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Co-existence of flocs and granules in aerobic granular sludge system: Performance, microbial community and proteomics

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ABSTRACT

In aerobic granular sludge (AGS) system, flocs typically coexist with granules. The inherent differences between flocs and granules can result in their different behaviors in the same reactor. In this study, a flocs/granules co-existence system was investigated and differences between the flocs and granules in co-existence system were compared on proteomic level, providing an in-depth insight into co-existence of flocs and granules. Results showed that the co-existence system obtained under 4.5 kg COD m^{-3} d^{-1} exhibited excellent nutrient removal (COD: 96–97 %, TN: 98–99 %) with approximately 9–11 % flocs. A comparison of flocs and granules in the co-existence system showed different functional bacteria in flocs and granules. The dominant bacteria in granules was *Candidatus_Competibacter*, while *Azoarcus* and *Thauera* were enriched in flocs. Furthermore, proteomic analysis suggested that the metabolic pathways associated with glycogen synthesis and denitrification were more active in granules than that in flocs, while the differentially expressed proteins (DEPs) related to aromatic compounds and xenobiotics metabolism in flocs were more abundant than that in granules. Species annotation revealed that the DEPs between granules and flocs were mainly contributed by *Candidatus_Competibacter* and *Thauera*. The in-depth insight into co-existence of flocs and granules provides important reference for the design, optimization and operation of AGS technology in further applications.

1. Introduction

Aerobic granular sludge (AGS) technology has drawn a great deal of attention all over the world in favor of its unique advantages such as excellent settleability, dense structure, high biomass retention and tolerance to toxicity [1–3]. To date, over 80 full-scale AGS system have been successfully applied and proved a great reduction of energy consumption, land area and operational costs compared with conventional activated sludge system [4–6].

In AGS system, flocs were typical and common components. According to previous studies, a certain amount of flocs coexisted in AGS system after the establishment of stable sludge settleability and nutrient removal, varying in the range of 3–20 % under different operational parameters [7–11]. Granules, with better settleability, are more likely to settle in the bottom of sludge bed, while flocs are always located on upper layer during non-aeration period. Such non-homogeneous distribution in height led to granules experiencing higher influent substrate concentration than flocs under plug-flow feeding condition, resulting in different biological niches [12]. In addition, granules possessed greater mass diffusion limitation than flocs due to their large size and compact structure [13], which resulted in the formation of different redox microenvironments (aerobic/anoxic/anaerobic) within granules, allowing simultaneous removal of chemical oxygen demand (COD), nitrogen and phosphorus to take place in a single reactor [14–16]. In flocs, on the other hand, loose structure and small size led to less diffusion limitation, creating fully aerobic microenvironment [17,18]. These distinctions may result in different functions of flocs and granules in co-existence system. However, the different behaviors between flocs and granules in co-existence system have not been investigated in detail yet.

Recently, the floc and granule co-existence system has attracted increasing research interest due to the fact of inevitable flocs in AGS system. According to previous studies, the presence of flocs, even at low concentration, had profound implications for reactor performance. Liu et al. [19] studied the impact of coexisting flocs in AGS system on nitrous oxide production and suggested that the presence of flocs decreased nitrous oxide (N_2O) production due to less oxygen transfer limitation within flocs. In addition to nitrogen transformation, flocs have been proved to have a competitive advantage in capturing particulate

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substrates compared with granules in co-existence system. Layer et al. [9] reported that particulate substrates preferentially and rapidly attached to flocs in co-existence system treating municipal wastewater. Nevertheless, most of the studies on co-existence of flocs and granules are conducted and discussed from macro-scale perspective, while the in-depth insight into the co-existence of flocs and granules at molecular level is still elusive.

Metaproteomics provides intracellular transcription information capable of reflecting the protein expression and metabolism pathway in microorganisms directly, which has emerged as a promising tool to acquire information at protein level and been successfully applied in the field of biological wastewater treatment such as understanding contaminant biodegradation mechanisms, functional protein or enzyme identification, etc [20]. Yan et al. [21] used proteomics to investigate the role of conducive materials in anaerobic digestion system and unveiled the reasons behind the effects on methanogenic performance at protein level. Azizan et al. [22] applied proteomics to explore and reveal the molecular mechanisms of low ammonium removal during wastewater treatment process. As such, it appears practically possible to employ proteomics to gain deeper insight into the co-existence of flocs and granules on protein level. Meanwhile, this may provide a molecular explanation for the potential impacts of floc presence on AGS system. To the best of our knowledge, this is the first time to apply proteomics in the study on floc and granule co-existence system, which can be a significant complement to molecular studies on AGS technology.

In this study, to gain deeper insight into the co-existence of flocs and granules, we (i) investigated the floc and granule co-existence system in terms of sludge properties and nutrient removal, (ii) compared the microbial community between the flocs and granules in co-existence system, (iii) applied label-free quantitative technique to further analyze the different behaviors between flocs and granules in co-existence system on proteomic level. This work greatly contributes to an in-depth understanding of the floc and granule co-existence system and provides a basis for the design, optimization and operation of AGS technology in further applications.

2. Materials and methods

2.1. Reactor operation

In this study, a sequencing batch reactor (SBR) with diameter of 5 cm and height of 60 cm was used. The operational cycle of reactor was 4 h, comprising 20-min feeding, 40-min non aeration, 165–170 min aeration, 5–10 min settling and 5-min discharging. Sludge retention time (SRT) was approximately 23 ± 2 days. SRT was calculated according to the equation provided in the Supplementary Materials (Text S1). Approximately 1.5 L/min air was introduced from reactor bottom and effluent was discharged at the middle of reactor with a volumetric exchange ratio of 50 %, resulting in hydraulic retention time (HRT) of 8 h.

2.2. Inoculum and feeding

4.5 g/L mature and stable granules with mean size of 3 mm and SVI_{10} of 40 mL/g were used as seed sludge in this study, whose culture method was described in previous study [23]. Organic loading rate (OLR) was controlled at 4.5 kg COD m^{-3} d^{-1} with carbon source of CH_3COONa. Besides that, the synthetic media fed for the reactor also included 60 mg/L NH_4^+-N, 10 mg/L total phosphorus (TP), 20 mg/L Ca^{2+}, 20 mg/L Fe^{2+}, 20 mg/L Mg^{2+}, and 1 mL/L microelements. The composition of trace element solution was described in Table S1.

2.3. Microbial community analysis

The flocs (denoted as Sf) and granules (Sg) samples in co-existence system were collected on day 70 and stored at −80 ℃ separately for microbial community analysis. The bacterial DNA from both Sf and Sg samples was extracted using E.Z.N.A.® soil DNA Kit according to manufacture protocol (Omega Bio-tek, Norcross, GA, U.S.). 338F and 806R were used to amplify the V3-V4 region of the 16S rRNA gene by polymerase chain reaction (PCR) system.

2.4. Label-Free quantitative technique proteomics

Proteins extracted from sludge samples (Sf, Sg) were analyzed using label-free quantitative technique proteomics. Three biological replicates were used for Sf and Sg. The procedure of label-free quantitative proteomics mainly included 7 steps: (i) protein extraction, (ii) protein quantification by Pierce™ BCA Protein Assay Kit, (iii) sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis (PAGE), (iv) reduction/alkylation, (v) protease digestion using trypsin, (vi) peptides quantification and (vii) LC-MS/MS analyses. The details for each step were described in the Supplementary Materials (Text S2).

2.5. Other analytical methods

Influent and effluent COD, NH_4^+-N, NO_2^--N and NO_3^--N concentrations, as well as sludge volume index (SVI) and mixed liquor suspended solids (MLSS) were determined by standard methods [24]. Average particle size and floc fraction (%) were measured using sieving methods [25].

3. Results and discussion

3.1. Characterization of floc and granule Co-existence system

Fig. 1 shows the variation of slduge characteristics during the operation of reactor. As illustrated in Fig. 1a, biomass concentration was gradually incerased and stabilized at approximatedly 7 g/L on day 40. A rapid increase of SVI_{10} from 40 mL/g to 55 mL/g and slight decrease of average particle size from 3 mm to 2.4 mm (day 0–10) were observed, followed by stabilizing at approximately 49 mL/g and 2.8 mm on day 40, respectively (Fig. 1b, c). Particle size distribution indicated that the fraction of flocs (<0.2 mm) exhibited an increase from approximately 1 % to 23 % during the initial start-up of reactor (day 0–10, Fig. 1d). Meanwhile, no particle breakage was observed, which was indicated by the increase in the biomass concentration of large-sized granules (>2 mm) from 3.5 ± 0.3 g/L to 3.8 ± 0.2 g/L on 0–10 days (Fig. 1a, d). With the extension of operational time, the proportion of flocs was gradually decreased and stabilized at 9–11 %. The fraction of large-sized granules was stabilized at 70–72 % on day 40 (Fig. 1d). Similar results were obtained in our previous study, where the fraction of flocs was stabilized at 3–6 % in AGS system under low OLR [11]. Besides that, a number of prior studies suggested the existence of a certain amount of flocs in AGS system, varying in the range of 3–20 % with changes of operational parameters [7,9,25]. Furthermore, the co-existence system where 16–23 % flocs were involved exhibited poorer settling performance than that obtained for 9–11 % flocs (Fig. 1c, d). Notably, the co-existence system containing 9–11 % flocs was stable in terms of sludge properties. It was known that a higher fraction of flocs weaken the settling performance of AGS system due to its fluffy and loose structure [26].

3.2. Nutrient removal in floc and granule Co-existence system

In addition to sludge characteristics, nutrient removal in floc and granule co-existence system was also investigated during the entire operation (Fig. 2). As shown in Fig. 2a, the floc and granule co-existence system exhibited excellent COD removal. COD removal efficiency was gradually increased and stabilized at 96–97 % along with reactor operation, which should be ascribed to the increased biomass concentration (Fig. 1a). Cyclic test of reactor performance showed the influent COD was mainly removed during anaerobic period, indicated by the rapid decrease of COD concentration from 760 mg/L to 198 mg/L during

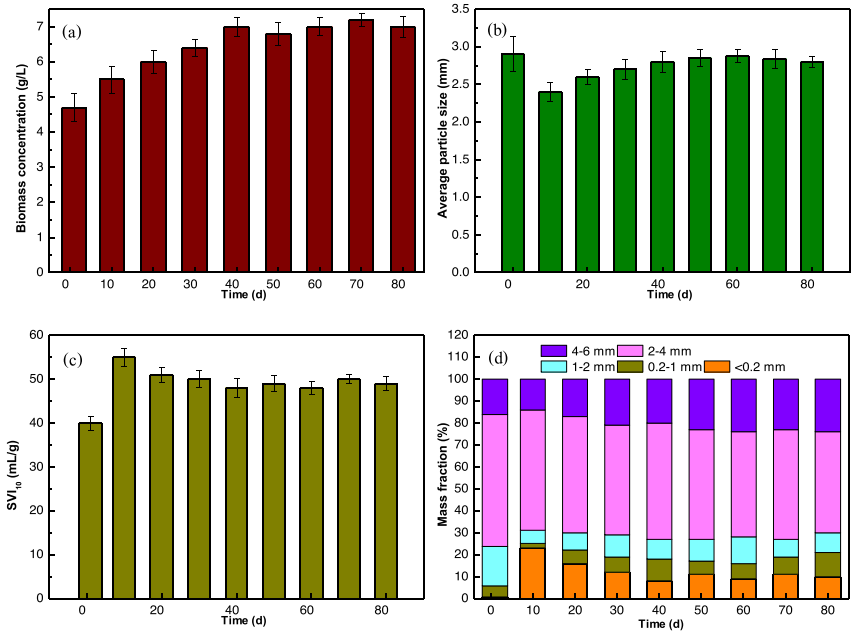

Fig. 1. Sludge properties during reactor operation: (a) biomass concentration, (b) average particle size, (c) sludge settleability (SVI_{10}) and (d) sludge size distribution.

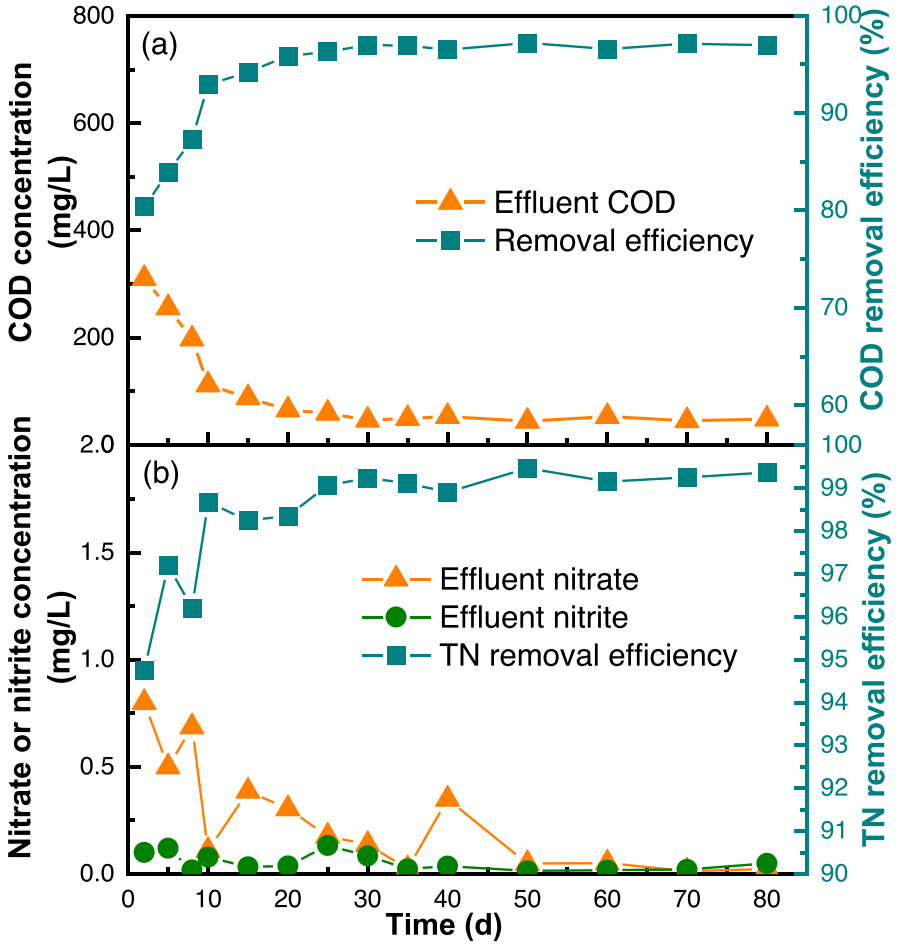

Fig. 2. Nutrient removal during reactor operation: (a) COD and (b) nitrogen removal.

0–60 min (Fig. S2). In general, anaerobic COD removal was typically caused by exogenous denitrification and intracellular polymer storage [27]. Considering the negligible nitrate/nitrite (NO_x-N) left in previous cycle and corresponding low NO_x-N concentration in anaerobic period (Fig. S2), it was reasonable to infer that the rapid anaerobic COD removal was a microbial polymer storage process. It is well-established that easy biodegradable substrates were able to be taken up and converted into storage polymers by microorganisms such as glycogen accumulating organisms (GAO) under anaerobic conditions, and thus facilitating COD removal. Besides that, the stored polymers were able to provide electron donors for denitrification in the subsequent aerobic period, which was beneficial for nitrogen removal [28,29]. At the end of anaerobic period, there was approximately 200 mg/L COD left (Fig. S2), suggesting incomplete internal storage of substrates under anaerobic condition and carbon available in aerobic period. This should be responsible for the development of flocs in co-existence system (Fig. 1d). Influent substrates were able to flow into aerobic phase when they were not totally taken up and converted by microorganisms during anaerobic period, which favored the growth of faster-growing ordinary heterotrophic organisms (OHO) [30]. Rapid growth of OHO was reported to reduce the aggregation potential of biomass, and thus facilitating the formation and presence of flocs in AGS system [6,29,31–33]. With the extension of operational time, the COD leaked into aerobic period was quickly removed in the first 20 min (Fig. S2). This indicated that the microorganisms were subject to long famine indicated by the low COD concentration (<50 mg/L) during 80–240 min (Fig. S2). In addition, low effluent NO_3^--N, NO_2^--N concentration (<0.5 mg/L) and high nitrogen removal efficiency (98–99 %) were observed, which suggested excellent nitrogen removal in the floc and granule co-existence system (Fig. 2b). Cyclic test of reactor performance showed that there was a slight decrease of NH_4^+-N (30–25 mg/L) concentrations during anaerobic period (0–60 min, Fig. S2), which should be attributed to microbial assimilation. It was known that microbes were able to consume NH_4^+-N to synthesize cellular substances under feast condition [34]. Notably, NH_4^+-N concentration was sharply decreased from 25 mg/L to below 0.5 mg/L, accompanied by a slight increase of NO_3^--N concentration from 0.4 mg/L to 4.3 mg/L in the subsequent aerobic period (Fig. S2). With the extension of operational time, NO_3^--N concentration gradually recovered to a low level again (0.2 mg/L, Fig. S2).

3.3. Bacterial community between flocs and granules in Co-existence system

In order to further understand the co-existence of flocs and granules, we next analyzed the microbial community in the flocs (denoted as Sf) and granules (Sg) separately. Fig. 3a shows the microbial community of Sf and Sg at phylum level. Clearly visible is that both Sf and Sg samples were dominated by *Proteobacteria* and *Bacteroidota*, with *Proteobacteria*

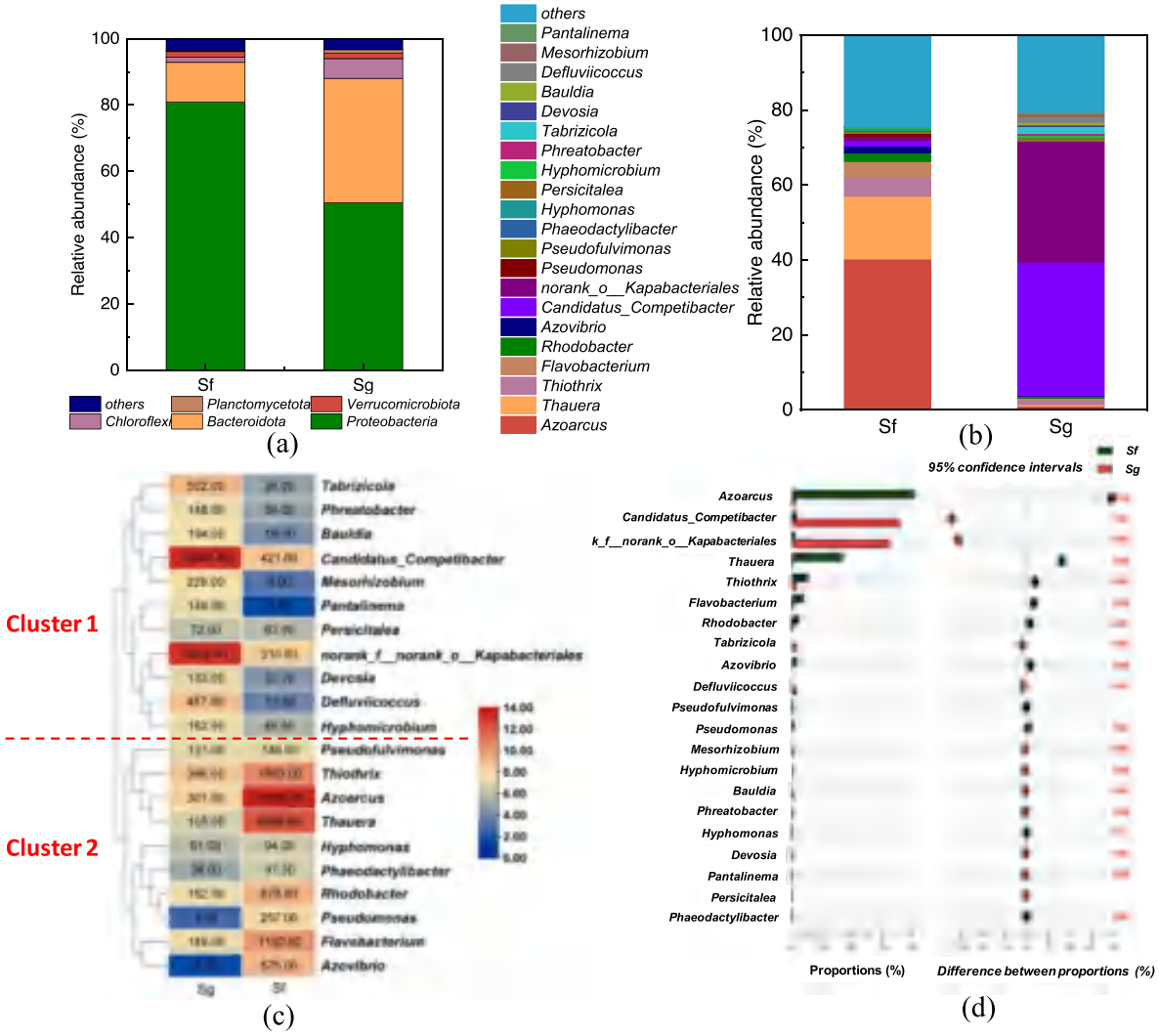

Fig. 3. Microbial community analysis of the flocs and granules in co-existence system at (a) phylum and (b-d) genus level.

being at the highest abundance followed by *Bacteroidota*. Specifically, the relative abundances of *Proteobacteria* in flocs and granules were 80.9 % and 50.5 %, respectively. And the relative abundances of *Bacteroidota* phylum in flocs and granules were 11.9 % and 37.5 %, respectively. This suggested that flocs contained more *Proteobacteria* and less *Bacteroidota* than granules. Similar results were found by Liu et al. [35] who analyzed bacterial populations from a wastewater treatment plant and found much more abundant *Proteobacteria* in flocs (51.9 %) than that in granules (28.2 %).

Bacterial communities in Sf and Sg at genus level are represented in Fig. 3b-d. As shown in Fig. 3c, microorganisms in cluster 1 were enriched in granules, while those in cluster 2 were predominant in flocs. In Sg, *Candidatus_Competibacter* was the most abundant bacteria, accounting for 35.7 %, much higher than 1.4 % in Sf (Fig. 3). As a kind of well-known glycogen-accumulating organisms (GAOs), *Candidatus_Competibacter* is able to store easily biodegradable substrates as intracellular polymers during anaerobic period [18]. Besides that, the pre-stored polymers can be utilized as electron donors to reduce NO_x-N during famine period [36,37]. In addition, the microorganism that stored substrates as intracellular polymers first would grow at a slow rate compared with the heterotrophic organisms utilizing COD without prior storage, reducing the outgrowth velocity of granule surface and favoring granule stability [33,38]. As such, the high abundance of *Candidatus_Competibacter* in granules should be responsible for the COD removal in anaerobic period and stable granules in co-existence system (Figs. 1, 2). On the contrary, *Azoarcus* and *Thauera* emerged as the dominant bacteria in Sf, accounting for 40.2 % and 16.8 %, respectively (Fig. 3). *Azoarcus* and *Thauera* were assumed to be main organisms responsible for polymer storage with denitrification capacity [39–44]. This revealed the dominance of ordinary heterotrophic organisms involved in polymer storage and denitrification in flocs. As such, the function of flocs could be removing COD and nitrogen through storing polymers and denitrification by functional bacteria such as *Azoarcus* and *Thauera*. The selection of polymer storage organisms in co-existence system (granules: *Candidatus_Competibacter*, flocs: *Azoarcus* and *Thauera*) should be attirbuted to the feast/famine regime (Fig. S2) [29]. Furthermore, different selection of functional bacteria in flocs and granules was probably due to long retention of granules. It was known that flocs was more likely to be washed out and wasted, exhibiting shorter average retention time compared with granules in the same reactor [45]. The long retention of granules was beneficial for the enrichment of slow-growing organisms. In addition, a remarkable fraction of genus *Thiothrix* (5.3 %), a reorganized filamentous miroorganism, was observed in Sf, which was four times more than that of granules (1.3 %) (Fig. 3). A function frequently attributed to filaments was their deteriorating effect on sludge settling performance. The abundant *Thiothrix* in flocs is consistent with the deterioration of reactor settleability after the appearance of flocs.

3.4. Comparative protein expression between flocs and granules in Co-existence system

In order to further unveil the different behaviors between flocs and granules at molecular level, we next applied label-free quantitative technique proteomics to analyze the differentially expressed proteins (DEPs) between the flocs and granules in co-existence system. The selection criterion for DEPs was $p < 0.05$ and ($FC < 0.5$ or $FC > 2$). Fig. 4a-c shows the classification of DEPs based on Gene Ontology (GO) secondary level, providing an overview of the DEPs between floc and granule samples. GO is a description of gene function based on a comprehensive database. According to GO annotation, the DEPs between flocs and granules were classified into molecular function, cellular component, and biological process [46,47].

Molecular function analyses indicated that the DEPs were mainly associated with catalytic activity, binding, transporter activity, structural molecule activity, translation regulator activity, transcription regulator activity, antioxidant activity, small molecule sensor activity, and molecular transducer activity (Fig. 4a). Of these, catalytic activity and binding proteins were the most abundant, accounting for 45 % and 38 % respectively (Fig. S3), in line with the results obtained by Zhang et al. [48]. Further analysis indicated that 221 and 223 hydrolase activity proteins were up- and down-regulated between Sg and Sf (Sf: control group, Sg vs Sf), respectively (Fig. S4). The expression of hydrolase activity proteins in both flocs and granules was likely due to the presence of starvation period (80–240 min, Fig. S2). It has been documented that microorganisms experiencing starvation hydrolyzed macromolecules for growth and maintenance [49]. In addition, 302 and 321 anion binding proteins were up- and down-regulated in Sg vs Sf, respectively (Fig. S4), suggesting more active expression of anion binding proteins in flocs than that of granules. Anion binding proteins

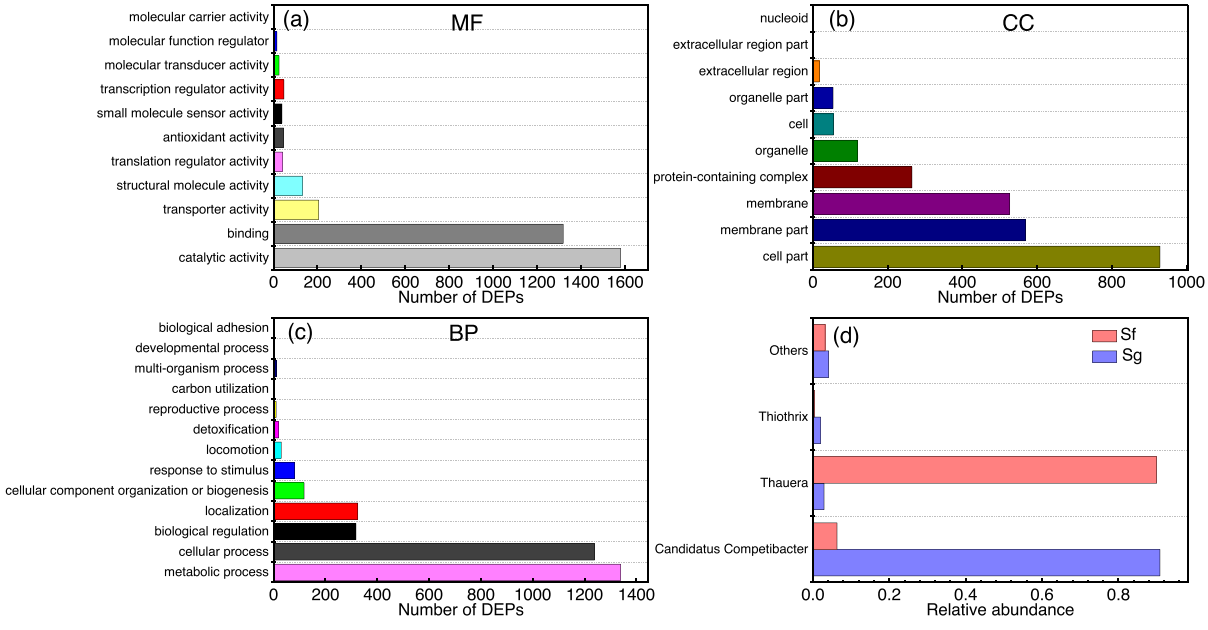

Fig. 4. GO annotation (a-c) and species annotation (d) of the differentially expressed proteins between flocs and granules in co-existence system.

were reported to negatively affect cell hydrophobicity because of the presence of free amino groups [50–52], and thus adversely affected microbial aggregation capacity [2,53,54]. The more anion binding proteins in flocs than granules supported the fact of poor microbial aggregation capacity in flocs than that in granules. Cellular component analysis revealed that the DEPs between flocs and granules mainly located at cell part, membrane part, membrane, protein-containing complex, organelle, organelle part, cell, and extracellular region (Fig. 4b). Furthermore, most of the DEPs were located in cell part, membrane part, and membrane, accounting for 37 %, 22 %, and 20 % respectively (Fig. S3). In terms of biological processes, the DEPs mainly participated in metabolic process, cellular process, localization, biological regulation, cellular component organization or biogenesis, response to stimulus, locomotion, and detoxification (Fig. 4c). Of these, the DEPs participated in metabolic process and cellular process were the most abundant, accounting for 39 % and 36 %, respectively (Fig. S3). Based on these results, most of the DEPs between flocs and granules within co-existence system were associated with the function of catalytic activity and binding, locating in cell and membrane part, and participating in metabolic and cellular process.

To investigate the contributors to these DEPs between flocs and granules, we next compared the DEP sequences with NR database to analyze the origin of the DEPs between flocs and granules. Fig. 4d shows the distribution of microbial community at genus level calculated according to the corresponding protein abundance. It demonstrated that *Candidatus Competibacter* and *Thauera* were the main contributors to the DEPs between flocs and granules in co-existence system. Specifically, 90.9 % DEPs in granules were originated from *Candidatus Competibacter*, while 90.0 % DEPs in flocs were produced by *Thauera* (Fig. 4d).

3.5. Comparative metabolic pathways between flocs and granules in Co-existence system

To further elucidate different behaviors of flocs and granules in co-existence system at molecular level, it is necessary to perform the enrichment analysis of metabolic pathways in which these DEPs are involved. As illustrated in Fig. 5, the DEPs between flocs and granules were identified to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. It was found that 31 pathways were sifnificantly enriched ($p < 0.05$, Fig. 5a). Among the enriched pathways, 9 pathways were shared by Sg and Sf, involving in carbon metabolism (ko01200), biosynthesis of amino acids (ko01230), glyoxylate and dicarboxylate metabolism (ko00630), glycolysis/gluconeogenesis (ko00010), nitrogen metabolism (ko00910), methane metabolism (ko00680), RNA degradation (ko03018), benzoate degradation (ko00362) and degradation of aromatic compounds (ko01220) (Fig. 5a, b). In most of the shared metabolic pathways (7/9), the up-regulated proteins were more than down-regulated proteins (Fig. 5b, c), indicating that the 7 metabolic pathways were more active in granules than that in flocs. In addition, 9 pathways were unique in granules (ko00620, ko00500, ko00270, ko00051, ko00050, ko02025, ko02026, ko02040, ko02020), and 13 pathways were enriched only in flocs (ko00720, ko01210, ko00220, ko00622, ko00430, ko00364, ko00540, ko00633, ko02010, ko03060, ko04714, ko04212, ko04216, Fig. 6).

Carbohydrate Metabolism. In the present study, carbohydrate metabolism exhibited differently between granules and flocs. Specifically, gluconeogenesis, starch and sucrose metabolism, fructose and mannose metabolism, glyoxylate and dicarboxylate metabolism, pyruvate metabolism were more active in granules than flocs. Of these, gluconeogenesis, starch and sucrose metabolism pathways were only remarkably up-regulated in Sg vs Sf (Fig. 6). As shown in Fig. 7 and Table S2, the expression of UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) and glucose-1-phosphate adenylyltransferase (EC 2.7.7.27) was up-regulated in Sg vs Sf (Fig. 7). UTP-glucose-1-phosphate uridylyltransferase and glucose-1-phosphate adenylyltransferase are the key enzymes that catalyze the conversion of α-D-glucose-1-phosphate into UDP-glucose (UDPG) and α-D-glucose-1-phosphate into ADP-glucose (ADPG), respectively [55]. Both UDPG and ADPG are able to be utilized as glycosyl donors for the elongation of α-1,4-glucosidic chain during glycogen synthesis process [56,57]. However, glycogen

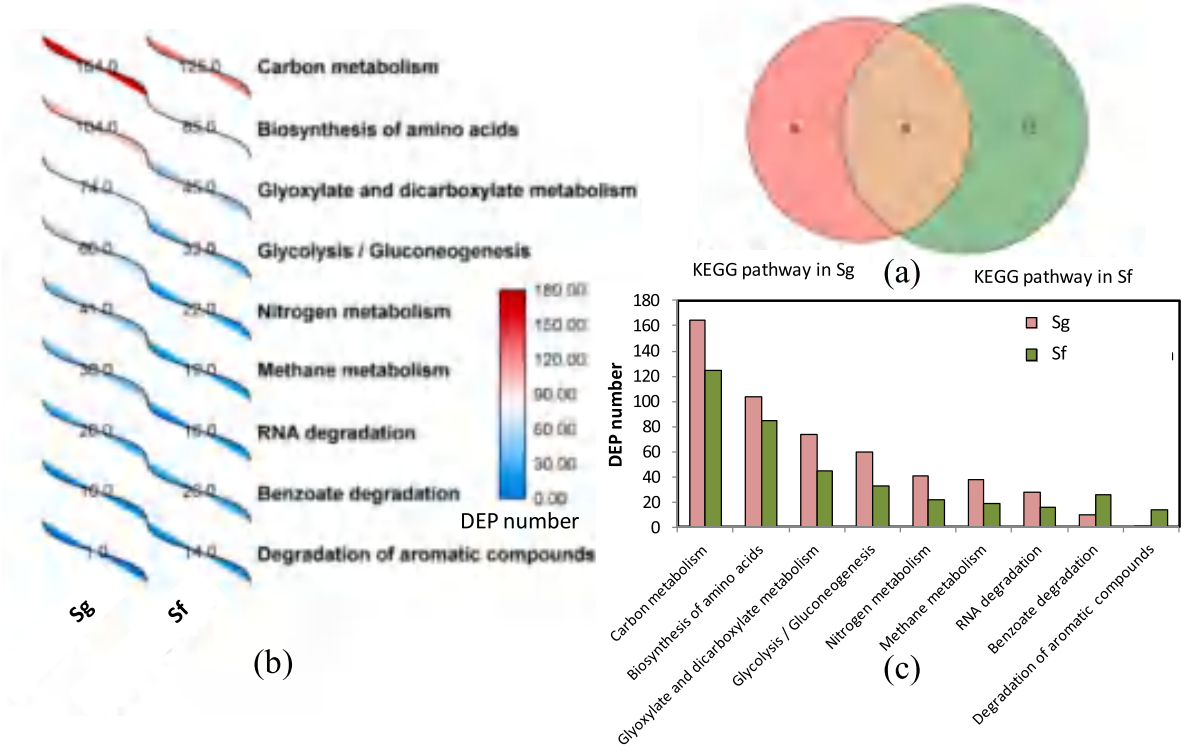

Fig. 5. KEGG enrichment analyses of the differentially expressed proteins (DEPs) between flocs and granules in co-existence system: (a) Venn diagram illustrating the classification of enriched KEGG pathways; (b) 9 shared KEGG pathways between flocs and granules; (c) number of DEPs involved in the 9 shared KEGG pathways.

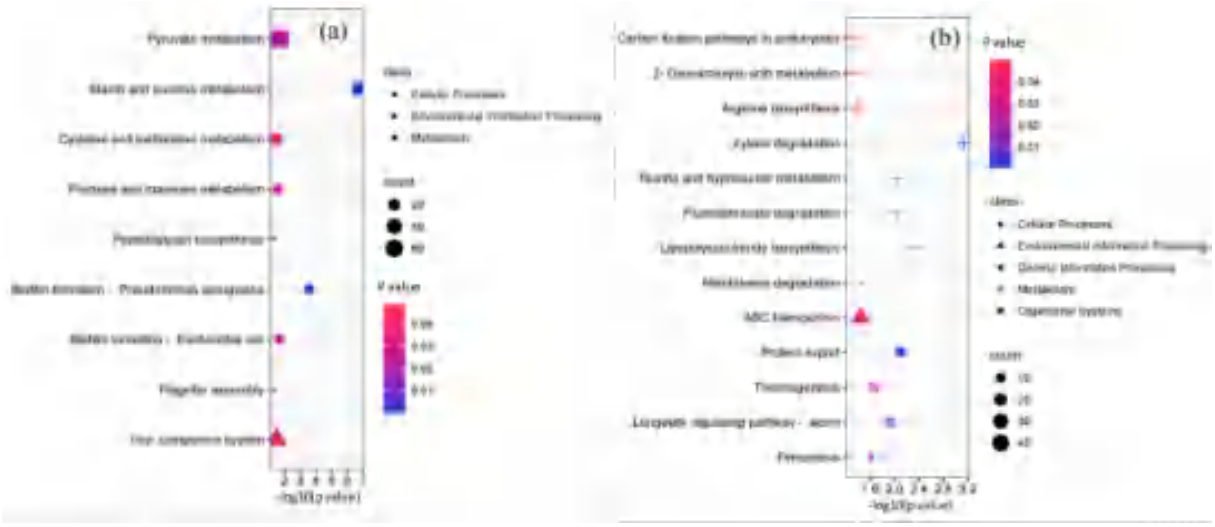

Fig. 6. Unique KEGG pathways enriched in granules (a) and flocs (b).

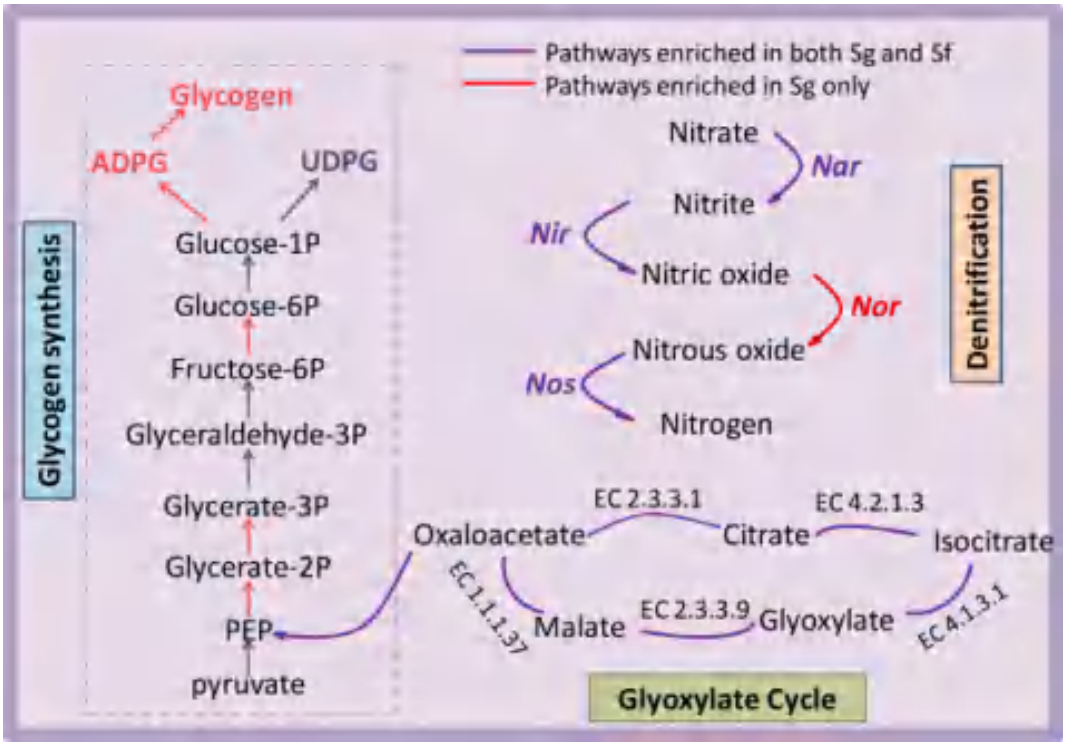

Fig. 7. Network of the enriched KEGG pathways in co-existence system of granules and flocs.

synthases (EC 2.4.1.242, EC 2.4.1.11), key enzymes catalyzing UDPG to glycogen, were not identified in the DEPs between Sg and Sf. Notably, EC 2.4.1.21, a kind of recognized glycogen synthase similar with EC 2.4.1.11, was up-regulated. These results suggested that ADPG could be the main glycosyl donors for glycogen synthesis in granules because of the absence of EC 2.4.1.242/EC 2.4.1.11 and up-regulation of EC 2.4.1.21 (Fig. 7, Table S2). According to species annotation, we found the up-regulated enzymes catalyzing glycogen synthesis were mainly governed by genus *Candidatus_Competibacter* (Table S2). It is well acknowledged that *Candidatus_Competibacter* is capable of hydrolyzing stored polymers accompanied by glycogen synthesis during starvation period [29]. However, there are to our knowledge fewer available data describing the complete pathway of glycogen synthesis in floc and granule co-existence system by applying proteomics. Besides that, all the key enzymes involved in glyoxylate cycle (EC 2.3.3.1, EC 4.2.1.3, EC 4.1.3.1, EC 2.3.3.9, EC 1.1.1.37) were significantly up-regulated between granules and flocs (Table S2), implying that the pathway of glyoxylate cycle was more active in granules than flocs. Similar result was obtained by Wilmes et al. [58], who found high expression of the proteins involved in glyoxylate cycle in EBPR system and suggested that glyoxylate shunt was critical for balancing substrate utilization and glycogen synthesis during aerobic period. These results confirmed that glycogen synthesis through glyoxylate cycle and gluconeogenesis was the active carbohydrate metabolism in granules. Similarly, key enzymes involved in gluconeogenesis and glyoxylate cycle were also enriched in flocs, which were mainly originated from genus *Thauera* based on species annotation (Fig. 7, Table S3). Notably, the DEPs involved in UDPG synthesis was identified in flocs, while no glycogen synthase was

observed in flocs (Table S3).

Nitrogen Metabolism. As shown in Fig. 5, the DEPs associated with nitrogen metabolism were enriched in both granules and flocs. 41 DEPs involved in nitrogen metabolism were identified in granules, which were more than 22 DEPs in flocs (Fig. 5b, c). Denitrification reducing NO_3^--N to gaseous nitrogen (N_2) is an important process in nitrogen metabolism. The key enzymes catalyzing conversion of NO_3^--N to N_2 mainly include nitrate reductase (*Nar*, *Nap*), nitrite reductase (*Nir*), nitric oxide reductase (*Nor*) and nitrous oxide reductase (*Nos*). In the present study, the expression of *Nar*, *Nap*, *Nir*, *Nor* and *Nos* behaved differently between granules and flocs in co-existence system (Fig. 7, Table S4). 24 DEPs were assigned to denitrification in granules, which was more than 8 DEPs in flocs (Table S4). Species annotation analysis demonstrated that the DEPs related to denitrification process in granules were originated from abundant denitrifiers such as *Candidatus_Competibacter*, *Thauera*, *Amaricoccus*, *Flavobacterium*, etc (Table S4). On the contrary, less denitrifiers (*Thauera* and *Amaricoccus*) worked in flocs (Table S4). Both abundant DEPs and denitrifiers in granules than flocs supported previous reports of effective denitrification in aerobic granular sludge compared with activated sludge [59]. Microorganism located deeper within granules experienced anoxic/anaerobic condition due to mass transfer limitation, which was beneficial for the growth of denitrifiers and enabled denitrification enzymes expression. On the contrary, flocs can be penetrated by oxygen due to their less diffusion limitation, which was adverse for the growth and behaviors of denitrifiers in flocs. Nevertheless, 8 DEPs were assigned to denitrification process in Sf, and most of them (88 %) were originated from *Thauera* (Table S4). It is known that *Thauera* is capable of utilizing NO_3^--N as electron acceptor under aerobic condition [60]. Moreover, *Thauera* can convert volatile fatty acid (VFA) to intercellular polymer substances [44,61]. The stored polymers could be utilized as electron donors when *Thauera* experienced famine period [62,63]. These results may well explain the identification of DEPs associated with denitrification in flocs.

Other Relevant KEGG Pathway. As shown in Fig. 6, two-component system and ATP-binding cassette (ABC) transporters were enriched in granules and flocs, respectively. Both two-component system and ABC transporters were classified as environmental information processing. In general, bacterial organisms need to regulate their metabolism in response to environmental stress, such as oligotrophy. Two-component system is a basic mechanism utilized by bacterial microorganisms to regulate their physiological metabolism to adapt environment [64]. In addition, ABC transporters are responsible for the translocation (import or export) of intermediate metabolite across cytoplasmic membranes, such as amino acids, sugars, ions and lipids, etc. As shown in Table S5, 48 % of the DEPs related to ABC transporter were classified as *Liv* (K01995). Studies have revealed that the pathway ABC transporters associated with *Liv* may help microorganisms adapt environment [65,66]. Interestingly, metabolic pathways related to aromatic compounds degradation (ko01220) and xenobiotics metabolism such as benzoate degradation (ko00362), xylene degradation (ko00622) and fluorobenzoate degradation (ko00364) were more active in flocs than granules (Figs. 5-6). Species annotation analysis suggested that most of the DEPs involved in aromatic compounds and xenobiotics metabolism (86 %) were originated from genus *Thauera* (Table S5). Previous studies have reported that genus *Thauera* was capable of utilizing benzoic acid, phenol and other aromatic compounds as carbon source for denitrification process [67,68].

4. Conclusions

A co-existence system with approximately 9–11 % flocs and excellent nutrient removal was obtained. Although the flocs and granules were in the same reactor, different microbial community and metabolic pathways were observed between flocs and granules. Glycogen synthesis and denitrification process were more active in granules, while the DEPs involved in aromatic compounds and xenobiotics biodegradation were more abundant in flocs. Species annotation demonstrated that *Candidatus_Competibacter* and *Thauera* were the main contributors to the DEPs between granules and flocs in co-existence system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2022.139011.

References

[1] S.S. Adav, D.-J. Lee, K.-Y. Show, J.-H. Tay, Aerobic granular sludge: Recent advances, Biotechnol. Adv. 26 (2008) 411–423, https://doi.org/10.1016/j.biotechadv.2008.05.002.
[2] S. Wang, X. Ma, Y. Wang, G. Du, J.-H. Tay, J. Li, Piggery wastewater treatment by aerobic granular sludge: Granulation process and antibiotics and antibiotic-resistant bacteria removal and transport, Bioresour. Technol. 273 (2019) 350–357, https://doi.org/10.1016/j.biortech.2018.11.023.
[3] Y. Jiang, X. Shi, H.Y. Ng, Aerobic granular sludge systems for treating hypersaline pharmaceutical wastewater: Start-up, long-term performances and metabolic function, J. Hazard. Mater. 412 (2021), 125229, https://doi.org/10.1016/j.jhazmat.2021.125229.
[4] L. Strubbe, M. Pennewaerde, J.E. Baeten, E.I.P. Volcke, Continuous aerobic granular sludge plants: Better settling versus diffusion limitation, Chem. Eng. J. 428 (2022), 131427, https://doi.org/10.1016/j.cej.2021.131427.
[5] M. Pronk, M.K. de Kreuk, B. de Bruin, P. Kamminga, R. Kleerebezem, M.C.M. van Loosdrecht, Full scale performance of the aerobic granular sludge process for sewage treatment, Water Res. 84 (2015) 207–217, https://doi.org/10.1016/j.watres.2015.07.011.
[6] M. Pronk, A. Giesen, A. Thompson, S. Robertson, M. van Loosdrecht, Aerobic granular biomass technology: advancements in design, applications and further developments, Water Pract. Technol. 12 (2017) 987–996, https://doi.org/10.2166/wpt.2017.101.
[7] N. Hubaux, G. Wells, E. Morgenroth, Impact of coexistence of flocs and biofilm on performance of combined nitritation-anammox granular sludge reactors, Water Res. 68 (2015) 127–139, https://doi.org/10.1016/j.watres.2014.09.036.
[8] Y. Shi, G. Wells, E. Morgenroth, Microbial activity balance in size fractionated suspended growth biomass from full-scale sidestream combined nitritation-anammox reactors, Bioresour. Technol. 218 (2016) 38–45, https://doi.org/10.1016/j.biortech.2016.06.041.
[9] M. Layer, K. Bock, F. Ranzinger, H. Horn, E. Morgenroth, N. Derlon, Particulate substrate retention in plug-flow and fully-mixed conditions during operation of aerobic granular sludge systems, Water Res. X 9 (2020), 100075, https://doi.org/10.1016/j.wroa.2020.100075.
[10] E.J.H. van Dijk, M. Pronk, M.C.M. van Loosdrecht, Controlling effluent suspended solids in the aerobic granular sludge process, Water Res. 147 (2018) 50–59, https://doi.org/10.1016/j.watres.2018.09.052.
[11] M. Geng, S. You, H. Guo, F. Ma, X. Xiao, J. Zhang, X. Ma, Response of aerobic granular sludge to loading shock: Performance and proteomic study, Chem. Eng. J. 444 (2022), 136458, https://doi.org/10.1016/j.cej.2022.136458.
[12] E.J.H. van Dijk, V.A. Haaksman, M.C.M. van Loosdrecht, M. Pronk, On the mechanisms for aerobic granulation - model based evaluation, Water Res. 216 (2022), 118365, https://doi.org/10.1016/j.watres.2022.118365.
[13] F. Fang, L.-L. Qiao, B.-J. Ni, J.-S. Cao, H.-Q. Yu, Quantitative evaluation on the characteristics of activated sludge granules and flocs using a fuzzy entropy-based approach, Sci. Rep. 7 (2017) 42910, https://doi.org/10.1038/srep42910.
[14] M.K.D. Kreuk, J.J. Heijnen, M.C.M.V. Loosdrecht, Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge, Biotechnol. Bioeng. 90 (2005) 761–769, https://doi.org/10.1002/bit.20470.

[15] Q. He, W. Zhang, S. Zhang, H. Wang, Enhanced nitrogen removal in an aerobic granular sequencing batch reactor performing simultaneous nitrification, endogenous denitrification and phosphorus removal with low superficial gas velocity, Chem. Eng. J. 326 (2017) 1223–1231, https://doi.org/10.1016/j.cej.2017.06.071.
[16] L. Berg, C.M. Kirkland, J.D. Seymour, S.L. Codd, M.C.M. Loosdrecht, M.K. Kreuk, Heterogeneous diffusion in aerobic granular sludge, Biotechnol. Bioeng. 117 (12) (2020) 3809–3819, https://doi.org/10.1002/bit.27522.
[17] B. Li, Y. Wang, X. Li, Z. Zhang, H. Wang, Y. Li, L. Wu, J. Li, Comparing the nitrogen removal performance and microbial communities of flocs-granules hybrid and granule-based CANON systems, Sci. Total Environ. 703 (2020), 134949, https://doi.org/10.1016/j.scitotenv.2019.134949.
[18] L. Zhang, M. Liu, S. Zhang, Y. Yang, Y. Peng, Integrated fixed-biofilm activated sludge reactor as a powerful tool to enrich anammox biofilm and granular sludge, Chemosphere 140 (2015) 114–118, https://doi.org/10.1016/j.chemosphere.2015.02.001.
[19] Y. Liu, H. Ngo, W. Guo, D. Wang, L. Peng, W. Wei, B. Ni, Impact of coexistence of sludge flocs on nitrous oxide production in a granule-based nitrification system: A model-based evaluation, Water Res. 170 (2020), 115312, https://doi.org/10.1016/j.watres.2019.115312.
[20] Shixia Chen, Lei Wang, Z. Han, Applications of metaproteomics in the study of wastewater biotreatment, Chinese J. Ecol. 25 (2014) 3056-3066, https://doi.org/10.13287/j.1001-9332.2014.0182.
[21] W. Yan, M. Mukherjee, Y. Zhou, Direct interspecies electron transfer (DIET) can be suppressed under ammonia-stressed condition – Reevaluate the role of conductive materials, Water Res. 183 (2020), 116094, https://doi.org/10.1016/j.watres.2020.116094.
[22] A. Azizan, F. Kaschani, H. Barinas, S. Blaskowski, M. Kaiser, M. Denecke, Using proteomics for an insight into the performance of activated sludge in a lab-scale WWTP, Int. Biodeterior. Biodegradation 149 (2020), 104934, https://doi.org/10.1016/j.ibiod.2020.104934.
[23] M. Geng, S. You, H. Guo, F. Ma, X. Xiao, J. Zhang, Impact of Fungal Pellets Dosage on Long-term Stability of Aerobic Granular Sludge, Bioresour. Technol. 332 (2021), 125106, https://doi.org/10.1016/j.biortech.2021.125106.
[24] APHA, Standard methods for the examination of water and wastewater, American Public Health Association, Washington DC, (2005).
[25] N. Derlon, J. Wagner, R.H.R. da Costa, E. Morgenroth, Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume, Water Res. 105 (2016) 341–350, https://doi.org/10.1016/j.watres.2016.09.007.
[26] A.M.P. Martins, Ö. Karahan, M.C.M. van Loosdrecht, Effect of polymeric substrate on sludge settleability, Water Res. 45 (2011) 263–273, https://doi.org/10.1016/j.watres.2010.07.055.
[27] Dongbo Liang, Wei Guo, Dongyue Li, Fan Ding, Peilin Li, Zhaoming Zheng, J. Li, Enhanced aerobic granulation for treating low-strength wastewater in an anaerobic-aerobic-anoxic sequencing batch reactor by selecting slow-growing organisms and adding carriers, Environ Res 205 (2022) 112547, Doi: 10.1016/j.envres.2021.112547.
[28] M. Pronk, B. Abbas, S.H.K. Al-zuhairy, R. Kraan, R. Kleerebezem, M.C.M. van Loosdrecht, Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge, Appl. Microbiol. Biotechnol 99 (2015) 5257–5268, https://doi.org/10.1007/s00253-014-6358-3.
[29] M.K. de Kreuk, M.C.M.v. Loosdrecht, Selection of slow growing organisms as a means for improving aerobic granular sludge stability, Water Sci. Technol. 49 (2004) 9–17, https://doi.org/10.2166/wst.2004.0792.
[30] M. Layer, A. Adler, E. Reynaert, A. Hernandez, M. Pagni, E. Morgenroth, C. Holliger, N. Derlon, Organic substrate diffusibility governs microbial community composition, nutrient removal performance and kinetics of granulation of aerobic granular sludge, Water Res. X 4 (2019) 100033, https://doi.org/10.1016/j.wroa.2019.100033.
[31] R.A. Hamza, O.T. Iorhemen, M.S. Zaghloul, J.H. Tay, Rapid formation and characterization of aerobic granules in pilot-scale sequential batch reactor for high-strength organic wastewater treatment, J Water Process Eng. 22 (2018) 27–33, https://doi.org/10.1016/j.jwpe.2018.01.002.
[32] L.B. Guimarães, J. Wagner, T.R.V. Akaboci, G.C. Daudt, P.H. Nielsen, M.C.M. van Loosdrecht, D.G. Weissbrodt, d.C. RHR, Elucidating performance failures in use of granular sludge for nutrient removal from domestic wastewater in a warm coastal climate region, Environ Technol 41 (2020) 1896–1911, https://doi.org/10.1080/09593330.2018.1551938.
[33] C. Fall, L.M. Barrón-Hernández, V.E. Gonzaga-Galeana, M.T. Olguín, Ordinary heterotrophic organisms with aerobic storage capacity provide stable aerobic granular sludge for C and N removal, J Environ Manage 308 (2022), 114662, https://doi.org/10.1016/j.jenvman.2022.114662.
[34] L. Qin, L. Liu, Z. Wang, W. Chen, D. Wei, Efficient resource recycling from liquid digestate by microalgae-yeast mixed culture and the assessment of key gene transcription related to nitrogen assimilation in microalgae, Bioresour. Technol. 264 (2018) 90–97, https://doi.org/10.1016/j.biortech.2018.05.061.
[35] J. Liu, J. Li, Y. Tao, B. Sellamuthu, R. Walsh, Analysis of bacterial, fungal and archaeal populations from a municipal wastewater treatment plant developing an innovative aerobic granular sludge process, Word J Microb Biot 33 (2016) 14, https://doi.org/10.1007/s11274-016-2179-0.
[36] J.P. Bassin, R. Kleerebezem, M. Dezotti, M.C.M.v. Loosdrecht, Simultaneous nitrogen and phosphate removal in aerobic granular sludge reactors operated at different temperatures, Water Res. 46 (2012) 3805–3816, https://doi.org/10.1016/j.watres.2012.04.015.
[37] M.I. Hossain, L. Cheng, R. Cord-Ruwisch, Sustained and enhanced anaerobic removal of COD and nitrogen in a zeolite amended glycogen accumulating organism dominated biofilm process, Sci. Total Environ. 807 (2022) 150602, https://doi.org/10.1016/j.scitotenv.2021.150602.
[38] V.A. Haaksman, M. Mirghorayshi, M.C.M. van Loosdrecht, M. Pronk, Impact of aerobic availability of readily biodegradable COD on morphological stability of aerobic granular sludge, Water Res. 187 (2020), 116402, https://doi.org/10.1016/j.watres.2020.116402.
[39] X. Zhou, Z. Zhang, X. Zhang, Y. Liu, A novel single-stage process integrating simultaneous COD oxidation, partial nitritation-denitritation and anammox (SCONDA) for treating ammonia-rich organic wastewater, Bioresour. Technol. 254 (2018) 50–55, https://doi.org/10.1016/j.biortech.2018.01.057.
[40] R. Pishgar, J.A. Dominic, Z. Sheng, J.H. Tay, Influence of operation mode and wastewater strength on aerobic granulation at pilot scale: Startup period, granular sludge characteristics, and effluent quality, Water Res. 160 (2019) 81–96, https://doi.org/10.1016/j.watres.2019.05.026.
[41] Q. He, L. Chen, S. Zhang, R. Chen, H. Wang, Hydrodynamic shear force shaped the microbial community and function in the aerobic granular sequencing batch reactors for low carbon to nitrogen (C/N) municipal wastewater treatment, Bioresour. Technol. 271 (2019) 48–58, https://doi.org/10.1016/j.biortech.2018.09.102.
[42] H. Wang, Q. Song, J. Wang, H. Zhang, Q. He, Z. Wei, J. Song, J. Zhou, L. Hui, Simultaneous nitrification, denitrification and phosphorus removal in an aerobic granular sludge sequencing batch reactor with high dissolved oxygen Effects of carbon to nitrogen ratios, Sci. Total Environ. 642 (2018) 1145–1152, https://doi.org/10.1016/j.scitotenv.2018.06.081.
[43] M. Wagner, A. Loy, Bacterial community composition and function in sewage treatment systems, Curr. Opin. Biotech 13 (2002) 218–227, https://doi.org/10.1016/S0958-1669(02)00315-4.
[44] A.S. Çiğgın, D. Orhon, S. Rossetti, M. Majone, Short-term and long-term effects on carbon storage of pulse feeding on acclimated or unacclimated activated sludge, Water Res. 45 (2011) 3119–3128, https://doi.org/10.1016/j.watres.2011.03.026.
[45] M. Ali, Z. Wang, K.W. Salam, A.R. Hari, M. Pronk, M.C.M. van Loosdrecht, P. E. Saikaly, Importance of Species Sorting and Immigration on the Bacterial Assembly of Different-Sized Aggregates in a Full-Scale Aerobic Granular Sludge Plant, Environ. Sci. Technol. 53 (2019) 8291–8301, https://doi.org/10.1021/acs.est.8b07303.
[46] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene ontology: tool for the unification of biology, Nat Genet. 25 (1) (2000) 25–29, https://doi.org/10.1038/75556.
[47] C.-Y. Xing, T.-F. Ma, J.-S. Guo, Y. Shen, P. Yan, F. Fang, Y.-P. Chen, Bacterially self-assembled encapsulin nanocompartment for removing silver from water, Water Res. 191 (2021), 116800, https://doi.org/10.1016/j.watres.2020.116800.
[48] P. Zhang, J.-S. Guo, Y. Shen, P. Yan, Y.-P. Chen, H. Wang, J.-X. Yang, F. Fang, C. Li, Microbial communities, extracellular proteomics and polysaccharides: A comparative investigation on biofilm and suspended sludge, Bioresour. Technol. 190 (2015) 21–28, https://doi.org/10.1016/j.biortech.2015.04.058.
[49] R.D.G. Franca, H.M. Pinheiro, M.C.M. van Loosdrecht, N.D. Lourenço, Stability of aerobic granules during long-term bioreactor operation, Synth. Syst. Biotechnol. 36 (2018) 228–246, https://doi.org/10.1016/j.biotechadv.2017.11.005.
[50] J.D. Watson, E.J. Milner-White, A novel main-chain anion-binding site in proteins: the nest. A particular combination of φ, ψ values in successive residues gives rise to anion-binding sites that occur commonly and are found often at functionally important regions, J. Mol. Biol. 315 (2002) 171–182, https://doi.org/10.1006/jmbi.2001.5227.
[51] W. Zhang, X. Dai, B. Dong, L. Dai, New insights into the effect of sludge proteins on the hydrophilic/hydrophobic properties that improve sludge dewaterability during anaerobic digestion, Water Res. 173 (2020), 115503, https://doi.org/10.1016/j.watres.2020.115503.
[52] A.F.S.A. Habeeb, Determination of free amino groups in proteins by trinitrobenzenesulfonic acid, Anal. Biochem. 14 (1966) 328–336, https://doi.org/10.1016/0003-2697(66)90275-2.
[53] Z. Zhang, J. Qiu, R. Xiang, H. Yu, X. Xu, L. Zhu, Organic loading rate (OLR) regulation for enhancement of aerobic sludge granulation: Role of key microorganism and their function, Sci. Total Environ. 653 (2019) 630–637, https://doi.org/10.1016/j.scitotenv.2018.10.418.
[54] Y.-M. Zheng, H.-Q. Yu, S.-J. Liu, X.-Z. Liu, Formation and instability of aerobic granules under high organic loading conditions, Chemosphere 63 (2006) 1791–1800, https://doi.org/10.1016/j.chemosphere.2005.08.055.
[55] G. Eydallin, M.T. Morán-Zorzano, F.J. Muñoz, E. Baroja-Fernández, M. Montero, N. Alonso-Casajús, A.M. Viale, J. Pozueta-Romero, An Escherichia coli mutant producing a truncated inactive form of GlgC synthesizes glycogen: Further evidences for the occurrence of various important sources of ADPglucose in enterobacteria, FEBS Letters 581 (2007) 4417–4422, https://doi.org/10.1016/j.febslet.2007.08.016.
[56] M.A. Ballicora, A.A.P.J. Iglesias, ADP-glucose pyrophosphorylase, a regulatory enzyme for bacterial glycogen synthesis, Microbiol. Mol. Biol. Rev. 67 (2003) 213–225, https://doi.org/10.1128/MMBR.67.2.213-225.2003.
[57] A.A. Iglesias, J. Preiss, Bacterial glycogen and plant starch biosynthesis, Biochem. Educ 20 (1992) 196–203, https://doi.org/10.1016/0307-4412(92)90191-N.
[58] P. Wilmes, M. Wexler, P.L. Bond, A.-L. Reysenbach, Metaproteomics provides functional insight into activated sludge wastewater treatment, PloS one 3 (3) (2008) e1778, https://doi.org/10.1371/journal.pone.0001778.

[59] J. Li, K. Garny, T. Neu, M. He, C.H.H. Lindenblatt, Comparison of some characteristics of aerobic granules and sludge flocs from sequencing batch reactors, Water Sci. Technol. 55 (2007) 403–411, https://doi.org/10.2166/wst.2007.284.
[60] P. Świątczak, A. Cydzik-Kwiatkowska, Treatment of Ammonium-Rich Digestate from Methane Fermentation Using Aerobic Granular Sludge, Water Air Soil Pollut. 229 (2018) 247, https://doi.org/10.1007/s11270-018-3887-x.
[61] D. Sruamsiri, P. Thayanukul, B.B. Suwannasilp, In situ identification of polyhydroxyalkanoate (PHA)-accumulating microorganisms in mixed microbial cultures under feast/famine conditions, Sci. Rep. 10 (2020) 3752, https://doi.org/10.1038/s41598-020-60727-7.
[62] H.T.B. Truong, P.V. Nguyen, P.T.T. Nguyen, H.M. Bui, Treatment of tapioca processing wastewater in a sequencing batch reactor: Mechanism of granule formation and performance, J. Environ. Manage. 218 (2018) 39–49, https://doi.org/10.1016/j.jenvman.2018.04.041.
[63] O.T. Iorhemen, M.S. Zaghloul, R.A. Hamza, J.H. Tay, Long-term aerobic granular sludge stability through anaerobic slow feeding, fixed feast-famine period ratio, and fixed SRT, J. Environ. Chem. Eng. 8 (2) (2020) 103681, https://doi.org/10.1016/j.jece.2020.103681.
[64] J. Luo, L. Zhang, W. Du, X. Cheng, F. Fang, J. Cao, Y. Wu, Y. Su, Metagenomic approach reveals the fates and mechanisms of antibiotic resistance genes exposed to allicins during waste activated sludge fermentation: Insight of the microbial community, cellular status and gene regulation, Bioresour. Technol. 342 (2021), 125998, https://doi.org/10.1016/j.biortech.2021.125998.
[65] Yingning Wang, Fang Ma, Jixian Yang, Haijuan Guo, Delin Su, L. Yu, Adaption and Degradation Strategies of Methylotrophic 1,4-Dioxane Degrading Strain Xanthobacter sp. YN2 Revealed by Transcriptome-Scale Analysis, Iny. J. Mol. Sci. 22 (2021) 10435, https://doi.org/10.3390/ijms221910435.
[66] J.F. Nilsson, L.G. Castellani, W.O. Draghi, J. Pérez-Giménez, G.A. Torres Tejerizo, M. Pistorio, Proteomic Analysis of Rhizobium favelukesii LPU83 in Response to Acid Stress, J. Proteome Res. 18 (10) (2019) 3615–3629, https://doi.org/10.1021/acs.jproteome.9b00275.
[67] Tong Ren, Yulei Chi, Yu Wang, Xuan Shi, Xin Jin, P. Jin, Diversified metabolism makes novel Thauera strain highly competitive in low carbon wastewater treatment, Water Res. 206 (2021) 117742, https://doi.org/10.1016/j.watres.2021.117742.
[68] B. Jin, Y.e. Liu, X. Li, J. Hou, Z. Bai, J. Niu, L. Wang, J. Zhao, New insights into denitrification and phosphorus removal with degradation of polycyclic aromatic hydrocarbons in two-sludge system, Bioresource Technology 346 (2022) 126610, https://doi.org/10.1016/j.biortech.2021.126610.