The treatment of solvent recovery raffinate by aerobic granular sludge in a pilot-scale sequencing batch reactor

Bei Long, Chang-zhu Yang, Wen-hong Pu, Jia-kuan Yang, Guo-sheng Jiang, Jing-feng Dan, Jing Zhang and Li Zhang

ABSTRACT

Mature aerobic granular sludge (AGS) was inoculated for the start-up of a pilot-scale sequencing batch reactor for the treatment of high concentration solvent recovery raffinate (SRR). The proportion of simulated wastewater (SW) (w/w) in the influent gradually decreased to zero during the operation, while volume of SRR gradually increased from zero to 10.84 L. AGS was successfully domesticated after 48 days, which maintained its structure during the operation. The domesticated AGS was orange, irregular, smooth and compact. Sludge volume index (SVI), SV30/SV5, mixed liquor volatile suspended solids/mixed liquor suspended solids (MLVSS/MLSS), extracellular polymeric substances, proteins/polysaccharides, average particle size, granulation rate, specific oxygen utilization rates (SOUR)H and (SOUR)N of AGS were about 38 mL/g, 0.97, 0.52, 39.73 mg/g MLVSS, 1.17, 1.51 mm, 96.66%, 47.40 mg O2/h g volatile suspended solids (VSS) and 8.96 mg O2/h g VSS, respectively. Good removal effect was achieved by the reactor. Finally, the removal rates of chemical oxygen demand (COD), total inorganic nitrogen (TIN), NH4\textsuperscript{+}-N and total phosphorus (TP) were more than 98%, 96%, 97% and 97%, respectively. The result indicated gradually increasing the proportion of real wastewater in influent was a useful domestication method, and the feasibility of AGS for treatment of high C/N ratio industrial wastewater.

Key words | aerobic granular sludge, high C/N ratio, pilot scale, sequencing batch reactor, solvent recovery raffinate

INTRODUCTION

Ethyl acetate is a fast drying industrial solvent with excellent soluble properties, which has been widely used in industrial processes. With the rapid development of the chemical and pharmaceutical industries in China, the consumption of ethyl acetate has increased greatly, which has led to a large amount of waste solvent being generated during the process of production. To reduce the cost of production, recovery and recycling of waste solvent has received more and more attention. However, the recovery rate cannot reach 100% via current technologies, especially for azeotrope containing ethyl acetate and water (boiling point 70 °C). Thus, a new environmental problem is created during the recovery process – solvent recovery raffinate (SRR). Typically, the raffinate is a kind of wastewater with a small amount, high concentration, single composition, and unharmonious C: N: P ratio. At present, this kind of high concentration organic wastewater can be treated separately, or diluted and treated together with domestic wastewater. Anaerobic granular sludge reactors, such as up-flow anaerobic sludge blanket and expanded granular sludge bed are widely used in treating this kind of wastewater. However, the obvious defects of anaerobic granular bioreactors are the long time for start-up and poor nitrogen and phosphorus removal rate. Although treating together with domestic wastewater is simple and practicable, it would increase the operational cost and risk the effluent quality. With this dilemma, research into economic and efficient treatment technology of SRR has practical significance.

Aerobic granular sludge (AGS) is a granular biological polymer formed by microbial self-agglomeration and proliferation in a specific environment (de Kreuk et al. 2007), which
has many incomparable advantages compared with activated sludge (Show et al. 2002), such as regular shape, compact structure, high settling velocity and high tolerance to toxicity. Owing to these advantages, AGS has become a hot research topic in the field of wastewater treatment. However, according to a large number of studies (e.g., Lee et al. 2010), it was found that AGS would be unstable and even disintegrate in long-term operational bioreactors which was the biggest bottleneck for application of AGS. Although there are many challenges, some research about AGS treatment of many kinds of real wastewater has been reported (Adav et al. 2008; Gao et al. 2011). These studies indicated that AGS had good removal rate of the pollutants, and the granules were more stable than simulated wastewater (SW) as the media. The results greatly promoted the development of AGS technology, which also proved AGS’s good potential for engineering application. To test AGS’s feasibility for real wastewater treatment, there have been some pilot projects (Ni et al. 2009; Liu et al. 2010, 2011; Su et al. 2012; Morales et al. 2013; Wei et al. 2013). To reduce the operational risk, medium or low concentration wastewater was usually chosen to be treated by most of the pilot studies, as high organic load was considered to be adverse for the stability of AGS (Jungles et al. 2011).

It has been shown that C/N ratio of wastewater as substrate for AGS is usually less than 20:1. In addition, researchers have usually preferred the treatment of high ammonia nitrogen or low C/N ratio wastewater by AGS (Isanta et al. 2013; Li et al. 2013; Long et al. 2014a). By contrast, research on AGS treating industrial wastewater with high C/N ratio is comparatively scarce, and the stability of AGS feeding by this kind of wastewater still needs to be inspected. That is because nitrogen load is an important biological selection pressure for enrichment of nitrifying bacteria, while the enrichment of autotrophic bacteria has proven to be beneficial for stability of AGS (de Kreuk & van Loosdrecht 2004). Owing to AGS’s unique advantages, it has superior adaptability to many special industrial wastewaters (Adav et al. 2009), and better technical and economic benefits can be achieved. However, efficient treatment of any real wastewater by AGS requires an adaptation process, because microbials in AGS would be uncomfortable or even collapse when directly exposed to high concentration real wastewater in a short time without adjustment. For this dilemma, gradually increasing the proportion of real wastewater in the influent for the selection of adaptive bacteria was not a bad choice, while this strategy has proven to be an effective domestication method for real complex wastewater treatment (Long et al. 2014a; Rosman et al. 2014; Zhu et al. 2014).

In this paper, inoculated with mature AGS in a pilot-scale sequencing batch reactor (SBR) and gradually increasing the proportion of real wastewater in the influent, the performance of the bioreactor on pollutants and stability of AGS for the treatment of SRR was studied. The research aimed to provide technological support for efficient biological treatment of similar industrial wastewater.

**METHODS**

**Equipment**

The working volume of the pilot-SBR was 105.5 L (27.7 cm in diameter, 175 cm in height and H/D ratio of 6.3) with an exchange ratio of 60%. SRR and SW were added into the reactor separately from two head tanks, while tap water was filled directly from the pipeline. Fine air bubbles provided by an air compressor for aeration and mixing were supplied through a dispenser at the bottom of the reactor. Superficial gas velocity was controlled between 1.25 and 2.40 cm/s. The reactor was located in semi-closed space (north and west sides were close to the fence) and worked at room temperature. The reactor operated sequentially for 6 hours per cycle, including 4 minutes of influent filling, 90 minutes of anaerobic period (no stirring), 260 minutes of aeration, 2 minutes of settling and 4 minutes of effluent withdrawal.

**Seed sludge**

Mature AGS formerly cultivated was inoculated for reactor start-up. The AGS was irregular and pale yellow (Figure 1(a)), average particle size, sludge volume index (SVI), mixed liquor volatile suspended solids/mixed liquor suspended solids (MLVSS/MLSS), SV30/SV5, proteins/polysaccharides (PN/PS) and extracellular polymeric substances (EPS) were 2.33 mm, 37.29 mL/g, 0.65, 0.95, 1.50, and 30.90 mg/g MLVSS, respectively. Initial MLSS of the reactor was about 9,000 mg/L.
SRR (Table 1) was from a distillation workshop of a pharmaceutical factory in Wuxi, China, and was generated during the recovery of waste ethyl acetate. The main organic chemicals of SRR were the residual ethyl acetate and a small amount of its hydrolysis products – acetic acid and ethanol. The influent was mixed wastewater of SRR, SW and tap water (Table 2). The proportion of SW (w/w) gradually decreased to zero during the operation, while the volume of SRR in the influent gradually increased. Owing to high chemical oxygen demand (COD) concentration of SRR, the real wastewater was diluted by about five

**Figure 1** | Macrostructure of AGS: (a) seed sludge (granular cluster in circle); (b)–(d) AGS domesticated on the 11th, 25th and 48th day; microstructure of the domesticated AGS: (c) epistyles; (d)–(f) bacillus, coccus and filamentous fungus.

**Media**

The treatment of SRR by AGS in a pilot-scale SBR
times volume of tap water from the 34th day. As no phosphorus was contained in the SRR, therefore total phosphorus (TP) of the influent gradually decreased during the operation. After the 24th day, external KH$_2$PO$_4$ was added into the influent as it was necessary for microbial growth.

### Analysis method

COD, NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, TP, suspended solids (SS), conductivity, SV, SVI, MLSS, MLVSS and water content were carried out according to *Standard Methods* (APHA 1998). The content of total inorganic nitrogen (TIN) could be calculated as $[\text{TIN}] = [\text{NH}_4^+\cdot\text{N}] + [\text{NO}_3^-\cdot\text{N}] + [\text{NO}_2^-\cdot\text{N}]$. Size distribution, average particle size and settling velocity of the granules were measured using the methods suggested by Long et al. (2014b). Granulation rate was the proportion of the MLSS larger than 0.3 mm of the total sludge's MLSS. A heat extraction method was modified to extract EPS from granular sludge as suggested by Morgan et al. (1990). PS content was determined using the phenol-sulphuric acid method (Gerhardt et al. 1994) with glucose as the standard. PN content was analysed by a UV spectrophotometer (UV-1600 (PC), MAPADA, China) following the modified Lowry method (Lowry et al. 1951), and EPS was the sum of PS and PN. The specific oxygen utilization rate (SOUR) by heterotrophic bacteria and specific oxygen utilization rates by ammonium oxidizers and nitrite oxidizers, namely (SOUR)$_{\text{NH}_4}$ and (SOUR)$_{\text{NO}_2}$, were determined using the methods developed by Moreau et al. (1994) and Ochoa et al. (2002). The sum of (SOUR)$_{\text{NH}_4}$ and (SOUR)$_{\text{NO}_2}$ was used to represent the overall activity of nitrifying populations, namely (SOUR)$_N$. Granular morphology was qualitatively observed using a scanning electron microscope (SEM, FEI, Sirion200, The Netherlands) (Tay et al. 2001).

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<th>Composition of SW and SRR</th>
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*The corresponding COD, NH$_4^+$-N and TP concentration were 1,666.67, 111.12 and 16.67 mg/L.*

*Minor element’s supplementation was 1 mL per 1 L SW.*

*The unit of conductivity was mS/cm.*
RESULTS AND DISCUSSION

Morphological changes of AGS

The inoculated AGS had a clear outline, whose shape was spherical, rodlike and ellipsoid. A large number of granular clusters could be observed in the reactor (Figure 1(a)), which was mainly ascribed to microbial cells becoming more hydrophobic and secreting more EPS leading to mutual adhesion between granules under high selection pressure (Guo et al. 2014). With the increase in the proportion of SRR in influent from the 5th day, the colour of the AGS progressively got deeper, which changed to yellow on the 13th day. Meanwhile, proportion of the clusters gradually decreased. A few large particles with cracks were observed on the 28th day, which indicated that larger particles were not stable and easily breakable. There appeared some flat particles on the 33rd day, which were the product of breakage of large particles. The colour of the AGS became obviously brown on the 34th day, and no large changes in morphology were observed during the following time. The appearance of the domesticated AGS was smooth and irregular, while its structure was more compact (Figure 1(b)).

The detailed microstructure of the domesticated AGS was examined using SEM (Figures 1(c)–1(f)). It can be seen that the granules have rich biological species inside, which include bacillus, coccus, filamentous fungus and protozoa (epistyles). The indicative protozoa – epistyles – usually appears when good effluent quality is monitored, which shows that the microbial system is in good condition. The filamentous fungus mainly resided in the outer space of the granule, while bacillus and coccus adhered tightly to each other and were embedded in quantities of inert substances. The unique structure improved the microbials to resist impact of outside toxicity (Zhu et al. 2013), and the reason could be ascribed to mass transfer limitation, the protective effects of EPS, etc.

Physico-chemical properties of AGS

Settling property

SVI is an index to evaluate settling performance of activated sludge, which can reflect the degree of the loose structure and coagulation performance. SVI of the AGS was always less than 48 mL/g during the operation (Figure 2(a)). It fluctuated during the former 37 days, and was between 31 and 48 mL/g. Then, SVI tended to be stable after the 38th day, and was about 40 mL/g.

SV is an index which can roughly reflect the amount of sludge in the reactor, which can also timely indicate sludge bulking and other abnormal conditions. Research showed that deviation between SV30 and SV5 of mature AGS was less than 10% (Liu & Tay 2007). The ratio of SV30/SV5 was always above 0.90 during the operation. It fluctuated slightly in the former 21 days (0.92–0.99). Then, it tended to be stable after the 22nd day, and was basically around 0.96-0.99.

Settling velocity of the inoculated AGS was 49.76 m/h, while settling velocity of the granule on the 46th day was 61.45 m/h. Discrete settling was the main kind of sludge sedimentation, and no obvious stratified settling was observed during the operation. Thus, according to the SVI, SV30/SV5 and settling velocity, the domesticated AGS represented better settling ability compared with the inoculated AGS.

MLSS and MLVSS

MLSS of the reactor fluctuated during the operation, and the range was between 8,404 and 13,320 mg/L (Figure 2(b)). MLSS was always above 10,000 mg/L after the 19th day. The result indicated that a large amount of biomass was maintained in the reactor.

MLVSS is part of the organic solid component of MLSS. Overall profile of MLVSS/MLSS declined in the former 33 days, with the minimum reaching 0.50 on the 33rd day. Then, there was a slight increase between the 34th and 36th days, which was eventually maintained between 0.51 and 0.52 after the 36th day. The domesticated AGS had higher inorganic composition compared with inoculated granules. The reason for this is associated with high metal ion concentration in the influent, which can transform into inorganic salts precipitated inside the granules.

EPS and PN/PS

EPS is viscous material secreted by micro-organisms to resist outside influence, which mainly consists of PS and PN. The overall profile of the amount of EPS was rising in the former
37 days (Figure 2(c)), and it reached a maximum of 52.0 mg/g MLVSS on the 37th day. The amount of EPS fluctuated violently during the 16th to the 38th day (29.23–52.0 mg/g MLVSS). Then, the amount of EPS tended to be stable after the 42nd day, and was between 38.73 and 43.88 mg/g MLVSS. The ratio of PN/PS was always above 1.0 during the operation, although it fluctuated in the former 40 days, but the range was very small (1.05–1.47). Then, the ratio of
PN/PS tended to be stable after the 42nd day (1.14–1.18), which was consistent with the change of EPS. Research has shown that the amount and components of EPS play an important role in AGS’s characteristics and stability (Liu et al. 2004; Mcswain et al. 2005). Thus, microbial cells secreted more EPS to resist adverse shock of SRR, especially when the proportion of SRR increased in influent, which prompted granules to maintain their structure (Adav et al. 2007). As AGS gradually adapted to SRR, extra nutrients were utilized for microbial growth, leading to the decline of EPS ultimately. As PN contributed most of the EPS, therefore, it played a more important role during the operation (Lv et al. 2014).

Size distribution and average particle size

The overall profile of the proportion of particles in the range of 0–2 mm was increased (Figure 2(d)), while the profile of larger particles in the range of 2–4 mm followed a downward trend. The range of 2–4 mm particles accounted for the largest proportion, which meant it was the advantaged section on the 22nd day. However, the range of the 1.43–2 mm section kept rising during the operation (30.78–42.34%), which eventually became the new advantaged section after the 34th day. As observed from AGS’s morphological changes during operation, larger granules were not stable and easy to break, and eventually broke into small particles under strong hydraulic shear force (Liu & Tay 2002).

The average particle size of AGS kept declining (2.33–1.51 mm) during the operation (Figure 2(e)). The overall profile of granulation rate followed a downward trend, but the degree of reduction was small (98.73–96.39%). The result indicated that medium size granules especially in the range of 1.43–2.0 mm were preferred by the reactor, and was the equilibrium between cohesion and disintegration under corresponding selection pressure (Liu et al. 2005).

Water content

Although water content fluctuated during the operation, the overall profile of the water content was increasing in the previous 31 days (Figure 2(f)). Water content tended to be stable from the 32nd day, and was maintained at about 98%. The result indicated that water content of the domesticated AGS was higher than inoculated sludge, which might relate to variation in the composition of the substrate. However, compared with activated sludge (water content was usually between 99.2 and 99.8%), the cultivated aerobic granules had a much lower water content. The result indicated that large amount of hydrophobic cells in granules were maintained in the reactor.

SOUR

(SOUR)H/(SOUR)N, (SOUR)H, (SOUR)N, (SOUR)NH4 and (SOUR)NO2 of the inoculated AGS were 3.98, 106.59 mg O2/h g VSS, 26.81 mg O2/h g VSS, 15.23 mg O2/h g VSS and 11.58 mg O2/h g VSS. However, (SOUR)H/(SOUR)N, (SOUR)H, (SOUR)N, (SOUR)NH4 and (SOUR)NO2 of the domesticated AGS on the 48th day were 5.29, 47.40 mg O2/h g VSS, 8.96 mg O2/h g VSS, 4.75 mg O2/h g VSS and 4.21 mg O2/h g MLVSS. This shows that activity of heterotrophic bacteria and nitrifying bacteria dropped sharply during the operation. The reasons include: (1) MLVSS/MLSS of the domesticated AGS was much lower than inoculated AGS, thus less active biomass was contained in the domesticated granules; (2) variation of the substrate led to the change of microbial species, which had a significant influence on bioactivity; and (3) as little floculent sludge was discharged from the reactor leading to a long sludge retention time during the domestication, so the ageing of the granules also led to a decrease of SOUR. Results indicate that the fraction of active biomass in the microbial community was found to be proportional to respirometric activity measured in terms of SOUR (Moreau et al. 1994; Ochoa et al. 2002). As (SOUR)H/(SOUR)N increased from 3.98 to 5.29, therefore, the proportion of nitrifying bacteria decreased significantly along with the increasing C/N ratio of the influent.

Reactor performances

COD and TP removal

The reactor had good performance for SRR during the operation (Figure 3(a)). Effluent COD was always less than 82.08 mg/L, and the removal rate was always above 95.08%. Although effluent TP fluctuated slightly during the
operation (Figure 3(b)), the overall profile of effluent TP followed a downward trend. The removal rate of TP was poor in the former 9 days, as the effluent TP was more than 0.88 mg/L, which reached a maximum of 2.13 mg/L on the 3rd day. As AGS gradually adapted to the quality of SRR, TP of the effluent remained below 0.28 mg/L after the 27th day.

Nitrogen removal

The overall profile of effluent TIN and NH$_4^+$-N was in decline (Figure 3(c)), although they fluctuated sharply in the former 24 days. Effluent TIN and NH$_4^+$-N tended to be stable after the 34th day. TIN was basically less than 2.32 mg/L and NH$_4^+$-N was no more than 1.8 mg/L after the 34th day, while their removal rates were more than 95.98 and 97.46%. NH$_3^-$-N of the effluent was consistently at a low level (less than 1.08 mg/L), which was almost under the detection limit after the 38th day. Obvious accumulation of NH$_3^-$-N (1.08–4.46 mg/L) was observed during the 23rd to the 29th day, while it was always less than 0.92 mg/L during other days. NH$_3^-$-N tended to be stable after the 32nd day, as it was maintained between 0.17 and 0.32 mg/L. Although C/N ratio gradually increased to 29:1 during the operation, no obvious disintegration of AGS was observed. The result indicated the unique advantages of AGS for treating high C/N ratio industrial wastewater.

SS of the effluent

The overall profile of effluent SS was rising in the former 19 days (Figure 3(d)), which reached a maximum of 410 mg/L.
on the 19th day. Then, SS fluctuated greatly between the 20th and 41st day (105–547 mg/L). SS tended to be stable after the 42nd day, and was between 95 and 115 mg/L. With the increase of the proportion of SRR, AGS was inadaptable to real wastewater quality in the former 41 days. A few larger particles disintegrated leading to the increase of SS. With the adaption of AGS to the influent, SS of the effluent gradually tended to become stable.

**Pollutant degradation in typical cycles**

The profiles of COD, TIN, NH$_4^+$-N and TP that degraded in typical cycles were approximately the same (Figure 4(a)), as mass transfer limitation caused by no mixing meant their variations were gentle during the anaerobic period; however, they decreased quickly after aeration. COD almost depleted to the minimum after the 150th minutes, which remained under 84.33 mg/L during the following time. The reactor then ran into an aerobic starvation stage (about 78% of the aeration period). Results indicated that a proper starvation time (60% of the aeration period), while degradation rate of NH$_4^+$-N was much slower, ORP rose rapidly at first and then slowed down. Although DO was close to saturated at the end of the cycle, ORP was still negative. The reason was mainly ascribed to SRR containing many reducing ions which were introduced by additives during the process of production.

Variation of pH was gentle during the anaerobic period (6.54–6.80). The reason was mainly ascribed to acetic hydrolysis of ethyl acetate. However, pH increased quickly during the aerobic period, and reached a maximum of 9.30 at the 180th minute. The overall profile of pH was to increase first (90th–180th minutes) and then decrease (180th–260th minutes). pH tended to be stable after the 270th minutes, and remained above 9.0 mg/L for the following time. Oxidation reduction potential (ORP) is a comprehensive index which represents redox ability of the solution. ORP of mixed liquors of the reactor represented strong reducibility at the beginning. It decreased slightly during the anaerobic period, which might relate to hydrolysis of ethyl acetate. Then, it kept rising during the aerobic period, and increased from −453 to −191 mV at the end of the cycle. However, the rising velocity slowed down after the 130th minutes. As COD degraded quickly in a short time, while degradation rate of NH$_4^+$-N was much slower, ORP rose rapidly at first and then slowed down. Although DO was close to saturated at the end of the cycle, ORP was still negative. The reason was mainly ascribed to SRR containing many reducing ions which were introduced by additives during the process of production.

Dissolved oxygen (DO) kept declining during the anaerobic period (Figure 4(b)), which reached a minimum of 0.36 mg/L at the 90th minutes, and then rose quickly after aeration. DO fluctuated during the 100th–280th minutes (6.0–9.11 mg/L), which could mainly be ascribed to aerobic metabolism and changed aeration rate. Large amounts of oxygen were consumed during the aerobic metabolism process, especially under high COD concentration environment, however periodic changes of aeration rate could result in uneven oxygenation. Thus, the two aspects eventually led to the fluctuation of DO. Then, DO tended to be stable after the 290th minutes, and remained above 9.0 mg/L for the following time. Oxidation reduction potential (ORP) is a comprehensive index which represents redox ability of the solution. ORP of mixed liquors of the reactor represented strong reducibility at the beginning. It decreased slightly during the anaerobic period, which might relate to hydrolysis of ethyl acetate. Then, it kept rising during the aerobic period, and increased from −453 to −191 mV at the end of the cycle. However, the rising velocity slowed down after the 130th minutes. As COD degraded quickly in a short time, while degradation rate of NH$_4^+$-N was much slower, ORP rose rapidly at first and then slowed down. Although DO was close to saturated at the end of the cycle, ORP was still negative. The reason was mainly ascribed to SRR containing many reducing ions which were introduced by additives during the process of production.

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Figure 4 | Degradation of pollutants, pH, DO and ORP in typical cycle on the 47th day: (a) variation of COD, TIN, NH₄⁺-N, NO₃⁻-N and NO₂⁻-N in typical cycle; (b) variation of pH, DO and ORP in typical cycle.
CONCLUSION

AGS was successfully domesticated by feeding with SRR in a pilot-scale SBR after 48 days, which maintained its structure during the operation. Results indicated that AGS could maintain stability for treating high C/N ratio industrial wastewater. The domesticated AGS had a smooth outline and compact structure, and had a good removal rate of pollutants.

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