The influence of additives (Ca^{2+}, Al^{3+}, and Fe^{3+}) on the interaction energy and loosely bound extracellular polymeric substances (EPS) of activated sludge and their flocculation mechanisms

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ABSTRACT

The activated sludge (AS) flocculability markedly improved after the addition of Al^{3+} and Fe^{3+} compared to Ca^{2+} at a concentration of 2 mEq/L. Though the energy barrier decreased about 30% when Ca^{2+} was added, the AS flocculability did not improve substantially. This indicates that extended DLVO theory can explain AS flocculation with Al^{3+} and Fe^{3+} as additives but is not appropriate for Ca^{2+}. In addition, no matter which cation was added, the AS flocculability was highly correlated to the loosely bound extracellular polymeric substances (LB-EPS) content. The majority of added Ca^{2+} remained in the bulk solution (about 92%), whereas almost all of the Al^{3+} and Fe^{3+} added was found in the pellet (about 98%). The cation' ability to bind to the AS is closely related to the energy barrier and LB-EPS contents, therefore it is the core reason behind the AS flocculation changes observed upon the addition of multivalent cations.

1. Introduction

The activated sludge (AS) process is commonly adopted in most wastewater treatment plants (WWTPs), and the effluent suspended solids (ESS) are strongly affected by the performance of AS flocculation and settling (Schmid et al., 2003). Increases in the quantity of ESS not only lead to increases in pollutants like chemical oxygen demand (COD), total nitrogen (TN), and total phosphate (TP), but also to heavy metals and potential pathogens (Chang et al., 2006). Thus, improvements in AS flocculability and settleability will have a major impact on the effluent quality.

AS is composed of various microorganisms that are sensitive to environmental conditions; thus, almost any fluctuation (such as temperature and organic load) in the surrounding system can cause changes in the physical, chemical, and biological properties of AS. Therefore, the flocculation mechanisms of AS are much more complex than those of typical colloidal particles. All commonly recognized intermolecular interaction theories have been used to explain the binding of AS entities (Wilen et al., 2000), such as polymer bridging, Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, hydrophobic interactions, multivalent bridging theory, and

steric interactions (Wilen et al., 2000; Sobeck and Higgins, 2002; Sheng et al., 2010).

Classical DLVO theory is often used to interpret colloid stability and to describe the microorganisms and AS flocculation. It is also thought that the interaction energy calculated according to this theory has a considerable effect on AS flocculation (Sobeck and Higgins, 2002; Liu et al., 2007). The total interaction energy (W_{tot}) in classical DLVO theory involves energy contributions from the Van der Waals force (W_A) and electric double layer (W_R). Later, contributions from Lewis acid–base interactions (W_{AB}) were added to extended DLVO theory (Wu et al., 1999). Thus W_{tot} in extended DLVO theory can be expressed as: $W_{tot} = W_R + W_A + W_{AB}$. The microbial flocculability and the contributions of extracellular polymeric substances (EPS) to AS aggregation were also recently explained by extended DLVO theory (Liu et al., 2008, 2010).

Furthermore, each of the aforementioned flocculation theories emphasizes the importance of EPS because, firstly, they mainly accumulate on the cell surface (Yuan et al., 2011) and hence will always be involved in interactions between flocs and, secondly, EPS are the critical organic part of AS (Park and Novak, 2007) and as such determine the physical and chemical characteristics of AS (Morgan et al., 1990). Because of the vital importance of EPS, many studies have focused on EPS in order to understand the mechanisms underlying AS flocculation and dewaterability. Most investigators believe that surface properties, surface charge, EPS composition, and the hydrophobicity of AS, rather than the quantities of EPS, govern its flocculability and settleability (Liao et al., 2001; Ni

et al., 2009; Badireddy et al., 2010; Liao et al., 2011). Recently, researchers have used various methods (such as ultrasonic irradiation, heat and cation exchange resin) to separate EPS into two parts, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). They then investigated the correlations of LB-EPS and TB-EPS with AS flocculation (Li and Yang, 2007; Yang and Li, 2009; Yu et al., 2009). These studies showed that the sludge aggregate structure and binding capacity of AS with small floc constituents were weakened by excessive LB-EPS, which diminished the flocculability. The data indicated that, the lower the LB-EPS content of the AS, the less ESS and the lower the sludge volume index (SVI) value. However, the exact role of EPS in biosolid–liquid separation is not well understood (Liao et al., 2001; Sheng et al., 2010).

In addition to EPS, cations are also a crucial element in AS flocculation (Sobeck and Higgins, 2002; Nguyen et al., 2008). The aforementioned DLVO theory and multivalent bridging theory are closely related to cations because multivalent cations can bridge EPS and neutralize the negative charges on the AS surface (Kara et al., 2008; Wilen et al., 2008). Traditionally, divalent cations such as Ca^{2+} and Mg^{2+} have been shown to be crucial in AS flocculation (Sobeck and Higgins, 2002; Nguyen et al., 2008; Park et al., 2010), but the impact of Al^{3+} and Fe^{3+} on AS flocculation has not been extensively studied (Park et al. 2006; Park et al., 2010). Al^{3+} and Fe^{3+} are widely used in WWTPs as coagulating agents for their remarkable effects in promoting AS flocculation and settleability (Subramanian et al., 2010). A close relationship was found between flocculation and the content of trivalent cations in the AS and, therefore, the higher the concentrations of trivalent cations, the better the effluent quality (Murthy and Novak, 2001; Park et al., 2006). However, as far as we know, there have been no literature reports with respect to the theoretical study of the different impacts of bivalent and trivalent cations on AS flocculation. Therefore, the mechanisms governing the different flocculation performances of cations are not well understood, although they should be highly related to the physico-chemical properties of AS and the interactions between AS and cations.

The aforementioned studies indicated that W_{tot} and the contents of LB-EPS and trivalent cations in AS all correlate with the flocculability. This finding leads to the questions, "Is there any connection among these three items? If so, how does the connection work, and which item is the key factor?" Obviously, the answers will provide a new perspective on the flocculation mechanisms of AS with multivalent cations (Ca^{2+}, Al^{3+}, and Fe^{3+}) as additives, which is also the objective of this study.

In order to answer these questions, AS with sludge retention times (SRTs) of 15 d was cultivated in a lab-scale reactor. Subsequently, Ca^{2+} ($CaCl_2$), Al^{3+} ($AlCl_3$), or Fe^{3+} ($FeCl_3$) was added in separate experiments, each at a concentration of 2 mEq/L, before the flocculation process. Next, the AS was separated into four parts: bulk solution, LB-EPS, TB-EPS, and pellet. The total interaction energy (W_{tot}) curves were obtained based on extended DLVO theory, and the total organic carbon (TOC) and cation contents of the four parts of the AS were analyzed. Then, the relationships between the AS flocculability, W_{tot}, LB-EPS content, and concentrations of multivalent cations (Ca^{3+}, Al^{3+}, and Fe^{3+}) were analyzed and revealed the mechanisms controlling AS flocculation with multivalent cations as additives. To the best of our knowledge, this may be the first time that the distribution of cations in the AS has been used to explain AS flocculation with different multivalent cations as additives.

2. Methods

2.1. AS cultivation

The AS used in this research was cultivated in a sequencing batch reactor (SBR) with a volume of 4 L. The reactor was seeded with AS from Qu Yang WWTP (in Shanghai, China) with a nitrogen and phosphorus removal process. The AS was mixed with a paddle mixer at 100 rpm to prevent settling, the temperature of the reactor was maintained at 20 ± 1 °C, and air was introduced through stone air diffusers in order to maintain dissolved oxygen (DO) within the range of 2–3 mg/L. SRT of 15 d was achieved by wasting of the AS at the end of the last phase of aeration.

The SBR was operated with a cycling time of 4 h; hence, six cycles were performed each day. Each cycle was divided into six phases: (1) mixing + filling (anaerobic) for 20 min; (2) mixing + aerating (aerobic) for 120 min; (3) mixing (anoxic) for 30 min; (4) mixing + aerating (aerobic) for 30 min; (5) settling for 30 min; and (6) decanting for 10 min. Glucose, NH_4Cl, and KH_2PO_4 were the carbon, nitrogen, and phosphorus sources, respectively. The COD:N:P ratio of the influent was maintained at 100:7.5:1.5, and the feeding of micronutrients was in accordance with the recipe that was given by Liao et al. (2001). The pH of the reactor was controlled within a range of 6.8–7.2 by adding $NaHCO_3$ to the influent. The mixed liquor suspended solids (MLSS) were kept at the level of about 2300 mg/L by adjusting the influent COD (the value was 327.5 ± 6.1). After 45 d of operation under good conditions, parameters such as COD, MLSS, SVI, and ESS were monitored on a daily basis for 10 d, and AS was collected for further experiments on the condition that the fluctuation of these parameters was less than 20%. AS samples were taken from the reactor at the last 10 min of the second aeration phase.

2.2. The flocculation and fractionation protocol of AS

The AS sample (about 2300 mg/L) was collected and evenly distributed to four 250 mL beakers, and each was filled with 100 mL AS without any dilution. Ca^{2+} ($CaCl_2$), Al^{3+} ($AlCl_3$), or Fe^{3+} ($FeCl_3$) was added to three of the four beakers, respectively, with the remaining one as a control. The cations were added at a concentration of 2 mEq/L (40.0, 18.0, and 37.3 mg/L for Ca^{2+}, Al^{3+}, and Fe^{3+}, respectively) in order to demonstrate distinct effects (Higgins and Novak, 1997). Subsequently, the four AS samples underwent a two-step flocculation process: rapid mixing with a stirrer at a speed of 117 rpm for 5 min followed by slow mixing at 50 rpm for 5 min; all of the operational conditions were exactly the same for the four beakers. After flocculation, bulk solution, loosely bound EPS (LB-EPS), tightly bound EPS (TB-EPS), and pellet were extracted using the methods described below.

The EPS extraction protocol in this article was modified based on the research of Morgan et al. (1990), Li and Yang (2007), and Yu et al. (2008). According to this method, the AS samples were separated into four parts: bulk solution, LB-EPS, TB-EPS, and pellet. First, 25 mL of sludge suspension was centrifuged at 4000g for 5 min at 4 °C, and the carefully collected supernatant was the bulk solution. A NaCl solution with the same conductivity as the AS sample was made and preheated to 70 °C; then, after complete removal of the bulk solution, it was used to immediately re-suspend the AS sample in the tube at its original volume. With no delay, the AS suspension was sheared by a vortex mixer (S25, IKA, Germany) for 1 min; then, it was centrifuged at 4000g for 10 min at 4 °C, and the supernatant was collected as LB-EPS. The AS sample left in the tube was re-suspended again to its original volume of 25 mL by adding more NaCl solution, and then it was put into a water bath at 60 °C for 30 min. It was centrifuged at 4000g for 15 min at 4 °C, and the supernatant collected was TB-EPS, and the AS sample left was the pellet.

2.3. Analytical techniques

The total organic carbon contents (TOC) of the bulk solution, LB-EPS, TB-EPS, and pellet were determined using a TOC analyzer