Effect of high ammonia on granular stability and phosphorus recovery of algal-bacterial granules in treatment of synthetic biogas slurry

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ABSTRACT
The aim of the study was to investigate the application of algal-bacterial granules in treatment of high ammonia wastewater. Two identical cylindrical reactors, i.e., Rc and Rs was used to develop granular sludge system with synthetic biogas slurry. Rs was run under an artificial solar lamp controlled at 12 h power on and 12 h power off (~10,000 lux); Rc was operated as control (no light). Results showed that algal-bacterial granules (ABGS) developed in Rs exhibited better structural stability in the face of high ammonia in influent. Compared with aerobic granules (AGS), ABGS possessed high proteins (PN) content (145.3 mg/g-VSS) in extracellular polymeric substances (EPS) and better O2 mass transfer inner granules. Higher phosphorus (P) removal capacity was obtained in Rs even under 400 mg/L NH3–N which resulted in higher P content in ABGS biomass (56.4 mg/g-TSS). Bioavailable P in ABGS was 44 mg P/g-SS on day 160, approximately 1.53-times higher than that in AGS.

1. Introduction
With the development of biogas project, a large amount of biogas slurry is produced which still containing high concentrations of chemical oxygen demand (COD), nitrogen (N) and phosphorus (P). If not properly treated, it will cause serious environmental pollution problems (Jiang et al., 2018; Krustok et al., 2015; Uggetti et al., 2014). Aerobic granules (AGS) technology which possess excellent settle-ability, high organic loading rate, tolerant to toxic substances, have been studied in various wastewater treatment, such as sewage wastewater, livestock wastewater, biogas slurry, landfill leachate, etc. (Figueras et al., 2011; Jiang et al., 2021; Nanchanaih and Reddy, 2018). During long-time running, however, granular instability always occurs in AGS system (Franca et al., 2018). Up to now, the mechanisms behind instability of AGS during long-time running have not been fully understood. O2 mass transfer limitation and extracellular polymeric substances (EPS) consumption has been thought as main reasons (Liu et al., 2016; Long et al., 2019; Zhu et al., 2013). Due to O2 mass transfer limitation, endogenous respiration will occur inner granules which will consume EPS and further disintegrate AGS (Franca et al., 2018; Long et al., 2019). So far, several methods have been presented to enhance AGS stability, i.e., controlling granular size (Di Iaconi et al., 2006; Long et al., 2019; Zhou et al., 2016; Zhu et al., 2013), promoting the EPS secretion (Huang et al., 2014; Liu et al., 2016), and strengthening the inner core (Cai et al., 2018; Liu et al., 2010).

Recently, algal-bacterial granule (ABGS) was cultured when shift AGS reactor to sun light or artificial light source (Huang et al., 2015; Wang et al., 2022; Yang et al., 2021; Zhang et al., 2018). Liu et al. (2018) detected that ABGS possessed better nutrients removal efficiencies than AGS under the same operating conditions. Algal-bacterial symbiosis increased granular adaptability to the fluctuation of sewage water quality (Zhao et al., 2018). Beside higher removal efficiency, ABGS proved better structure stability than AGS during long-time running due to algae and bacteria symbiosis. Ahmad et al. (2017) reported the role of algae could act as the framework of ABGS which was thought as the reason for high stability of ABGS during long-time running. In ABGS,
algal can provide organics, N, P and O2 to bacteria and facilitate its growth and activity which might benefit for granular stability (Tang et al., 2018). Zhao et al. (2018) found that the AGS exhibited excellent accumulation capacity of P even face low C/N wastewater, and the bioavailable P reached up to 98%. And in our previous study (Cai et al., 2019), the AGS exhibited excellent P removal capacity (11.5 mg P/g-VS-d) and higher P content (36.0 mg P/g-TS) indicated great potential in P recovery of granules.

All of these studies focused on domestic wastewater which was low in ammonia and very little information can be retrieved on the treatment of high ammonia wastewater i.e., biogas slurry. In traditional oxidation ponds, high ammonia (higher than 200 mg/L) have been reported high inhibition to algae growth (Abeliovich and Azov, 1976; Krustok et al., 2015). In the medium experiment, free ammonia (FA) has been proved a strong toxic effect on the physiological activities of microalgae even under relatively low concentrations (Jiang et al., 2018; Uggetti et al., 2014). In AGS, the effect of ammonia on granular stability and activity should be further studied before this AGS system applied in biogas slurry treatment.

In this study, AGS system was applied to treat synthetic biogas slurry and the effect of ammonia concentration (from 200 mg/L to 400 mg/L) on the granular stability, nutrients removal and P accumulation were investigated. The granules’ morphology, average size, strength and nutrients removal were analyzed to represent granular stability. EPS content and O2 mass transfer inner granules were detected to clear-cut the stable mechanisms. Finally, for the purpose of resource recovery, phosphorus accumulation and its bioavailability in granules were compared between the AGS and ABGS.

2. Materials and methods

2.1. Experiment setup and reactors operation

Two cylindrical column sequencing batch reactors (SBRs) (Rc and Rs) made of transparent acrylic plastic with an effect working volume of 2.5 L (diameter was 8 cm, effective height of 50 cm) was used in this study. A typical operating cycle (6 h) was included 2 min feeding, 90 min anaerobic, 240 min aeration, 3 min settling, 2min decanting and 23 min idling. During the aeration phase, dissolved oxygen (DO) of aqueous solution was controlled at 6–8 mg/L by an aeration system and the superficial air velocity was set at 1.0 cm/s. After 3 min’ settling of each cycle, 1.25 L supernatant was discharged from the central water outlet and HRT was controlled at 12 h.

For Rs, an artificial solar lamp (10,000 lx) (CDM-R, PHILIPS, Netherlands) was employed to create a photo-period (12 h) and a dark-period (12 h) in one day. Rc was covered by a black film to shield light. The whole experiment was conducted at room temperature (25 ± 2 °C).

2.2. Seeding and synthetic biogas slurry

Cryo-stored AGS after 2 months under –20 °C was employed as seed sludge in this study. After unfreezing at room temperature, the AGS was filtered through sieves with pore size of 1.4 mm and 0.6 mm to remove oversized and too small granules, and then equally divided into two reactors. The initial concentration of mixed liquor suspended solids (MLSS) in two reactors was almost same, around 3800 mg/L.

Synthetic biogas slurry consisted by 380 mg/L dissolved organic carbon (DOC) (190 mg/L from glucose and 190 mg/L from sodium acetate (total DOC = 380 mg/L)), 20 mg P/L (KH2PO4), 10 mg Ca2+/L (CaCl2·2H2O), 5 mg Mg2+/L (MgSO4·7H2O), 5 mg Fe2+/L (FeSO4·7H2O) and 1 μL/L of trace element solution. The composition of the trace elements solution was the same as Huang et al. (2015). Besides, NH4Cl was employed as nitrogen source. According to influence NH3–N concentration, the experiment was divided into two stages, i.e., stage I (from day 0–90, NH3–N = 200 mg N/L) and stage II (from day 91–160, NH3–N = 400 mg N/L).

2.3. Analytical methods

Granular size was measured by a stereo microscope (ES-18TZLED, MOTIC, China) equipped with a microscope camera (MOTICAMS5, MOTIC, China). MLSS, MLVSS and SVI (sludge volume index) of mixed liquor were detected in accordance with APHA (2012). SVIS was measured in a similar manner as SVI by modifying the settling time from 30 min to 5 min.

Mixed liquor was sampled at 5 min before the end of the aeration phase. After filtered through 0.45 μm membrane, the sludge samples were used for measurement of granules’ properties: Chlorophyll a was extracted and evaluated according to standard method (APHA, 2012); integrity coefficient, metal ions content, EPS extraction and determination, TP content and fractionation (organic phosphorus (OP), non-apatite phosphorus (NAIP) and apatite phosphorus (AP)) in granules were the same as those in a previous work Cai et al. (2018). The filtrates were used for NH3–N, total nitrogen (TN) and total phosphorus (TP) analysis (APHA, 2012). DOC was measured by a TOC analyzer (VarioTOC, Elemental, Germany). The P fractionations in sludge were evaluated according to Standard, Measurements and Testing (SMT) method as describing of Cai et al. (2018).

DO distributions inner AGS and ABGS were measured according to the description of Meng et al. (2019). Three spherical granules with similar size (~1.5 mm) were taken from two reactors: the first from Rc (AGS), the second from Rs during photo period (ABGS-P); the third from Rs during dark period (ABGS-D) at the end of one cycle and placed in DO saturated synthetic biogas slurry (DO = 8 mg/L). ABGS-P was exposed to the artificial solar lamp same with Rs reactor; AGS and ABGS-D were without light illumination. The DO concentration at different depths of the granules were detected by a 10 μm diameter DO microelectrode (DO-10, Unisense, Denmark). DO values were measured every 30 μm, starting from the surface of the granules.

Nutrients’ removal efficiencies (DOC, NH3–N and TP) and statistics analysis were calculated according to Cai et al. (2018), FA concentration was calculated according to Huang et al. (2020).

3. Results and discussion

3.1. Morphological changes of granules

During 160 days operation, the changes of granular morphology in Rc and Rs were recorded in Figure 1. After 2 months' cryostorage under –20 °C, the stored mature AGS displayed in brown and irregular shape. The structure looks loose and hollow. The granules would disintegrate due to destructive effect of the freezing and thawing process (Gao et al., 2012). During stage I, several granules in Rs appeared green after 5 days without inoculating. Due to open system of the photo-reactor, microalgae spores in the air could be as microalgae resource (He et al., 2018; Kumari and Singh, 2021). At end of stage I, stable ABGS were cultured in Rs and most exhibited sphere- and ellipsoid-shape indicated that cryo-stored AGS can be used as inoculum for ABGS culture. In Rc, more granules exhibited yellowish and smaller size than Rs. After that, the structure of ABGS kept stable and much smoother and smaller than Rs during dark period (ABGS-D).

3.2. Size variation of granules

Average granular size was significantly different in two reactors. After 30 days’ operation, average granular size rapidly increased from 0.83 mm to 1.01 mm and 0.93 mm in Rc and Rs, respectively. After that, the average size of granules in Rs kept increasing and fluctuated around 1.31 mm at the end of stage I. In Rc, it gradually decreased to approximately
0.84 mm. During stage II, the average size of granules in Rs rapidly increased to 1.57 mm at day 120 and fluctuated around 1.60 mm at day 160 which might due to the growing of filamentous bacteria on the granules' surface. While the granule size in Rc gradually minished to only 0.51 mm on day 160 might due to high NH₃–N concentration. Figure 2a also gives the size distribution of two reactors. In Rs, it decreased in smaller particles (<0.9 mm) and increased in larger particles (>1.5 mm). On day 160, more than 77% of the granules were bigger than 1.5 mm and small particles (<0.3 mm) almost disappeared. In Rc, small granules (<0.3 mm) more than 18.6% which significantly increased. Higher ammonia was reported favoring the enrichment of autotrophic nitrifiers in granules and leading to small granular size. This was different with the result of He et al. (2018) that the growth of the water-born algae slightly decreased the mean sizes of the granules. The reason might be better structure stable of ABGS which resulted bigger granular size. Higher ammonia was reported favoring the enrichment of autotrophic nitrifiers in granules and leading to small granular size (Kocaturk and Erguder, 2016).

Figure 1. The morphological changes of AGSs and ABGSs.

Figure 2. Changes in granular size (a), MLSS and MLVSS/MLSS (b) and integrity coefficients (c) of two reactors during 160 days operation. Rc: the reactor without illumination, Rs: the reactor under illumination of an artificial solar light.
3.3. Biomass variation of granules

After startup, the MLSS increased rapidly from 3.8 g/L (MLVSS/MLSS = 0.71) to 10.1 g/L (MLVSS/MLSS = 0.79) in Rc and 9.92 g/L (MLVSS/MLSS = 0.78) in Rs, indicating AGS reserved highly microbial activities after cryostorage. During stage II, the MLSS in Rc and Rs remained relatively stable fluctuated at 9.8–11.5 mg/L, respectively, attributable to good settleability (SVI₅ = 25.5 mL/g and 23.5 mL/g). MLVSS/MLSS of Rs was lower than Rc due to more inorganics (Mg, Ca and Fe) were accumulated in ABGS (Figure S1) (Cai et al., 2018).

3.4. Strength of granular

After 2 months’ cryostorage, the integrity coefficient increased from 5.3% to 10.4% indicating granule strength of AGS decreased. Cryostorage could damage granular structure and diminish the strength of granules (Gao et al., 2012). During stage I, lower integrity coefficients of ABGS than AGS illustrated that ABGS possessed higher granular strength than AGS. From sectional drawing (Figure S2), algae not only existed on granule surface also embedded inside ABGS. Algae could grow inner granules and serve as the backbone and enhanced the granular strength (Ahmad et al., 2017; He et al., 2018; Meng et al., 2019). During stage II, the integrity coefficient of Rc showed a slight increase from 5.5% to 6.2% on day 160 while it kept stable ~4% for ABGS indicated ABGS possessed better adaptability to high NH₃–N concentration than AGS. This observation indicated that the symbiosis of bacteria and algae in ABGS is benefit for granular structure stability.

Chl-a content decreased from 3.78 μg/g-VSS on day 90 to 2.46 on day 160 indicating algae growth were inhibited in stage II under 400 mg/L NH₃–N. Krustok et al. (2015) reported algae could grow under NH₃–N concentrations up to 200 mg/L and the inhibition effect is obvious with NH₃–N concentration higher than 200 mg/L. Some others suggested that FA is highly toxic to the growth of microalgae even at very low concentrations (Jiang et al., 2018; Uggetti et al., 2014). There are still number of algae in granules on day 160 with NH₃–N increased to 400 mg/L (FA = 13.84 mg N/L), indicated algal-bacterial symbiotic in Rs enhanced the adaptation of algae to NH₃–N (Figure S2).

3.5. Extracellular polymeric substances of granules

EPS is considered to be one of the main factors affecting the structural stability of granular sludge (Ahmad et al., 2017; Huang et al., 2020). From Figure 3, an obvious decrease in EPS content (from 173.7 mg/g-VSS to 62.8 mg/g-VSS) and PN/PS (from 2.9 to 1.2) was noticed after 2 months’ cryostorage probably due to the endogenous respiration of bacteria which indicated that EPS, especially PN, could as energy source for bacteria during long starvation period (Gao et al., 2012).

During stage I, the total EPS content rapidly increased to 183.5 mg/g-VSS and 201.1 mg/g-VSS on day 40, and after that it maintained ~181 mg/g-VSS (PN/PS = ~2.5) and ~207 mg/g-VSS (PN/PS = ~2.9) in Rc and Rs, respectively. With respect to constitute (PN and PS), ABGS possessed higher PN content (157.9 mg/g-VS) than AGS (125.0 mg/g-VS). During stage II, EPS contents sharply decreased in both reactors and maintained 138.1 mg/g-VSS (PN/PS = 0.80) and 184.3 mg/g-VSS (PN/PS = 1.91) on day 160 in Rc and Rs, respectively. This was different with the conclusions of Huang et al. (2020) that EPS content decreased with NH₃–N increasing. The increased EPS in this study might because that NH₃–N was suddenly increase not stepwise increase. According to the review of Franca et al. (2018), the suddenly increase in NH₃–N might result in EPS decrease and further damage granular stability while a stepwise increase in NH₃–N concentration was benefit for the slow-growing nitrifying bacteria which could avoid this damaging effect. As for constitute, PN contents in Rc and Rs decreased to 61.2 mg/g-VSS and 121.1 mg/g-VSS, respectively; while PS increased to 76.9 mg/g-VSS and 63.3 in Rc and Rs, respectively, indicating that higher NH₃–N could stimulate PS secretion.

ABGS possessed higher EPS content, especially PN content, during the whole experiment indicated that the symbiosis of algae and bacteria in ABGS could secret more EPS (Huang et al., 2020; Zhang et al., 2018). Restated, the EPS, especially PN, can enhance the connection between adjacent microbial cells and formed a cross-linking network by adsorb

![Figure 3. Variations of EPS and PN/PS ratios during 160 days’ operation.](image-url)
environmental matrices which benefit to the granule stability (Franca et al., 2018).

3.6. Dissolved oxygen distribution of granules

DO mass transfer limitation inner granules is also considered to be one of important reasons for granule unstable due to the hydrolysis of anaerobic microorganisms (Franca et al., 2018). According to the DO concentrations at various depths inner granules (Figure 4), the deepest depth that DO penetrated in AGS was 580 μm and there was no anaerobic core were detected in ABGS indicated that ABGS possessed a better O2 transfer than AGS. In algal-bacterial symbiosis system, microalga photosynthesis can consume the CO2 and provide O2 to bacterial (Tang et al., 2018). It's worth noting that the DO transfer depth in ABGS was 680 μm without illumination which was also deeper than AGS indicated that the symbiotic relationship between algae and bacteria could promote O2 transfer inner ABGS.

3.7. Reactor performance

3.7.1. Overall performance on pollutants removal

After 10 days' acclimatization, DOC and NH4-N removal efficiencies higher than 95% indicating that the stored AGS still possess high microbial activity. The DOC and NH3-N removal efficiency was higher than 96% averagely in both two reactors. During stage II, the DOC removal kept high efficiency (>96%). The NH3-N removal was inhibited by higher influent NH3-N (400 mg/l), however, which was 77.0% and 71.3% in Rc and Rs, respectively. Average NH3-N removal efficiencies in Rc was slightly higher than that in Rs.

3.7.2. Total phosphorus removal

TP removal were gradually stable after 40 days' operation and the average removal efficiencies were 57.7% and 73.8%. Significantly higher TP removal was observed in Rs during stage I. During stage II, the average TP removal efficiencies of two reactors were comparable (p = 0.63 > 0.05) and fluctuated at 40.4–42.1%. As for MLVSS-based P removal capacity (Figure S3d), the average P removal capacity, (7.3 mg P/g-VSS-d) in Rs was significantly higher than that in Rc (6.6 mg P/g-VSS-d) (p = 0.03 < 0.05) during stage I; while the P removal capacity was noticeably decreased to almost same level (p = 0.8 > 0.05) and fluctuated around 2.5–3.1 mg P/g-VSS-d after day 120. Interestingly, significant difference in P removal was detected in photo-period and dark-period of ABGS, especially during stage I. In dark-period, the TP removal efficiency and P removal capacities was 51.3% and 5.5 mg P/g-VSS-d, which was much lower than that in photo-period (p = 0.002 < 0.01). As discussion of section 3.6, photosynthesis of microalgae in photo-period promote O2 transformation depth in ABGS which could inhibit the P anaerobic release, and further decreased P removal (Cai et al., 2019; Huang et al., 2015).

3.8. Phosphorus content and bioavailability in granules

The granules were sampled every 10 days for the measurement of biomass TP content as shown in Figure 5. During stage I, TP content in AGS and ABGS increased rapidly from initial 29.9 mg P/g-TSS to 51.4 mg P/g-TSS and 49.6 mg P/g-TSS on day 40 with the increasing P removal.
After that the biomass TP content reached a platform which was 51.7 in AGS and 57.2 mg P/g-TSS in ABGS on day 90. Higher P content was measured in ABGS due to higher P removal capacity. During stage II, TP content kept stable and fluctuated from 50.5 mg P/g-TSS to 56.4 mg P/g-TSS in ABGS which was significantly higher than AGS (33.7 mg P/g-TSS on day 160) although the two reactors shared similar P removal capacity. Zhao et al. (2018) also discovered that ABGS possessed high and stable TP content even in the face of low carbon wastewater (COD/N = 1).

The P fractionsation of AGS and ABGS during experiment were evaluated according SMT method and the NAIP + OP was thought as bioavailable constituents. From Figure 5, NAIP was the dominant P fractionation in both granules followed with OP and AP during the whole experiment. During stage I, P bioavailability ((NAIP + OP)/TP) changes slightly from initial 78.6 % to 79.1 % in AGS and 83.6% in ABGS on day 90. During stage II, P bioavailability in AGS increased to 86.8% while it decreased to 80.8% in ABGS. Due to higher TP content, the net content of bioavailable P (NAIP + OP) was 44.4 mg P/g-TSS while it was only 28.7 mg P/g-TSS.

Lower P bioavailability in ABGS indicating that more AP accumulated, that is to say more Ca content in ABGS. Ca content in ABGS was 24.29 mg/g-TSS which was 1.5 times higher than AGS (Figure S1). In addition to Ca, Mg and Fe content in ABGS (21.22 mg/g-TSS and 9.61 mg/g-TSS) was also significantly higher than those in AGS (14.77 mg/g-TSS and 5.21 mg/g-TSS). The reason might be the large granular size and stable structure of ABGS which was beneficial to the precipitation of metal ions (Mg, Ca and Fe) and lead to a higher TP content and NAIP and AP content. Moreover, more divalent and trivalent cations have positive effects on granular stability (Cai et al., 2018; Liu et al., 2010, 2016).

Restated, due to algal-bacterial symbiotic, ABGS possessed higher EPS content and better DO transformation depth which resulted in larger size and more stable structure than AGS.

4. Conclusions

In this study, ABGS was successfully cultured and operated to treat synthetic biogas slurry during 160 days' operation. ABGS exhibited large granular size and stable structure than AGS when influent NH4–N increased from 200 mg/L to 400 mg/L. Compared with AGS, ABGS contained higher PN content (145.3 mg/g-VSS) in EPS and better O2 mass transfer inner granules. In the face of 400 mg/L NH4–N, ABGS possessed higher TP content (56.4 mg P/g-TSS) and higher bioavailable P content (44.4 mg P/g-TSS), which reflected great potential for P recovery.

Declarations

Author contribution statement

Wei Cai: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Peiqi Hu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zhaohua Li: Shujing Zhu; Qun Kang & Hongbing Chen: Contributed reagents, materials, analysis tools or data.

Jin Zhang: Conceived and designed the experiments.

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Data availability statement

Data included in article supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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