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Production of polyhydroxyalkanoates and enrichment of associated microbes in bioreactors fed with rice winery wastewater at various organic loading rates

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Abstract

This study aimed to explore the production of polyhydroxyalkanoates (PHA) and selection of PHA-accumulating microorganisms in bioreactors fed with rice winery wastewater at various organic loading rates (OLRs). The substrate utilization, sludge properties, PHA synthesis and microbial community structure of three sequencing batch reactors were monitored. The results show the highest PHA yield (0.23 g/g) was achieved in one of the three reactors with an OLR of 2.4 g COD/L/d, in which *Zoogloea* was the most dominant PHA-accumulating microorganism. To quantify the PHA production and track the population changing profiles of the PHA-accumulating microorganisms in the long-term reactor operation, the Activated Sludge Model No. 3 was modified with two different heterotrophic microorganisms responding differently with the same substrate. The modeling results indicate that a moderate OLR (>2.4 gCOD/L/d) was beneficial for PHA production. The results are useful for understanding the PHA production from industrial wastewaters and selection of PHA-accumulating microorganisms.

Keywords: polyhydroxyalkanoates (PHA); organic loading rate (OLR); rice winery wastewater; microbial enrichment; mathematical model

1. Introduction

Due to the chemical resistance and thermostability, conventional plastics produced from fossil resources are widely used in daily life and industries at present, leading to a severe environmental pollution problem, which is called “white pollution” (Colombo et al., 2016). To tackle this issue, great efforts have been made to search for biopolymers that are physiochemically similar to the conventional plastics but are environmentally friendly and sustainable (Rodriguez-Perez et al., 2018). Produced by prokaryotic microbes as intra-cellular storage materials, polyhydroxyalkanoates (PHA) are biopolymers with similar characteristics to the conventional plastics, but are biodegradable and biocompatible (Obruca et al., 2018). The excellent properties of PHA make them an ideal choice for medical and industrial applications (Pakalapati et al., 2018).

However, the currently limited use of PHA is the result of their relatively high production price, which is mainly dependent on the substrate sources, PHA-accumulating microorganisms, accumulation conditions and the subsequent recovery process. To lower the PHA production costs, many efforts have been made. For example, mixed microbial cultures (MMCs) have been used to replace pure cultures to produce PHA (Fradinho et al., 2013; Huang et al., 2017; Uma and Gandhimathi, 2019). Furthermore, low-cost agricultural and industrial waste feedstocks have been used as alternative substrates to reduce the PHA production costs (Burniol-Figols et

al., 2018; Grazia et al., 2017).

The rice winery industry, one of the traditional industries in China, has not only created huge revenues but also generated plenty of pollutants. Large amounts of rice winery wastewater, which is mainly composed of sugars, ethanol, glycerol, esters and polyphenolic compounds, is produced in the brewing process (Mosse et al., 2011). If poorly disposed of, rice winery wastewater would cause a severe environmental pollution problem. Therefore, effective ways for rice winery wastewater treatment should be developed. Recently, several approaches for rice winery wastewater reutilization have been explored. For example, rice winery wastewater was converted to high-concentration industrial xanthan by specific microorganisms (Roncevic et al., 2017). It can also be used as a co-substrate with waste activated sludge to produce short-chain fatty acids via anaerobic fermentation (Luo et al., 2018). Since its main component is biodegradable organic matter, rice winery wastewater can also be used as the potential substrate for PHA production.

Aerobic dynamic feeding (ADF) is the most commonly used strategy for enriching PHA-accumulating microorganisms under the feast-famine regime (Coats et al., 2016; Li et al., 2011; Marang et al., 2016). In the feast period, PHA-accumulating microorganisms store external substrates as PHA, while the intracellular PHA is used by PHA-accumulating microorganisms as the carbon and energy source for cell growth and maintenance in the subsequent famine period. In this manner, PHA-accumulating microorganisms have a strong competitive advantage over other

counterparts without the PHA-accumulating capacity (van Loosdrecht et al., 1997).

However, the enrichment of PHA-accumulating microorganisms is affected by many operational factors, among which organic loading rate (OLR) is recognized as a key one as OLR is tightly related to the substrate costs and significantly influences the metabolic process of microbial community (Oliveira et al., 2017). Moreover, OLR governs the feast-to-famine ratio (F/F), which affects the extent of selective pressure in microbial culture selection. In some previous studies the effect of OLR on the microbial culture selection process has been investigated. Campanari et al. (2014) found that the optimal PHA production occurred at an OLR of 4.70 gCOD/L/d and that the highest PHA storage rate and yield reached 339 ± 48 mgCOD/gCOD/h and 0.56 ± 0.05 gCOD/gCOD, respectively. Farghaly et al. (2016) observed the maximum PHA content of 67.44 ± 5.88 % at an OLR of 1.5 gCOD/L/d with dark fermented paperboard mill wastewater as the substrate. Wen et al. (2018) achieved the maximum PHA content of 33.4% at 2.5 g/L NaCl with waste fermentation leachate as the substrate. However, no information about the effects of OLR on the selection of PHA-accumulating microorganisms and PHA production from rice winery wastewater is available so far.

Furthermore, due to the complexity of operation conditions in PHA production process, many factors should be optimized for PHA production. However, at the present time, experiments have to be repeated to determine the optimal operation conditions, which is time-consuming and costly. Thus, mathematical models are

proposed to describe PHA production processes and optimize operational parameters.

With the calibrated and validated mathematical models for PHA storage, the optimal operation conditions in PHA production process can be obtained. Microbial PHA formation as a complex biological process has been simulated by different mathematical models (Chen et al., 2016; Das and Grover, 2018; Elsayed et al., 2018; Li et al., 2018). However, mostly existing mathematical models for PHA production are based on batch experiments or short-term experiments. Moreover, long-term simulation of microbial selection process can provide useful information to optimize actual microbial selection process at the initial stage of PHA production by MMC.

Therefore, the objective of this study was to explore the enrichment of the PHA-accumulating microorganisms and production of PHA with rice winery wastewater as the substrate at various OLRs through combining experimental and mathematical modeling methods. The substrate utilization, sludge properties, and PHA synthesis at various OLRs were evaluated. Also, the microbial community was investigated by 16S rDNA gene high-throughput sequencing to further understand the effects of OLR on the PHA-accumulating microorganisms selection process. Finally, a modified mathematical model was established to simulate the substrate consumption, PHA production and long-term selection of PHA-accumulating microorganisms.

2. Materials and Methods

2.1 Rice winery wastewater characterization and reactor long-term experimental setup

To enrich the PHA-accumulating microorganisms under the feast-famine regime, three 3-L sequencing batch reactors (SBRs, named as A, B and C) were operated with different OLRs (1.2, 2.4 and 3.6 gCOD/L/d) in 6-h cycles, consisting of feeding (5 min), aeration (320 min), settling (15 min), withdrawal (5 min) and idle phase (15 min). Seeding sludge was collected from a municipal wastewater treatment plant in Nanjing City, China. Before inoculation, the sludge was washed twice by pure water and filtrated through the 0.6-mm screen to remove particles. The initial sludge concentration in each SBR was about 3000 mg/L. Supernatant of 1.5 L was discharged in every withdrawal period. Then, 1.5-L diluted rice winery wastewater was fed in every feeding period. The hydraulic retention time (HRT) was kept at 12 h. Air was supplied at the bottom of the SBRs by an air pump through gravel diffusers and controlled at 100 L/h with dissolved oxygen (DO) concentration of >2 mg/L. The three reactors were operated at room temperature (25-30°C). Sludge retention time (SRT) was kept at 10 days through wasting mixed liquor at the end of aeration periods.

The raw rice winery wastewater was obtained from Shuanggou Rice Winery Plant in Huaiyin City, China. The concentrations of total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total nitrogen and total phosphorus in the raw rice winery wastewater were 170336 ± 1080 mgCOD/L, 150123 ± 965 mgCOD/L, 2274 ± 28 mgN/L and 872 ± 34 mgP/L, respectively. The

initial pH of the raw rice winery wastewater was 3.4 ± 0.2 . The raw wastewater was diluted at different ratios by deionized water and used as the influent of the three SBRs. The pH of diluted rice winery wastewater was adjusted to 7.0 ± 0.2 by adding NaOH (5 M).

2.2 Batch experiment setup

After 45-d reactor operation at various OLRs, batch experiment was conducted. The OLRs in the three batch experiments were controlled at 1.2, 2.4 and 3.6 gCOD/L/d. The operation conditions, such as temperature, DO, cycle time and the pH of feeding, were as same as those for the long-term experiment. The COD, total nitrogen (TN), total phosphorus (TP) and PHA were monitored in the batch experiment process.

2.3 Model-based evaluations

The mathematical model applied in this study was modified based on the Activated Sludge Model No. 3 (ASM3) (Gujer et al., 1999). The modified ASM3 focuses on the aerobic storage process involving three dissolved model components and four particulate model components (Table 1). In this modified model, the microorganisms were divided into PHA-accumulating microorganism (X_{PAB}) and non-PHA-accumulation microorganism (X_H). Five component processes, including aerobic growth of active heterotrophic bacteria without PHA accumulation (X_H) on external

substrate, aerobic storage of PHA (X_{PHA}) in active PHA accumulating bacteria (X_{PAB}), aerobic growth of active PHA accumulating bacteria (X_{PAB}) on X_{PHA} , aerobic decay of X_H and X_{PAB} , were taken into consideration. In these processes, all substrates (S_S) in rice winery wastewater were assumed to be biodegradable.

Sensitivity analysis was performed for all parameters used in this modified model with two key variables, X_{PHA} and S_S . The modified model was first calibrated with data of substrate consumption and PHA accumulation in the batch experiment of SBR-B on Day 45. Some parameters were set as those reported by Gujer et al. (1999), while other parameters (Y_{PHA} , Y_{PAB} , $Y_{H,S}$, $\mu_{H,S}$, μ_{PAB} , q_{PHA} and K_{PHA}) were estimated in this study as they significantly affect the PHA production and microbial selection process. The function of parameter estimation in AQUASIM V2.1 was conducted through minimizing the chi-squared criterion χ^2 (weighted least squares) between the experimental and simulated results (Reichert et al., 1998). Afterwards, the modified ASM3 model was used to simulate the long-term PHA-accumulating microbial selection process. The initial inoculation conditions applied in the microbial selection experiment were used as the initial conditions of the modified model (Table 4). Then, the modified model was run to simulate the long-term microbial selection of PHA-accumulating microorganisms, and PHA production performance at various OLRs. The simulation results were compared with the experimental measurements.

2.4 PHA extraction and quantification

The mixed sludge samples of 10-mL aliquot (one centrifuge tube) was collected from each SBR with 2 drops of sulfuric acid to stop all biological activity and then was centrifuged at 6000 rpm and 4°C for 10 min. The supernatant was discarded and 2.5 mL acetone was added into the centrifuge tube. After the sludge pellet was completely mixed with acetone, the sample was washed twice with deionized water and freeze dried by vacuum freeze dryer (Labconco, USA).

The quantity and composition of PHA were determined by a gas chromatography (GC, 7820A, Agilent Co., USA). 20-mg lyophilized sample was mixed with 2 mL chloroform, 2 mL 10% (v/v) sulfuric acid in methanol and 0.2 mL (2 g/L) benzoic acid in methanol. Then, the solution was heated for 6 h at 100°C in a 5 mL Wheaton tube. After the sample cooled down, 1-mL deionized water was added. The solution was shaken violently and 1-mL lower organic phase was collected for GC analysis. Following the procedure described by Wang et al. (2014), the GC analysis of PHA samples was performed on GC equipped with a 30 m $\times$ 0.32 mm $\times$ 0.25 μ m HP-5 column. The concentrations of PHB and PHV were calibrated through standard curves with a solution made of a commercial co-polymer of PHB-PHV (95:5 w:w) (Sigma Co., USA) and benzoic acid as internal standard. The PHA content (%) was calculated as PHA weight (g) divided by the cell dry weight (g) of GC samples. The yield of PHA was calculated as PHA weight (g) divided by the consumed COD (g) in the batch experiments.

2.5 Analytic methods

The COD, NH_4^+ , TN and TP in the influent and effluent were monitored every five days. The PHA samples were collected at the end of feast period and the end of the famine period every 5 days. Mixed liquid volatile suspended solids (MLVSS) and sludge volume index (SVI) were also measured every 5 days.

The DO concentration in three reactors was measured with a HQ30d DO electrode (HACH Inc., USA) while the pH was monitored with a 6010M pH electrode (JENCO, USA). COD, NH_4^+ , TN, TP, MLVSS and SVI were measured according to the Standard Methods (APHA., 2005). Simulations were done in AQUASIM V2.1 (Reichert et al., 1998).

2.6 Microbial community analysis

Microbial samples were collected from the three SBRs for microbial community analysis on 0 d, 15 d, 30 d and 45 d. Total genomic DNA from the activated sludge samples was extracted using CTAB/SDS method. Sequencing libraries were generated using NEB Next®Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit®2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. DNA was amplified using the 341F/806R primer set (341F: 5'-CCTAYGGGRBGCASCAG-3', 806R: 5'-GGACTACNNGGGTATCTAAT-3'), which targets at the V3–V4 region of the bacterial 16S rDNA. Sequencing analysis

was performed by UPARSE software package using the UPARSE-OTU and UPARSE-OTUref algorithms. Sequences with $\geq 97\%$ similarity were assigned to the same OTUs.

3. Results and Discussion

3.1 Effluent quality, sludge properties and PHA content in three SBRs

In order to investigate the effects of OLR on PHA content and PHA-accumulating microorganisms selection, the three reactors (SBR-A, SBR-B and SBR-C) with various OLRs (1.2, 2.4, and 3.6 gCOD/L/d) were used. The sludge properties, substrate utilization, and PHA synthesis in the three reactors were monitored. The influent and effluent concentrations of COD, NH_4^+ , TN and TP in the experiments under various OLR are shown in Fig. 1. The influent NH_4^+ , TN and TP in the three reactors were almost exhausted. The mean COD removal efficiency in the three reactors reached 91.8%, 96.2% and 93.2%, respectively, demonstrating that rice winery wastewater could be easily degraded by the microorganisms in activated sludge.

The MLVSS and SVI values of the activated sludge in the three reactors are presented in Fig. 2. In SBR-A, the MLVSS decreased from 2750 mg/L to 920 mg/L while the SVI value increased from 77 to 173, suggesting the deterioration of settling

performance of activated sludge and the loss of mass sludge from the reactor (Fig. 2A). In SBR-B, the values of MLVSS and SVI increased from 2320 mg/L and 83 to 5230 mg/L and 169, respectively, indicating that carbon source was sufficient for microbial growth and maintenance at OLR of 2.4 gCOD/L/d (Fig. 2B). In SBR-C, the MLVSS decreased while the SVI increased sharply due to the filamentous sludge bulking, implying that a high OLR of 3.6 gCOD/L/d and relatively low concentrations of nitrogen and phosphorus stimulated the growth of filamentous bacteria (Fig. 2C).

The contents of PHA in the three reactors in the experiments are illustrated in Fig. 3. The maximum PHA content in SBR-C (5.31-10.86%) was higher than that in SBR-A (0.34-4.06%) and SBR-B (1.08-4.01%) due to the high OLR (3.6 gCOD/L/d) in the SBR-C. The maximum PHV contents in the three reactors increased from 0.32%, 1.25% and 0.99% to 2.70%, 2.36% and 1.50%, respectively (Fig. 3E). The maximum PHB content in SBR-C was high (5.95%-8.32%) in the initial 20 days of experiments and then decreased to 3.81% because of the filamentous sludge bulking (Fig. 3C). However, fewer PHB (<1.16%) was produced in the end of feast period in SBR-A and SBR-B. These results indicate that OLR had a significant effect on PHA production and rice winery wastewater was preferentially used for PHV synthesis. The high OLR in SBR-C and unbalanced C/N ratio (COD/TN=74.9) of the rice winery wastewater resulted in sludge bulking. Our previous study also show that a high C/N ratio led to the outgrowth of filamentous microorganisms and a drop in sludge concentration (Fang et al., 2009). In addition, the increased C/N ratio brought

about a decrease in the observed coefficient of active-biomass yield ($Y_{\text{obs,BA}}$) and an increase in the observed coefficient of PHA yield ($Y_{\text{obs,PHA}}$) (Pokoj et al., 2019).

However, Sepehri and Sarrafzadeh (2019) found that the minimum C/N ratio remarkably reduced the production of microbial metabolites and enhanced nitrification efficiency in the activated sludge process. Therefore, a proper OLR and C/N ratio should be found to achieve microbial selection of PHA-accumulating microorganisms.

After 45-d operation, batch experiments were conducted to investigate the substrate consumption and PHA production in one cycle in the three reactors. As shown in Fig. 4, COD, TN and TP were quickly consumed by microorganisms in the three reactors. The PHA production in the batch experiments is listed in Table 2. The highest PHA yield of 0.23 gCOD/gCOD was achieved in SBR-B, which achieved the same level of PHB yield produced by *Burkholderia cepacia* using straw hydrolysates as substrate (0.25 gCOD/gCOD) (Cesario et al., 2014). The PHA yield was the lowest (0.115 gCOD/gCOD) in SBR-A, probably due to its low concentrations of substrate and MLVSS. At a high OLR of 3.6 gCOD/L/d, the PHA yield was also relatively low (0.152 gCOD/gCOD), indicating that the storage ability of the PHA-accumulating microorganisms was inhibited. This result is consistent with the results reported by Jia et al. (2013), who found that the PHA production was inhibited by a high OLR. Therefore, our results suggest that OLR of 2.4 gCOD/L/d was favorable for PHA accumulation.

3.2 Shifts of microbial community at various OLR

The high-throughput sequencing of the sludge samples from the three reactors in 45-d operation was performed. The taxonomic composition of bacterial populations in the three reactors was dominated by *Proteobacteria*, followed by *Bacteroidetes*, *Verrucomicrobia*, *Patescibacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes* and *Planctomycetes*. Similarity analysis indicate that SBR-A and SBR-B were similar in terms of bacterial distribution after 45-d operation, while they were different from the SBR-C and the seeding sludge.

Genus level distribution of bacterial populations within three reactors is presented in Fig. 5. Numerous typical PHA-accumulating microorganisms were detected in the three reactors. *Zoogloea*, *Rhodobacter* and *Rubrivivax* present in all the three reactors were identified as PHA-accumulating microorganisms (Ciesielski et al., 2010, Jiang et al., 2011, Wieczorek et al., 1996). Besides, *Leptothrix*, *Burkholderiaceae*, *Comamonas*, *Haliscomenobacter*, *Rhodobacteraceae*, *Amaricoccus* and *Plasticicumulans*, reported as PHA-accumulating microorganisms in previous literature, were also detected in the three reactors (Dionisi et al., 2002, Koller et al., 2010, Lemos et al., 2008, Obruca et al., 2018, Tamis et al., 2014). However, their abundances were variable in the three reactors with various OLR.

The relative abundances of PHA-accumulating microorganisms within the three SBRs are summarized in Table 3. *Zoogloea* was the main PHA-accumulating

microorganisms in all three reactors, while other PHA-accumulating microorganisms were relatively low in abundance. The total percentage of PHA-accumulating microorganisms within the three reactors reached 18.2%, 29.6% and 17.3%, respectively, after 45-d operation. The highest abundance of PHA-accumulating microorganisms in SBR-B was consistent with the highest PHA yield (0.23 g/g), implying that a moderate OLR (e.g., 2.4 gCOD/L/d of this work) was favorable for PHA-accumulating microorganisms enrichment, while too low or too high OLR would lead to PHA-accumulating microorganisms out-competitive. For example, Sepehri and Sarrafzadeh (2018) found that the growth of nitrifying bacteria at a low OLR was greatly accelerated, which was not favorable for the selection of PHA-accumulating microorganisms.

3.3 Modeling results of the batch and long-term experiments

The sensitivity analysis of all parameters was conducted to identify the most determinant parameters on S_S and X_{PHA} . The parameters Y_{PHA} , $Y_{H,S}$, q_{PHA} , and $miu_{H,S}$ had a significant effect on S_S , while the parameters Y_{PHA} , $Y_{H,S}$, Y_{PAB} , q_{PHA} , miu_{PAB} , and K_{PHA} had a considerable influence on X_{PHA} . Therefore, seven parameters (Y_{PHA} , Y_{PAB} , $Y_{H,S}$, $\mu_{H,S}$, μ_{PAB} , q_{PHA} and K_{PHA}) were chosen to be calibrated in this study.

The data from the batch experiments of SBR-B were chosen for parameter estimation. The experimental data and the results predicted with the calibrated model for SBR-B were compared. The good agreement between the experimental

measurements and the model predictions of the substrate consumption process (S_S) was observed. Moreover, the model results and measured data of PHA production and utilization process (X_{PHA}) in the batch experiment also exhibited good consistency. The estimated average regression coefficient (R^2) reached 0.89 and 0.83, respectively.

In this modified model, the concentration change of X_{PAB} and X_H was an important indicator of PHA-accumulating microorganism selection process. In addition, the percentage of PHA-accumulating microorganisms in the total microorganisms (f_{PAB}), which was equal to X_{PAB} divided by the sum of X_{PAB} and X_H , was also a significant factor affecting PHA production. Therefore, the modified model was further used to simulate the long-term microbial selection of PHA-accumulating microorganisms and performance of PHA production at OLR of 2.4 gCOD/L/d. As illustrated in Fig. 6, the concentrations of X_H and X_{PAB} in the modeling process gradually reached steady-state values of 4257 and 1844 mg/L, respectively. Furthermore, the abundance of PHA-accumulating microorganisms in total microorganisms (f_{PAB}) predicted by the model changed from 14.7% to 30.2%, indicating the results of the microbial selection process of PHA-accumulating microorganisms.

The comparison between the model results and measured data at day 45 is listed in Table 4. The simulation and measured results of f_{PAB} were 30.2% and 29.6%, respectively. Moreover, the maximum X_{PHA} reached 123.7 and 135.6 mgCOD/L, respectively. The error percentages between model results and measured data of f_{PAB}

and the maximum X_{PHA} were 2.0% and 8.7%, respectively. The low error percentage and good consistency between the long-term simulation results and measured data indicate that the modified model could effectively describe the long-term PHA-accumulating microorganism selection process.

The estimated yield coefficient of PHA storage (Y_{PHA}) on rice winery wastewater was 0.41 mg COD/mg COD in our study, indicating that nearly 40% of consumed COD was transformed as PHA. However, the yield coefficient was lower than that (0.54) reported by Dias et al (2005). Different model structures, carbon sources, operation settings and microbial community might be responsible for the difference in yield coefficient. Although the Y_{PHA} on rice winery wastewater was not high as that on sodium acetate, the negative environmental value of rice winery wastewater and its need of treatment endow it a good substrate for PHA production. The values of some important parameter, such as OLR, X_H , X_{PAB} , and PHA yield, etc., are useful for further mathematical model improvement and PHA production with rice winery wastewater in full scale applications.

3.4 Modeling the impact of OLR on microbial selection of PHA-accumulating microorganisms

To further understand the effect of OLR on PHA-accumulating microorganisms, the above established model was used to simulate the variations of X_H , X_{PAB} and maximum X_{PHA} at various OLRs (ranging from 0.3 to 3.0 gCOD/L/d). Due to the

filamentous bulking at high OLRs (>3.0 gCOD/L/d), the results of high OLRs (>3.0 gCOD/L/d) was not considered in this modified model. The identical initial microbial community data (Table 4) with various OLRs were used in the long-term modeling, and the results of X_H , X_{PAB} and max X_{PHA} at various OLRs are summarized in Table 5. The modeling results show the modified ASM3 model successfully simulated the microbial selection process of PHA-accumulating microorganisms at low and moderate OLRs (i.e., <2.4 gCOD/L/d) and moderate OLR (2.4 gCOD/L/d) was beneficial for microbial selection and PHA production.

The OLR had an influence on microbial selection process of PHA-accumulating microorganisms through altering the feast/famine ratio and provided both the physiological and ecological selective pressures (Chen et al., 2017). Increased OLR leads to an extension of the feast phase and an increase in the F/F ratio, which would change the microbial community (Sepehri and Sarrafzadeh, 2019). Albuquerque et al. (2010) proposed that non-storing microorganisms might be able to endure these shorter famine phases, which was unbeneficial for the selection of PHA-accumulating microorganisms. The shorter famine phase might not ensure the decrease of growth cofactors to a point that limits microbial growth and favors PHA accumulation in the subsequent feast phase (Albuquerque et al., 2010). In addition, the high OLR with low N and P concentrations may cause filamentous bulking, which deteriorates the settling and PHA production of activated sludge and impairs the subsequent PHA recovery

process. Therefore, the upper limit of the OLR provided by different substrates suitable for microbial selection and PHA production warrants further investigations.

4. Conclusions

The effects of OLR on PHA accumulation and PHA-accumulating microorganisms selection with rice winery wastewater as substrate were investigated in this study. The highest PHA yield of 0.23 gCOD/gCOD was achieved in SBR-B. *Zoogloea* was the most dominant PHA-accumulating microorganisms in three reactors and the highest content of PHA-accumulating microorganisms (29.6%) was presented in SBR-B. The modified ASM3 model was established to evaluate and explore the effects of different OLR on the microbial selection and PHA accumulation. The experimental and model results implied that moderate OLR was beneficial for PHA accumulation and microbial selection of PHA-accumulating microorganisms.

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Appendix A. Supplementary data

E-Supplementary data for this work can be found in e-version of this paper online.

References

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Figure Captions

Fig. 1. Influent and effluent quality in the three reactors at various OLRs in the experiments: (A, D) 1.2 gCOD/L/d; (B, E) 2.4 gCOD/L/d; and (C, F) 3.6 gCOD/L/d.

Fig. 2. Variations of MLVSS and SVI in the three reactors at various OLR during the experiments: (A) 1.2 gCOD/L/d; (B) 2.4 gCOD/L/d; and (C) 3.6 gCOD/L/d.

Fig. 3. PHA content in the sludge from the three reactors during the experiments: (A, C, E) total PHA, PHB, and PHV content at the end of feast phase; and (B, D, F) total PHA, PHB, and PHV content at the end of famine phase.

Fig. 4. Concentrations of COD, TN and TP in batch experiments: (A, D) 1.2 gCOD/L/d; (B, E) 2.4 gCOD/L/d; and (C, F) 3.6 gCOD/L/d.

Fig. 5. Genus level distribution of bacterial populations within three reactors: (A) 1.2 gCOD/L/d; (B) 2.4 gCOD/L/d; and (C) 3.6 gCOD/L/d.

Fig. 6. Long-term simulated results of (A) X_H , X_{PAB} , and (B) $\max f_{PAB}$ at OLR of 2.4 gCOD/L/d using the modified ASM3 model.

Table 1 Nomenclature of variables of modified PHA model used in this study

Component	Definition	Unit
Model dissolved components		
S_s	External substrate from rice winery wastewater	mgCOD/L
S_O	Dissolved oxygen	mgO ₂ /L
S_{NH4}	Ammonia nitrogen	mgN/L
Model particulate components		
X_H	Active heterotrophic bacteria without PHA accumulation	mgCOD/L
X_{PAB}	Active PHA-accumulating microorganisms	mgCOD/L
X_{PHA}	PHA accumulated in X_{PAB}	mgCOD/L
X_I	Residual inert organics	mgCOD/L

Table 2 Performance of PHA production by selected sludge at day 45 in batch experiments

SBR	MLVSS (g/L)	WV (L)	OLR (gCOD/L/d)	Max PHA content (%)	PHA yield (g/g)
A	0.92	3	1.2	3.76	0.12
B	5.23	3	2.4	2.59	0.23
C	3.43	3	3.6	3.99	0.15

WV: working volume (L)

PHA yield: g per PHA yield per g COD consumed (g/g)

Table 3 Relative abundances of PHA-accumulating microorganisms within the three reactors in the experiments

PHA producers %	SBR- A			SBR-B			SBR-C		
Genus	15 d	30 d	45 d	15 d	30 d	45 d	15 d	30 d	45 d
<i>Zoogloea</i> ^a	14.4	5.0	12.9	30.3	7.0	25.0	41.1	31.6	11.9
<i>Rhodobacter</i> ^a	2.0	3.0	1.0	2.9	3.2	0.6	1.3	1.3	1.1
<i>Rubrivivax</i> ^a	2.6	0.6	1.0	2.1	1.1	1.0	0.6	0.1	0.1
<i>Leptothrix</i> ^a	0.9	0.5	0.2	1.0	0.2	0.1	2.5	2.0	0.1
<i>α-proteobacteria</i> ^b	2.5	0.9	0.4	1.5	1.5	0.4	0.3	0.7	0.3
<i>Burkholderiaceae</i> ^c	0.7	0.8	0.5	0.9	1.1	1.1	0.8	0.9	0.6
<i>Comamonas</i> ^a	1.1	1.0	0.9	0.7	0.7	0.3	0.5	0.2	0.1
<i>Haliscomenobacter</i> ^d	0.8	0.4	0.8	0.5	0	0.2	0.3	1.5	1.0
<i>Rhodobacteraceae</i> ^a	0.5	0.8	0.3	1.0	1.5	0.4	0.9	0.7	0.4
<i>Amaricoccus</i> ^e	0.1	0.1	0.2	0.2	0.2	0.3	0.1	0.9	1.7
<i>Plasticicumulans</i> ^f	0.3	0	0	0.2	0	0	0.9	1.3	0
Total	25.9	13.1	18.2	41.3	16.5	29.6	49.3	41.2	17.3

^a Koller et al. (2010) ^b Morgan-Sagastume (2016) ^c Obruca et al. (2018) ^d Dionisi et al.

(2002) ^e Lemos et al. (2008) ^f Tamis et al. (2014)

Table 4 Model conditions and results of the long-term microbial selection of PHA-accumulating microorganisms

	X_H	X_{PAB}	X_{total}	f_{PAB}	$Max X_{PHA}$
	(mg/L)	(mg/L)	(mg/L)	(%)	(mg/L)
Initial conditions	2664	336	3000	11.2 ^a	-
Measured results in batch experiment on day 45	3685	1549	5234	29.6 ^a	135.6
Model results on day 45	4257	1844	6101	30.2 ^b	123.7

X_{total} : the sum of X_H and X_{PAB}

f_{PAB} : the percentage of X_{PAB} in X_{total}

^a, the value of f_{PAB} determined by 16s rDNA

^b, the value of f_{PAB} determined by model simulation

Table 5 Model results of long-term microbial selection of PHA-accumulating microorganisms at various OLRs on Day 45

OLR	X_H	X_{PAB}	X_{total}	f_{PAB}	Max X_{PHA}
(gCOD/L/d)	(mg/L)	(mg/L)	(mg/L)	(%)	(mg/L)
0.3	491	201	692	29.0	15.2
0.6	987	408	1395	29.2	30.6
1.8	3109	1325	4434	29.8	95.9
2.4	4257	1844	6101	30.2	123.7
3.0	5472	2411	7883	30.6	158.5

Fig. 1. Influent and effluent quality in the three reactors at different OLRs in the experiments: (A, D) 1.2 gCOD/L/d; (B, E) 2.4 gCOD/L/d; and (C, F) 3.6 gCOD/L/d.

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Research Highlights

- Rice winery wastewater was a proper substrate for PHA production by MMC
- Effects of OLR on the PHA microbial selection were investigated
- Moderate OLR was beneficial to microbial selection and PHA accumulation
- *Zoogloea* was the most dominant PHA-accumulating microbe in the reactors
- Modified ASM3 model was built to describe long-term PHA microbial selection