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Global warming readiness: Feasibility of enhanced biological phosphorus removal at 35 °C

Guanglei Qiu [a,b,*], Yingyu Law [b], Rogelio Zuniga-Montanez [b,c], Xuhan Deng [a], Yang Lu [b], Samarpita Roy [b], Sara Swa Thi [b], Hui Yi Hoon [b], Thi Quynh Ngoc Nguyen [b], Kaliyamoorthy Eganathan [d], Xianghui Liu [b], Per H. Nielsen [b,e], Rohan B.H. Williams [d], Stefan Wuertz [b,c,f,*]

[a] School of Environment and Energy, South China University of Technology, Guangzhou 510006, China
[b] Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, 637551, Singapore
[c] Department of Civil and Environmental Engineering, University of California, One Shields Avenue, Davis, CA 95616, United States
[d] Singapore Centre for Environmental Life Sciences Engineering, National University of Singapore, 119077, Singapore
[e] Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Aalborg DK-9220, Denmark
[f] School of Civil and Environmental Engineering, Nanyang Technological University, 639798, Singapore

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ABSTRACT

Recent research has shown enhanced biological phosphorus removal (EBPR) from municipal wastewater at warmer temperatures around 30 °C to be achievable in both laboratory-scale reactors and full-scale treatment plants. In the context of a changing climate, the feasibility of EBPR at even higher temperatures is of interest. We operated two lab-scale EBPR sequencing batch reactors for > 300 days at 30 °C and 35 °C, respectively, and followed the dynamics of the communities of polyphosphate accumulating organisms (PAOs) and competing glycogen accumulating organisms (GAOs) using a combination of 16S rRNA gene metabarcoding, quantitative PCR and fluorescence *in situ* hybridization analyses. Stable and nearly complete phosphorus (P) removal was achieved at 30 °C; similarly, long term P removal was stable at 35 °C with effluent PO_4^{3-}-P concentrations < 0.5 mg/L on half of all monitored days. Diverse and abundant *Candidatus* Accumulibacter amplicon sequence variants were closely related to those found in temperate environments, suggesting that EBPR at this temperature does not require a highly specialized PAO community. A slow-feeding strategy effectively limited the carbon uptake rates of GAOs, allowing PAOs to outcompete GAOs at both temperatures. *Candidatus* Competibacter was the main GAO, along with cluster III *Defluviicoccus* members. These organisms withstood the slow-feeding regime, suggesting that their bioenergetic characteristics of carbon uptake differ from those of their tetrad-forming relatives. Comparative cycle studies revealed higher carbon and P cycling activity of *Ca.* Accumulibacter when the temperature was increased from 30 °C to 35 °C, implying that the lowered P removal performance at 35 °C was not a direct effect of temperature, but a result of higher metabolic rates of carbon (and/or P) utilization of PAOs and GAOs, the resultant carbon deficiency, and escalated community competition. An increase in the TOC-to-PO_4^{3-}-P ratio (from 25:1 to 40:1) effectively eased the carbon deficiency and benefited PAOs. In general, a slow-feeding strategy and sufficiently high carbon input benefited a high and stable EBPR at 35 °C, representing basic conditions suitable for full-scale treatment plants experiencing higher water temperatures.

1. Introduction

Enhanced biological phosphorus removal (EBPR) is a process widely employed for P removal in municipal wastewater treatment plants (WWTPs), because it is relatively cheap and sustainable and is desirable for downstream nutrient recovery (García Martín et al., 2006; Simoes et al., 2020; Roy et al., 2021). Despite the advantages, its application in warm climates has long been considered unfavorable, owing to

* Corresponding authors at: Singapore Center for Environmental Life Sciences Engineering, Nanyang Technological University, 637551, Singapore.
E-mail addresses: qiugl@scut.edu.cn (G. Qiu), swuertz@ntu.edu.sg (S. Wuertz).

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observations that the functional bacteria (i.e., polyphosphate accumulating organisms, PAOs) in the process were outcompeted by their rivals (glycogen accumulating organisms, GAOs) at high temperatures (> 25 °C, Whang and Park, 2002; Panswad et al., 2003; López-Vázquez et al., 2009). Deteriorated EBPR performance and the dominance of GAOs were reported at full-scale WWTPs with average yearly temperatures above 25 °C (Wong et al., 2005; Cao, 2011). Seasonal deterioration in EBPR performance as wastewater temperature increased during the summer has also been reported (Gu et al., 2005). In a pilot-scale membrane bioreactor employing the University of Cape Town (UCT) configuration for raw municipal wastewater treatment, total phosphorus removal dropped from 95% to below 50% when the temperature increased from 24 °C to above 35 °C. At temperatures above 30 °C, volatile fatty acids (VFAs) were completely utilized; however, phosphorus was not released, suggesting competition with GAOs (Sayi-Ucar et al., 2015). In batch tests, the maximum acetate uptake rates of *Candidatus* Accumulibacter - PAOs and *Candidatus* Competibacter - GAOs were similar at temperatures < 20 °C (Brdjanovic et al., 1997). With increasing temperature, the rates of *Ca.* Competibacter continued to increase following the extended Arrhenius equation (López-Vázquez et al., 2007), while those of *Ca.* Accumulibacter remained relatively constant in the range of 20–30 °C. Additionally, significantly increased anaerobic maintenance coefficients were observed for *Ca.* Accumulibacter. These results helped to explain the EBPR instability reported in WWTPs treating warm effluents (López-Vázquez et al., 2007). However, these experiments were performed by exposing the enrichment culture obtained at 20 °C to other temperatures, and hence it is not clear if the observed short-term effects were partially a stress response by *Ca.* Accumulibacter to acute temperature changes.

On the other hand, there have been efforts to explore the long-term feasibility of EBPR at temperatures > 25 °C. Robust EBPR was obtained at 30 °C for an extended period (> 100 days) by applying a short EBPR cycle (Freitas et al., 2009). Selectively removing biomass from the top of the sludge bed allowed to maintain complete P removal for nearly three months in an aerobic granular sludge system operated at 30 °C (Winkler et al., 2011). Long-term EBPR (160 days) stability has been demonstrated in a lab-scale reactor at 28 °C in the absence of a short EBPR cycle or selective sludge removal (Ong et al., 2014). However, as the temperature increased to 32 °C, EBPR activities were significantly compromised, with a reduction in the average abundance (based on quantitative polymerase chain reaction (qPCR) analyses) of *Ca.* Accumulibacter. Shen et al. (2017) further showed that having multiple anaerobic/aerobic stages in one EBPR cycle aided EBPR at 30 °C.

In addition to these laboratory-scale studies, which included either specific operational control or the presence of a specifically enriched PAO community, there are reports of stable EBPR in full-scale systems in warm climates (Law et al., 2016; Cao et al., 2017; Cokro et al., 2017). The presence of GAOs had no apparent effect on the long-term stability of EBPR at 30 °C (Law et al., 2016). A recent field study in Singapore further showed a wide range of PAOs exhibiting high micro-diversity in carbon usage, which might be important for the observed EBPR stability in these full-scale WWTPs operating at water temperatures of 28–32 °C (Qiu et al., 2019).

Even higher water temperatures are expected due to global warming. The global surface temperatures are likely to rise by up to 4.8 °C by the end of this century in a high-emission scenario (Intergovernmental Panel on Climate Change (IPCC), 2018). More pronounced temperature increases have been observed in highly urbanized areas; for example, temperatures in Singapore have risen at a rate (0.25 °C per decade since 1948, and currently around 0.5 °C per decade) more than double that of the global average during the same period (Meteorological Service Singapore, 2021).

The aim of this work was to explore the feasibility of EBPR at 35 °C. Two lab-scale sequencing batch reactors (SBRs) were operated in parallel at 30 °C and 35 °C, respectively, for over 300 days. We employed a slow-feeding strategy to mimic the operating conditions typically found in the field (Qiu et al., 2019), with a mixture of acetate and propionate as carbon source. Consecutive cycle studies involved switching temperatures between 30 °C and 35 °C to investigate the effect of temperature on carbon and P transformation kinetics and stoichiometry. The fine-scale dynamics of the bacterial community was monitored using 16S rRNA gene metabarcoding, fluorescence *in situ* hybridization (FISH) and qPCR.

2. Materials and methods

2.1. SBR operation

Two SBRs with a working volume of 1.59 L each were inoculated with activated sludge from a local WWTP in Singapore with an ambient water temperature of 28–31 °C (Qiu et al., 2019). A slow feeding strategy mimicking the actual feeding conditions in full-scale plants was applied for reactor operation (Tu and Schuler, 2013; Qiu et al., 2020; Tian et al., 2022). Additionally, a mixture of acetate and propionate (molar ratio of 8.4:1) was used as a carbon source. Acetate and propionate (acetate predominantly) have been reported as the major VFAs in municipal wastewater in different geographical regions (López-Vázquez et al., 2008a; Qiu et al., 2019). The SBRs were operated with 6 h cycles, including a 60 min feeding, a 20 min anaerobic, a 180 min aerobic, and a 100 min settling/decant stage. In each cycle, 0.74 L of synthetic wastewater (containing 193.8 mg/L acetate, 17.2 mg/L propionate and 8.8 mg/L PO_4^{3-}-P, detailed in the Supplementary Materials) was introduced into the reactor, with a resultant TOC/P molar ratio of 25:1. Two reactors were operated in parallel at 30 °C (R30) and 35 °C (R35), respectively. Temperature control was achieved using a proportional-integral-derivative temperature controller connected to heating jackets wrapping the reactors. The HRT and SRT in both reactors were 12.9 h and 25 d, respectively. The pH was automatically controlled at 6.80–7.50 by using a M200 transmitter (Mettler-Toledo, Switzerland) connected to an acid/base (0.5 M HCl/NaOH) dosing system. The DO was maintained at 0.8–1.2 mg/L during the aerobic phase by using the same transmitter connected to a solenoid valve in the aeration system. During Days 175–220, sodium acetate and propionic acid concentrations in the feed (Supplementary Materials) for R35 were increased 1.6 times (resultant TOC/P molar ratio of 40:1), which was reduced to 1.2 times (TOC/P molar ratio of 30:1) from Day 220 onwards until the end of the experiment. The resultant mixed liquor suspended solids (MLSS) and mixed liquor suspended volatile solids (MLVSS) concentrations were 1.57–2.00 and 1.07–1.57 g/L, respectively, in R30, and 1.51–2.15 and 1.25–1.93 g/L, respectively, in R35 during the experiment (Fig. S1).

2.2. Consecutive cycle study

A consecutive cycle study was performed on Days 55, 77, 105, 203 and 294 to investigate the effect of temperature on the carbon and P cycling kinetics and stoichiometry. Cycle studies were done in adjacent SBR cycles in both reactors with the first cycle at their respective original temperature, i.e., R30 at 30 °C and R35 at 35 °C. In the subsequent cycle, the temperature in the two reactors was switched, i.e., in R30 to 35 °C and in R35 to 30 °C. This strategy allowed the interrogation of temperature effects in both reactors by effectively discounting any potential bias arising from the acute response of the microbial communities to temperature changes. In the cycle study, filtered water (through 0.45 μm membrane filters) and activated sludge samples were collected at regular time intervals for PO_4^{3-}-P, VFAs, polyhydroxyalkanoate (PHA) and glycogen analyses.

2.3. Chemical analysis

PO_4^{3-}-P levels were measured using test kits (HACH, USA) following Standard Methods as were mixed liquor suspended solids (MLSS) and mixed liquor suspended volatile solids (MLVSS) concentrations (Fig. S1,

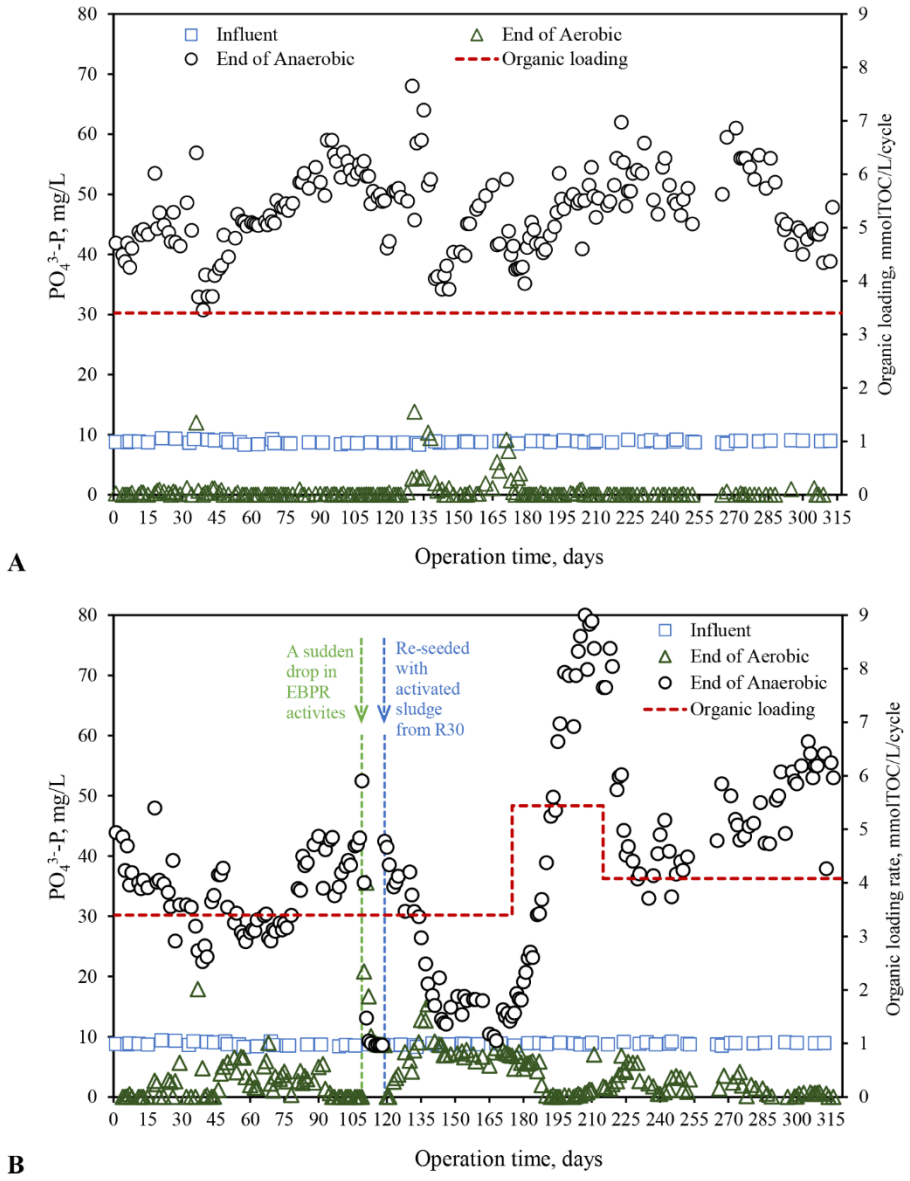


Fig. 1. Phosphorus removal performance in the EBPR reactors at 30 °C (A. R30) and 35 °C (B. R35). Slow-feeding regime was used with the influent wastewater being introduced into reactors at fix speeds in 60 min. The carbon source was a mixture of acetate and propionate (a molar ratio of around 8.4:1). The resultant TOC/P molar ratio was 25:1. The TOC/P molar ratio in R35 was increased to 40:1 during Days 175–220, which was further reduced to 30:1 from Day 220 onwards.

APHA, 1999). Acetate and propionate concentrations were measured using a gas chromatograph (Shimadzu, Japan) equipped with a DB-FFAP column (Agilent Technology, U.S.) and an FID detector. PHA analyses were performed using a gas chromatograph (Shimadzu, Japan) equipped with a DB-5MS Ultra Inert column (Agilent Technology, USA) and an FID detector (Oehmen et al., 2005). Glycogen analyses were carried out by measuring glucose after acid digestion of the freeze-dried sludge according to Kristiansen et al. (2013).

2.4. Fluorescence in situ hybridization

Activated sludge samples collected from both reactors during the operation were immediately fixed using 4% paraformaldehyde at 4 °C for 2 h. The fixed samples were washed with 1 × phosphate-buffered saline (PBS) solution and stored in a mixture of 1 × PBS and ethanol (1:1) at -20 °C before FISH analysis. Microorganisms of interest were detected using EUBmix (EUB338, EUB338II and EUB338III) targeting most bacteria (Daims et al., 1999), PAOmix (PAO651, PAO462 and PAO846) targeting *Ca.* Accumulibacter (Crocetti et al., 2000), GAOmix (GAO431 and GAO989) targeting *Ca.* Competibacter (Crocetti et al., 2002; Kong et al., 2002), and TFOmix (DF218 and DF618, Wong et al., 2004) and DFmix (DF988, DF1020, Meyer et al., 2006) targeting cluster I and cluster II *Defluviicoccus*, respectively (DF988 also targets cluster III members, Nittami et al., 2009). FISH images were collected using a LSM780 confocal laser scanning microscope installed with Zen (Carl Zeiss, German) software.

2.5. DNA extraction, 16S rRNA gene metabarcoding and qPCR

Genomic DNA was extracted using the FastDNA^{TM} SPIN Kit for Soil (MP Biomedicals, USA) (Albertsen et al. 2015). Bacterial 16S rRNA gene metabarcoding was carried out, targeting the V1-V3 region with primer set: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 534R (5'-ATTACCGCGGCTGCTGG-3'). Extracted genomic DNA were subjected to 16S rRNA gene metabarcoding analysis by Miseq (Illumina, USA) at the Australian Center for Ecogenomics. The generated data was analyzed using the DADA2 pipeline (Version 1.12, Callahan et al., 2016) and MiDAS 3.6 (Nierychlo et al., 2020) as reference for taxonomy

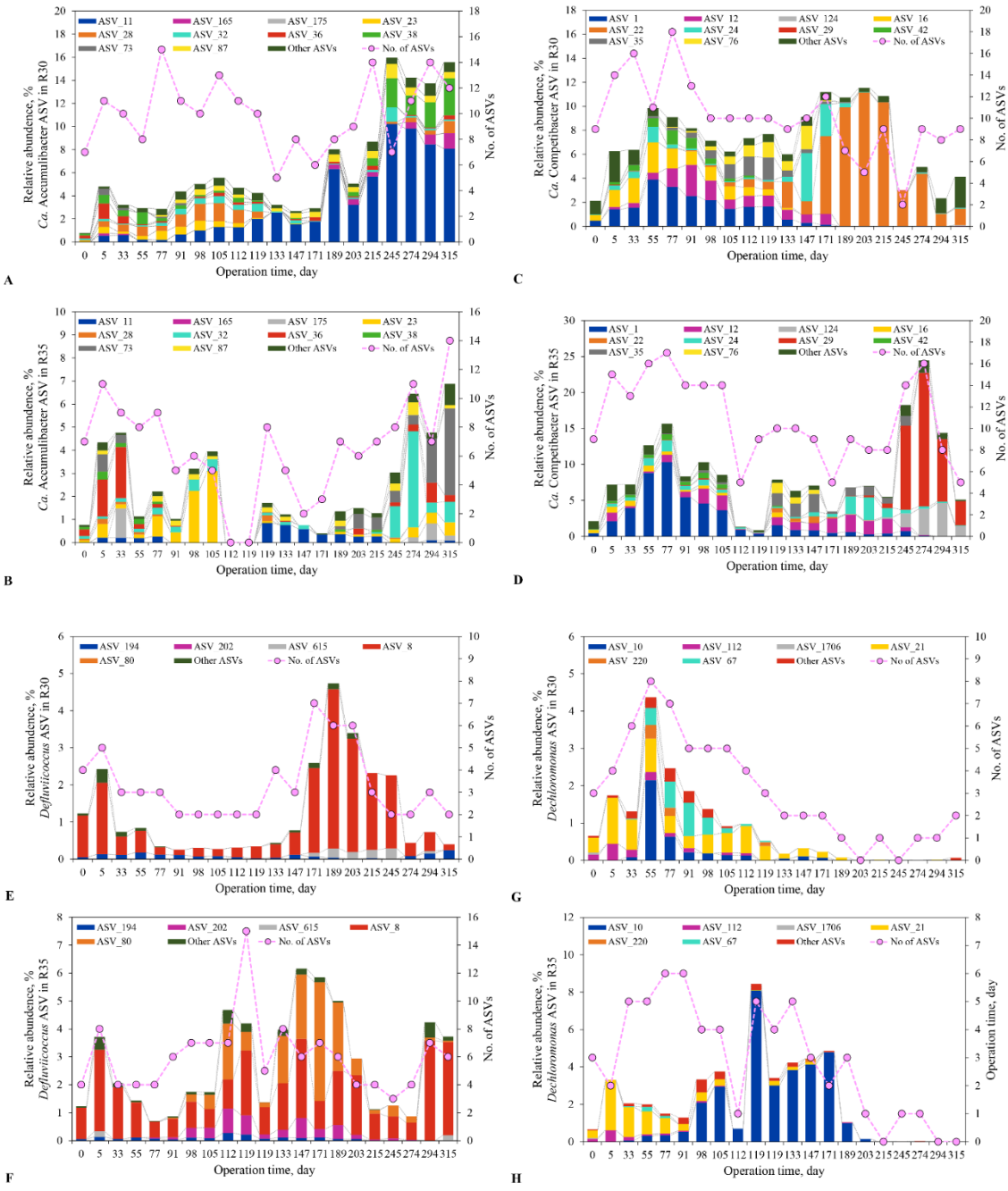


Fig. 2. Community composition and dynamics revealed by 16S rRNA gene metabarcoding: (A) *Ca.* Accumulibacter in R30; (B) *Ca.* Accumulibacter in R35; (C) *Ca.* Competibacter in R30; (D) *Ca.* Competibacter in R35; (E) *Defluviicoccus* in R30; (F) *Defluviicoccus* in R35; (G) *Dechloromonas* in R30; (H) *Dechloromonas* in R35. The second Day 119 sample in R35 was collected immediately after re-seeding the reactor.

assignment. The obtained data were deposited in the NCBI database under the BioProject No. PRJNA807832.

A 16S rRNA gene clone library was prepared on certain genomic DNA samples (R30 Day5, R35 Day5, R30 Day105 and R35 Day105) for the recovery of the full-length 16S rRNA gene sequences of dominant *Ca.* Accumulibacter taxa after PCR amplification with primer set 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'- GGTTACCTTGTTACGACTT-3') (Qiu et al. 2013). Phylogenic analysis (MEGA X, Kumar et al., 2018) was performed to compare the *Ca.* Accumulibacter sequences in the clone library and those from metabarcoding to further confirm their phylogenic relationships with existing *Ca.* Accumulibacter sequences. Additionally, qPCR was used to analyze the clade level distribution of *Ca.* Accumulibacter, according to He et al. (2007). See Supplementary Materials for additional information about 16S rRNA gene metabarcoding and qPCR.

2.6. Statistical analysis

Statistical analyses were performed using R version 3.6.3 (R Core Team, 2020). The alpha diversity calculation was performed using R packages "hillR" version 0.5.0 (Chao et al., 2014). Canonical-correlation analysis (CCA) was performed to relate the EBPR kinetics and stoichiometry (obtained in cycle studies) and the community dynamics using R packages "vegan" version 2.5-6. A permutation test was performed with function "anova.cca" to assess the significance of CCA. Microbial ecological network analysis was done using Cytoscape version 3.7.2.

3. Results and discussion

3.1. EBPR works at 35 °C

With influent TOC and PO_4^{3-}-P concentrations of 86 and 8.8 mg/L, respectively (TOC/P molar ratio of 25:1), R30 (the reactor operated at 30 °C) showed high and stable EBPR performance for > 300 days (Fig. 1A). P release was maintained at > 35 mg/L and complete P removal was achieved on most days. A technical failure occurred on Day 35, resulting in no carbon source being supplied for two days. Good EBPR activity was restored immediately after the system had returned to normal operation, showing the high resilience of the system to unexpected disturbances. Previous studies reported significantly reduced EBPR activity at 32 °C (Ong et al., 2014). Similarly, Shen et al. (2017) observed fluctuating EBPR performance at 30 °C using a multi-cycle operational strategy. It is possible that the slow feeding strategy used in this work benefited a high and stable EBPR at 30 °C (discussed later). As for carbon sources, a modeling study suggested PAOs could outcompete GAOs at 30 °C when fed with acetate and propionate at a molar ratio of 3:1, compared to a ratio of 1:1 or pure acetate or propionate (López-Vázquez et al., 2009). Similarly, acetate-fed and propionate-fed bioreactors operating at 30 °C displayed higher and more stable EBPR with acetate as feed (Shen et al., 2017). Wang et al. (2020) further showed that, propionate was always the preferred carbon source of *Ca.* Accumulibacter enriched with acetate or propionate at high temperature, but the addition of this carbon source alone led to aerobic glycogen replenishment instead of high P uptake. In conclusion, a mixture of VFAs dominated by acetate appears more desirable than one dominated by propionate to achieve EBPR at high temperatures.

Complete P removal was also achieved in R35 (the reactor operated at 35 °C) during the first two weeks (Fig. 1B). The same technical fault as for R30 occurred on Day 35, and the following two-day starvation period had no lasting impact on R35. EBPR activity was restored within two days. Complete P removal was maintained during Days 91–109, until there was a sudden drop in P release and uptake activities from 40 to 0 mg/L within a day. The reason for this decrease in EBPR activity is unknown and may not be a result of GAO competition, since the relative abundances of GAOs also decreased significantly during this period (Fig. 2). The reactor R35 did not recover on its own from this complete loss of EBPR activity (Days 109–119, Fig. 1B). Re-seeding with waste sludge collected from R30 instead of fresh field sludge, for the sake of experimental consistency and comparability of the two reactors, led to a restoration of EBPR activities, although at a lower level (P release and uptake values around 10 mg/L, Days 135–175). An increase in the influent TOC concentration to 137 mg/L (TOC/P ratio of 40:1) led to a rapid increase in EBPR activities. Complete P removal was achieved on Day 187 and maintained until Day 219, when the influent TOC was reduced to 103 mg/L (TOC/P ratio of 30:1). After a period of low-level fluctuation, near-complete P removal was achieved again (Day 285), and was maintained until the end of the experiment (Day 316). In general, although the overall performance was lower compared to R30, appreciable EBPR activities were achieved in R35 for an extended period. Near complete P removal was achieved (effluent PO_4^{3-}-P < 0.5 mg/L) more than half the time. The present study is the first report of sustained EBPR at this temperature, although the process stability and reliability need to be further assessed at different scales. Although VFAs are major components of dissolved organics in municipal wastewater (López-Vázquez et al., 2008a), a diverse range of carbon sources can contribute to EBPR in the field (Qiu et al., 2019). Diversified carbon sources may result in increased diversity of the EBPR microbial community, which is expected to benefit system stability.

3.2. Community composition and dynamics

In the activated sludge inoculum, *Ca.* Accumulibacter was the predominant PAO at a relative abundance of 0.76% followed by *Tetrasphaera* at 0.3%; *Ca.* Competibacter (2.12%) and *Defluviicoccus* (1.23%) were the predominated GAOs followed by *Micropruina* (0.05%). During reactor operation, PAOs and GAOs were top-ranked community members in both reactors (Fig. S2). *Ca.* Accumulibacter remained as the predominant PAO, with relative abundance values significantly increased in both reactors (up to 16.0% and 6.88% in R30 and R35, respectively). *Tetrasphaera* disappeared within the first month. No *Microlunatus phosphovorus* (Nakamura et al., 1995), *Ca.* Methylophosphatis (Singleton et al., 2021), *Ca.* Obscuribacter (Soo et al., 2014) or *Ca.* Accumulimonas (Nguyen et al., 2012) (putative PAOs) were detected in the seed sludge or during reactor operation. *Ca.* Competibacter remained the predominant GAO (2.12–11.5% in R30 and 1.35–24.4% in R35) followed by *Defluviicoccus* (0.25–4.73% in R30 and 0.70–6.16% in R35). *Ca.* Contendobacter (a member of the *Ca.* Competibacter lineage, McIlroy et al., 2014) appeared in both reactors only during a limited period (Days 98–133 in R30 and Days 119–203 in R35). *Micropruina* was only present in the seed sludge (0.05%). No *Propionivibrio* (Albertsen et al., 2016) related GAOs were detected in either reactor. Despite the different ranking in relative abundances, most (23 out of 30) dominant genera were shared between the two reactors. Detailed dynamics of each major PAO/GAO lineage are discussed in the subsequent sections.

3.2.1. *Ca.* Accumulibacter in the reactors are closely related to those commonly found in temperate systems

Thirty-nine *Ca.* Accumulibacter amplicon sequence variants (ASVs, each ASV was uniquely numbered) were detected, 27 and 28 of which occurred in R30 and R35, respectively (Fig. 2A and B). The two reactors shared 16 ASVs, with 9 of them (ASVs 11, 23, 28, 32, 36, 38, 73, 87, 353) being detected in > 4 samples in both reactors, representing the core *Ca.* Accumulibacter community. Among these 9 core ASVs, 6 (ASVs 23, 28, 32, 36, 38, 353) were originally present in the seed sludge. The remaining 3 ASVs (11, 73 and 87) started to emerge in both reactors from Day 5, implying that they were probably also present in the seed sludge but at undetectable relative abundances. All these ASVs flourished at the beginning of the experiment, concomitant with the presence of desirable EBPR activities in both reactors, during the first few weeks (Fig. 1), suggesting that the operation strategy employed in this study effectively retained the majority of *Ca.* Accumulibacter species originally present in the field, which is key to sustained EBPR activities in the long term. Additionally, these results demonstrated that most *Ca.* Accumulibacter in the full-scale sludge could effectively survive an elevated temperature of 35 °C.

Despite the extended presence of these core ASVs, their relative abundances and status of predominance differed in the two reactors (Fig. 2A and B). In R30, ASVs 11, 23, 28, 32, 36, 38 stayed at comparable relative abundances ranging from 0.07% to 1.57%. Later, ASV11 started to dominate until the end of operation. ASV11 is closely related (100% identity) to *Ca.* Accumulibacter clade IIF strain SCELSE-1 (Qiu et al. 2020). Similarly, Ong et al. (2014) reported the predominance of clade IIF *Ca.* Accumulibacter in a bioreactor at 32 °C. It seems that certain metabolic characteristics enable members of this clade to dominate at high temperatures (Qiu et al., 2020). In contrast, ASV11 was less abundant in R35 throughout the operation. The sudden disruption of EBPR activities in R35 and subsequent inoculation with R30 sludge on Day 119 presented an opportunity to test the growth potential of ASV11 at 35 °C. Its decreasing relative abundance (to < 0.1%) suggested that ASV 11 may not have a significant growth advantage at 35 °C. Compared to R30, R35 exhibited dominance of single ASVs during each period of operation, which might be a reason for the lower EBPR stability in R35 than in R30 (Fig. 1). ASV36 prevailed during the first 50 days, followed by ASV23 and ASV11 and then ASV32 and ASV73. A whole-genus phylogenetic analysis suggested that these ASVs are widely distributed in the *Ca.* Accumulibacter lineage (Table S1 and Fig. S3). For example, ASV36 is closely related (100% identity) to clade IID sequences EF565158/EF565159 recovered from a full-scale WWTP in US. ASV 32

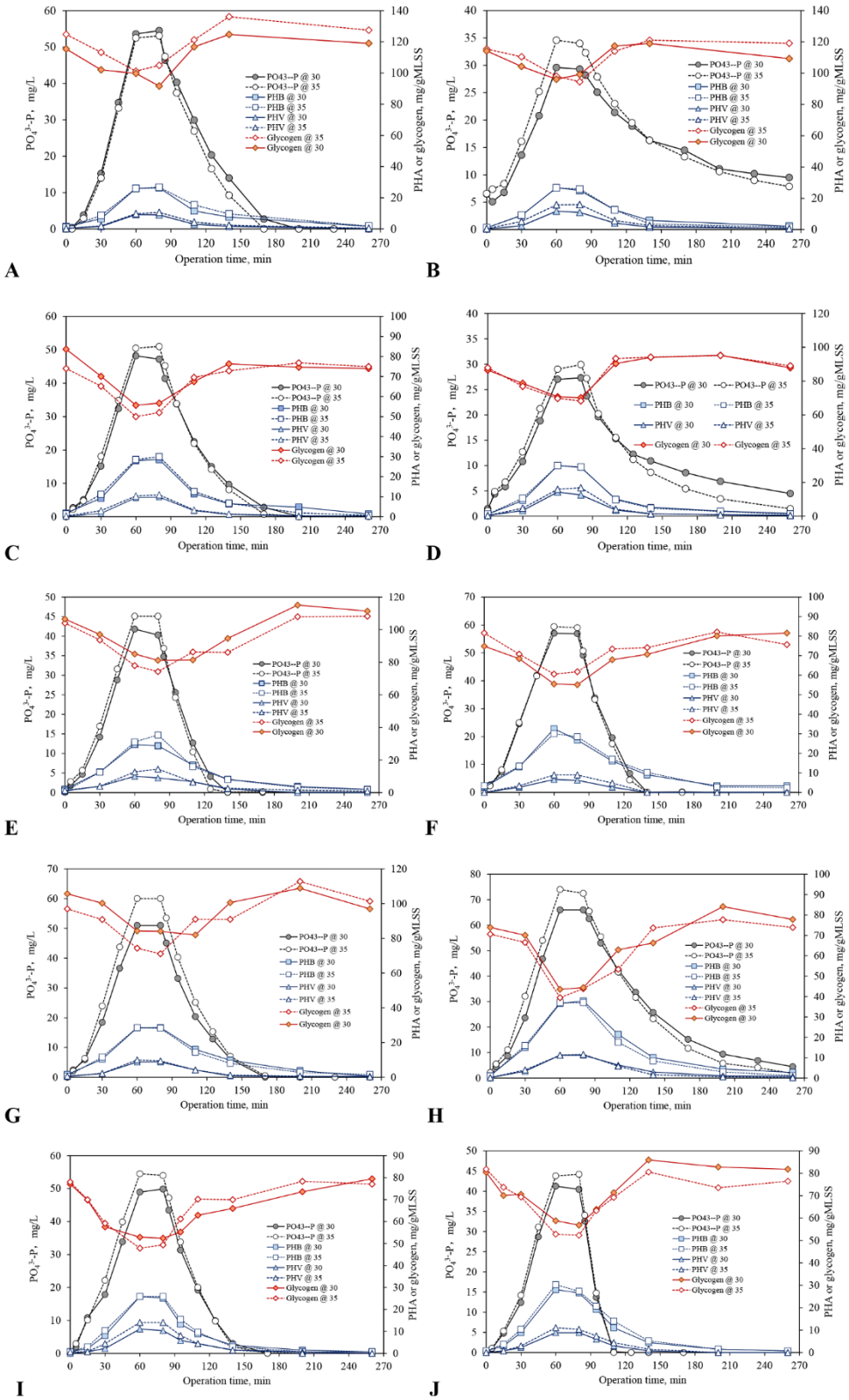


Fig. 3. Representative carbon and phosphorus cycling characteristics in (A) R30 on Day55, (B)R35 on Day55, (C) R30 on Day77, (D)R35 on Day77, (E) R30 on Day105, (F) R35 on Day105, (G) R30 on Day215, (H) R35 on Day215, (I) R30 on Day 294, and (J) R35 on Day 294.

is closely related (100% identity) to clade IIB sequence AB736230 from a lab-scale EBPR system operated at 20 ± 4 °C (Satoh et al., 2013); ASV87 is closely affiliated (100% identity) with a clade IIF member (JQ726366) from an SBR operated at 20 °C (Kim et al., 2013); and ASV28 is closely related (100% identity) to Clade IIC sequence KJ807999 from a full-scale WWTP in China. The closed genome of ASV28 was recovered using Nanopore long read sequencing as reported in our previous work (Arumugam et al., 2021). A clone library was established to recover the full-length 16S rRNA gene sequences of the dominant ASVs (Fig. S3). Subsequent phylogenetic analysis supported

Table 1
P and carbon transformation kinetics obtained in this study and a comparison with literature values

Origin		T [a]	$r_{C\text{-}upt}$ [b]	$r_{P\text{-}rel}$ [c]	$r_{PHA\text{-}form}$ [d]	$r_{P\text{-}upt}$ [e]	$r_{PHA\text{-}cons}$ [f]	m^{An} [g]	m^{O} [h]	Refs.
Ca. Competibacter (Acetate)		20	4.52	–[i]	–	–	2.71 ± 0.22	0.075	0.029	López-Vázquez et al. (2007, 2008b)
		30	6.23	–	–	–	–	0.086	–	
		35	6.78	–	–	–	–	0.099	–	
Ca. Accumulibacter (Acetate)		20	3.84	–	–	–	–	–	–	
		30	3.84	–	–	–	–	–	–	
Ca. Accumulibacter (Acetate)		20	3.28 ± 0.40	2.45 ± 0.71	3.36	1.41 ± 0.22	0.89 ± 0.05	0.033	0.070	Brdjanovic et al. (1997)
		30	2.48 ± 0.57	1.77 ± 0.61	2.51	1.77 ± 0.16	1.02 ± 0.08	0.082	0.163	
PAO model (Acetate)		20	5.03	2.84	3.08	1.71	0.68	0.057	0.090	Smolders et al. (1995)
Mixed enrichment culture (Acetate)		24	–	0.365	–	0.200	–	–	–	Ong et al. (2014)
		28	–	0.300	–	0.197	–	–	–	
		32	–	0.232	–	0.226	–	–	–	
Defluviicocous vanus (Actate)		30	4.04	–	–	–	–	–	–	Wang et al. (2021)
Defluviicocous vanus (Propoinate)		30	4.01	–	–	–	–	–	–	
Enrichment culture (Acetate)		30	4.96	3.44-5.15	–	0.57- 0.736	–	–	–	Shen et al. (2017)
Enrichment culture (Propionate)		30	4.74	1.87-3.31	–	0.457-0.537	–	–	–	
Ca. Accumulibacter (Acetate)		30	6.91	3.37	4.46	1.58	2.32	0.042	–	Qiu et al. (2020)
R30	Normal cycle study	30	1.04-1.85[j]	1.07-1.71	1.00-1.76	0.86-2.79	0.82-1.47	–	–	This study
	Consecutive cycle study	30	1.31-1.67[j]	1.36-1.69	1.05-1.43	1.08-1.92	1.05-1.38	–	–	
		35	1.31-1.67[j]	1.69-2.33	1.13-1.48	1.15-2.14	1.10-1.49	–	–	
R35	Normal cycle study	35	1.03-2.27[j]	0-1.32	1.01-2.66	0-1.61	0.79-2.45	–	–	
	Consecutive cycle study	35	1.04-1.62[j]	0.51-1.72	0.81-1.09	0.36-1.71	0.84-1.13	–	–	
		30	1.04-1.62[j]	0.51-1.47	0.81-1.02	0.26-1.55	0.77-0.99	–	–	

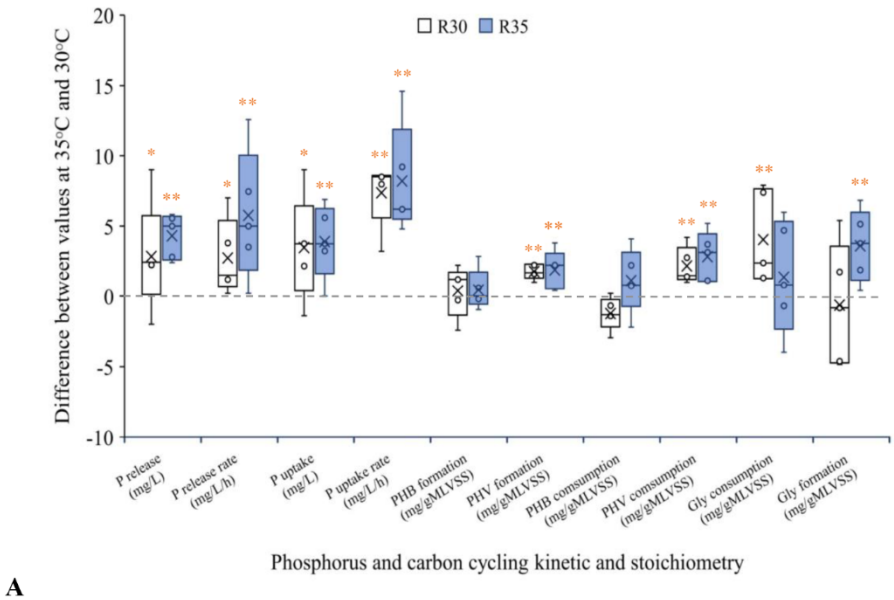
[a] Temperature, °C.
[b] Anaerobic carbon uptake rate, mmol C/g VSS/h.
[c] Anaerobic P release rate, mmol P/g VSS/h.
[d] Anaerobic PHA formation rate, mmol C/g VSS/h.
[e] Aerobic P uptake rate, mmol P/g VSS/h.
[f] Aerobic PHA consumption rate, mmol C/g VSS/h.
[g] Anaerobic maintenance coefficient, mmol ATP/g VSS/h.
[h] Aerobic maintenance coefficient, mmol ATP/g VSS/h.
[i] Values not applied or available.
[j] Slow feeding was used in this study, the carbon uptake rates were determined by the feeding rate.

their identification. qPCR analysis was also employed to analyze the clade-level distribution and dynamics (Fig. S4). No clade I member was detected in either reactor. The dynamics of clades IIB, IIC and IID generally agree with the 16S rRNA gene-based identification of these dominant ASVs. Collectively, these results suggested that a diverse range of *Ca.* Accumulibacter members could survive at 35 °C. These ASVs are not unique but have also been found in laboratory- and full-scale EBPR systems in temperate climates, implying that successful EBPR at elevated temperatures does not require a highly specialized *Ca.* Accumulibacter community.

3.2.2. *Rare Ca. Compatibacter ASVs in the seed sludge thrived in reactors together with Defluviicoccus cluster III members*

Fifty-four *Ca.* Competibacter sequences were detected, 40 of which were found in each reactor (Fig. 2C and D). Similar to what was observed for *Ca.* Accumulibacter, multiple ASVs (1, 12, 16, 24, 42) dominated R30 for the first 150 days and ASV 22 proliferated during the remainder of reactor operation, while single ASVs dominated R35 during each period of operation (ASVs 1, 29 and 124). Different from the core *Ca.* Accumulibacter ASVs, which were all derived from the seed sludge, a great number of *Ca.* Competibacter ASVs were present in the seed sludge but decreased in relative abundance in both reactors after initially thriving during the first 100 days). The remaining predominant ASVs (e.g., ASV 29 and ASV 124 in R35 and ASV 22 in R30) had not been detected in the seed-sludge. They appeared only after Day 77. No closely related relatives of these ASVs were found in the NCBI database. The best matches were LR650365 (94.12% identity to ASV 29), HQ467835 (96.73% identity to ASV 124), and DQ201883 (96.73% identity to ASV 22). In contrast, close relatives of ASVs, which dominated the seed sludge, were commonly detected in temperate WWTPs; e.g., the top three most dominant *Ca.* Competibacter ASVs in the seed sludge, ASV16, ASV1 and ASV132 showed 100%, 98.91% and 99.64% similarity to sequences KF428043 (Wang et al., 2014a), HQ476545 (Kim et al., 2013) and KJ807854 (Wang et al., 2014b), respectively. Since *Ca.* Competibacter in full-scale systems have not been as well studied, it is reasonable that no close relatives were found in databases for these ASVs that were rare in the seed sludge but thrived in both reactors. Since the same was observed in R30 and R35, we cannot exclude the possibility that lab-scale reactors tend to selectively benefit certain rare *Ca.* Competibacter ASVs in the long-term (Nielsen et al., 2019). Overall, a higher predominance of *Ca.* Competibacter was observed at 35 °C (Fig. 2), which corresponded to the lower EBPR performance of R35 (Fig. 1).

Defluviicoccus-related GAOs showed lower relative abundance compared to *Ca.* Competibacter (Fig. 2E and F). ASV 8 dominated R30, with other ASVs staying at low levels throughout the operation. The dominant ASV in R35 was also ASV 8 before Day 112. Afterwards, ASV80 and ASV202 increased but then declined as the EBPR activity recovered to a high level (Fig. 1). ASV 8 is closely related (100% identity) to AB445107 (Nittami et al., 2009), a Cluster III member having a '*Nostocoida limicola*-like' filamentous morphology commonly found in full-scale systems (McIlroy, et al., 2010; Stokholm-Bjerregaard et al., 2017; Chen et al., 2022). FISH analysis confirmed their presence and morphology (Fig. S5). ASV 80 showed 99.63% similarity to DQ250533, a cluster II member having the typical tetrad-forming morphology (Wong and Liu, 2007). FISH analysis allowed their detection in R35 together with ASV 202 (cocci in clumps) (Fig. S5J). Neither ASV 80 nor ASV 202 were detected in the seed sludge. Their proliferation in R35 coincided with reduced EBPR activity (Fig. 1) and a reduction in the relative abundance of *Ca.* Competibacter (Fig. 2D), which could have opened up an ecological niche. Tetrad-forming *Defluviicoccus* were believed to be unable to compete with *Ca.* Accumulibacter under low-substrate conditions (Tu and Schuler, 2013) due to their inability to effectively take up low-concentration substrates (Burow et al., 2008). Recent research further suggested lower uptake rates of VFAs of *Defluviicoccus vanus* (cluster II member) than *Ca.* Accumulibacter at 30 °C (Wang et al., 2020). Their decrease after the restoration of the EBPR



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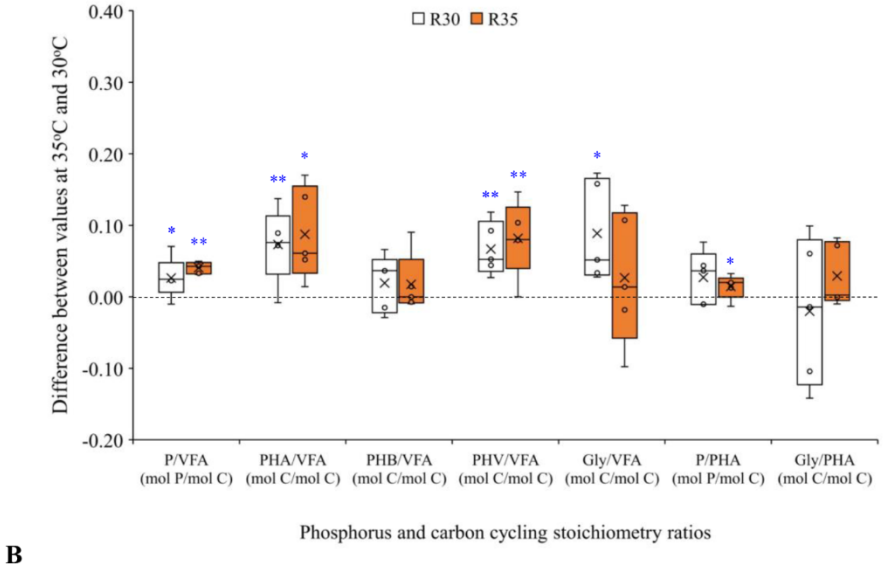

B

Fig. 4. Differences in (A) the P and carbon cycling kinetic and stoichiometry parameters and (B) the stoichiometry ratios in the consecutive cycle study ($n = 5$). A Z-test was used to test if the mean difference values were significantly different from 0 (* indicates $P < 0.05$; ** indicates $P < 0.01$). For each kinetic or stoichiometry parameter, each consecutive cycle study generated one difference value after subtracting the value obtained at 30 °C from that obtained at 35 °C.

activities verified these hypotheses. However, the ability of Cluster III member ASV 8 to co-exist with *Ca.* Accumulibacter and *Ca.* Competibacter in the long term under the slow-feeding condition suggested that they are metabolically different from their Cluster II relatives in terms of carbon uptake bioenergetics (Chen et al., 2022).

Apart from these well-characterized PAOs and GAOs, *Dechloromonas*-related sequences were also detected in both reactors (Fig. 3G and H). ASVs in R30 showed high inter-lineage dynamics in the first 125 days. Alternating predominance was observed for ASVs 10, 67, 112 and 166. In R35, a significant predominance of single ASVs (10 and 21) was observed during different stages of operation, similar to the patterns observed for *Ca.* Accumulibacter and *Ca.* Competibacter communities. Recent research allowed the identification of two novel *Dechloromonas*-related PAOs in full-scale WWTPs (Petriglieri et al., 2021). In R35, ASVs 10 and 21 were present during days 108–119, when there was no detectable EBPR activity, suggesting that both ASVs acted as GAOs. We did not find well-characterized relatives of other ASVs in the database (the highest similarity score was 97.45%, which is between ASV67 and *Candidatus* Dechloromonas phosphoritropha (midas_s_96) (Petriglieri et al., 2021)). Their ecological roles in the systems need further confirmation.

3.2.3. *Regulation of PAO-GAO interactions via controlled organic carbon supply benefits a higher EBPR stability at elevated temperature*

Almost all core ASVs in each lineage (both PAOs and GAOs) were thriving (Fig. S6), and the appearance of rare species originally undetectable in the seed sludge suggests that the operational conditions (including the composition of the feed) in this work effectively benefited the growth of each community. Hence, an elevated temperature of 35 °C does not present an obstacle for any of these single ASVs to survive and multiply. Within each lineage of GAOs and PAOs, certain ASVs fared better in R35 than in R30, with lower community richness (reflected by the Hill number ^{1}D) and evenness (reflected by ^{2}D, Fig. S7), implying that both PAO and GAO communities were stressed at 35 °C. Previous research suggested that both PAOs and GAOs have faster carbon metabolic rates and maintenance coefficients as the temperature increases

Table 2
P and carbon transformation stoichiometry ratios obtained in this study and a comparison with literature and model values

Origin			Anaerobic						Aerobic		Refs.
Reactor		T[a]	P/VFA[b]	PHA/VFA[c]	PHB/VFA[d]	PHV/VFA[e]	PHB/PHV[f]	Gly/VFA[g]	P/PHA[h]	Gly/PHA[i]	
R30	Normal cycle study	30	0.35-0.52	1.02-1.31	0.76-1.01	0.25-0.41	2.20-3.35	0.40-0.57	0.19-0.33	0.39-0.55	This study
	Consecutive cycle study	30	0.38-0.53	1.04-1.23	0.75-0.95	0.23-0.39	2.60-3.82	0.34-0.51	0.23-0.33	0.45-0.59	
		35	0.40-0.56	1.13-1.30	0.79-0.96	0.34-0.46	2.31-2.77	0.39-0.62	0.28-0.35	0.47-0.52	
R35	Normal cycle study	35	0.00-0.39	1.07-2.03	0.79-1.26	0.27-0.77	1.64-2.97	0.46-1.07	0.00-0.22	0.35-0.61	
	Consecutive cycle study	35	0.26-0.43	1.01-1.43	0.64-0.96	0.37-0.66	1.55-3.04	0.45-0.68	0.12-0.28	0.45-0.57	
		30	0.22-0.39	0.95-1.46	0.64-0.95	0.37-0.55	1.87-2.99	0.44-0.59	0.10-0.27	0.33-0.58	
PAO model (Acetate)		20	0.5	1.33	1.33	0.00	0	0.5	0.41	0.42	Smolder et al. (1995)
PAO model (Propionate)		20	0.42	1.22	0	0.56	–[j]	0.33	–	–	Oehmen et al. (2005)
GAO model (*Ca.* Competibacter)		20	0	1.86	1.36	0.46	2.96	1.12	0	0.65	Zeng et al. (2003)
GAO model (*Defluviicoccus*)		20	0	1.50	0	0.83	0	0.67	–	–	Oehmen et al. (2006)
Ca. Competibacter (Acetate)		20[k]	0	1.97 ± 0.13	1.28 ± 0.06	0.54 ± 0.04	1.46 ± 0.21	1.20 ± 0.19	0	0.98	López-Vázquez et al. (2007, 2008b)
Enrichment culture (Acetate)		30	0.54-0.64	–	0.82-0.96	0.07-0.19	–	0.24-0.41	0.75-0.98	0.21-0.28	Shen et al. (2017)
Enrichment culture (propionate)		30	0.25-0.45	–	0.03-0.05	0.49-0.71	–	0.16-0.30	0.74-0.84	0.19-0.26	Shen et al. (2017)
Enrichment culture (Acetate)		28-32	0.38-0.57	–	–	–	–	–	–	–	Ong et al. (2014)
Enrichment culture of PAOs (Acetate)		30	0.36 ± 0.11	1.01 ± 0.15	–	–	–	–	–	–	Brdjanovic et al. (1997)
Ca. Accumulibacter (Acetate)		30	0.51-0.59	1.32-1.34	1.22-1.26	0.11-0.13	9.7-11.1	0.43-0.46	0.39-0.43	0.35-0.41	Qiu et al. (2020)

[a] Temperature, °C.
[b] P-release to VFA-uptake molar ratio, mol P/mol C.
[c] PHA-formation to VFA-uptake molar ratio, mol C/mol C.
[d] PHB-formation to VFA-uptake molar ratio, mol C/mol C.
[e] PHV-formation to VFA-uptake molar ratio, mol C/mol C.
[f] PHB to PHV molar ratio in the PHA, mol C/mol C.
[g] Glycogen-consumption to VFA-uptake molar ratio, mol C/mol C.
[h] P-uptake to PHA-consumption molar ratio, mol P/mol C.
[i] Glycogen-formation to PHA-consumption molar ratio, mol C/mol C.
[j] Values not available.
[k] The stoichiometry ratios was found insensitive to temperature changes from 20 to 30 °C.

(Brdjanovic et al., 1997; López-Vázquez et al., 2007, 2008b, Table 1). This would result in increased carbon requirements of the EBPR community at 35 °C, causing stress due to a carbon deficiency. To test this hypothesis, the TOC/P ratio in R35 was increased from 25:1 to 40:1 on Day 175 (Fig. 1). A subsequent increase in EBPR activity was observed together with increases in the diversity and evenness of all respective lineage members to values comparable to those in R30 (Fig. S7), supporting the notion that the supply of carbon was a limiting factor in R35. The lower relative abundance of PAOs and higher abundance of GAOs at 35 °C suggests that PAOs are more sensitive to carbon deficient conditions. Increasing the concentration of carbon sources effectively allowed *Ca.* Accumulibacter to outcompete *Ca.* Competibacter in terms of relative abundance by the end of the experiment (Fig. 2B and D).

There was no clear anticorrelation between relative abundance values of PAOs and GAOs in either reactor (Fig. S8). Microbial ecological network analysis failed to identify any direct interaction between PAOs and GAOs (Fig. S9), implying that the variation in EBPR activities was not directly triggered by preferential growth of GAOs. Previous research suggested superior (anaerobic) carbon uptake rates of GAOs at temperatures above 25 °C. However, these apparent anaerobic carbon uptake rates were obtained under conditions where carbon sources were fed to the reactor as a pulse (López-Vázquez et al., 2007). To date, almost all laboratory-scale reactor experiments reported in the literature employed a fast-feeding regime. Modeling studies also used the maximal anaerobic carbon uptake rates to evaluate PAO-GAO competition (López-Vázquez et al., 2009). In full-scale WWTPs (Law et al., 2016; Qiu et al., 2019; Chen et al., 2022) and in this work the anaerobic carbon uptake was not determined by the maximal uptake rates of both organisms but by the feeding rate (Table 1). The average carbon feeding rate was 1.04–1.85 mmol C/g VSS/h in R30 and up to 2.27 mmol C/g VSS/h in R35 (higher volumetric loading rates at increased influent TOC/P molar ratios in R35, which also resulted in slightly higher MLSS and MLVSS concentrations in R35 than in R30 at an average of 1.96 versus 1.76 g/L for MLSS, and 1.55 versus 1.26 g/L for MLVSS, respectively, during Day 175–220, Fig. S1), which is far below the maximum carbon uptake rates of around 4.0 mmol C/g VSS/h for *Ca.* Accumulibacter and above 6.0 mmol C/g VSS/h for *Ca.* Competibacter at 35 °C (López-Vázquez et al., 2007, Table 1). The capability of *Ca.* Accumulibacter to generate luxury PMF via the efflux of protons in symport with PO_4^{3-}-P (Saunders et al., 2007; Burow et al., 2008; Qiu et al., 2020; Chen et al., 2022) likely confers an advantage in competing with GAOs at low substrate concentrations.

3.3. PAO kinetics and stoichiometry are not compromised at 35 °C

Previous research suggested lower anaerobic carbon-uptake rates and a higher maintenance coefficient of *Ca.* Accumulibacter-PAOs compared to *Ca.* Competibacter-GAOs at temperatures above 20 °C (López-Vázquez et al., 2007). Regarding their aerobic metabolism, *Ca.* Competibacter showed no advantage over *Ca.* Accumulibacter

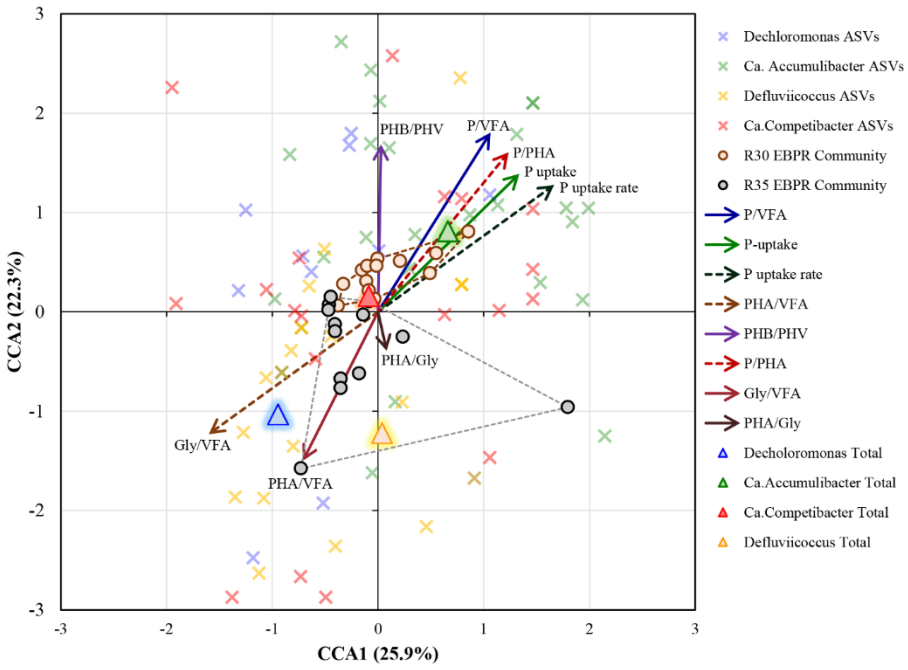

Fig. 5. Canonical Correlation Analysis (CCA) analysis revealing the relationship between the EBPR community and the kinetic and stoichiometry parameters. The data have been transformed initially by applying the Hellinger transformation. The relative contribution (eigenvalue) of each axis to the constrained space is indicated in percent in the axis title. A permutation test of the CCA analysis showed a *p*-value 0.001.

(López-Vázquez et al., 2008b). On the contrary, the aerobic carbon conversion rates of *Ca.* Competibacter decreased significantly above 30 °C with significantly reduced aerobic maximum yields, and increased PHA and oxygen requirements for glycogen production (López-Vázquez et al., 2008b). It is still not clear whether the kinetics of aerobic metabolism in *Ca.* Accumulibacter continue to increase or decline at temperatures above 30 °C, as it does for *Ca.* Competibacter. Additionally, the previous understanding was mainly obtained by exposing enrichment cultures obtained at 20 °C to high temperatures. It is essential to know whether EBPR communities growing at high temperatures have a similar response to temperature changes. To test previous observations and further understand the temperature effects on EBPR at temperature above 30 °C, the P- and carbon-cycling activities in both reactors were therefore monitored via consecutive cycle studies.

Consecutive cycle studies were performed five times throughout the long-term operation (on Days 55, 77, 105, 203 and 294) (Fig. 3). Generally, higher P release occurred at 35 °C in both reactors but pronouncedly higher P uptake rates were observed at 35 °C than at 30 °C in both reactors in all cases regardless of community composition, showing higher aerobic P uptake activities of *Ca.* Accumulibacter at 35 °C (Fig. 3). This was not restricted to the presence of specific species/clades. Additionally, higher PHV formation ($P < 0.01$) was observed at 35 °C than at 30 °C in both reactors (Fig. 4). GAOs produce more PHV than PAOs with either acetate or propionate as a carbon source (Table 2). Since there was no change in the influent between the consecutive SBR cycles, increased PHV formation suggested an increase in carbon uptake by GAOs at 35 °C. This hypothesis was also supported by the higher amount of glycogenolysis ($P < 0.01$ in R30, Fig. 4). A previous study suggested a higher anaerobic carbon-uptake rate of *Ca.* Competibacter with increasing temperature (López-Vázquez et al., 2007). In view of the slightly higher relative abundance of *Defluviicoccus* in R35 than in R30, *Defluviicoccus* probably also has a similar temperature dependency. The aerobic carbon conversion related kinetics and stoichiometry parameters showed a less significant increase as compared to the aerobic P uptake related parameters (Fig. 4), indicating a lower aerobic carbon metabolism of GAOs compared to *Ca.* Accumulibacter. This result is in line with the previous finding that the aerobic metabolic efficiency of *Ca.* Competibacter decreased at temperatures above 30 °C (López-Vázquez et al., 2008b).

In general, these results suggest that a temperature increase from 30 °C to 35 °C did not have a detrimental effect on the activity of *Ca.* Accumulibacter. In contrast, the aerobic activity, especially *P* metabolism, increased significantly (Figs. 3 and 4). This advantage in aerobic metabolism may partially offset its weakness in anaerobic metabolism as compared to GAOs. Yet the increased anaerobic maintenance coefficient and the higher aerobic carbon consumption might have resulted in a carbon imbalance in the long-term, again underlining the importance of sufficient carbon supply to sustain long-term activity. These results explain the observation that *Ca.* Accumulibacter effectively co-existed with GAOs at 35 °C and that a higher TOC/P ratio benefited its competitiveness vis-à-vis GAOs. In fast-feeding systems, an increased TOC/P ratio may impair EBPR activities at high temperature, as shown by Ong et al. (2013), owing to the higher carbon uptake rates of GAOs compared to those of PAOs at high temperature. The more carbon is added, the more carbon can be taken up by GAOs. The slow-feeding strategy effectively pegged the carbon uptake rates of GAOs and PAOs at the same level (i.e., the carbon feeding rate, Table 1), thus allowing PAOs to obtain sufficient carbon for EBPR.

In addition to the consecutive cycle study, normal cycle studies were performed to monitor the changes in P and carbon cycling kinetics and stoichiometry during the long-term operation (Table 2). CCA was used to relate these parameters to the community dynamics in both reactors (Fig. 5). Generally, the *Ca.* Accumulibacter population was positively related to the anaerobic P release/VFA uptake (P/VFA) ratios, the aerobic P uptake rates, and the aerobic P uptake/PHA consumption (P/PHA) ratios; these parameters are effective indicators of a higher EBPR activity. The population of GAOs was positively related to the anaerobic VFA uptake/PHA formation and glycogen consumption/VFA uptake ratios, higher values of which are indications of the thriving of GAOs. *Dechloromonas* was also positively related to these factors, implying that they probably performed as GAOs. In contrast to the high correlation between the *Ca.* Accumulibacter population and the P uptake rate, a low correlation was observed between the *Ca.* Accumulibacter population and the P release rate, owing to the slow-feeding strategy, which limited

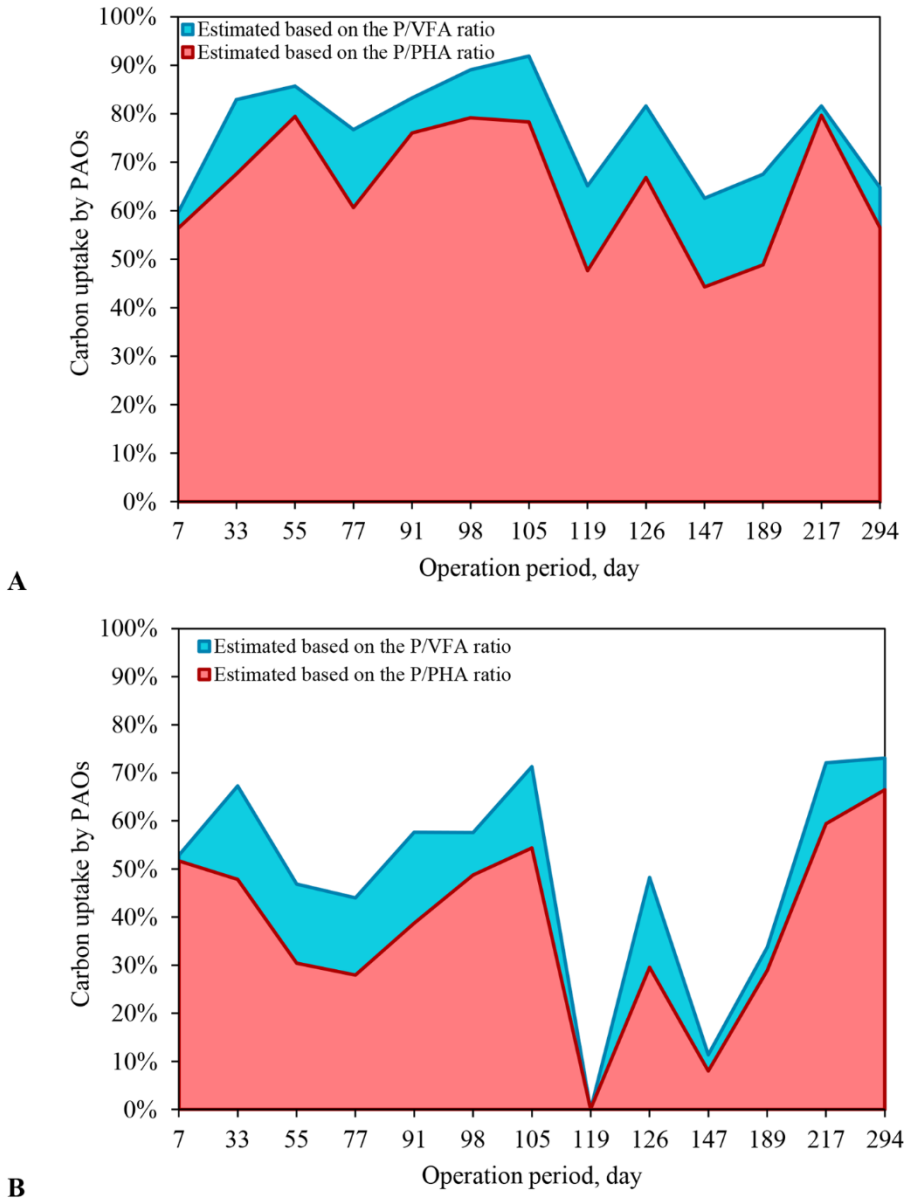

Fig. 6. Estimated carbon uptake by PAOs in (A) R30 and (B) R35 based on the anaerobic P-release/carbon-uptake (P/VFA) and the aerobic P uptake/PHA consumption (P/PHA) ratios measured in the cycle studies.

the carbon uptake rates and P release rates of *Ca.* Accumulibacter. The strategy also effectively restricted the advantage of *Ca.* Competibacter in anaerobic carbon uptake. We estimated the percentage of carbon taken up by PAOs in both systems based on the anaerobic P/VFA ratios and aerobic P/PHA ratios. Our previous research suggested a P/VFA ratio of a highly enriched *Ca.* Accumulibacter culture at 30 °C in the range of 0.51–0.59 (Qiu et al., 2020) and based on the metabolic model, the P/PHA ratio of *Ca.* Accumulibacter is around 0.42. By comparing the anaerobic P/VFA and aerobic P/PHA ratios observed in this work and the model values (Table 2), around 70% (59.6–91.9% with an average of 76.3% based on the P/VFA ratios, or 44.3–79.7% with an average of 64.7% based on the P/PHA ratios) of the carbon sources were taken up by *Ca.* Accumulibacter in R30. In R35, it was around 50% (33.8–73.1% with an average of 56.8% based on the P/VFA ratios, or 27.9–66.5% with an average of 44.0% based on the P/PHA ratio) with the highest values observed at high TOC/P ratios (Fig. 6), showing that the slow-feeding strategy could effectively prevent excessive uptake of carbon sources by GAOs even at high TOC/P ratios.

4. Conclusions

The following findings will have important implications for a successful operation of EBPR systems at elevated temperatures:

- EBPR works at 35 °C. A wide range of *Ca.* Accumulibacter ASVs closely related to those commonly found in full-scale and lab-scale EBPR systems operated at temperate conditions survived and proliferated for a prolonged period (over 300 days), suggesting that EBPR at elevated temperature might not require a highly specialized *Ca.* Accumulibacter community.
- Short-term temperature tests showed increased activity of *Ca.* Accumulibacter (especially P uptake rates) when the temperature increased from 30 to 35 °C, suggesting that elevated temperature does not have a direct adverse effect on *Ca.* Accumulibacter.
- Low richness and evenness were observed at 35 °C for each specific PAO/GAO linage, showing that the EBPR community was stressed because of higher carbon (and/or P) metabolic rates and the resultant carbon deficiency. Increasing the TOC/P ratio eased the

community competition and benefited EBPR at 35 °C. Increased carbon input might be necessary for stable EBPR at this temperature.
- Slow feeding effectively pegged the carbon uptake rates of GAOs and PAOs at the same level, which may have allowed *Ca.* Accumulibacter to co-exist with GAOs and outcompete them at 35°C to achieve complete P removal.
- *Ca.* Competibacter was the predominant GAOat 35 °C together with Cluster III *Defluviicoccus*. Cluster III *Defluviicoccus* members effectively survived the slow-feeding condition, implying that their bioenergetic characteristics for carbon uptake are different from those of their tetrad-forming relatives.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118301.

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