Granulation of activated sludge using butyrate and valerate as additional carbon source and granular phosphorus removal capacity during wastewater treatment

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Abstract

As an efficient and low-cost phosphorus (P) removal method from wastewater, enhanced biological phosphorus removal process always faces the insufficient carbon source issue. In this study, two identical sequencing batch reactors were used to cultivate aerobic granular sludge, in which butyrate(Rb) and valerate(Rv), two major volatile fatty acids that can be produced from anaerobic fermentation of waste biomass, were respectively applied as additional carbon source. Both reactors exhibited almost same excellent organics and total nitrogen removals during 120 days' operation, about 95.2-95.7% and 67.9-68.0% respectively with noticeable difference in P removal. Compared to the granules in Rv (24.3 mg P/g-total solids), bigger and more stable ones with higher P removal capacity (11.5 mg P/g-volatile solids·d) were finally achieved in Rb, containing higher P content (36.0 mg P/g-total solids) with more orthophosphate and polyphosphate accumulated. Microbial community analysis reflected more polyphosphate-accumulating organisms (Rhodocyclus-related bacteria and Actinobacteria) in the granules from Rb.

Keywords: Enhanced biological phosphorus removal (EBPR); Aerobic granular sludge (AGS); Butyrate; Valerate; Polyphosphate-accumulating organisms (PAOs)

1. Introduction

As an efficient and low-cost phosphorus (P) removal technology, enhanced biological phosphorus removal (EBPR) process has been recognized its promising potential for P removal in wastewater treatment plants (WWTPs) (Oehmen et al., 2007). It also offers promise for P recovery from wastewater if being appropriately integrated with subsequent P extraction/purification steps. The success of EBPR process is mainly attributed to polyphosphate-accumulating organisms (PAOs) which can uptake carbon sources and release phosphate into wastewater under anaerobic condition, and alternatively accumulate excessive amount of phosphate into cells under aerobic condition (Cai et al., 2013; Hollender et al., 2002). On the other hand, owing to its excellent settleability, high biomass retention, ability to endure organic loading fluctuation and high tolerance to toxicants, aerobic granular sludge (AGS) has been successfully applied in practice and paid more attention nowadays (Adav et al., 2008; Pronk et al., 2015; Tiwari et al., 2018). In addition, AGS has also been reported to integrate with EBPR process, exhibiting excellent P removal efficiency with the formation of P-rich granular sludge (Cai et al., 2016; Wu et al., 2010).

However, in the real world of wastewater treatment, P removal is not so efficient by using EBPR process, most probably due to lack of sufficient carbon source in wastewater (Gong et al., 2018; Guerrero et al., 2015; Yang et al., 2018). Additional carbon sources, such as volatile fatty acids (VFAs), glucose, methanol and ethanol are usually tested for EBPR process (Hollender et al., 2002; Oehmen et al., 2007; Taya et al., 2013; Wang et al., 2013). Among them, VFAs have been regarded as the most

suitable and commonly used carbon source for EBPR (Ji and Chen, 2010; Oehmen et al., 2007). While addition of commercial VFAs would directly increase the overall operation costs and carbon footprint of the WWTPs, which is not in line with their sustainable development goal(SDG) and management. As it is known, VFAs-rich fermentation liquor can be efficiently and purposefully produced from waste biomass such as livestock manure, sewage sludge in the WWTP itself, etc. which might be potentially utilized as additional carbon source for biological P removal processes (Huang et al., 2016; Ji and Chen, 2010; Liu et al., 2018; Esteban-Gutiérrez et al., 2018). In general, acetic (HAc), propionic (HPr), butyric (HBu) and valeric (HVa) acids are the main VFA species in the VFAs-rich fermentation liquor. Among these four VFAs, acetate and propionate are usually concerned and used as carbon source to investigate their impact on EBPR systems (Oehmen et al., 2007; Wan et al., 2015; Cai et al., 2016). In most cases, HAc and HPr occupy high proportions of the total VFAs in the VFAs-rich fermentation liquor. As a matter of fact, the amount of HBu and HVa may account for 20 - 40% of the total VFAs after anaerobic fermentation of swine manure or waste activated sludge (Huang et al., 2016; Yuan et al., 2009), and HBu may even reach 60% during the acidic fermentation of winery wastewater at 55°C (Esteban-Gutiérrez et al., 2018). Up to now, however, little information is available on the effect of HBu and HVa on EBPR process or AGS biotechnology. Restated, different carbon sources may have different profound influences on the granulation process, granule morphology, structural strength, P accumulation and microbial communities of AGS (Tay et al. 2002; Wei et al., 2014; Pronk et al., 2015; Wan et al.,

2015; Cai et al., 2016). Thus, before the VFAs-rich fermentation liquor being utilized as additional carbon source for EBPR and AGS processes in WWTPs, the effect of HBu and HVr on them should be clarified.

In this study, AGS-based EBPR process was applied to treat high P concentration wastewater (~ 50 mg P/L). In addition to glucose as the co-existing biodegradable organic substance, the two VFAs, namely HBu and HVa, about 500 mg of chemical oxygen demand (COD) per unit liter (mg COD/L) were used as additional carbon source and added into two identical sequencing batch reactors (SBRs), respectively. Aerobic granulation was first performed and then nutrients removals by the two reactors were evaluated. P accumulation and P species were analyzed and compared between the seed activated sludge and cultivated granules. Besides, microbial biodiversity in the granules cultivated with HBu and HVa were also compared to probe the mechanisms involved.

2. Materials and methods

2.1. Reactors and operation

Two identical laboratory-scale cylindrical column SBRs were used in this study, each with a working volume of 1.30 L (60 mm in diameter and 460 mm in effective height). The two SBRs were operated automatically at room temperature (25±2°C) by using almost the same operation strategy as described in Cai et al. (2016). Initially, one 6-h cycle consisted of feeding for 2 min, non-aeration for 60 min, aeration for 276 min, settling for 20 min and decanting for 2 min. During the first 5 days' operation,

the settling time was gradually reduced to 3 min with the remaining 17 min being used for aeration. The aeration strength, volumetric exchange ratio, hydraulic retention time (HRT) and sludge retention time (SRT) were controlled at 0.6 cm/s, 50%, 12 h, and ~10 days, respectively. Dissolved oxygen (DO) was kept between 6-8 mg/L during the aeration period. The whole experiment lasted for 120 days.

2.2 Influent characteristics and seed sludge

The reactors were inoculated with 1.0 L activated sludge sampled from the secondary sedimentation tank of the Shimodate Sewage Treatment Plant, Ibaraki Prefecture, Japan. The initial concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the two reactors were 4.76 g/L and 3.52 g/L (MLVSS/MLSS=0.74), respectively. Except the different VFAs used in this study (HBu and HVr), the composition of the synthetic wastewater was as the same as that in Cai et al. (2016), with a COD:N:P=20:2:1. In addition to 500 mg COD/L of glucose in the influent, sodium butyrate (~500 mg COD/L) and sodium valerate (~ 500 mg COD/L) were added into the two reactors as additional carbon source, which were labeled as Rb and Rv, respectively, to explore their effect on P accumulation and granule formation.

2.3. Analytical methods

All the parameters related with reactor performance, including ML(V)SS, total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), and sludge settleability indicated by sludge volume index (SVI) were measured using the same methods described elsewhere (Cai et al., 2016; Cai et al., 2018).

Granular size was monitored every 10 days by a stereo microscope (STZ-40TBa, SHIMADZU, Japan). A scanning electron microscope (SEM, JSM6330F, Japan) was used to observe the morphology of granules at the end of experiment after being pretreated with the method described by Wu et al. (2010). Settling velocity was determined according to Cai et al. (2018), in which about 100 granules were randomly sampled from the two reactors respectively for this determination with their average values being reported in this work. The strength of granules was estimated by the method described previously (Cai et al., 2016).

The methods and conditions for extracellular polymeric substances (EPS) extraction and analysis, metal ions content, TP content and fractionation in granules were the same as those in a previous work (Cai et al., 2016). In this study, ICP-OES (Perkin-Elmer Optima 7300DV, USA) and Bruker Avance-600MHz NMR Spectrometer were also used to quantify metal ions and P species in granules.

Microbial community analysis was conducted on the granular sludge sampled on day 120 by using the same methods and operation conditions as described in Cai et al. (2016). In brief, the total DNA of granular sludge sample was firstly extracted by using Mo-Bio PowerMax® Soil DNA Isolation Kit (MoBio Laboratories, Inc., USA). Then polymerase chain reaction (PCR) was performed, and the rough full-length 16S rDNA gene was amplified by PCR with a forward primer V4F, 5-AYTGGGYDTAAAGNG-3 and an equimolar mixture of four reverse primers, i.e. V4R1 5-TACCRGGGTHTCTAATCC-3, V4R2 5-TACCAGAGTATCTAATTC-3, V4R3 5-CTACDSRGGTMTCTAATC-3, and V4R4 5-TACNVGGGTATCTAATCC-3

based on the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/pyro/help.jsp). The PCR conditions were as follows: 95°C for 7 min, followed by 32 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final extension step at 72°C for 10 min. The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany). Finally, after quantification using Qubit® 2.0 Fluorometer (Invitrogen, USA), the PCR products of all samples were taken for high-throughput sequencing on Ion Torrent PGM System (Life Technology, USA). Mothur (version: 1.31.2) was used for analysis of microbial biodiversity in the granules.

Nutrients removal efficiencies, including DOC, NH_3-N, TN, and TP removal capacities were calculated according to Cai et al. (2016). All the analyses (except microbial community) were conducted in triplicate and the results were expressed as mean ± standard deviation.

3. Results and discussion

3.1. Granulation and characterization of granules in the two reactors

3.1.1 Change in morphology

The two bioreactors were continuously operated for 120 days. On day 10, granules appeared and gradually became the main existing form of biomass in the reactors. After 120 days' operation, the reactors were dominated by irregular granules with a compact and dense structure and little flocs were observed, indicating that butyrate and valerate could also be used as carbon source for the cultivation of AGS. Seen from the digital images, some granules in both reactors reflected light green in

color, most probably due to the growth of microalgae as the reactors were placed close to the window, resulting in their exposure to sunlight during the daytime. SEM observations on the cross section of the granules from the two reactors disclose that the compact structure was composed of various types of bacteria tightly linked to each other. In the core of Rb, more sarciniform-like bacteria were noticed, while Rv was dominated by coccus-shaped bacteria.

3.1.2 Granule size

The sludge was sampled every 10 days for the measurement of particle size as shown in Fig. 1a. On an average, the sludge particle size increased from the initial 0.16 mm to 1.10 mm in Rb and to 0.93 mm in Rv on day 90, respectively. During the subsequent operation, their granule size kept relatively stable, with their average granule diameter being 1.18 (Rb) and 0.92 mm (Rv), respectively. More specifically, after 120 days' operation, > 98% granules were larger than 0.2 mm, indicating a complete granulation according to the definition by Liu et al. (2010) (with no flocs observed and granules smaller than 0.3 mm being below 5%). Bigger granules were always observed in Rb (with butyrate addition), most probably due to its stronger granular strength (as shown in Fig. 1b with lower ΔTurbidity being obtained in the sludge suspension after shaking for 5-20 min). Namely, the granules in Rv were easily to disintegrate to smaller particles. As a result, from the digital images of the granules, small pellets or particles were always observed in Rv, which might come from the broken manure granules.

Fig. 1

3.1.3 Biomass growth

Fig. 2 shows the variations of MLSS and MLVSS/MLSS ratio in the two reactors during the operation. An obvious decrease in MLSS was noticed in both reactors during the first 10 days, probably due to sludge domestication and reduction in settling time (from 20 min to 3 min on day 5), then the biomass in both reactors gradually increased. After day 70, the MLSS in Rb kept increasing to 8.3 g/L on day 90, and later fluctuated at 8.1-8.4 g/L; in contrast, the MLSS in Rv kept relatively stable and fluctuated at 6.8-7.3 g/L during the subsequent operation.

Fig. 2

On the other hand, the MLVSS/MLSS ratios in Rb and Rv sharply decreased to 0.64 and 0.67, respectively on day 10, and recovered to around 0.77 on day 70. After that, the MLVSS/MLSS ratio in Rb slightly decreased (from 0.76 to 0.72), implying that more mineral substances were accumulated (Oehmen et al., 2005). More metals, especially Mg and Ca were detected to accumulate in the granules of Rb, which might have some positive effect on the granular stability (Franca et al., 2018). Table 1 lists the main characteristics of the seed activated sludge and granules obtained in the two reactors on day 120, including settling velocity, moisture and EPS contents, etc. Clearly seen, the granules in Rb exhibited a relatively lower SVI, higher settling velocity and lower moisture content in comparison to those in Rv. These results also imply that butyrate might be more favorable for granule cultivation in comparison to valerate under the tested operation strategy, and the granules in Rb might have better nutrients removal and P accumulation capacity.

Table 1

3.2. C, N and P removals and P accumulation

3.2.1. C and N removals

The two SBRs were evaluated on their performance of C, N, and P removals during the 120 days' operation. Both reactors exhibited excellent DOC and NH_3-N removals under the test conditions. The DOC and NH_3-N removal efficiencies were averagely 95.7% and 99.5% in Rb, and 95.2% and 99.7% in Rv, respectively. After 30 days' domestication, Rb and Rv also demonstrated stable TN removal, averagely 68.0% (Rb) and 67.9% (Rv).

3.2.2 P removal

Much difference was observed in effluent TP concentration and TP removal efficiency between the two reactors during the experiment. The initial 15 days seemed to be the domestication period for both reactors, during which very low TP removal was noticed, averagely 37.9% and 38.1% in Rb and Rv, respectively. After day 15, however, both reactors showed an increasing trend of TP removal, averagely about 61.0% (Rb) and 54.4% (Rv) from day 70 to day 80. Rb with butyrate addition exhibited slightly higher TP removal than Rv with valerate addition. After day 80 with mature granule formed, much lower effluent TP concentration was detected in Rb than in Rv, and the TP removal by Rb kept increasing and stabilized at around 70-75% till the end of experiment. While in Rv, the TP removal seemed to be deteriorated, which decreased to 43.9% (in average) during the operation from day 80 to day 120. To further compare the P removal by the two reactors, MLVSS-based P removal

capacity was also calculated every 20 days as illustrated in Fig. 3a. Clearly seen, enhanced P removal was realized in both reactors under the designed operation conditions. During the initial 40 days, the average P removal capacity was significantly enhanced from 5.2 mg P/g-VSS·d to 12.2 mg P/g-VSS·d in Rb and to 11.2 mg P/g-VSS·d in Rv, implying that the supplementation of butyrate or valerate is beneficial for enhanced P removal (Oehmen et al., 2007). During the operation from day 40 to day 60, some decrease in P removal capacity was detected in the two reactors. Compared to Rv, Rb showed relatively stable P removal capacity. The change in P removal capacity in Rv might be associated with the deficiency in nutrients and decreased bioactivity of PAOs (Hu et al., 2017). From a viewpoint of long-term operation, butyrate is more beneficial for biological P accumulation into AGS.

Fig. 3

3.2.3. P accumulation and fractionation

In EBPR processes, P is finally accumulated into the biomass and a net P removal from wastewater can be realized by discharging the P-rich sludge. To reuse the P accumulated in the sludge, P content and fractionation should be further performed (Huang et al., 2015). The change of TP content in the granules during the experiment is shown in Fig. 3b. Before the appearance of stable granules (day 15), the TP content in sludge decreased to some extent, possibly due to the loss of PAOs bioactivity as they need to adapt to the new environment. After granule appeared, P was detected to gradually accumulate into the granular sludge; at the end of

experiment, the P contents in the granules of Rb and Rv were averagely 36.0 mg P/g-TSS and 24.3 mg P/g-TSS, respectively. The higher P removal capacity and more P accumulation in the granules of Rb (with butyrate addition) signal that more PAOs were enriched in the biomass of Rb. This observation implies that butyrate is a more suitable carbon source for PAOs than valerate. The higher P content in the sludge might be the reason of the lower MLVSS/MLSS in the granules of Rb (section 3.1.3, Table 1). It is worth noting that during the final 30 days' operation, the granular P content in Rv slightly decreased from 25.8 mg P/g-TSS (day 90) to 24.3 mg P/g-TSS (day 120), which is in accordance with the decrease in TP removal capacity of Rv (Figs. 3a and 3b).

P species, including IP (orthophosphate (ortho-P), polyphosphate (poly-P) and pyrophosphorus (pyro-P)) and OP (monoester phosphorus (monoester-P) and diester phosphorus (diester-P)) were also identified and quantified in this work, and the major results are presented in Table 2. The TP contents in the extracts were 17.6 mg P/g-TSS, 33.2 mg P/g-TSS and 26.1 mg P/g-TSS for the seed activated sludge, and the granules from Rb and Rv, with the recovery ratios about 94.8%, 92.3% and 95.1%, respectively. These results are comparable to 97.1% obtained by Huang et al. (2015) who used the same extraction method.

Table 2

In the extracts of the seed sludge, and the granules from Rb and Rv, the ortho-P contents were 8.3 mg P/g-TSS, 12.7 mg P/g-TSS and 10.4 mg P/g-TSS, respectively. Clearly, granulation could help to accumulate more ortho-P into the granules, and

more cations (especially Fe, Al and Ca) may precipitate with ortho-P to form the core of granules (Franca et al., 2018). On the other hand, the higher EPS content (Table 1) in the granules can also adsorb more ortho-P (Cai et al., 2016; Huang et al., 2015). Poly-P contents in the extracts were 2.9 mg P/g-TSS, 14.8 mg P/g-TSS and 8.5 mg P/g-TSS, respectively. Compared to the granules from Rv, a remarkable accumulation of poly-P was detected in the granules from Rb, implying the accumulation of more PAOs in the granules, as PAOs can take up ortho-P and synthesize as poly-P. In addition, small amounts of Pyro-P were detected, which might come from the hydrolysis of poly-P (Zhang et al., 2013).

Organic P compounds, on the other hand, are now known to contain many labile species which may play an important role in the P circulation and eutrophication processes in the aquatic ecosystems. In the extracts of the seed sludge, and the granules from Rb and Rv, monoester-P, i.e. the dominant OP component, was about 5.5 mg P/g-TSS, 4.8 mg P/g-TSS and 6.4 mg P/g-TSS, respectively. A small amount of diester-P was also measured in the three extracts, showing some decrease from 0.9 mg P/g-TSS (seed sludge) to 0.5 mg P/g-TSS (Rb) and to 0.6 mg P/g-TSS (Rv), respectively. Monoester-P is mainly associated with the production of nucleotides such as glycerol-6-phoshate (found in cell membrane), and diester-P is closely related to deoxyribonucleic acid (DNA-P), lipid (lipids-P) and teichoic acid (Teichoic-P) in cells (Ahlgren et al., 2011; Huang et al., 2015). Thus, the quantity of these two P species can be used to indicate the concentration of cells in aerobic granules,

suggesting that the relative mass of microorganisms might decrease to some extent due to the accumulation of P.

Restated, the granular systems, especially Rb exhibited excellent organics and nutrients removal.

3.3. Changes in microbial community in the granules

The difference in P removal and accumulation between the granules in Rb and Rv might be resulted from the different microbial communities established in the two reactors after 120 days' operation. Based on the results from high-throughput sequencing, the main classes in the seed sludge and the two granule samples at the end of experiment are shown in Fig. 4. In the seed sludge, the Betaproteobacteria (20.62%), Flavobacteria (18.54%), Alphaproteobacteria (16.29%), Gammaproteobacteria (16.46%) and Sphingobacteria (7.63%) were the dominant five classes that amounted to almost 80% species. Apparently, the initial seed sludge had high microbial diversity, with numerous groups of known microorganisms co-existing in a relatively evenly distributed manner. However, during the cultivation with butyrate and valerate as the additional carbon source, the microbial communities of aerobic granules were largely sifted: Betaproteobacteria and Sphingobacteria were significantly enriched to 26.23%, 29.80% and 13.11%, 12.03%, respectively in Rb and Rv; while the abundances of Flavobacteria and Gammaproteobacteria were decreased. All the other classes (including Deltaproteobacteria, TM7, Actinobacteria, Bacteroidetes, Nitrospira, Acidobacteria and Verrucomicrobiae) experienced shift in abundance during the granulation process. It could be concluded that the microbial

community structure was reshaped at class level mainly in terms of the relative abundances in the two reactors when butyrate and valerate were applied as additional carbon source. Besides, the above microbes have been always reported to be dominant in both activated sludge and AGS systems for biological nutrients removal, suggesting the ubiquitous nature of these microorganisms (Huang et al., 2014; Mehlig et al., 2013; Zengin et al., 2010).

PAOs associated with Accumulibacter have been always detected in Betaproteobacteria species which are regarded as the most important PAOs groups (Kong et al., 2005; Shen and Zhou, 2016). The abundance of Betaproteobacteria increased from 20.62% (seed sludge) to 26.23% (Rb) and 29.80% (Rv) after granulation, indicating that more PAOs were enriched in the granules under the designed operation condition. Rv contained more Betaproteobacteria than Rb, however, it exhibited a lower TP removal capacity than Rb (Fig. 3a). The subclass of Betaproteobacteria closely associated with Rhodocyclus-related bacteria are regarded as candidatus PAOs, as they possess good P removal performance (Mehlig et al., 2013; Oehmen et al., 2007). More Rhodocyclus-related bacteria were detected in Rb than Rv (17.5% versus 12.6%), which might contribute to the higher P removal capacity in Rb.

Besides the Betaproteobacteria, PAOs have been also found in the species of Actinobacteria (Kong et al., 2005; Marques et al., 2017; Mehlig et al., 2013). The abundance of Actinobacteria in the seed sludge was only 0.61%, while it slightly increased to 1.37% (Rb) and 0.92% (Rv) after granulation. Actinobacteria are also

considered as candidatus PAOs that can take up P from wastewater under alternative anaerobic/aerobic conditions (Marques et al., 2017; Mehlig et al., 2013). Kong et al. (2005) claimed that Actinobacteria could not uptake VFAs (e.g., acetate) and glucose under anaerobic or aerobic conditions, which only thrive on organic matters like amino acids. In the synthetic wastewater used in this study, only glucose and butyrate or valerate were added. However, Actinobacteria were enriched in the granules in both reactors, especially Rb. This observation is to some extent in agreement with the finding by Marques et al. (2017) who observed that some Actinobacteria like Tetrasphaera could uptake P anaerobically by using the energy generated from glucose fermentation. The other classes, like Sphingobacteria which have been noticed to exhibit strong adhesion to the surface of calcium precipitates (Wan et al., 2015), were 7.63% in the seed sludge and increased to 13.11% and 12.03% in the granules of Rb and Rv, respectively.

Fig. 4

4. Conclusions

In this study, butyrate and valerate were applied as additional carbon source to test their influence on aerobic granulation and then granules' performance and stability. Mature granules were achieved around 80-90 days in both reactors, and the granules in Rb possessed higher P removal capacity (11.5 mg P/g-VSS·d) and higher granular P content (36.0 mg P/g-TSS) than Rv (8.9 mg P/g-VSS·d and 24.3 mg P/g-TSS). Microbial biodiversity analysis revealed that the addition of butyrate was more

beneficial for the enrichment of PAOs, especially Rhodocyclus-related bacteria and Actinobacteria.

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Appendix A. Supplementary data

E-supplementary data associated with this article can be found in the online version.

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Tables

Table 1

Main characteristics of the seed activated sludge and aerobic granular sludge from the two reactors on day 120.

Parameters	Seed sludge	Rb	Rv
Particle size (mm, average)	0.16	1.18	0.92
MLVSS/MLSS	0.74±0.01	0.72±0.01	0.76±0.01
SVI_5 (mL/g)	120.5±3.9	32.5±2.1	38.2±1.5
Settling velocity (m/h, average)	7.9	27.8	21.5
Moisture content (%)	>99	94.3±0.4	95.7±0.2
EPS (mg/g-VSS)	26.6±0.6	80.3±2.8	94.7±4.6
PN/PS	1.8±0.2	2.6±0.1	3.1±0.3

Note: Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate. ML(V)SS- mixed liquor (volatile) suspended solids, SVI_5- sludge volume index in 5 min, EPS- extracellular polymeric substances, PN- proteins in EPS, PS- polysaccharides in EPS.

Table 2

Average contents of different P fractions in the seed activated sludge, and the granules from the two reactors, which were extracted by PCA + NaOH method and their relative proportions (unit: mg P/g-TSS)

	TP in extract	IP			OP	
		Ortho-P	Poly-P	Pyro-P	Monoester-P	Diester-P
Seed sludge	17.6	8.3	2.9	0.1	5.5	0.9
Rb	33.2	12.7	14.8	0.5	4.8	0.5
Rv	26.1	10.4	8.5	0.2	6.4	0.6

Note: Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate, TP- total phosphorus, IP-inorganic phosphorus, OP- organic phosphorus.

Figures

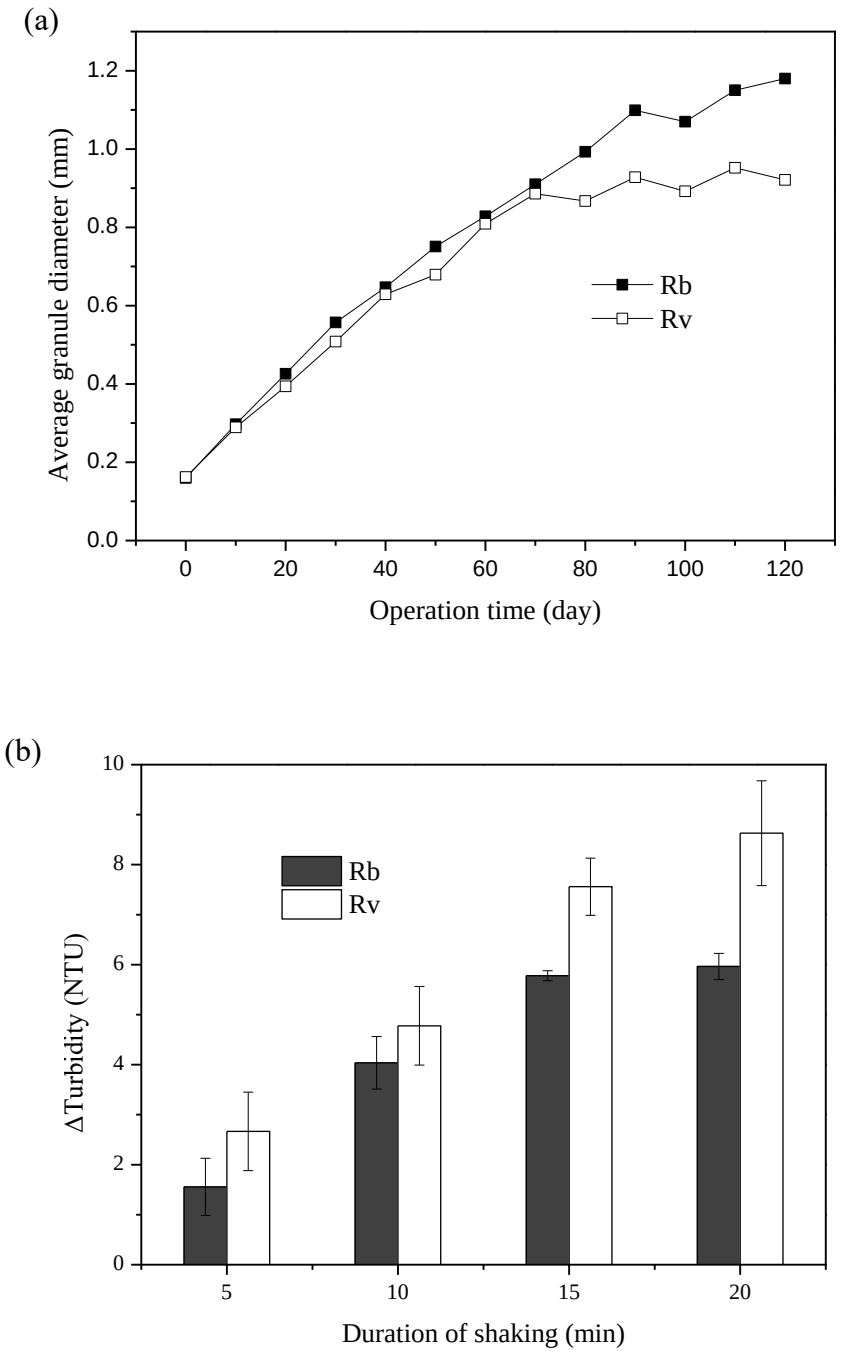

Fig. 1. Variations in average granular size during the 120 days' operation (a), and granular strength on day 120 (b). Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

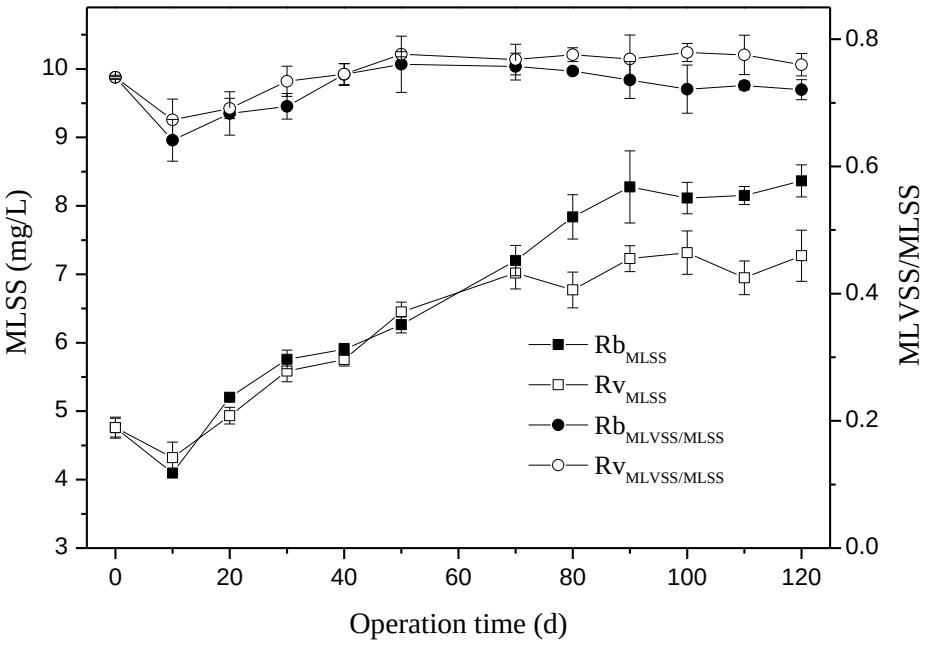

Fig. 2. Changes in MLSS and MLVSS/MLSS ratio during the 120 days' operation. ML(V)SS- mixed liquor (volatile) suspended solids, Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

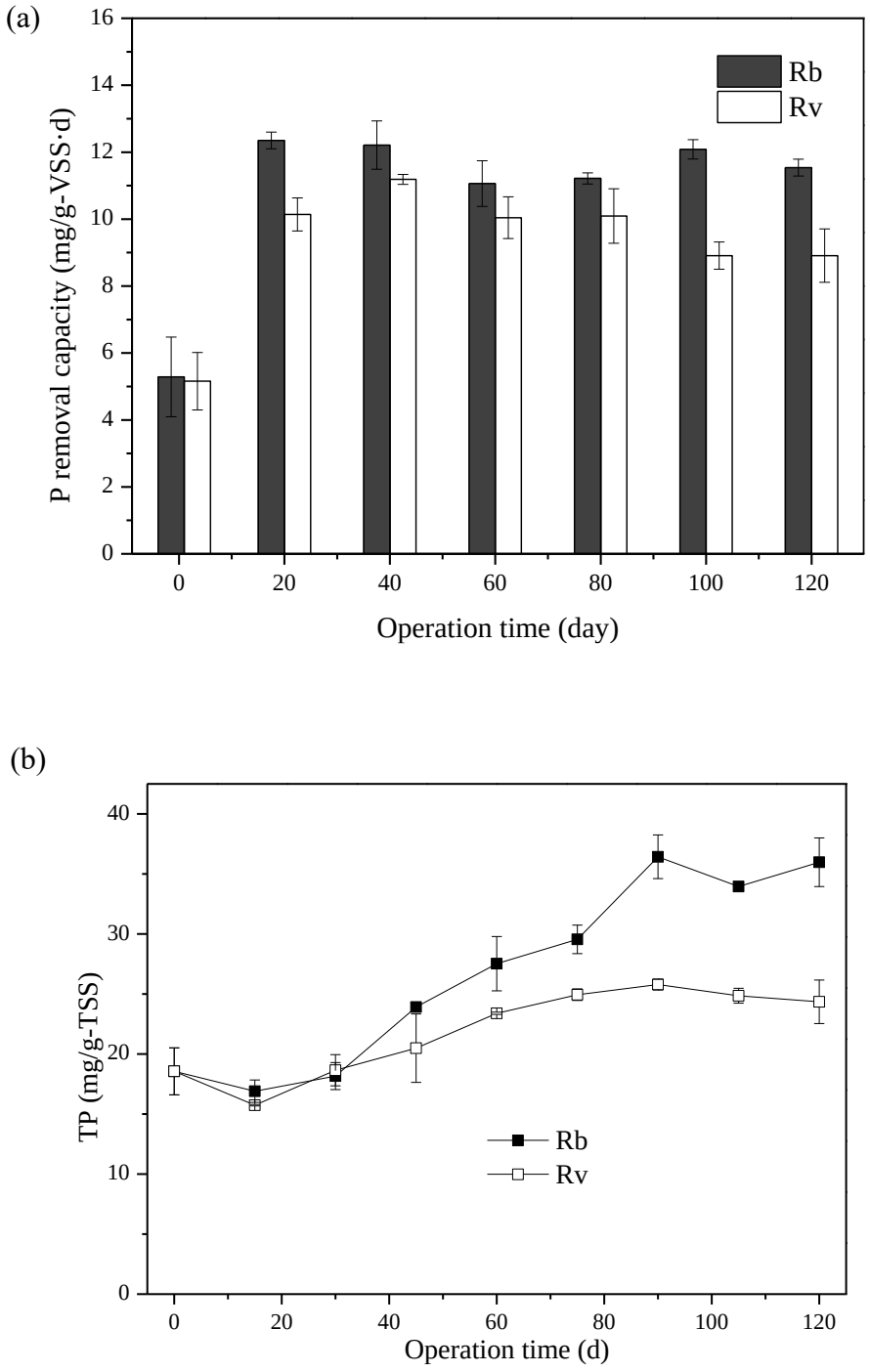

Fig. 3. Changes in P removal capacity (a) and P content in the granules from the two reactors (b). Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

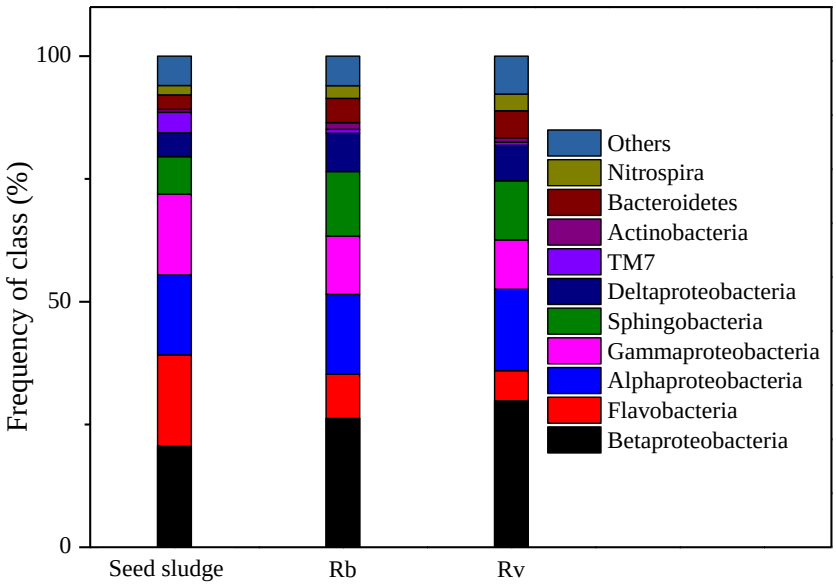

Fig. 4. Abundance of main classes in the seed activated sludge, and the granules from Rb and Rv on day 120, respectively. Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

Supplementary Materials

Granulation of activated sludge using butyrate and valerate as additional carbon source and granular phosphorus removal capacity during wastewater treatment

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Table S1

Average metal contents in the seed activated sludge and the granules from the two reactors (unit: mg/g-TSS).

	Na	K	Mg	Ca	Fe	Mn	Al
Seed sludge	4.5±0.2	3.2±0.3	4.2±0.5	13.3±0.6	3.6±0.4	0.2±0.1	0.3±0.1
Rb	4.6±0.3	8.6±1.1	7.9±0.8	21.3±1.3	15.4±1.8	0.3±0.1	0.9±0.1
Rv	3.9± 1.0	4.6±1.6	4.6±0.1	18.9±1.8	16.5±1.6	0.4±0.1	1.2±0.1

Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

Table S2

Composition of the microbial community in the seed activated sludge and the granules from the two reactors on day 120 based on high-throughput sequencing (unit: %).

Class	Seed sludge	Rb	Rv
Betaproteobacteria	20.62	26.23	29.80
Flavobacteria	18.54	9.02	6.14
Alphaproteobacteria	16.29	16.26	16.60
Gammaproteobacteria	16.46	11.89	10.07
Sphingobacteria	7.63	13.11	12.03
Deltaproteobacteria	4.85	7.79	7.19
TM7	4.16	0.82	0.65
Actinobacteria	0.61	1.37	0.92
Bacteroidetes	2.95	4.92	5.49
Nitrospira	1.91	2.60	3.40
Others	5.98	6.01	7.71

Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

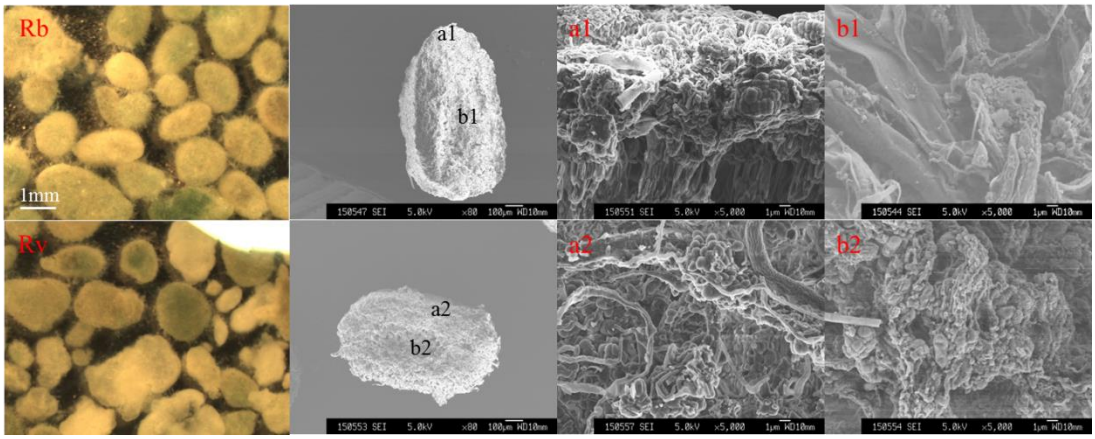
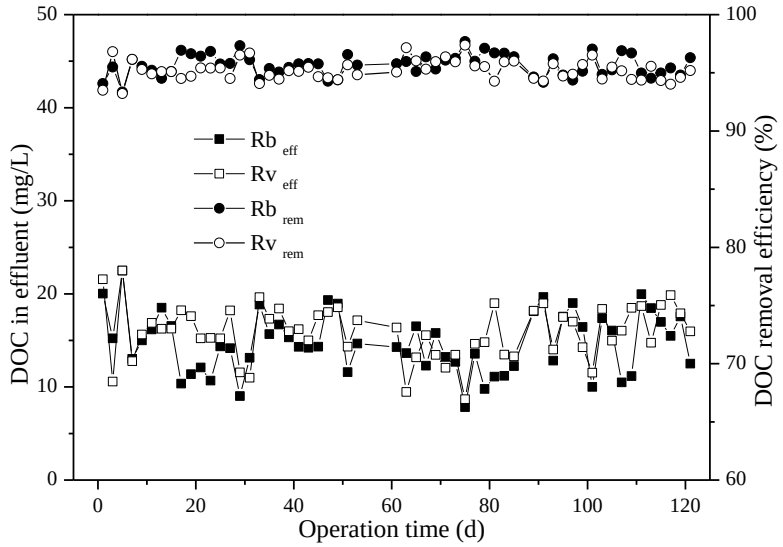
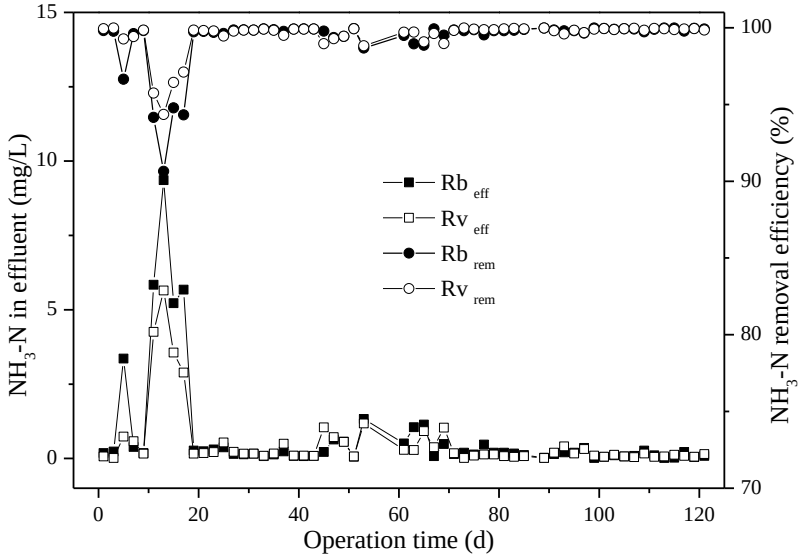


Fig. S1. Appearance and morphology of the aerobic granules at day 120 from the two reactors, Rb and Rv. a1 and a2 are the edge of the granule, and b1 and b2 are the core of the granules from the two reactors, respectively. Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

(a)


DOC in effluent (mg/L)
DOC removal efficiency (%)
Operation time (d)
Rb eff
Rv eff
Rb rem
Rv rem
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120

(b)


NH3-N in effluent (mg/L)
NH3-N removal efficiency (%)
Operation time (d)
Rb eff
Rv eff
Rb rem
Rv rem
0
5
10
15
70
80
90
100
0
20
40
60
80
100
120

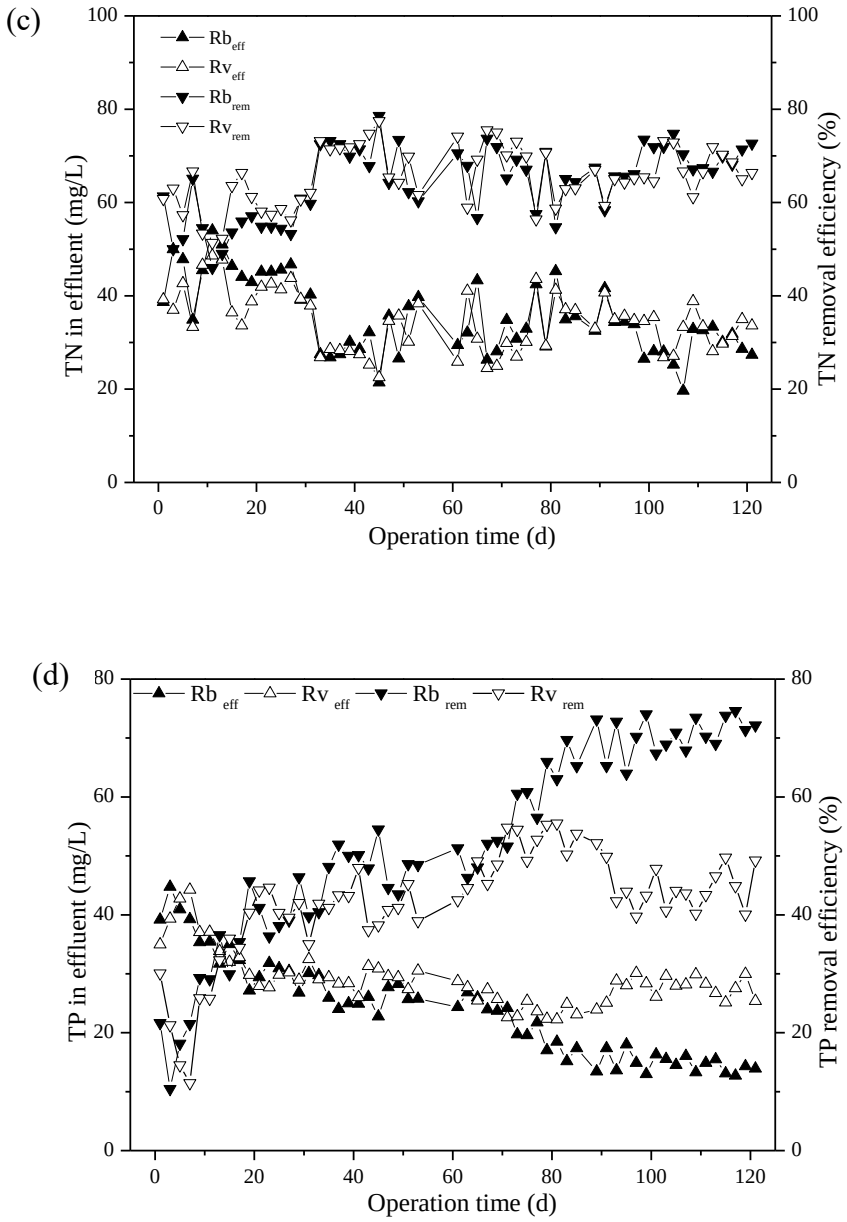


Fig. S2. DOC (a), NH_3-N (b), TN (c) and TP (d) removals by the two reactors during the 120 days' operation. Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.

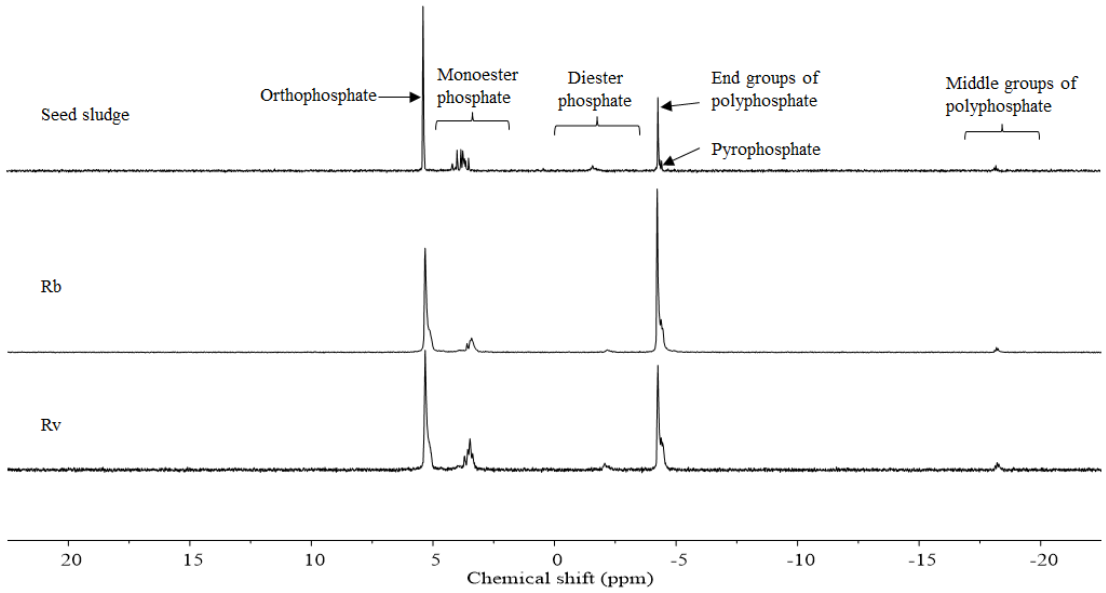


Fig. S3. Typical ^{31}P NMR spectra of NaOH + Na_2EDTA extracts from the seed activated sludge and the granular sludges from Rb and Rv on day 120. Rb- the reactor fed with glucose and butyrate, Rv- the reactor fed with glucose and valerate.