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RESEARCH ARTICLE

Isolation and application of predatory *Bdellovibrio*-and-like organisms for municipal waste sludge biolysis and dewaterability enhancement

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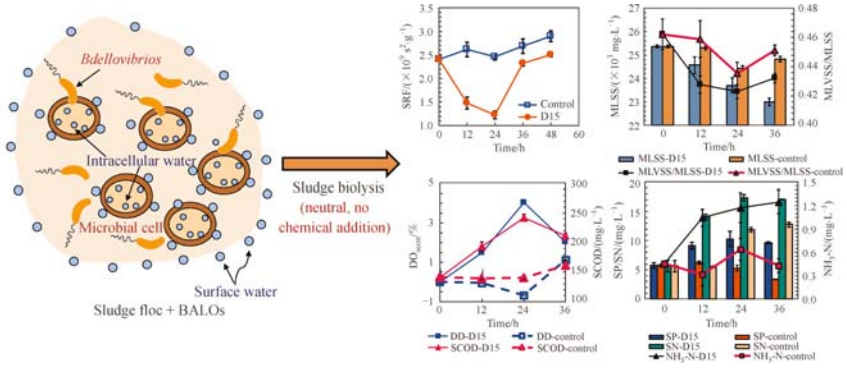
HIGHLIGHTS

- Indigenous predatory BALO strains were successfully isolated from activated sludge.
- Sludge SRF and CST were significantly reduced by BALOs induced biolysis process.
- The increase of BALO input dosage promoted the sludge biolysis efficiency.
- Sludge biolysis disintegrated flocs and lysed cells for internal water release.
- The optimal sludge biolysis time was 24 h and no pH adjustment was needed.

GRAPHIC ABSTRACT



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ABSTRACT

Bdellovibrio-and-like organisms (BALOs) are a group of ubiquitous and obligate predatory bacteria and commonly used as biocontrol agents. In this study, an efficient, environmental-friendly, and convenient BALOs encouraged municipal waste sludge biolysis pretreatment technique was developed and investigated for dewaterability enhancement of excess waste sludge. The indigenous predatory BALOs were successfully isolated from the sludge for biolysis treatment. Without any chemical addition or pH adjustment, the sludge specific resistance (SRF) and capillary suction time (CST) were significantly reduced by as high as 53.4% and 23.8%, respectively within 24 h's treatment, which would further be lowered with the increase of BALOs input dosage. However, the continuous extension of reaction time would worsen the sludge dewaterability. The decreases of SRF and CST accompanied with the increases of sludge disintegration degree and soluble chemical oxygen demand, nitrogen, and phosphorus concentrations all emphasized the contributions of BALOs' predation activities to sludge disturbance, cell lysis, and consequently the release of sludge intracellular water to finally effectively improve the sludge dewaterability and disposal efficiency.

1 Introduction

The most widely applied activated sludge process for biological wastewater treatment normally generates huge amounts of excess waste sludge with a high moisture content of over 95% after gravity thickening [1]. Incineration, composting, land application, and landfill disposal are the most commonly used approaches for sludge management [2]. In consideration of storage space, energy input, and cost requirements for subsequent sludge transport and handling, the efficient sludge dewatering process to reduce sludge in volume is highly required for municipal wastewater treatment plant (WWTP) [2,3].

Free water, interstitial water, surface water, and intracellular water are the general water physical states in sludge [4]. The traditional mechanical dewatering methods, such as vacuum or belt filter and plate-and-frame filter

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press can only remove free, interstitial, and partial surface water and reduce the sludge moisture content to around 70% [5]. The remaining intracellular water accounts for 70%–80% of the packed cell mass [6]. To enhance sludge dewaterability, the pretreatment technologies such as ultrasonication, microwave irradiation, and electrolysis are generally applied and the conditioning chemical reagents are commonly added to disrupt sludge's floc structure and lyse cells for internal water release [7]. The according sludge treatment expense usually account for as high as 25%–65% of total WWTP operation costs [7]. Meanwhile, the added chemical reagents would decrease sludge settling performance and cause secondary pollutions. Therefore, the development of a more efficient, economical, and environmental-friendly sludge pretreatment method before mechanical dewatering is highly demanded.

Bdellovibrio-and-like organisms (BALOs) are a group of motile and obligate predatory Gram-negative bacteria [8]. They have a distinct parasitic biphasic life cycle [8] and are ubiquitous in nature. The free-swimming BALO cells first attack, invade, and lodge within the periplasm of the prey cell and inactivate its potential to synthesize RNA, protein and DNA. BALOs then utilize the prey's macromolecules as a source of nutrients, secrete proteases and nucleases synthesized in their own cytoplasms, and target the cytoplasm of the prey cell for release and initiate new life cycles [9]. BALOs have been isolated from a variety of habitats including soil, fresh and brackish water, sewage, seawater, and rhizosphere [10–12]. Since BALOs mainly predate on a diversity of Gram-negative cells, especially most Gram-negative pathogens [13], they have been applied as potential "living antibiotics" and biocontrol agents in agriculture, animal husbandry and water purification [14]. More importantly, BALOs can selectively parasite and lyse most of the pathogenic Gram-negative bacteria and leave no residue or infection problems, which are the general concern for bacteriophage usage [15]. Because of sludge's higher cell density and abundant organic and nutrient contents [16], BALOs are commonly present in activated sludge of WWTP [17]. Their predation activities should not only induce cell lysis [18] but also disturb the sludge's floc composition and structure [19] for internal water release and sludge dewaterability improvement. Therefore, BALOs promoted sludge pretreatment is expected to be promising for sludge dewaterability improvement although the relevant exploration has seldom been addressed yet.

The objectives of this study were to investigate the feasibility and efficiency of BALOs induced biolysis treatment to promote sludge dewaterability. The indigenous predatory BALO strains and their hosts were first isolated and screened from the activated sludge. BALO's sludge disturbance and cell lysis performances were evaluated based on the measurement of sludge disintegration degree, specific resistance to filtration (SRF) and capillary suction time (CST). Meanwhile, the conditions for sludge-BALOs interactions were optimized to facilitate further practical BALOs application in sludge dewatering efficiency improvement.

2 Materials and methods

2.1 Sludge sampling

The raw activated sludge used in this study was collected from the secondary clarifier of a municipal WWTP (Nanjing, China), transported to the laboratory within 2 h after the sampling, and used after an overnight gravity concentration at room temperature. The soluble chemical oxygen demand (SCOD) and the mixed liquid suspended solids (MLSS) concentrations of the tested sludges were 140–160 $mg \cdot L^{-1}$ and 21,000–25,000 $mg \cdot L^{-1}$, respectively. The ratios of mixed liquor volatile suspended solid concentrations to MLSS concentrations (MLVSS/MLSS) were 0.48–0.51. The sludge SRF values were 1.0×10^9–4.8×10^9 $s^2 \cdot g^{-1}$. The sludge pH and moistures were 6.7–6.9 and 97.5%–98.2%, respectively.

2.2 Strain isolation and cultivation

2.2.1 Isolation and cultivation of prey bacteria

As obligate predators, BALOs require co-cultivation with prey bacteria for growth. The collected sludge was first vigorously oscillated for 30 min before 60-min quiescent settlement. A series of 10-fold dilutions were then made for the supernatant with 0.01 $mol \cdot L^{-1}$ sterile Phosphate Buffered Saline (PBS) buffer and spread onto a series of sterile lysogeny broth (LB) agar plates for incubation at 30°C. The obtained colonies were repeatedly streaked on the LB agar plates for three times for purification. The obtained colonies were examined by gram staining for Gram negative ones, which were then incubated as the candidate prey bacteria in the nutrient broth (NB, pH = 7.0–7.4) in a thermostatic shaker (30°C, 150 $r \cdot min^{-1}$) and stored at 4°C before use.

2.2.2 Isolation and cultivation of BALOs

The isolation and purification of BALOs relied on the double-layered agar plate made of a 500-fold diluted nutrient broth (DNB) (0.6% and 1.2% agar in the top and bottom layers, respectively) [20]. The sludge was first vigorously oscillated and centrifuged at a speed of 3,000 $r \cdot min^{-1}$ for 10 min. The supernatant was further centrifuged at 13,000 $r \cdot min^{-1}$ for 20 min to collect cell pellet, which was re-suspended in a 2-mL sterile PBS, and 10-fold diluted for BALOs isolation. Each dilution was mixed with fresh prey culture and melted top level medium in a volume ratio of 1:1:10 before immediately overlaying onto

the bottom layer medium in a petri dish prepared in advance. The solidified double-layer plates were incubated at 30°C to form plaques, which were randomly collected and inoculated into 70 mL DNB liquid medium mixed with 1 mL prey culture for enrichment (30°C, 150 r·min^{-1}). When the incubation medium became clear, two successive platings followed by liquid enrichment were performed to obtain pure BALO strains.

2.3 Screening of BALO strains and hosts

The isolated BALOs and prey bacteria were further screened for efficient sludge biolysis. The preferred prey bacteria were selected mainly according to the reduction rate of spectrum absorbance at the wavelength of 600 nm (OD_{600}) during their co-cultivation with BALOs. The fresh BALO enrichment was mixed with the sludge in a volume ratio of 1:10 for sludge biolysis (30°C, 150 r·min^{-1}). The BALO, which could induce the highest SRF reduction rate during the sludge biolysis was chosen for further study.

2.4 BALO interaction with sludge

A series of 10-fold diluted BALO cultures were added into the sludge samples in a volume ratio of 1:10 for sludge biolysis (30°C, 150 r·min^{-1}). The SRF, CST and sludge characteristics were monitored in a 12-h interval during the incubation. The optimal reaction time, sludge pH (adjusted with 0.1 mol·L^{-1} HCl or NaOH) and BALO input dose were explored, respectively for BALO-sludge interaction.

2.5 Analytical methods

SRF was assessed using a Buchner funnel-vacuum suction method. The sludge sample (150 mL) was suction-filtrated through a pre-wetted medium speed quantitative filter paper (Whatman, Dassel, Germany) in a 9 mm diameter Buchner funnel under a constant pressure of 0.045 MPa with a vacuum pump (2RK-1,Boerkang Co., China). The CST was measured with a capillary suction timer (Type 304M, Triton Electronics, UK).

The concentrations of soluble chemical oxygen demand (SCOD), soluble phosphorus (SP), soluble nitrogen (SN), ammonia nitrogen (NH_3-N), MLSS and MLVSS were measured during the sludge biolysis process according to the standard methods [21] The pH was measured with a pH meter (PHS-3C, Leici Co., China). The degree of sludge disintegration (DD_{SCOD}) was calculated according to the following equation [22].

$$DD_{SCOD}(\%) = \frac{SCOD_S - SCOD_{S_0}}{SCOD_{\mathrm{NaOH}} - SCOD_{S_0}} \times 100, \quad (1)$$

where $SCODs$ and $SCOD_{S_0}$ referred to the $SCOD$ of treated and untreated sludge samples, respectively. Alkaline hydrolysis procedure was applied to obtain $SCOD_{\mathrm{NaOH}}$, in which 0.5 mol·L^{-1} NaOH was added to an untreated sludge sample and mixed for 24 h at room temperature.

2.6 Environmental scanning electron microscopy (ESEM)

The sludge morphology was examined with a Philips XL30 Environmental Scanning Electron Microscope (Philips, Eindhoven, Netherlands). The sludge sample was fixed in 2.5% glutaraldehyde solution overnight at 4°C. Then, it was rinsed with 1M PBS (pH = 7.2) for 3 times (15 min each) and dehydrated in an ethanol series (50%–70%–80%–90%–95% in sequence; 3 min each). Finally, it was immersed in 100% ethanol twice (2 min each), and dried in a vacuum freeze-dryer at –40°C (FD-1A-50, Boyikang Laboratory Instruments Co.,Ltd, Beijing, China) before scanning.

2.7 Identification of BALO strain and host

The genomic DNA was extracted from the cell pellet using a PowerWater DNA Isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instruction and quantified with a NanoDrop ND-1000 (Thermo Scientific, Waltham, MA, USA). The 16S rRNA gene was amplified for 35 cycles of polymerase chain reaction (PCR, 1 min at 94°C, 45 s at 56°C, and 25 s at 72°C) preceded by a 3-min initial denaturation at 94°C and followed by a final 10-min extension at 72°C in a T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). A Takara PCR Mix kit (TaKaRa Bio Inc., Otsu, Shiga, Japan) and a pair of *Bdellovibrio*-specific primers (forward: 5′-CAGG CCTAACACATGCAAGTC-3′, reverse: 5′-CGWCACTGAAGGGGTCAA-3′) [12] for BALOs or a bacterial 16S rRNA gene universal primer set (forward: 5′-TCC TAC GGG AGG CAG CAG T –3′; reverse: 5′- GGA CTA CCA GGG TAT CTA ATC CTG TT –3′) [23] for prey bacteria were used for PCR operation. The PCR amplicons were sent to Shanghai Sangon Biotechnology Co, Ltd (Shanghai, China) for sequencing and the obtained sequences were aligned against GenBank (http://www.ncbi.nlm.nih.gov) for classification and then were deposited in the DDBJ/EMBL/GenBank nucleotide sequence database.

3 Results

3.1 Isolation and preliminary screening of BALOs and prey

Fifteen Gram negative prey strains were isolated from the municipal sewage sludge as the candidate hosts for BALO cultivation. However, five of them did not induce the formation of BALO plaques and were abandoned. The genomic DNA of the remaining 10 prey strains were later individually extracted for 16S rRNA gene sequencing and alignment. The results indicated that these 10 strains

belonged to five genus of *γ-proteobacteria: Aeromonas* (accession numbers of KX640106 and KX640107), *Klebsiella* (KX636139 and KX640112), *Enterobacter* (KX640108, KX640113, and KX640114), *Raoultella* (KX640110), and *Escherichia* (KX640109 and KX640111). They were later randomly chosen for BALO isolation and 30 pure BALO strains were finally retrieved.

To first screen BALOs with high proliferation activities, the *Escherichia* culture (KX640109, $(1.0\pm0.1)\times10^9$ CFU $\cdot mL^{-1}$) was applied for BALOs enrichment. According to Table 1, the reduction rates of OD_{600} value varied from (39.3±0.6) % to (90.9 + 3.0) % during the BALO enrichments and D8 strain caused the highest OD_{600} reduction rate. Ten strains with the OD_{600} reduction rates lower than 50% were discarded and the remaining 20 ones were further examined for sludge biolysis.

3.2 Screening of prey

To efficiently cultivate BALOs, the screening of the desired prey strain was performed via the monitoring of the OD_{600} variation during the co-incubation of D8 strain with 10 previously isolated prey strains, respectively. All prey strains were lysed but in different extents during the co-cultivation with D8 (Fig. 1). The OD_{600} value decreased gradually along with the incubation time and the medium finally became clear because of the prey cells' lysis under the tiny BALOs' predation [10,12]. The OD_{600} values of two *Klebsiella* strains inoculated media were generally lower than the other 8 at any sampling time and they finally declined by 99.5%–99.6%. Therefore, these two *Klebsiella* strains were chosen for further BALOs cultivations.

3.3 Secondary screening of BALOs

The desired BALO strain with satisfying sludge biolysis performance was screened from 20 previously isolated and screened ones based on the SRF assessment during the BALO-sludge interaction. The 20 BALO strains were divided into 4 groups for evaluation. The four groups were examined separately and the tested slugdes were collected at the different times from the WWTP. The control samples with no BALO addition showed irregular variations in SRF reduction rates during the incubation periods, which were also incomparable among themselves probably because of their varied sludge properties and microbial compositions. Almost all tested BALOs with the similar initial concentrations in the sludges ($(2.0\pm0.5)\times10^7$ PFU$\cdot mL^{-1}$–$(4.5\pm0.5)\times10^7$ PFU$\cdot mL^{-1}$) had certain degrees of sludge lysis abilities and the smallest sludge SRF values were

Table 1 The reduction of OD_{600} during BALOs' liquid enrichments co-incubated with $(1.0\pm0.1)\times10^9$ CFU$\cdot mL^{-1}$ prey culture

sample No.	reduction rate/%	sample No.	reduction rate/%	sample No.	reduction rate/%
D1	71.7±0.7	D11	64.1±1.2	D21	80.6±3.0
D2	80.9±0.2	D12	70.4±0.63	D22	67.8±1.6
D3	75.4±1.0	D13	63.4±1.0	D23	45.9±0.4
D4	68.9±1.3	D14	71.6±2.5	D24	49.6±0.8
D5	47.7±0.4	D15	65.2±2.2	D25	46.5±0.7
D6	66.1±1.2	D16	48.5±0.7	D26	70.0±1.9
D7	40.9±0.5	D17	39.3±0.6	D27	48.8±0.8
D8	90.9 + 3.0	D18	82.9±3.7	D28	76.9±1.9
D9	73.4±0.8	D19	39.9±0.4	D29	86.2±0.5
D10	44.5±0.3	D20	69.1±1.7	D30	68.8±1.6

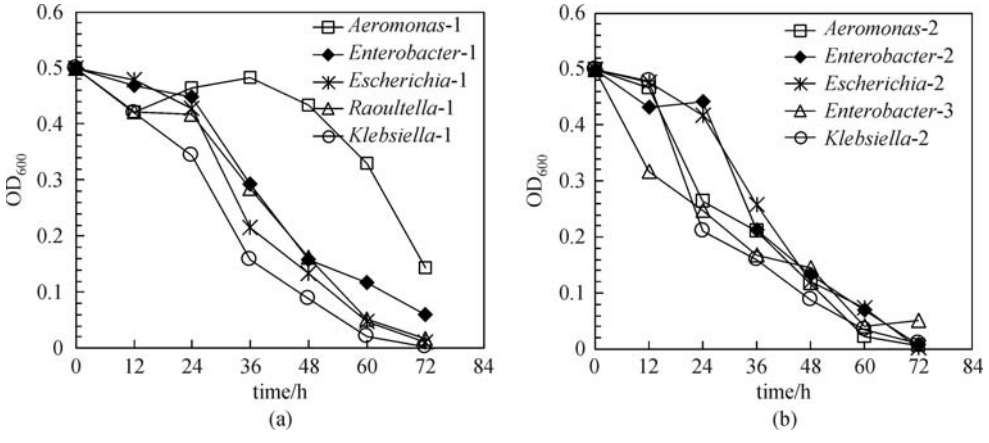

Fig. 1 D8 BALO strain enrichments with different prey species

normally obtained after 24 h's treatment (Table 2). However, the further increase of sludge biolysis time boosted the SRF value. D15 induced the highest SRF reduction rate ((47.2 + 1.7) %) after 24 h's sludge biolysis, and D3, D4, D18, and D20 generally caused the reduction of SRF by over 40% in different test sets. Therefore, all these 5 strains were considered as the promising BALOs for sludge dewatering performance enhancement.

3.4 Sludge biolysis with BALOs

3.4.1 Effects of BALO species

The five screened BALO strains were first co-incubated with the prey bacteria *Klebsiella*-1 (KX636139) (Fig. 2 (a)). D15 inoculated medium displayed the highest OD_{600} reduction rate of (89.6±0.1) % at the end of incubation, while the other four caused 68.3% – 73.3% reduction. The results suggested that D15 were probably more efficient than the other four for prey lysis. Then, the five enriched BALO cultures with the similar concentrations were mixed with the same sludge samples, respectively for biolysis treatment to evaluate their capabilities to promote sludge dewaterabilities (Fig. 2(b)). The SRFs of all the treated sludges were generally smaller than that in the control at any sampling time. The smallest SRF values were generally obtained at 24 h and D15 was observed to induce the largest SRF reduction rate of (51.5±5.9) %. Therefore, D15 was expected to possess higher cell lysis ability than the other four under the same sludge biolysis conditions and was applied in the following studies. The phylogenetic analysis of D15 via 16S rRNA gene sequencing and alignment identified it in the genus of *Bdellovibrio* with the accession number of KX440969.

3.4.2 Optimization of sludge pH

Since BALOs are generally active in the neutral environments [24], the pH of the sludge samples were adjusted to 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0, respectively before D15 culture addition to explore the pH impacts on sludge biolysis efficiency (Fig. 3). The pH values of all treated

Table 2 The variations of SRF during the BALO-sludge interaction processes

group	strain number	SRF reduction rate/%			
		12 h	24 h	36 h	48 h
1	control-1	15.8±2.8	16.6±5.7	6.8±2.8	–9.8±4.2
	D1	13.5±2.8	8.6±2.8	4.3±1.4	–5.3±1.4
	D2	33.5±1.4	39.2±3.1	29.5±2.8	–1.9±3.4
	D3	24.7±7.1	41.6±4.2	30.0±5.7	14.8±5.6
	D4	23.6±5.7	41.7±2.8	23.4±1.4	5.6±2.8
	D6	35.8±2.8	38.5±7.1	34.7±4.2	21.4±1.4
2	control-2	–1.5±4.7	–1.8±5.4	5.8±5.7	–12.6±2.8
	D8	–14.6±5.1	4.6±4.2	6.8±4.2	–18.6±3.1
	D9	4.8±2.4	6.3±2.8	17.6±1.4	–1.1±4.7
	D11	–35.3±6.1	–47.4±4.2	2.5±2.6	–22.7±3.4
	D12	7.7±5.7	13.6±3.7	18.1±3.3	1.5±5.7
	D13	6.9±7.1	18.1±5.7	17.8±4.4	2.6±2.1
3	control-3	19.2±4.2	15.2±2.8	1.3±2.5	–3.0±1.2
	D14	6.1±8.5	30.7±5.7	33.1±2.8	30.3±2.8
	D15	23.0±4.2	47.2±1.7	39.4±3.8	25.5±4.1
	D18	27.7±5.1	43.9±2.5	38.1±2.6	21.1±5.7
	D20	23.8±5.7	40.7±3.7	38.2±7.1	–3.9±3.9
	D21	28.7±2.8	35.6±1.8	38.3±1.4	28.4±3.7
4	control-4	4.5±3.7	2.4±1.4	–7.0±5.7	–15.0±7.1
	D22	23.0±3.4	16.7±5.7	9.8±6.4	3.7±5.7
	D26	5.8±7.1	4.2±7.1	11.1±2.8	–0.5±4.2
	D28	6.2±5.3	20.0±4.6	0.4±4.2	–4.3±3.4
	D29	30.8±2.8	9.2±6.2	5.7±4.2	–0.1±5.0
	D30	–0.1±4.2	–3.0±4.2	–7.8±3.6	–18.4±2.8

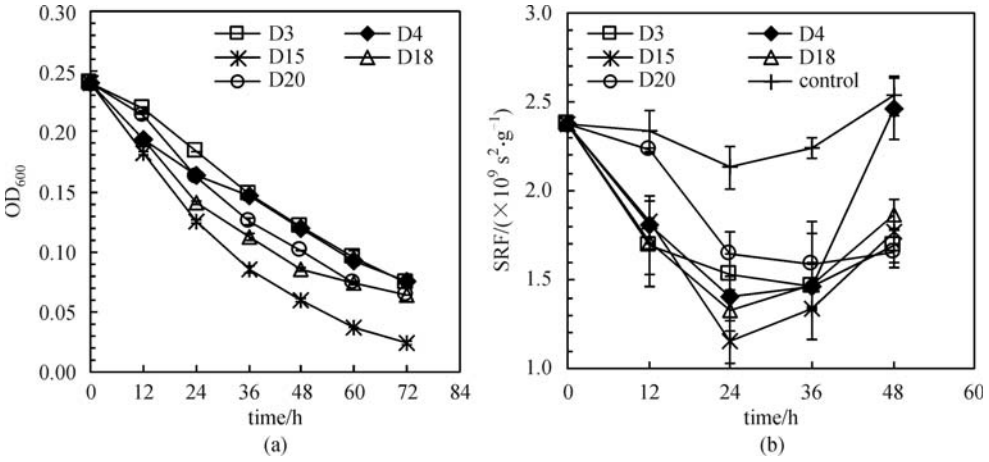

Fig. 2 The predation performances of the five BALO strains during their co-incubations with *Klebsiella*-1 ((1.2±0.1) × 10^9 CFU ·mL^{-1}), respectively (a) and the SRF variation profiles of the sludge lysed by the five BALO cultures of the similar concentrations (6.2±0.1) × 10^6 PFU·mL^{-1}) in the sludges, respectively (b)

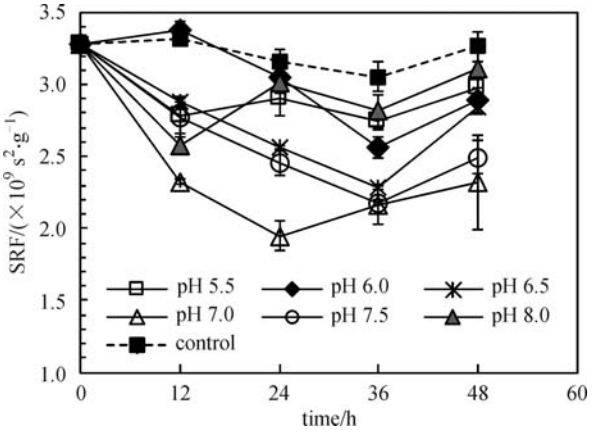

Fig. 3 The impacts of sludge pH on SRF during the sludge biolysis with D15 culture ((4.8±0.3) × 10^6 PFU·mL^{-1})

sludges only decreased by 0.2–0.3 at the end of the treatments (data not shown). The SRF in the neutral sludge sample (pH = 7.0) was always smaller than those in the acidic or basic sludge samples at any sampling time, except when at 36 h, the similar SRF values were noticed in the sludge samples with pH between 6.5 and 7.5, which were 25.0%–29.1% lower than that in the control (Fig. 3). The smallest SRF in the neutral sludge sample was still detected at 24 h, which was 38.4% smaller than that in the control. The SRF values in the pH 5.5, 6.0 and 8.0 sludge samples declined by 0%–21.4% in the first 12 h and then kept relatively high during the whole biolysis period. Because the sampled raw sludge pH was generally neutral (pH = 6.8±0.1), no pH adjustment was made in our following studies.

3.4.3 Effects of BALO input dosage

To investigate the impact of BALO concentration on sludge biolysis efficiency, a series of 10-fold diluted fresh D15 cultures were prepared and mixed with the sludge samples of the same origin to reach the concentrations of (4.6±0.6) × 10^8 PFU·mL^{-1} ((1.8±0.2) × 10^{10} PFU·g $MLSS^{-1}$), (6.4±0.6) × 10^7 PFU·mL^{-1} ((2.6±0.2) × 10^9 PFU·g $MLSS^{-1}$), and (3.6±0.6) × 10^6 PFU·mL^{-1} ((1.4±0.2) × 10^8 PFU·g $MLSS^{-1}$), respectively. The SRF and CST of each treated sludge displayed the similar variation profiles during the sludge-BALOs interaction (Fig. 4). The lowest SRF and CST values for all the treated sludges were found at 24 h reaction time point, which were 27.3%–53.4% and 10.9%–23.8% lower than those in the control, respectively (Fig. 4). In addition, the SRF and CST of all treated samples were smaller than those of the control and their values were in the opposite rank order of the samples' initial D15 concentrations at any sampling time. Therefore, the BALOs stimulated biolysis treatment greatly reduced the sludge's SRF and CST and the increase of the input BALO dosage enhanced the sludge dewater-

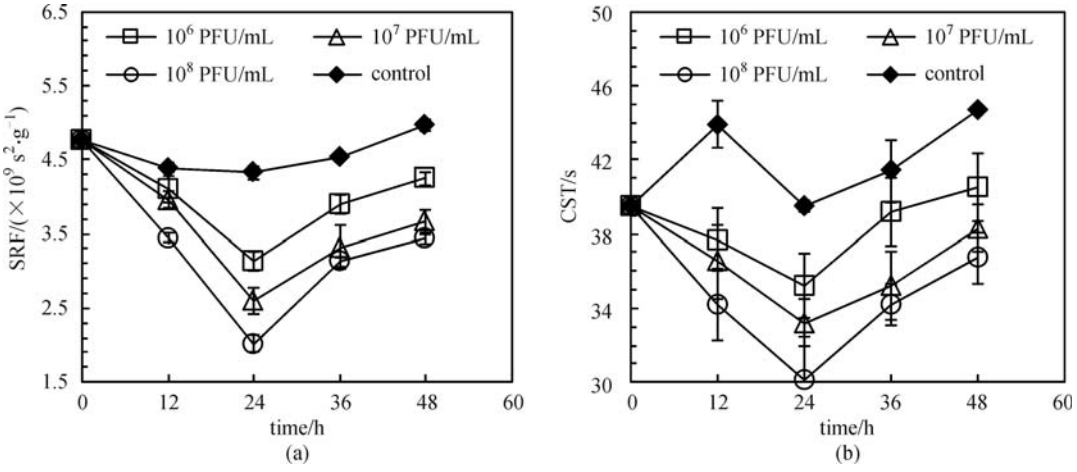

Fig. 4 The effects of BALO input dosage on sludge dewatering performance improvement in terms of SRF (a) and CST (b) (Note: the legends indicate the magnitudes of the initial D15 concentrations in the sludge samples which were (3.6±0.6) × 10^6 PFU·mL^{-1} ((1.4±0.2) × 10^8 PFU·g $MLSS^{-1}$), (6.4±0.6) × 10^7 PFU·mL^{-1} ((2.6±0.2) × 10^9 PFU·g $MLSS^{-1}$), and (4.6±0.6) × 10^8 PFU·mL^{-1} ((1.8±0.2) × 10^{10} PFU·g $MLSS^{-1}$), respectively)

ability. In addition, this study again indicated that the extension of the reaction time to longer than 24 h would not help improve the sludge dewaterability and 24 h was considered as the optimal biolysis reaction time.

3.4.4 Sludge properties during biolysis

To better understand the BALO predation disturbance to sludge flocs, the sludge' characteristics were evaluated during the D15 promoted biolysis process (Fig. 5). Although the variations of MLSS concentration and MLVSS/MLSS ratio were both observed in either the BALOs treated sludge and the control during the incubation period probably due to the metabolic activities of the surviving microbial populations, their values in the BALOs treated sludge were consistently lower than those in the control at any sampling time. The MLSS concentration continuously declined during the 36-h BALO-sludge interaction and the final MLSS concentration was (7.4±0.1) % lower than that in the control (Fig. 5 (a)). In contrast, the MLVSS/MLSS ratio displayed a downward tendency during the first 24 h's biolysis process followed by a slight recovery (Fig. 5(a)) and the minimum MLVSS/MLSS ratio of 0.42±0.01 was achieved at 24 h, which was consistent with the SRF and CST's variation trends (Fig. 4). The MLVSS/MLSS ratio is considered as an index of the sludge activity [25]. The significant reductions of the MLSS concentration and the MLVSS/MLSS ratio in the treated sludge were probably contributed by the D15 induced lysis of microbial cells in the sludge for the release of intracellular organic contents. The growth of D15 and the remaining living microbes in the sludge would consume the released organic matters and even cause the restoration of MLVSS/MLSS ratio, which was supported by the SCOD, DD_{SCOD}, SP and SN variation profiles discussed next (Figs. 5(b) and 5(c)).

The SCOD and DD_{SCOD} in the D15 treated sludge generally showed the similar increasing trends over time until after 24 h's biolysis reaction when the peak DD_{SCOD} and SCOD concentration reached (Fig. 5(b)). In contrast, no dramatic variations of SCOD concentration and DD_{SCOD} occurred in the control, especially during the first 24 h. The peak SCOD concentration in the D15 treated sludge at 24 h was (241±6) mg·L^{-1}, which was almost twice of that in the control ((140±4) mg·L^{-1}). Meanwhile, the peak DD_{SCOD} of the D15 treated sludge was even 3.6 times of that in the control, which emphasized the contributions of BALO predations to cell lysis and sludge structure disruption.

The similar time-dependent SP and SN variation profiles were observed for D15 treated sludge (Fig. 5(c)) and they also matched those of SRF, CST, SCOD, and DD_{SCOD} (Fig. 4 and Fig. 5(b)). The highest concentrations of SP ((10.3±1.3) mg·L^{-1}) and SN ((17.1±0.2) mg·L^{-1}) were both found at the end of 24 h's biolysis treatment, which were 1.9-and 1.5-fold of those in the control, respectively. Meanwhile, the NH_3-N concentration in the sludge liquid phase was in a moderate increase mode over time during the sludge biolysis from (0.5±0.0) mg·L^{-1} (0 h) to the highest level of (1.3±0.0) mg·L^{-1} (36 h) (Fig. 5(c)).

The extent of the sludge disintegration (DD_{SCOD}) was found to generally have a positive relationship with the sludge dewaterability in terms of SRF or CST, although a plateau occurred when DD_{SCOD} was between 1.5%–2% (Fig. 5(d)). The lowest SRF or CST level was observed when the highest DD_{SCOD} of (4.0±0.1) % was achieved.

3.5 ESEM

The morphology of the D15 treated sludge was explored via ESEM imaging as compared to the control (Fig. 6). The control sludge was compact and flocculent and was mainly

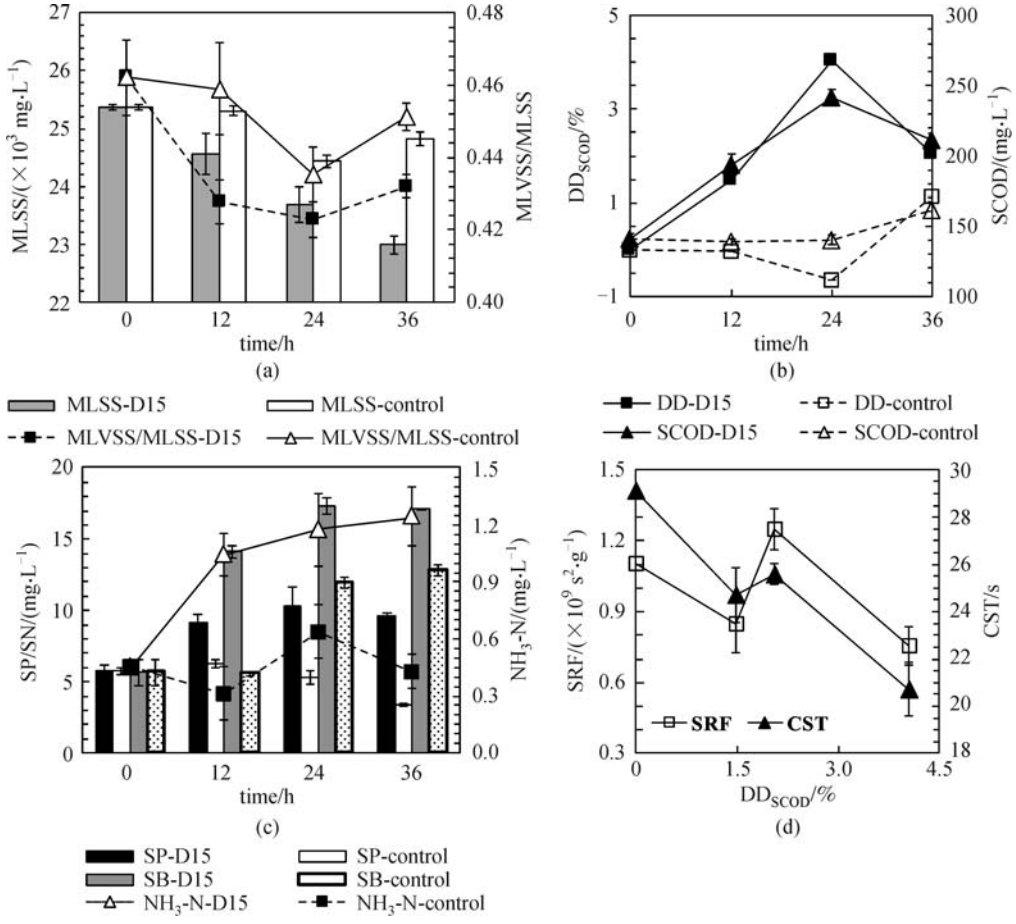

Fig. 5 The variations of MLSS concentration and MLVSS/MLSS ratio (a), DD_{SCOD} and SCOD concentrations (b), and SP, SN, and NH_3-N concentrations (c), and the relationship between DD_{SCOD} and SRF or CST (d) during the sludge biolysis in the presence of $(4.2\pm0.3) \times 10^7$ PFU·mL^{-1} D15 culture

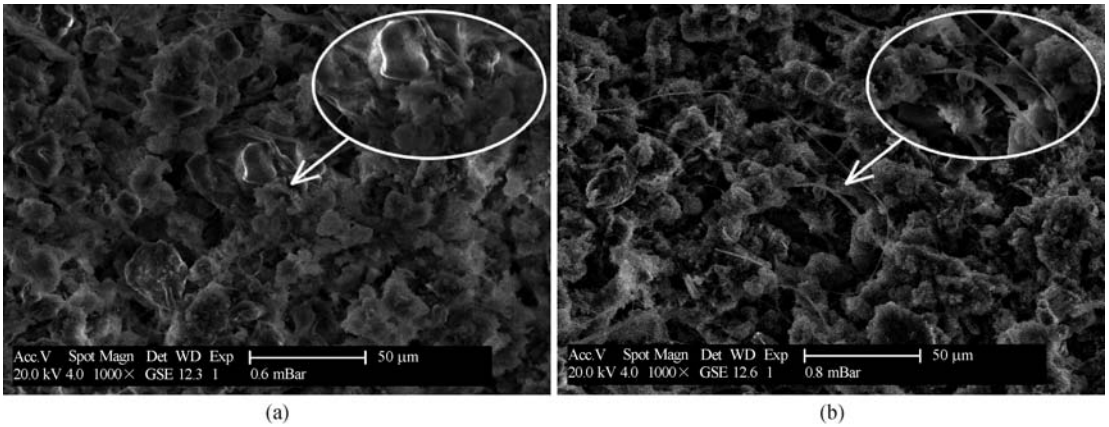

Fig. 6 ESEM images of the control sludge sample (a) and the one (b) treated with D15 for 24 h with 1,000 fold of magnification. The images in the oval white frame was the further enlargement of the area pointed by the arrow with 3,000 fold of magnification

composed of large rough floc blocks (Fig. 6(a)). In contrast, the flocs of the D15 treated sludge were smaller, the pore sizes in the sludge appeared larger, and several filaments became clearly observed interspersed in the sludge, which were generally considered to serve as the "backbone" of the microbial flocs [26] (Fig. 6(b)). Therefore, BALO predation activities during the biolysis treatment disrupted the sludge's flocculent structures and

caused the apparent morphology changes as compared with that of the control.

4 Discussion

BALOs are pervasive and highly adaptive to the environment [10]. However, their isolation from activated sludge in municipal WWTP has seldom been reported yet. The biological activated sludge mainly consists of microorganisms, either as single cells, filamentous bacteria or microcolonies, and organic fibers associated with inorganic particles (salt and sand) [16]. Thus, they should provide BALOs sufficient prey cells and nutrients for survival and proliferation. Since BALOs have been demonstrated to preferentially prey on bacteria from the same habitat over other environments [27], we first tried to isolate indigenous BALO strains and their hosts from the municipal activated sludge for further biolysis investigation. Thirty predatory BALO strains and ten hosts from five genus were successfully retrieved (Fig. 1 & Table 2), which suggested that BALOs were active members in the sludge and were capable of invading a diversity of γ-proteobacterial prey organisms commonly present in WWTPs [10,28].

The BALOs stimulated biolysis process was revealed to greatly enhance the sludge dewatering performance (Figs. 3–4). The sludge SRF and CST were reduced by as high as 53.4% and 23.8%, respectively after 24 h's treatment and the declines of SRF and CST were generally accompanied with the increase of DD_{SCOD}. The highest SCOD, SP and SN concentrations and the DD_{SCOD} value were detected when the sludge SRF and CSF were the smallest (Figs. 4 and 5). The MLVSS/MLSS profiles mirrored the DD_{SCOD} ones (Fig. 5). All these findings and the ESEM imaging of sludge morphology changes (Fig. 6) strongly implied that the BALOs' predation activities disturbed the sludge floc structure, increased the degree of sludge disintegration, impaired the cell integrity, and finally efficiently released the sludge's bound and internal water contents, which was expected to be critical for sludge dewaterability improvement [3,5,29]. Moreover, the increase of BALO concentration enhanced the sludge dewatering performance (Fig. 4) probably because the high density of predators could ensure their sufficient collisions with the prey bacteria and their effective adhesion and invasion to the prey cells [14,28] to speed up the cell lysis rates.

However, the extension of the biolysis time would not always improve the sludge dewatering performance but worsen it instead (Figs. 2–4). The re-growths and the metabolic activities of the survived living microbes in the treated sludge would consume the released SCOD and nutrients from the impaired prey cells [30] and probably re-stabilized the sludge floc structure in some extent to decrease the sludge dewaterability. In addition, the proliferation of BALOs based on their predation activities would enable them the important component of the sludge microbial community and might unavoidably contribute to the deterioration of sludge dewaterability. The similar phenomena have been documented when the bioleaching technique was applied for sludge dewaterability improvement [3].

Although there are various available physical, chemical and biological sludge pretreatment methods to promote sludge dewatering efficiencies, BALOs stimulated sludge biolysis process still displayed operational, economic, ecological, and hygienic advantages [15]. As the strict aerobes, BALOs were also found to survive under anoxic and microaerobic conditions [31], which enable them highly flexible and adaptable for sludge biolysis. In addition, as the solitary flagellum-propelled and chemotaxis-directed hunter cells [32], BALOs are sensitive to pH changes and their predation activities can be inhibited in the acidified or basified medium [33,34]. Therefore, the highest sludge biolysis efficiency was obtained when the sludge pH was 7 (Fig. 3) and no extra pH adjustment is generally needed when the neutral municipal activated sludge is handled, which not only simplifies the sludge biolysis treatment but also avoids several problems commonly occurred during the following sludge handling processes, such as pipeline corrosion and ecological environment impacts during landfill disposal. Furthermore, no conditioning chemical reagent addition during or before sludge biolysis would enable the treatment free of secondary pollution and lower the treatment costs. Solubilisation and release of organic components and nutrients during cell lysis would even improve the sludge biodegradability during the downstream digestion treatment [35]. More importantly, BALOs have been accepted to be nonpathogenic and nontoxic to animals and humans and capable of attacking most Gram-negative pathogens [13,14]. They depend on preys for survival and would not develop into dominant groups of sludge microbial communities [36]. All these BALOs' unique characteristics strongly supported their future practical application in sludge dewaterability improvement.

5 Conclusions

In this study, the efficient, environmental-friendly, simple and economical BALOs stimulated sludge biolysis pretreatment process was extensively explored for sludge dewaterability enhancement. The predatory BALO strains were successfully isolated from the activated sludge. The most significantly reduced sludge SRF and CST levels were generally obtained after 24 h's biolysis under neutral environment. The decreases of SRF and CST were accompanied with the decrease of MLSS concentration and the increase of DD_{SCOD} and SCOD, SP, NH_3-N, and SN concentrations, which strongly demonstrated the contributions of BALOs' predation activities to the release

of sludge intracellular water. The increase of BALO input dosage promoted the sludge biolysis efficiency, while the extension of reaction time did not always improved the sludge dewatering performance.

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