Efficient phosphate accumulation in the newly isolated *Acinetobacter junii* strain LH4

Yong-He Han^{1,2,3} · Ting Fu¹ · Shan-Shan Wang^{1,2,3} · Hong-Ting Yu¹ · Ping Xiang¹ · Wen-Xian Zhang¹ · Deng-Long Chen^{2,3,4} · Min Li¹

Received: 17 August 2017 / Accepted: 6 July 2018 / Published online: 11 July 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Phosphate (PO_4^{3-}) accumulation associated with bacteria contributes to efficient remediation of eutrophic waters and has attracted attention due to its low cost, high removal efficiency and environmental friendliness. In the present study, we isolated six strains from sludge with high concentrations of chemical oxygen demand, total nitrogen and total phosphorus levels. Among them, strain LH4 exhibited the greatest PO_4^{3-} removal ability. Strain LH4 is typical of *Acinetobacter junii* based on physiological, biochemical, and molecular analyses and is a PO_4^{3-} -accumulating organism (PAO) based on toluidine blue staining. The strain grew quickly when subjected to aerobic medium after pre-incubation under anaerobic condition, with a maximum OD_{600} of 1.429 after 8 h and PO_4^{3-} removal efficiency of 99%. Our data also indicated that this strain preferred utilizing the carbon (C) sources sodium formate and sodium acetate and the nitrogen (N) sources NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$ over other compounds. To achieve optimal PO_4^{3-} removal efficiency, a C:N ratio of 5:1, inoculation concentration of 3%, solution pH of 6, incubation temperature of 30 °C, and shaking speed of 100 rpm were recommended for *A. junii* strain LH4. By incubating this strain with different concentrations of PO_4^{3-} , we calculated that its relative PO_4^{3-} removal capacity ranged from 0.67 to 3.84 $\text{mg L}^{-1} \text{h}^{-1}$, ranking in the top three among reported PAOs. Our study provided a new PO_4^{3-} -accumulating bacterial strain that holds promise for remediating eutrophic waters, and its potential for large-scale use warrants further investigation.

Keywords Phosphate-accumulating organisms · Metachromatic granules · *Acinetobacter junii* · Eutrophication · Remediation

Introduction

Phosphorus (P) is an abundant element in the lithosphere and is essential for all living organisms (Liu et al. 2016). In aquatic systems, however, excessive P input can result in

eutrophication, leading to harmful algal blooms that impact fish growth and animal and human health (Ascott et al. 2016; Han et al. 2014). P-induced eutrophication has been frequently reported and has attracted widespread concern. When the P concentration exceeds 0.1 mg L^{-1} and the total P (TP) content is much lower than that of total nitrogen (TN), indicating P limitation (i.e., TN:TP > 15:1), eutrophication occurs (Han et al. 2014). In addition to eutrophication-triggered deterioration of water quality, phosphate (PO_4^{3-}) deposition is prevented by eutrophication, thereby reducing fertilizer production and global food security (Pantano et al. 2017).

Both natural and anthropogenic activities lead to P release into ecosystems. Biological transformation of P occurs ubiquitously and is often an important source of excess P in waters (Law al and Adeloju 2013). As a result, all types of non-labile PO_4^{3-} including ortho- PO_4^{3-} and condensed PO_4^{3-} (pyre-, meta- and poly- PO_4^{3-}) are eventually

Yong-He Han and Ting Fu contributed equally to this work.

✉ Min Li
mli@fjnu.edu.cn

¹ College of Life Science, Fujian Normal University, Fuzhou 350017, China

² Quangan Petrochemical Research Institute, Fujian Normal University, Quanzhou 362801, China

³ The Innovative Center for Eco-Friendly Polymeric Materials of Fujian Province, Quanzhou 362801, China

⁴ College of Environmental Science and Engineering, Fujian Normal University, Fuzhou 350007, China

converted into inorganic forms (Law al and Adeloju 2013). Recently, Meinikmann et al. (2015) reported a new natural source of P pollution in lakes during groundwater discharge, with P contaminants originating from wastewater. Similar to natural activities, human-induced imbalances between N and P have also been identified as an important factor altering natural and managed ecosystems across the globe (Peñuelas et al. 2013). Among anthropogenic activities, synthetic detergents are the largest source of inorganic P, while food and human wastes are the major sources of organic P (Law al and Adeloju 2013). For example, Liu et al. (2016) showed that intensification of P cycling in China since the 1600s is related to increasing population and demand for diets centered on animal proteins, leading to heavier P deposition in inland waters. Compared with the output from daily use or consumption of P-containing goods or foods, agricultural application results in slow P flux (Carpenter 2005). However, this activity may be more important for maintaining eutrophication in agricultural regions, as worldwide application of mineral P fertilizers to croplands is up to 17 Tg P per year, and such application can result in continuous transfer of P to adjacent water bodies (Carpenter 2005; Peñuelas et al. 2013). In addition to these activities, another source of the increasing P levels in drinking water is attributed to anthropogenic P addition in water treatment plants to reduce pipe corrosion, dissolved lead and copper concentrations in customers' taps and reduce the formation of iron and manganese precipitates (Ascott et al. 2016). Therefore, technologies for rapidly reducing P content in over-enriched waters and soils are necessary to improve water quality (Carpenter 2005).

(Bio)sorbent-mediated adsorption is a widely used method for remediating eutrophic waters. Several chemical materials such as Bephos™ (Zamparas et al. 2013), Al_2O_3 (Genz et al. 2004), zeolite (Gibbs and Özkundakci 2010), and iron compounds (Deppe and Benndorf 2002; Chen et al. 2016), as well as biomaterials such as sawdust (Pantano et al. 2017), biochar (Cai et al. 2017), corn straw (Wang et al. 2016a), and ostrich bone waste (Arshadi et al. 2015) have been investigated. Nano- and magnetic sorbents not only are efficient for P-removal but also can reclaim P from aqueous solutions (Cai et al. 2017). However, the major disadvantages of these materials are low efficiency, high cost, and potential toxicity to aquatic organisms (Zamparas et al. 2013). The environmentally friendly method of plant-based floating beds has been widely used for remediating eutrophic waters (Li et al. 2011). To enhance its effectiveness for N- and P-removal, denitrifying and PO_4^{3-} -accumulating organisms (PAOs) are often applied in the floating-bed system (Li et al. 2011). To date, several bacteria capable of efficient P accumulation have been reported, such as *Accumulibacter* (Acevedo et al. 2012; Tu and Schuler 2013), *Acinetobacter* (Hrenovic et al. 2010), *Alcaligenes* (Han et al. 2014; Joo

et al. 2006), *Alphaproteobacteria* (Nguyen et al. 2012), *Dechloromonas* (Tu and Schuler 2013), *Defluviicoccus* (Tu and Schuler 2013), and *Tetrasphaera* (Tu and Schuler 2013). Because PAOs can accumulate abundant PO_4^{3-} under aerobic conditions and transfer poly- PO_4^{3-} to ATP under anaerobic conditions to assist in the synthesis of polyhydroxyalkanoates, a growing number of studies have tried to use these bacteria to promote simultaneous removal of volatile fatty acids and P from wastewater (Yu and Li 2015).

By the 1980s, the P-accumulating characteristics of *Acinetobacter* had been reported by Fuhs and Chen (1975) and Deinema et al. (1980). Several studies have shown that *Acinetobacter* spp. play important roles in P-removal in activated sludge systems (Carr et al. 2003; Lötter et al. 1986). As a well-known PAO in the genus *Acinetobacter*, *A. junii* exhibits highly efficient P-removal from artificial wastewater and activated sludge (Hrenovic et al. 2010, 2011; Momba and Cloete 1996). However, its P accumulation characteristics under different conditions have not been fully elucidated. In this study, the new *A. junii* strain LH4 was isolated from sludge with high chemical oxygen demand (COD), TN and TP levels. The full objectives of this work are to evaluate bacterial growth and PO_4^{3-} removal efficiency under different physicochemical conditions and to evaluate the capacity of this strain to remove PO_4^{3-} , using different initial PO_4^{3-} concentrations.

Materials and methods

Bacteria isolation and PO_4^{3-} -accumulating ability identification

The sludge used for PAOs isolation was collected from a sewage outlet of a chemical plant located in Gaoqi Village, Shangjie County, Minhou Town, Fuzhou, China. Before chemical analyses, the sludge was freeze-dried (Chist Alpha1-2 LD, Marin Christ, Germany) for 48 h. Soil pH was determined in 0.01 M CaCl_2 (1:5 soil to solution). To determine the sludge properties, the dried samples were digested using USEPA method 3050B, filtered and diluted as required, followed by determination for COD, TN and TP concentrations (Han et al. 2014, 2016). The data showed that the sludge pH was 6.8, while the concentrations of COD, TN, and TP were 5324, 1430 and 1572 mg kg^{-1} , respectively.

To isolate potential PAOs, the fresh sludge was washed carefully 3 times by sterile Milli-Q water and centrifuged at 7015×g for 5 min (CR21G, Hitachi Koki Co., Ltd., Japan). After decanting the supernatant, 0.5 g of sludge was added to an enrichment medium (EM) according to Zhang et al. (2011). By incubating at 30 °C, 150 rpm for 12 h, 1.5 mL of established suspension was re-incubated in a new EM. For

each transfer, the component KH_2PO_4 in medium was gradually increased from 2 to 20 mg. Aliquots of the suspension containing 20 mg KH_2PO_4 were collected and diluted by sterile Milli-Q water, followed by streaking on a screening medium (SM) (Zhang et al. 2011). After 48 h of incubation, the colonies with specific morphology were picked out and re-incubated in Luria–Bertani (LB) medium for an additional enrichment. All bacterial suspensions were collected and mixed with 30% (v/v) glycerol (v:v, 1:1), followed by storing at -80°C for further use.

To determine PO_4^{3-} -accumulating ability in isolated strains, all bacteria were incubated in LB medium at 30°C , 150 rpm for 12 h. After centrifuging at $7015\times g$ for 5 min, the bacterial biomass was collected and re-incubated in a modified PO_4^{3-} -limited medium (MLM) consisting of 3.23 g $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$, 23.00 mg $\text{Na}_2\text{HPO}_4\cdot 2\text{H}_2\text{O}$, 152.80 mg NH_4Cl , 81.12 mg $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 11.00 mg $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ and 2 mL trace element solution in 1 L Milli-Q water at pH 7.0 ± 0.2 (Merzouki et al. 1999). The trace element solution consists of 63.70 g Na_2EDTA , 5.06 g $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$, 5.00 g $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$, 2.20 g ZnSO_4 , 5.50 g CaCl_2 , 1.10 g $\text{Na}_2\text{MoO}_4\cdot 4\text{H}_2\text{O}$, 1.57 g $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ and 1.61 g $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ in 1 L Milli-Q water at pH 7.2 ± 0.2 . After 12 h of incubation at 30°C and 150 rpm, the bacteria with 5% inoculation were transferred to a modified PO_4^{3-} -enriched medium (MEM) consisting of 3.23 g $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$, 35 mg KH_2PO_4 , 305.52 mg NH_4Cl , 91.26 mg $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 25.68 mg $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ and 2 mL trace element solution as previously described in 1 L Milli-Q water at pH 7.0 ± 0.2 (Merzouki et al. 1999). Bacterial PO_4^{3-} -accumulating ability was evaluated after 12 h of growth at 30°C and 150 rpm by determining the residual PO_4^{3-} concentrations in supernatant.

All media used in this study were autoclaved at 121°C for 20 min before use.

Strain LH4 identification

Before morphology observation, the strain was incubated in LB medium and centrifuged as previously described. The bacterial biomass was immobilized and dehydrated according to Wang et al. (2016b). In general, the biomass was immobilized by 2.5% glutaraldehyde (v/v, prepared by PBS consisting of 19 mL 0.2 M NaH_2PO_4 and 81 mL 0.2 M Na_2HPO_4 at pH 7.4) for 4 h, washed three times carefully by PBS, and dehydrated by ethyl alcohol with increasing concentrations of 30, 50, 70, 85, 90% (15 min per rinse), and 100% (twice, 15 min per rinse). After that, the biomass was washed by isoamyl acetate (twice, 20 min per rinse) to remove ethyl alcohol and stored at -20 , -40 , and -80°C for 12 h, respectively. Once being freeze-dried for 48 h, the bacterial morphology was observed by scanning electron microscopy (SEM; JSM-6500F, JEOL, Japan).

To identify whether the strain belonged to PAOs, the metachromatic granules were tested by toluidine blue according to Zhuang et al. (2014). Before test, the bacteria were incubated in LB medium as previously described. Aliquots of bacterial suspension were pipetted onto the glass slides, air-dried and hot-fixed. After that, the biomass was stained by toluidine blue for 5 min, followed by staining by potassium iodide for 1 min and observing by a DME microscopy (Leica, Germany). After each treatment, the slides were washed carefully with sterile Milli-Q water for 10 s to remove all chemicals. It is expected to observe the purple granules inside bacterial cells if poly- PO_4^{3-} presents.

The bacterial physiological and biochemical characteristics were tested by using a bioMerieux identification system (VITEK® 2, France) in Fujian Center for Disease Control and Prevention (Fuzhou, China). A total of 48 substrates for this purpose were included in Biolog-GN II microplate™ (Biolog, Inc., USA). Before molecular identification, the bacterial genomic DNA was extracted by using a DNA extraction kit (NEP021-1, Beijing Dingguo Changsheng Biotechnology Co. Ltd., China) according to the manufacturer's instructions. The 16S rRNA gene was amplified by the universal primers 27F (AGAGTTTGTATCTGGCTCAG) and 1492R (ACGGCTACCTTGTACGACTT) on a 2720 Thermal cycler (Applied Biosystems, USA). The polymerase chain reaction (PCR) mixtures consisted of 12.5 μL 2 $\times$ Taq PCR Colorless Mix (Beijing Dingguo Changsheng Biotechnology Co. Ltd., China), 0.5 μL of each 10 μM primers, 10 μL of PCR degrade water and 1 μL of DNA template. The PCR programs for 16S rRNA amplification consisted of pre-denaturation for 5 min at 94°C , followed by 35 cycles of denaturation and annealing (30 s at 94°C , 30 s at 55°C and 40 s at 72°C), and a final extension at 72°C for 10 min (Han et al. 2017). The PCR product was purified and sequenced by Beijing Dingguo Changsheng Biotechnology Co. Ltd., China. After an online analysis by BLAST similarity search against the known sequences in NCBI database, the phylogenetic tree was constructed by using the neighbor-joining algorithm (Saitou and Nei 1987) in MEGA 5.0 software (Tamura et al. 2011).

Batch experiments for PO_4^{3-} -accumulating ability identification

To test bacterial growth and PO_4^{3-} -accumulating ability over a long-term incubation, the bacteria with 5% inoculation concentration were incubated in LB medium at 30°C , 150 rpm for 12 h, followed by another 12 h of incubation in MLM under anaerobic condition (N control system). After that, the bacterial suspension with 5% inoculation concentration was transferred to a new MEM for a total incubation time of 120 h. The sampling was conducted at 1, 2, 3, 5, 8, and 12 h for the first 12 h, and every 12 h over the

left period. Bacterial growth was recorded as optical density (OD) at 600 nm and PO_4^{3-} concentration was determined by using molybdenum-blue reaction method (Anschutz and Deborde 2016). PO_4^{3-} removal efficiency was recorded as % of the final concentration to the initial concentration.

To investigate the impacts of different conditions on PO_4^{3-} removal efficiency, the bacteria were pre-incubated in LB medium and MLM as shown above for 12 h, respectively. The re-suspensions were incubated in MEM with different pHs (4–9), temperatures (25–40 °C), carbon (C) sources (sodium formate, sodium acetate, sodium propionate, sodium succinate, sodium citrate, glucose, and sucrose), N sources (NH_4Cl , NaNO_3 , NaNO_2 , peptone, NH_4NO_3 , and $(\text{NH}_4)_2\text{SO}_4$), C:N ratios (1:3–10:1), shaking speeds (0–250 rpm), inoculation concentrations (1–9%) and initial PO_4^{3-} concentrations (10–100 mg L^{-1}). The OD_{600} was used to record bacterial growth and the PO_4^{3-} concentrations in supernatant were determined by using molybdenum-blue method as previously described. To compare PO_4^{3-} removal efficiencies in different PAOs, i.e., the strain LH4 vs. other reported bacteria, the relative removal content of PO_4^{3-} ($\text{mg h}^{-1} \text{L}^{-1}$) was also calculated.

Statistical analysis

All experiments were performed in triplicate and the data were presented as the mean of triplicates with standard error. A two-way analysis of variance (ANOVA) was used to determine the significant differences by Tukey's multiple comparisons test at $P \leq 0.05$ using GraphPad Prism (Release 6.0, USA).

Results and discussion

Six strains were isolated from sludge

Through sequential enrichment in medium containing 2, 5, 8, 10, 15, and 20 $\text{mg KH}_2\text{PO}_4$, six potential PAOs were isolated based on colony morphology. To evaluate their PO_4^{3-} removal abilities, all isolates were incubated in LB medium for 12 h, followed by sequential anaerobic–aerobic incubation for 12 h each. As shown in Table 1, all strains were capable of PO_4^{3-} removal, with removal efficiencies ranging from 21% (LH2) to 91% (LH4). Because strain LH4

had the highest PO_4^{3-} removal efficiency, it was used for follow-up experiments.

Strain LH4 belongs to *A. junii*

Strain LH4 formed a round, pale yellow colony on LB agar medium with bright white coloration along the colony margin (Fig. 1a). The bacterial cells were rod-shaped without flagella (Fig. 1b). The size of each cell was approximately 0.7–1.5 μm long by 0.4–0.5 μm wide (Fig. 1b).

Strain LH4 was a Gram-negative bacterium based on the results of Gram staining (data not shown), and its physiological and biochemical characteristics were investigated using a Biolog-GN microplate. Among 48 substrates, glutamyl arylamidase pNA, tyrosine arylamidase, L-lactate alkalization, succinate alkalization, phosphatase, L-histidine assimilation, coumarate, L-malate assimilation and L-lactate assimilation displayed positive results (Table 2).

Fig. 1 Colony morphology of the strain LH4 grown on LB agar medium (a) and SEM observation of the bacterial cell morphology (b, $\times 5000$)

Table 1 Phosphate removal efficiency in PAOs isolated from the sludge

Strains	L4	L5	L7	L10	LH2	LH4
PO_4^{3-} concentration (mg L^{-1}) ^a	19.2	11.0	12.7	12.2	19.4	2.1
Removal efficiency (%)	21.6	55.1	48.0	50.2	20.8	91.4

^aInitial concentration was 24.5 mg L^{-1}

Table 2 Selected physiological and biochemical characteristics of strain LH4 analyzed by Biolog-GN II microplate™

Substrates	Abbreviations	Results	Substrates	Abbreviations	Results
Ala-Phe-Pro-arylamidase	APPA	— ^a	Saccharose/sucrose	SAC	—
Adonitol	ADO	—	D-Tagatose	dTAG	—
L-Pyrrolydonyl-arylamidase	PyrA	—	D-Trehalose	dTRE	—
L-Arabitol	IARL	—	Citrate (sodium)	CIT	—
D-Cellobiose	dCEL	—	Malonate	MNT	—
β-Galactosidase	BGAL	—	5-Keto-D-gluconate	5KG	—
H ₂ S production	H ₂ S	—	L-Lactate alkalisation	ILATK	+
β-N-acetyl-glucosaminidase	BNAG	—	α-Glucosidase	AGLU	—
Glutamyl arylamidase pNA	AGLTp	+ ^b	Succinate alkalisation	SUCT	+
D-Glucose	DGLU	—	β-N-acetyl-galactosaminidase	NAGA	—
γ-Glutamyl-transferase	GGT	—	α-Galactosidase	AGAL	—
Fermentation/glucose	OFF	—	Phosphatase	PHOS	+
β-Glucosidase	BGLU	—	Glycine arylamidase	GlyA	—
D-Maltose	dMAL	—	Ornithine decarboxylase	ODC	—
D-Mannitol	dMAN	—	Lysine decarboxylase	LDC	—
D-Mannose	dMNE	—	Decarboxylase base	ODEC	—
β-Xylosidase	BXYL	—	L-Histidine assimilation	IHISA	+
β-Alanine arylamidase pNA	BAlap	—	Courmarate	CMT	+
L-Proline arylamidase	ProA	—	β-Glucuronidase	BGUR	—
Lipase	LIP	—	O/129 resistance	O129R	—
Palatinose	PLE	—	Glu-Gly-Arg-arylamidase	GGAA	—
Tyrosine arylamidase	TyrA	+	L-Malate assimilation	IMLTa	+
Urease	URE	—	ELLMAN	ELLM	—
D-Sorbitol	dSOR	—	L-Lactate assimilation	ILATa	+

^aNegative^bPositive

With reference to the Taxonomic Outline of the Prokaryotes, Bergey's Manual of Systematic Bacteriology (Garrity et al. 2004), strain LH4 exhibited some similarities to *A. junii*. For example, *A. junii* cannot utilize most carbohydrates, in particular D-glucose, which was supported by our data (Table 2). Strain LH4 utilized L-histidine but exhibited a negative result in the ornithine decarboxylase analysis (Table 2), which was consistent with Garrity et al. (2004). Moreover, the characteristics of lactate, malate and tyrosine utilization by LH4 were also reported in a previous study (Bouvet and Grimont 1986). On the other hand, 82 and 95% of reported strains can utilize sodium citrate and display a positive result in the lysine decarboxylase analysis, respectively (Garrity et al. 2004; Bouvet and Grimont 1986), but LH4 did not (Table 2). Meanwhile, analyses of glutamyl arylamidase pNA, phosphatase, courmarate and succinate (positive results in LH4) have not yet been reported in *A. junii* (Table 2). To further clarify this bacterium's evolutionary position, 16S rRNA gene sequencing and sequence alignment were also conducted. The results showed that the 16S rRNA gene (GenBank accession number KY006113) of strain LH4 had 99% similarity to that of *A. junii* strain

WAB1940 with 99% bootstrap support (Fig. 2). Based on the physiological, biochemical and molecular evidence, we concluded that LH4 was a typical strain of *A. junii*.

A. junii strain LH4 is a typical PAO

As described previously, strain LH4 removed 91% of 24.5 mg L⁻¹ PO₄³⁻ in 12 h (Table 1), indicating that it might be a typical PAO. To support this hypothesis, the strain was stained with toluidine blue, and metachromatic granules were observed inside bacterial cells (Fig. 3a). As such, we concluded that the strain was a typical PAO, as reported previously (Hrenovic et al. 2010; Momba and Cloete 1996). Because *A. junii* is a commonly observed PAO in activated sludge systems, and to our knowledge, its PO₄³⁻-accumulating characteristics are not well-known, our study focused mainly on the bacterial growth and PO₄³⁻ removal efficiency of *A. junii* strain LH4 under different conditions.

Efficient PO₄³⁻ accumulation in PAOs is associated with both bacterial biomass and growth stage (Momba and Cloete 1996; Boswell et al. 2001). PO₄³⁻ release from cells under

Fig. 2 The neighbor-joining phylogenetic tree based on 16S rRNA sequences of the strain LH4 and the reference strains from NCBI database. The tree root was constructed with bootstrap values calculated from 1000 resamplings. The numbers at each node indicate the percentage of bootstrap support. The scale bar indicates 5 divergences per 1000 bases. The numbers in brackets are 16S rRNA gene sequence accession numbers in GenBank database. The sequences were aligned and the phylogenetic tree was constructed by Mega 5.0 software

anaerobic conditions is the key factor driving abundant PO_4^{3-} accumulation (Boswell et al. 2001; Mulkerrins et al. 2004). In our study, the bacteria were pre-incubated under anaerobic conditions for 12 h for PO_4^{3-} release and then transferred to fresh aerobic medium for PO_4^{3-} accumulation. In this process, strain LH4 removed 96% of 24.5 mg L^{-1} PO_4^{3-} after 3 h of growth (Fig. 3b). Correspondingly, the strain had an OD_{600} of 1.164 (Fig. 3b). The highest PO_4^{3-} removal efficiency (99%) and highest OD_{600} (1.429) were achieved after 8 h of growth (Fig. 3b). After 120 h of growth, the OD_{600} decreased to 1.139, with the PO_4^{3-} removal efficiency decreasing to 38% (Fig. 3b). In both the logarithmic and decline phases, the OD_{600} was well correlated with the PO_4^{3-} removal efficiency ($R^2 > 0.90$, $P < 0.001$; Fig. 3c), indicating a positive relationship between bacterial biomass and PO_4^{3-} accumulation (Momba and Cloete 1996). However, during the stationary phase, all OD_{600} measurements were similar, as were the PO_4^{3-} removal efficiencies (Fig. 3c). Our data indicated that the pre-incubation process under anaerobic conditions enhanced subsequent PO_4^{3-} accumulation, explaining why this strain grew so quickly and reached the logarithmic phase without exhibiting a lag phase (Fig. 3b). Moreover, the accumulated PO_4^{3-} was re-released into the medium once the bacteria died, resulting in a sharp decrease in PO_4^{3-} removal efficiency after 36 h of incubation (Fig. 3c). Because P pollution varies among environments, it is important to understand bacterial growth and PO_4^{3-} removal efficiency under different environmental conditions.

Effects of physicochemical conditions and key nutrients on bacterial growth and PO_4^{3-} removal

Studies have shown that pH and temperature are important environmental parameters that influence bacterial metabolic

activities, nutrient availability and gas-transfer rates in medium (Mulkerrins et al. 2004). Another environmental parameter affecting bacterial growth is the dissolved oxygen (DO) concentration (Olsvik and Kristiansen 1992; Peña et al. 2000). For optimal growth, bacteria often need nutrients such as C and N sources, although some bacteria can grow well in minimal salt medium (Ingraham et al. 1983). All of these factors are also important to the growth of, and PO_4^{3-} accumulation by, PAOs (Mulkerrins et al. 2004), which were also investigated in our study.

As shown in Fig. 4a, strain LH4 could not grow in medium with a pH < 6.0, while it had similar OD_{600} values of 1.320–1.352 when the medium pH ranged from 6 to 9. Similar to bacterial growth, low pH also completely inhibited PO_4^{3-} removal (Fig. 4a), supporting the close association between PO_4^{3-} removal and bacterial biomass (Momba and Cloete 1996). Our data indicated that strain LH4 preferred to grow at a high pH, consistent with the results reported by Filipe et al. (2001a) that the PO_4^{3-} uptake, polyhydroxyalkanoate consumption and bacterial growth rates observed at pH 6.5 were 42, 70 and 53%, respectively, of those observed at pH 7.0. Other studies have also reported negative effects of low pH on bacterial growth and PO_4^{3-} uptake (Zhuang et al. 2014; Filipe et al. 2001b).

In contrast to pH, better bacterial growth and PO_4^{3-} uptake in PAOs are achieved at lower temperatures (Whang and Park 2006). For example, Florentz et al. (1987) observed greater P-removal efficiency at low temperatures (5–15 °C) compared with high temperatures (> 15 °C). In our study, the OD_{600} (i.e., 1.753) in strain LH4 was highest at 30 °C, with 24% greater biomass than that at 25 °C (Fig. 4b). In addition, the strain grew well at 37 °C ($\text{OD}_{600} = 1.375$), but its OD_{600} decreased to 0.449 at 40 °C (Fig. 4b). Our data were consistent with the findings of Garrity et al. (2004) and

Fig. 3 Metachromatic granule staining (a, $\times 1000$), the growth curve and PO_4^{3-} removal efficiency over 120 h (b) and the correlations between bacterial biomass and PO_4^{3-} removal efficiency (c). The bacteria with 5% inoculation concentration were incubated at 30 °C, 150 rpm and pH 7.0 ± 0.2 . The initial concentration of PO_4^{3-} was 24.5 mg L^{-1}

Bouvet and Grimont (1986) that *A. junii* can only grow well at temperatures ≤ 37 °C, whereas 90% of reported strains can grow at temperatures up to 41 °C. Additionally, although this strain exhibited its highest OD₆₀₀ at 30 °C, that was not the case for PO_4^{3-} removal efficiency. As shown in Fig. 4b, the greatest PO_4^{3-} removal efficiency was 92% at 35 °C. This discrepancy was likely due to the differing effects of temperature on metabolic activities and bacterial growth (Mulkerrins et al. 2004). Greater PO_4^{3-} removal at higher temperatures (30–37 °C) was supported by Converti et al.

(1995) but not by Florentz et al. (1987), indicating that different PAOs exhibit different responses to temperature.

In addition to solution pH and temperature, we also determined the impact of shaking speed, a key factor influencing DO and substrate exchange, on bacterial growth and PO_4^{3-} removal efficiency. As expected, strain LH4 grew well and removed PO_4^{3-} from aqueous solution under both anoxic and aerobic conditions (Fig. 4c). Specifically, both bacterial growth (OD₆₀₀ = 0.440–1.447) and PO_4^{3-} removal efficiency (39–99%) increased with an increase in shaking speed from 0 to 100 rpm (Fig. 4c). This result indicated that higher DO concentration and greater substrate exchange increased nutrient utilization and promoted bacterial growth, thereby increasing PO_4^{3-} uptake into cells (Peña et al. 2000; Olsvik and Kristiansen 1992). Our data also showed that both bacterial growth and PO_4^{3-} removal efficiency were stable at shaking speeds up to 250 rpm (Fig. 4c), in contrast to previous findings that excessive aeration led to deterioration of biological P-removal in an activated sludge system (Peng et al. 2006). It has been shown that 4 mg L^{-1} DO is sufficient to stimulate PO_4^{3-} removal, but higher oxygen concentrations represent a waste of energy for aeration purposes (Mulkerrins et al. 2004). Therefore, maintaining a shaking speed of 100 rpm is adequate for efficient PO_4^{3-} uptake in strain LH4.

As noted previously, both C and N sources are very important for bacterial growth, and this is equally true for PAOs (Ingraham et al. 1983; Zhuang et al. 2014; Madigan et al. 2008). While C sources serve as substrates for polyhydroxybutyrate synthesis in PAOs, N sources are often involved in the synthesis of proteins, nucleic acids and other cell components (Yu and Li 2015; Madigan et al. 2008). In our study, C sources including sodium formate, sodium acetate, sodium propionate, sodium succinate, sodium citrate, glucose and sucrose, and N sources including NH_4Cl , NaNO_3 , NaNO_2 , peptone, NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ were investigated. Although the trait of acetate utilization has been widely documented in PAOs (Cokgor et al. 2004; Soejima et al. 2006), our data indicated that strain LH4 utilized only sodium formate and sodium acetate (Fig. 4d), with sodium formate resulting in both a higher OD₆₀₀ and PO_4^{3-} removal efficiency compared with sodium acetate (1.942 vs. 1.72 and 95 vs. 90%, respectively; Fig. 4d). On the other hand, sodium propionate was not well utilized (Fig. 4d). This may be expected because these compounds have different –COOH groups, implying that nutrients with low molecular weights are more easily degraded for growth (Zhuang et al. 2014). Interestingly, the strain had an OD₆₀₀ of 0.222 and removed 26% of 24.5 mg L^{-1} PO_4^{3-} in the presence of sodium succinate (Fig. 4d), in agreement with the results of physiological and biochemical characterization (Table 2). Unlike C sources, aside from peptone, all N sources tested were utilized by strain LH4 (Fig. 4e). Among them, NH_4Cl

Fig. 4 Bacterial growth and PO_4^{3-} removal efficiency of strain LH4 for 12 h of incubation under different pHs (a), temperatures (b), shaking speeds (c), C sources (d), N sources (e), C:N ratios (f) and inoculation concentrations (g). The bacteria with 5% inoculation concentration were incubated at 30 °C, 150 rpm and pH 7.0 ± 0.2 in medium containing sodium acetate and NH_4Cl unless otherwise noticed. The initial concentration of PO_4^{3-} was 24.5 mg L^{-1}

and $(\text{NH}_4)_2\text{SO}_4$ benefited bacterial growth to similar extents, leading to higher OD₆₀₀ values of 1.286 and 1.305, compared with other N sources (Fig. 4e). The corresponding PO_4^{3-} removal efficiencies were 94 and 93%, respectively (Fig. 4e). Although the presence of nitrite (NO_2^-), generally at concentrations of 8 mg L^{-1} or greater, often displays inhibitory effects on bacterial growth and PO_4^{3-} removal by

PAOs (Sin et al. 2008; Zeng et al. 2014), LH4 did not follow this trend. For example, strain LH4 had an OD₆₀₀ of 1.086 and removed 90% of $24.5 \text{ mg L}^{-1} \text{ PO}_4^{3-}$ in the presence of $394 \text{ mg L}^{-1} \text{ NaNO}_2$ (i.e., $263 \text{ mg L}^{-1} \text{ NO}_2^-$; Fig. 4e), showing a similar removal efficiency with 16% less biomass. Similar results were observed with the addition of NaNO_3 and NH_4NO_3 (Fig. 4e). Our data indicated that strain LH4

preferred to utilize N sourced from NH_4^+ rather than from NO_3^- or NO_2^- . Moreover, strain LH4 tolerated high concentrations of NO_3^- and NO_2^- , thus displaying good potential for the remediation of eutrophic waters with high N contents.

Most bacteria need more N than do fungi or actinomycetes, whereas fungi and actinomycetes need more C than do other bacteria (Madigan et al. 2008). Imbalances between C and N may impact bacterial growth (Goldman et al. 1987); for example, the PAO *Alcaligenes* sp. ZGED-12 grew better with increasing C:N ratios from 1:1 to 1:3, but its OD_{600} decreased at a C:N ratio of 5:1 or greater (Zhuang et al. 2014). This result was attributed to the inefficient use of N sources at high C:N ratios (Zhuang et al. 2014), which was supported by our data. As shown in Fig. 4f, while the OD_{600} of LH4 was 0.233 at a C:N ratio of 1:3, it increased to 1.753 at a C:N ratio of 7:1 and then decreased to 1.667 at a C:N ratio of 10:1. Therefore, in addition to the types of C and N sources available, C:N ratios are also important for bacterial growth and PO_4^{3-} removal.

Because bacterial biomass was correlated with PO_4^{3-} uptake (Figs. 3c, 4e, f) (Momba and Cloete 1996), we also determined bacterial growth and PO_4^{3-} removal efficiencies at different inoculation concentrations ranging from 1 to 10%. The results showed that a 1% inoculation concentration of strain LH4 exhibited an OD_{600} of 1.354 and took up 98% of spiked PO_4^{3-} after 12 h of incubation (Fig. 4g). With increasing inoculation concentrations, both bacterial growth ($\text{OD}_{600}=1.376$) and PO_4^{3-} (100%) removal efficiency first increased, followed by considerable decreases to 1.311 and 93%, respectively (Fig. 4g). This result again verified that, within a specific range, greater biomass led to higher PO_4^{3-} removal efficiency. Meanwhile, an excess concentration of the PAO suspension might result in uptake competition and thus decrease PO_4^{3-} accumulation.

A. junii strain LH4 efficiently removed PO_4^{3-} from aqueous solution

To fully clarify the PO_4^{3-} accumulation ability of strain LH4, the bacteria were grown in media spiked with 10, 25, 40, 70 or 100 mg L^{-1} PO_4^{3-} (P_{10} , P_{25} , P_{40} , P_{70} or P_{100} , respectively). In the P_{10} , the bacteria exhibited an OD_{600} of 1.27 and removed 81% of the PO_4^{3-} in 12 h (Fig. 5). Although the bacterial OD_{600} in P_{25} was similar to that in P_{10} (1.281 vs. 1.27), the PO_4^{3-} removal efficiency (92%) was 11% greater (Fig. 5). As PO_4^{3-} removal was associated with both utilization for bacterial growth and cell P-accumulation, the low removal efficiency in P_{10} might be because of dominant use of PO_4^{3-} for bacterial growth (Fig. 5), as observed using low-P medium, i.e., MLM (Merzouki et al. 1999). In contrast, it is likely that the PO_4^{3-} -accumulation system was activated by

Fig. 5 Bacterial growth and PO_4^{3-} removal efficiency of strain LH4 for 12 h of incubation with different initial concentrations of PO_4^{3-} . The bacteria with 3% inoculation concentration were incubated at 30 °C, 150 rpm and pH 7.0 $\pm$ 0.2 in MEM

25 mg L^{-1} PO_4^{3-} (Fig. 5), a typical concentration in MEM (Merzouki et al. 1999). While the OD_{600} increased by 9–18% (1.396–1.513) in P_{40} , P_{70} and P_{100} , the PO_4^{3-} removal efficiency decreased to 45–79% of that in P_{25} (Fig. 5). This result indicated that high PO_4^{3-} levels enhanced bacterial growth, which is also related to other factors such as C and N sources, pH and DO (Mulkerrins et al. 2004).

In addition to bacterial growth and PO_4^{3-} removal efficiency, we also calculated the relative removal capacities of strain LH4 and other PAOs. To make the data comparable, all data were presented as depleted PO_4^{3-} content ($\text{mg L}^{-1} \text{ h}^{-1}$) (Table 3). To date, dozens of PAOs have been reported, of which only about 30 strains have been fully investigated in terms of their relative PO_4^{3-} removal capacity at the laboratory scale, and some representative strains are listed in Table 3. Among them, only five strains had relative removal capacities of 3.0 $\text{mg L}^{-1} \text{ h}^{-1}$ or greater, with the highest value of 4.12 $\text{mg L}^{-1} \text{ h}^{-1}$ observed in *Pseudomonas fluorescens* (Table 3). Strain LH4 ranked among the top three (Table 3). In addition, six strains of *A. junii* (Momba and Cloete 1996), *A. denitrificans* (Sidat et al. 1999), *Alcaligenes* sp. (Zhuang et al. 2014), *B. cereus* (Sidat et al. 1999), *P. testosteroni* (Sidat et al. 1999) and *S. epidermidis* (Sidat et al. 1999) had relative removal capacities ranging from 2.0 to 3.0 $\text{mg L}^{-1} \text{ h}^{-1}$ (Table 3). Although strain LH4 displayed a strong ability for PO_4^{3-} accumulation, its potential use in eutrophic water remediation warrants further investigation. Future work should be performed at a large-scale, e.g., a sequencing batch reactor with an activated sludge process, to evaluate the industrial applications of PAOs.

Table 3 Phosphate removal efficiency and relative removal capacity in representative PAOs

PAOs	Initial concentration (mg L ⁻¹)	pH	Time (h)	Removal efficiency (%)	Relative removal capacity (mg L ⁻¹ h ⁻¹)	References
<i>A. calcoaceticus</i> var. <i>lwoffii</i>	38	7.5	5	48	3.68	Sidat et al. (1999)
<i>A. junii</i>	61.5	7.0	24	40	1.05	Hrenovic et al. (2010) ^a
<i>A. junii</i>	35	7.0	8	35	1.69	Momba and Cloete (1996)
<i>A. junii</i>	35	7.0	11	66	2.09	Momba and Cloete (1996)
<i>Aeromonas hydrophila</i>	38	7.5	5	23	1.72	Sidat et al. (1999)
<i>A. denitrificans</i>	38	7.5	5	36	2.76	Sidat et al. (1999)
<i>Alcaligenes</i> sp.	100	8.0	24	59	2.46	Zhuang et al. (2014)
<i>Bacillus cereus</i>	38	7.5	5	36	2.74	Sidat et al. (1999)
<i>Enterobacter</i> sp.	10	7.2	48	88	0.19	Zhang et al. (2011)
<i>Enterobacteriaceae</i> sp.	25.6	7.0	20	35	0.45	Bao et al. (2007) ^b
<i>Microlunatus phosphovor</i>	n.m. ^c	6.8–7.0	19	n.m	0.31–0.42	Onda and Takii (2002)
<i>Moraxella phenylpyruvica</i>	38	7.5	5	42	3.16	Sidat et al. (1999)
<i>P. acidovorans</i>	38	7.5	5	23	1.78	Sidat et al. (1999)
<i>P. fluorescens</i>	38	7.5	5	54	4.12	Sidat et al. (1999)
<i>P. mendocina</i>	38	7.5	5	52	3.92	Sidat et al. (1999)
<i>P. testosteroni</i>	38	7.5	5	35	2.66	Sidat et al. (1999)
<i>Pseudomonas</i> sp.	25.6	7.0	20	56	0.72	Bao et al. (2007) ^d
<i>Staphylococcus epidermidis</i>	38	7.5	5	27	2.08	Sidat et al. (1999)
<i>A. junii</i>	25	7.0	12	92	1.92	In this study
	70	7.0	12	66	3.84	

^aThe bacteria were immobilized onto sepiolite^bAerobic P accumulation capacity^cNot mentioned^dAnoxic P accumulation capacity

Conclusion

In this study, *A. junii* strain LH4, which is a PAO, was isolated from sludge with high COD, TN and TP levels and was characterized using physiological, biochemical and molecular tests. This strain preferred to utilize sodium formate and sodium acetate as C sources and to utilize NH₄Cl and (NH₄)₂SO₄ as N sources. The optimal physicochemical conditions included a C:N ratio of 5:1, inoculation concentration of 3%, solution pH of 6, incubation temperature of 30 °C and shaking speed of 100 rpm, which allowed ideal PO₄³⁻ removal by the strain. Because the relative PO₄³⁻ removal capacity (0.67–3.84 mg L⁻¹ h⁻¹) of strain LH4 ranked among the top three of the reported PAOs, we concluded that it may be a good candidate for potential use in the remediation of eutrophic waters.

Acknowledgements This work was financially supported in part by the Science and Technology Program of Fujian Province (2017Y0027), the Education Department Fund of Fujian Province (JAT170144), the Key Research and Development Platform of Advanced Polymer Materials (2016G003), the Key Technology Research and Development Platform of Synthetic Resin Functionalization of Fujian Province (2014H2003), the Science and Technology Program of Quanzhou

(2016Z019), the Science and Technology Program of Quangan (2016G16) and the Innovative Entrepreneurial Training Plan for College Students of Fujian Province (201510394020).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

- Acevedo B, Oehmen A, Carvalho G, Seco A, Borrás L, Barat R (2012) Metabolic shift of polyphosphate-accumulating organisms with different levels of polyphosphate storage. *Water Res* 46:1889–1900
- Anschutz P, Deborde J (2016) Spectrophotometric determination of phosphate in matrices from sequential leaching of sediments. *Limnol Oceanogr Methods* 14:245–256
- Arshadi M, Gholtash JE, Zandi H, Foroughifard S (2015) Phosphate removal by a nano-biosorbent from the synthetic and real (Persian Gulf) water samples. *RSC Adv* 5:43290–43302
- Ascott MJ, Gooddy DC, Lapworth DJ, Stuart ME (2016) Estimating the leakage contribution of phosphate dosed drinking water to environmental phosphorus pollution at the national-scale. *Sci Total Environ* 572:1534–1542

- Bao L-L, Li D, Li X-K, Huang R-X, Zhang J, Lv Y, Xia G-Q (2007) Phosphorus accumulation by bacteria isolated from a continuous-flow two-sludge system. *J Environ Sci China* 19:391–395
- Boswell CD, Dick RE, Eccles H, Macaskie LE (2001) Phosphate uptake and release by *Acinetobacter johnsonii* in continuous culture and coupling of phosphate release to heavy metal accumulation. *J Ind Microbiol Biotechnol* 26:333–340
- Bouvet PJM, Grimont PAD (1986) Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int J Syst Evol Microbiol* 36:228–240
- Cai R, Wang X, Ji X, Peng B, Tan C, Huang X (2017) Phosphate reclaim from simulated and real eutrophic water by magnetic biochar derived from water hyacinth. *J Environ Manag* 187:212–219
- Carpenter SR (2005) Eutrophication of aquatic ecosystems: bistability and soil phosphorus. *Proc Natl Acad Sci USA* 102:10002–10005
- Carr EL, Kämpfer P, Patel BKC, Gürtler V, Seviour RJ (2003) Seven novel species of *Acinetobacter* isolated from activated sludge. *Int J Syst Evol Microbiol* 53:953–963
- Chen M, Sun H-Q, Jiang H-L (2016) The addition of FeOOH binds phosphate in organic matter-rich sediments. *Chem Ecol* 32:432–445
- Cokgor EU, Yagci NO, Randall CW, Artan N, Orhon D (2004) Effects of pH and substrate on the competition between glycogen and phosphorus accumulating organisms. *J Environ Sci Health A* 39:1695–1704
- Converti A, Rovatti M, Borghi MD (1995) Biological removal of phosphorus from wastewaters by alternating aerobic and anaerobic conditions. *Water Res* 29:263–269
- Deinema MH, Habets LHA, Scholten J, Turkstra E, Webers HAAM (1980) The accumulation of polyphosphate in *Acinetobacter* spp. *FEMS Microbiol Lett* 9:275–279
- Deppe T, Benndorf J (2002) Phosphorus reduction in a shallow hypereutrophic reservoir by in-lake dosage of ferrous iron. *Water Res* 36:4525–4534
- Filipe CDM, Daigger GT, Grady CPL (2001a) Effects of pH on the rates of aerobic metabolism of phosphate-accumulating and glycogen-accumulating organisms. *Water Environ Res* 73:213–222
- Filipe CDM, Daigger GT, Grady CPL (2001b) pH as a key factor in the competition between glycogen-accumulating organisms and phosphorus-accumulating organisms. *Water Environ Res* 73:223–232
- Florentz M, Caille D, Bourdon F, Sibony J (1987) Biological phosphorus removal in France. *Water Sci Technol* 19:1171–1173
- Fuhs GW, Chen M (1975) Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. *Microb Ecol* 2:119–138
- Garrrity GM, Bell JA, Lilburn TG (2004) Taxonomic outline of the prokaryotes. *Bergey's manual® of systematic bacteriology*. Springer, Berlin
- Genz A, Kornmüller A, Jekel M (2004) Advanced phosphorus removal from membrane filtrates by adsorption on activated aluminium oxide and granulated ferric hydroxide. *Water Res* 38:3523–3530
- Gibbs M, Özkundakci D (2010) Effects of a modified zeolite on P and N processes and fluxes across the lake sediment-water interface using core incubations. *Hydrobiologia* 661:21–35
- Goldman JC, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol Oceanogr* 32:1239–1252
- Han Y, Zhang W, Lu W, Zhou Z, Zhuang Z, Li M (2014) Co-immobilization of *Pseudomonas stutzeri* YHA-13 and *Alcaligenes* sp. ZGED-12 with polyvinyl alcohol-alginate for removal of nitrogen and phosphorus from synthetic wastewater. *Environ Technol* 35:2813–2820
- Han Y-H, Yang G-M, Fu J-W, Guan D-X, Chen Y, Ma LQ (2016) Arsenic-induced plant growth of arsenic-hyperaccumulator *Pteris vittata*: impact of arsenic and phosphate rock. *Chemosphere* 149:366–372
- Han Y-H, Fu J-W, Xiang P, Cao Y, Rathinasabapathi B, Chen Y, Ma LQ (2017) Arsenic and phosphate rock impacted the abundance and diversity of bacterial arsenic oxidase and reductase genes in rhizosphere of As-hyperaccumulator *Pteris vittata*. *J Hazard Mater* 321:146–153
- Hrenovic J, Tibljas D, Ivankovic T, Kovacevic D, Sekovanic L (2010) Sepiolite as carrier of the phosphate-accumulating bacteria *Acinetobacter junii*. *Appl Clay Sci* 50:582–587
- Hrenovic J, Kovacevic D, Ivankovic T, Tibljas D (2011) Selective immobilization of *Acinetobacter junii* on the natural zeolitized tuff in municipal wastewater. *Colloid Surf B* 88:208–214
- Ingraham JL, Maaløe O, Neidhardt FC (1983) Growth of the bacterial cells. Sinauer Associates, Inc., Sunderland
- Joo H-S, Hirai M, Shoda M (2006) Piggery wastewater treatment using *Alcaligenes faecalis* strain No. 4 with heterotrophic nitrification and aerobic denitrification. *Water Res* 40:3029–3036
- Law al AT, Adeloju SB (2013) Progress and recent advances in phosphate sensors: a review. *Talanta* 114:191–203
- Li X-N, Song H-L, Li W, Lu X-W, Nishimura O (2011) An integrated ecological floating-bed employing plant, freshwater clam and biofilm carrier for purification of eutrophic water. *Ecol Eng* 36:382–390
- Liu X, Sheng H, Jiang S, Yuan Z, Zhang C, Elser JJ (2016) Intensification of phosphorous cycling in China since the 1600s. *Proc Natl Acad Sci USA* 113:2609–2614
- Lötter LH, Wentzel MC, Loewenthal RE, Ekama GA, Marais GvR (1986) A study of selected characteristics of *Acinetobacter* spp. isolated from activated sludge in anaerobic/anoxic/aerobic and aerobic systems. *Water SA* 12:203–208
- Madigan MT, Martinko JM, Dunlap PV, Clark DP (2008) Brock biology of microorganisms. Benjamin Cummings, San Francisco
- Meinikmann K, Hupfer M, Lewandowski J (2015) Phosphorus in groundwater discharge—a potential source for lake eutrophication. *J Hydrol* 524:214–226
- Merzouki M, Delgenès J-P, Bernet N, Moletta R, Benlemlih M (1999) Polyphosphate-accumulating and denitrifying bacteria isolated from anaerobic-anoxic and anaerobic-aerobic sequencing batch reactors. *Curr Microbiol* 38:9–17
- Momba MNB, Cloete TE (1996) The relationship of biomass to phosphate uptake by *Acinetobacter junii* activated sludge mixed liquor. *Water Res* 30:364–370
- Mulkerrins D, Dobson ADW, Collieran E (2004) Parameters affecting biological phosphate removal from wastewaters. *Environ Int* 30:249–259
- Nguyen HTT, Nielsen JL, Nielsen PH (2012) ‘*Candidatus Halomonas phosphatis*’, a novel polyphosphate-accumulating organism in full-scale enhanced biological phosphorus removal plants. *Environ Microbiol* 14:2826–2837
- Olsvik ES, Kristiansen B (1992) Influence of oxygen tension, biomass concentration, and specific growth rate on the rheological properties of a filamentous fermentation broth. *Biotechnol Bioeng* 40:1293–1299
- Onda S, Takii S (2002) Isolation and characterization of a Gram-positive polyphosphate-accumulating bacterium. *J Gen Appl Microbiol* 48:125–133
- Pantano G, Ferreira JS, Aquino FWB, Pereira-Filho ER, Mozeto AA, Fadini PS (2017) Biosorbent, a promising material for remediation of eutrophic environments: studies in microcosm. *Environ Sci Pollut Res* 24:2685–2696
- Peña C, Trujillo-Roldán MA, Galindo E (2000) Influence of dissolved oxygen tension and agitation speed on alginate production and its

- molecular weight in cultures of *Azotobacter vinelandii*. *Enzyme Microb Technol* 27:390–398
- Peng Y-z, Wang X-l, Li B-k (2006) Anoxic biological phosphorus uptake and the effect of excessive aeration on biological phosphorus removal in the A²O process. *Desalination* 189:155–164
- Peñuelas J, Poulter B, Sardans J, Ciais P, van der Velde M, Bopp L, Boucher O, Godderis Y, Hinsinger P, Llusia J, Nardin E, Vicca S, Obersteiner M, Janssens IA (2013) Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat Commun* 4:2934
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Sidat M, Bux F, Kasan H (1999) Polyphosphate accumulation by bacteria isolated from activated sludge. *Water SA* 25:175–180
- Sin G, Niville K, Bachis G, Jiang T, Nopens I, van Hulle S, Vanrolleghem PA (2008) Nitrite effect on the phosphorus uptake activity of phosphate accumulating organisms (PAOs) in pilot-scale SBR and MBR reactors. *Water SA* 34:249–260
- Soejima K, Oki K, Terada A, Tsuneda S, Hirata A (2006) Effects of acetate and nitrite addition on fraction of denitrifying phosphate-accumulating organisms and nutrient removal efficiency in anaerobic/aerobic/anoxic process. *Bioproc Biosyst Eng* 29:305–313
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
- Tu Y, Schuler AJ (2013) Low acetate concentrations favor polyphosphate-accumulating organisms over glycogen-accumulating organisms in enhanced biological phosphorus removal from wastewater. *Environ Sci Technol* 47:3816–3824
- Wang H, Xu X, Ren Z, Gao B (2016a) Removal of phosphate and chromium(VI) from liquids by an amine-crosslinked nano-Fe₃O₄ biosorbent derived from corn straw. *RSC Adv* 6:47237–47248
- Wang S-S, Ye S-L, Han Y-H, Shi X-X, Chen D-L, Li M (2016b) Biosorption and bioaccumulation of chromate from aqueous solution by a newly isolated *Bacillus mycoides* strain 200AsB1. *RSC Adv* 6:101153–101161
- Whang L-M, Park JK (2006) Competition between polyphosphate- and glycogen-accumulating organisms in enhanced-biological-phosphorus-removal systems: effect of temperature and sludge age. *Water Environ Res* 78:4–11
- Yu H, Li M (2015) Denitrifying and phosphorus accumulating mechanisms of denitrifying phosphorus accumulating organisms (DPAOs) for wastewater treatment—a review. *Acta Microbiol Sin* 55:264–272
- Zamparas M, Drosos M, Georgiou Y, Deligiannakis Y, Zacharias I (2013) A novel bentonite-humic acid composite material BephosTM for removal of phosphate and ammonium from eutrophic waters. *Chem Eng J* 225:43–51
- Zeng W, Li B, Yang Y, Wang X, Li L, Peng Y (2014) Impact of nitrite on aerobic phosphorus uptake by poly-phosphate accumulating organisms in enhanced biological phosphorus removal sludges. *Bioproc Biosyst Eng* 37:277–287
- Zhang P, Sun M, Zhang C (2011) Preliminary study on screening and phosphorus removal characteristics of a halophilic phosphate accumulating bacterial strain. *Acta Sci Circumst* 31:2368–2373
- Zhuang Z, Han Y, Zhang W, Zhou Z, Chen J, Li M (2014) Isolation, identification and phosphorus-removal characterization of bacteria *Alcaligenes* sp. strain ED-12 for phosphorus-accumulation. *Acta Sci Circumst* 34:678–687