fig. 1d(E)) than the scattered $NiFeCO_3$-LDH particles (Fig. 1d(D)). The results from EDX spectra clearly suggest that the characteristic peaks of P in *B. subtilis* (Fig. 1e) and Ni and Fe in LDH (Fig. 1f) all remained in the bacteria-LDH aggregates (Fig. 1g). The percentages of Pt and P presented in *B. subtilis*, $NiFeCO_3$-LDH, and *B. subtilis*-$NiFeCO_3$-LDH aggregates through SEM-EDX analysis were also provided, as shown in Table 1.

The LDH structure is stable after adsorption of bacteria because the same XRD patterns from the pristine LDH and the bacteria-LDH