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Investigation of measurement methods and characterization of zeta potential for aerobic granular sludge

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ABSTRACT

A measurement protocol and characterization of zeta potential for aerobic granules were investigated. The homogenization at 8000 rpm for 1.5 min was a good pretreatment method to break big aerobic granules into fine particles for directly measuring the zeta potential. There was no obvious variation of zeta potential for sludge concentration in the range of 0.1–8.0 g TSS/l. For mature aerobic granules, the zeta potential of fine particles after homogenization, LB-EPS, TB-EPS, and residual sludge after EPS extraction had different values of −10.2 mV, −10.7 mV, −18.2 mV, and −30.0 mV, respectively. The low and similar zeta potential features of fine particles after homogenization and LB-EPS suggested that LB-EPS should be a more important component than TB-EPS in influencing the electrochemical properties of the sludge. During the aerobic granulation process, the zeta potential of fine particles decreased gradually from –19.5 mV to −10.0 to −12.0 mV finally. The decrease of zeta potential might be a necessary condition for the formation and stability of aerobic granules.

Introduction

Aerobic granulation is a process of cell-to-cell self-immobilization and autoaggregation, which is accompanied with the change of microbial surface properties, hydrophobicity, surface charge (SC) and flocculating ability [1–3]. In the current theory to explain the phenomenon of microbial aggregation, the extended DLVO model and the polymer bridging model are widely accepted. The principle of these two theories is involved in the role of the surface charge and electrochemical properties of sludge [4]. According to the extended DLVO theory, decreased negative SC would lead to decreased repulsive electrostatic interactions between approaching surfaces and therefore also to stronger bonding between the various fractions of sludge [5–8]. Zeta potential is an important index to characterize the SC of sludge, which would tend to decrease gradually with the reduction of negative charge of sludge surface [9,10].

To date, it is still unclear for answering why and how aerobic granules form from the perspective of the mechanism [11]. It is also not possible to accurately say whether aerobic granules have evolved from smaller granules after outgrowths, or are derived from flocs due to either their coalescence, adherence to a solid substratum, and growth from EPS production, or some other transformation [12]. But in any case, change in electrochemical properties, such as surface charge and zeta potential, might be important and necessary during the aerobic granulation. Moreover, for mature aerobic granules, the stability also has a close relationship with its electrochemical properties [13]. Therefore, the information of the zeta potential probably provides a better understanding for the formation process and stability mechanism of aerobic granules.

Unfortunately, most results of zeta potential reported were about colloids and sludge flocs [14]. According to the measurement principles and method using the latest equipment, the small particles size (<100 μm) and good stability of the system are the prerequisites for the zeta potential test [15]. It is known that aerobic granules have compact structure, large particles size (0.2–5 mm), and good settleability [16]. Therefore, it is impossible to directly determine the zeta potential of aerobic granules now. The results and reports were quite scarce. In recent years, Xu et al. measured the zeta potential (11.5–33 mV) of aerobic granules at different pH 2–7 [17]. Zhang et al. also reported the zeta potential of bacterial cells from −32.4 to −13.3 mV during the aerobic granulation process [9]. However, the detailed test methods were all not mentioned explicitly. Even for anaerobic granules, only fine particles obtained by sieving (<50 μm) and settling for 30 s (<100 μm), as a representative, were used to characterize the zeta potential of anaerobic granules [18–20]. Although these results, to a certain extent, provided some useful information, the zeta potential properties of big aerobic or anaerobic granules were not obtained.

Aerobic granules could be regarded as a big microbial aggregate, which was composed of large number of small aggregates. Essentially,

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Nomenclature

SC	surface charge
DLVO	Derjaguin–Landau–Verwey–Overbeek
LB-EPS	loosely bound extracellular polymeric substances
TB-EPS	tightly bound extracellular polymeric substances
TSS	total suspended solids
VSS	volatile suspended solids
$d(0.1)$	cumulative particles size distribution (0–100%) corresponding to 10% in diameter
$d(0.5)$	cumulative particles size distribution (0–100%) corresponding to 50% in diameter
$d(0.9)$	cumulative particles size distribution (0–100%) corresponding to 90% in diameter

the characteristics of the granular sludge are determined by its internal structure and composition [21]. Imaginably, the investigation on zeta potential of these small aggregates than big granules could more deepen the understanding of the mechanism of formation and stability of granular sludge. Apparently, a prerequisite is that large aerobic granules must be broken into small particles using an appropriate breaking method. Till now, the measures for crushing aerobic granules were only used for investigating the hydrophobic property and enhancing the EPS extraction efficiency [22]. Moreover, the effect of crushing measures on aerobic granules and optimal conditions were never mentioned systematically.

Aerobic granular sludge is mainly composed of microorganisms and extracellular polymeric substances (EPS). Microorganisms in aerobic granules are surrounded by EPS, which construct the immediate environment of these cells [23]. EPS increase the softness of the cell surface and play a dominant role in all types of biofilm formations, including flocculation and granulation [9,24,25]. Therefore, EPS would influence electrochemical properties of sludge and be also thought to play an important role in aerobic granulation [26–28]. In nature, the change of sludge surface charge or the zeta potential of sludge primarily depends on the contribution of EPS [29,30]. According to the results reported in literature, the EPS of sludge is composed of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) [31,32]. Different chemical components have different contributions to the electrochemical properties of sludge [33]. However, the precise contribution of LB-EPS and TB-EPS to the aerobic granulation and stability is unclear, and some results reported in the literature were also inconsistent [34]. Also, the specific zeta potential characteristics of different chemical compositions of aerobic granules are still poorly understood.

In this experiment, a set of comprehensive and systematic pre-treatment method, based on homogenization for crushing the aerobic granules, was established and verified firstly in order to investigate the zeta potential characterization of aerobic granules. Furthermore, the discrepancy of zeta potential for different components of aerobic granules, primarily including small aggregates (or mixed sludge suspension) after homogenization, LB-EPS, TB-EPS, and residual sludge after EPS extraction, were compared and evaluated to deeply understand the roles on the aerobic granulation and mechanical stability. The variation of zeta potential was studied during the aerobic granulation. Meanwhile, the effectiveness and credibility of the established pre-treatment method were confirmed and proved by the results using sludge flocs as a control.

Materials and methods

Sludge samples and preparation

Aerobic granules and sludge flocs used in this experiment were sampled from the same sequencing batch reactors (SBRs) treating synthetic wastewater in different operation stages in the lab, respectively. After sampling, the sludge samples were first centrifuged to remove the supernatants at 2000 × *g* for 10 min. Next, the retained sludge was resuspended and washed with deionized water twice at the same centrifugation conditions for the removal of other impurities.

Sludge samples treatment and protocols

In this experiment, the sludge samples were mainly divided into three categories in order to meet the needs for measuring the particles size, zeta potential, and EPS extraction, respectively. Homogenizer (IKAT25, Genman) was used to break the sludge samples. For judging the change of particles size after homogenization, aerobic granules were homogenized at 6000, 8000, and 10,000 rpm (the angular velocity of homogenizer is only a quantitative parameter, which, to some extent, reflects the degree of decomposition of aerobic granules) for 0.5, 1.0, 1.5, and 2.0 min, respectively, to explore the suitable operation conditions. In order to compare the relationship between zeta potential values and sludge concentrations, aerobic granules and sludge flocs were first configured to different concentrations (0.1, 0.3, 0.5, 1.0, 3.0, 6.0, 8.0, 10.0, and 12.0 g TSS/L) with deionized water, and then were homogenized by homogenization at 8000 rpm for 1.5 min (the total volume of each sample was about 30 mL, which can meet the requirement of the homogenizer). Similarly, for EPS extraction and zeta potential measurement of EPS and sludge, the aerobic granules were homogenized at 8000 rpm for 1.5 min, with a selective sludge concentration of about 3.0 g TSS/L. In addition, for comparing the EPS release after homogenization at 8000 rpm for 1.5 min, the mixed sludge suspension was centrifuged at 2000 × *g* for 10 min. Then, the supernatants were collected for EPS analysis, and the sludge pellet was resuspended to a predetermined volume of 30 mL for second and third homogenization at the same conditions for obtaining the supernatants.

Particles size determination

Particles size distributions before and after homogenization were determined by Mastersizer 2000 (Malvern). Each sample was tested in triplicate, and the average value was used in this study.

EPS extraction and analysis

In order not to affect the composition of the sludge, a modified heat extraction method was used to extract the LB-EPS and TB-EPS of aerobic granules and sludge flocs after homogenization [33]. The sludge suspension was heated in a water bath. When the temperature reached at 50 °C, the sludge suspension was then sheared by a vortex mixer (Maxi Mix II, Thermolyne) for 1 min without any delay, and followed by centrifugation at 4000 × *g* for 10 min. The organic matter in the supernatant was readily extractable EPS, and was regarded as the LB-EPS of the biomass. For the extraction of the TB-EPS, the sludge pellet left in the centrifuge tube was resuspended with deionized water to its original volume. The sludge suspension was heated to 80 °C in a water bath for 30 min, and the sludge mixture was then centrifuged at 10,000 × *g* for 15 min. The supernatant collected was regarded as the TB-EPS extraction of the sludge.

The carbohydrate content in EPS was measured using the Anthrone method with glucose as the standard [35]. The protein content in EPS