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Phosphate substances transformation and vivianite formation in P-Fe containing sludge during the transition process of aerobic and anaerobic conditions

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\textbf{HIGHLIGHTS}

- Iron-phosphate compounds accounted for 93\%–97\% of TP in sludge.
- The P-Fe containing sludge realized vivianite generation in anaerobic process.
- ORP and pH maintained at \(-350\) mV and 7.5 met condition of vivianite formation.
- Phosphate transformation and vivianite formation mechanism was revealed.

\textbf{ABSTRACT}

Excess sludge was considered as a promising raw material for phosphorus recovery. In this study, the P-Fe containing sludge came from the aerobic membrane bioreactor with electrocoagulation (EC), which was refluxed to the anaerobic unit for iron reduction. Under anaerobic condition, the ORP and pH maintained at \(-350\) mV and 7.5, which exactly met the conditions for vivianite formation. According to the analysis of X-ray polycrystalline diffraction (XRD) and field emission scanning electron microscopy (FE-SEM), the final product of the sludge after anaerobic condition was mainly vivianite. Microbial analysis showed that there were iron reducing bacteria (IRB) in sludge before and after anaerobic process, including \textit{Dechloromonas}, \textit{Desulfovibrio}, \textit{Aeromonas} and \textit{Methanobacterium}. During the transition process of aerobic and anaerobic conditions, amorphous phosphate substances in P-Fe containing sludge could be transformed vivianite just with long term standing, which could promote the recovery of phosphate resource from wastewater.

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1. Introduction

Phosphorus is one of the main nutrients that cause eutrophication and water pollution (Yan et al., 2019). At present, the methods for phosphorus removal mainly include chemical precipitation, biological phosphorus removal and adsorption phosphorus removal (Prot et al., 2020; Yang et al., 2018). Chemical phosphorus removal (CPR) is mainly achieved by addition of iron or aluminum to produce precipitation with phosphate. It has the advantages of high phosphorus removal efficiency, simple operation and strong impact load resistance etc. (Ren et al., 2020). The most P can be concentrated in iron-based sludge through electrocoagulation (EC) so as to remove phosphorus from wastewater. It has the advantages of flexible process, strong controllability, avoiding the use of chemicals, avoiding secondary pollution, which can be easy to realize automation and high energy efficiency (Nguyen et al., 2016; Omwene et al., 2018). The biological method reduces phosphate content in wastewater by transferring phosphate to sludge phase (Liu et al., 2020). The above methods of phosphorus removal will produce large amounts of phosphorus-rich sludge that require additional treatment. As a result, residual sludge is considered a very promising phosphorus recovery raw material (Meng et al., 2019).

The recovery of phosphorus from sludge realizes sludge reduction and sustainable utilization of phos phorus resources. Therefore, phosphorus recovery from sludge is an important goal of sewage treatment research (Li et al., 2020). At present, the main ways of recovery are struvite precipitation and calcium phosphate precipitation (Zhang et al., 2020a). The recovery of phosphate from struvite is generally considered as a promising approach, but the recovery rate is relatively low (Zhang et al., 2020a). The struvite is typically recovered in enhanced biological phosphorus removal (EBPR), but the start-up of EBPR process may be difficult because it requires strict operating conditions and is often affected by poor operating stability (Li et al., 2018; Wu et al., 2019a).

In the phosphate rock method, the excess phosphorus-rich activated sludge is firstly discharged for phosphorus removal, and then produces calcium phosphate precipitation through sludge anaerobic digestion (Zou and Wang, 2016). Compared with the struvite method, the calcium phosphate precipitation method can obtain higher phosphorus recovery, but it is difficult to separate the solids in the precipitation. Also, the calcium phosphate is a poor fertilizer (Zou and Wang, 2016; Zou et al., 2014).

Vivianite (Fe₃(PO₄)₂·8H₂O), a new product of phosphorus recovery, has been found in the anaerobic digestion of sludge recently. It is a very stable (Ksp = 10⁻³⁰) iron phosphate crystal with relatively simple forming conditions and wide application range (Wang et al., 2019). In addition, vivianite takes precedence over struvite formation during anaerobic digestion (Carlill and Wheatley, 1997).

At present, the most commonly used method for recovering vivianite is achieved by addition of FeCl₃ under acidic conditions to recover phosphorus from waste activated sludge (Cao et al., 2019). However, this method requires large quantities of chemicals, which may cause secondary pollution to the environment. In addition, an excessive acidic environment may adversely affect microbial activity, resulting in the reduction of anaerobic performance (Li and Li, 2017). As a green emerging technology, EC is currently used in combination with other technologies as an auxiliary process. For example, introduction of EC into MBR can not only remove P from wastewater, but also help to alleviate membrane pollution (Liu et al., 2019a). In the whole process, a large amount of the P-Fe containing sludge needs to be disposed further. The transformation of P-Fe in wastewater treatment system is a complex process. For example, the composition and percentage of phosphates in vivianite, as well as the microbial and chemical reactions related to the transformation of iron and phosphorus substances in the aqueous and solid phases have not been well uncovered deeply.

So in this study, phosphate substances transformation and vivianite formation in P-Fe containing sludge during the transition process of aerobic and anaerobic conditions was investigated. The P-Fe containing sludge was generated in the aerobic unit and then refluxed into the anaerobic unit to achieve dissimilated iron reduction at low oxidation-reduction potential (ORP). The changes of iron and phosphorus in aerobic and anaerobic sludge were studied by Field emission scanning electron microscopy (FE-SEM), X-ray polycrystalline diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and microorganism structure characterization. The phosphate transformation and vivianite formation mechanism under aerobic and anaerobic conditions were revealed in detail.

2. Materials and methods

2.1. Experimental operation

The reactor used in this study was composed of cell 1–4, with a total effective volume of 37.5 L. The cell 1–3 mainly produced Fe²⁺ by electrolysis of iron plate, and the effective volume of each cell was 7 L (Supplementary Information). Fe²⁺ oxidation and flocculation occurred in cell 4 (effective volume: 16.5 L). When EC-MBR operation was ended, the aerobic P-Fe containing sludge was generated in cell 4 with mixed liquid suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) of 7100 mg/L and 3463 mg/L (Wu et al., 2020), respectively, which was continuously refluxed to the cell 1 with peri-staltic pump (5.12 L/h, Long pump YZ1515x). This procedure lasted for three days and the aeration in cell 4 was maintained during refluxing. Due to the special structure of this baffled reactor, most of the P-Fe containing sludge was trapped by cell 1, and small part of which was injected into cell 2 because of sludge flotation in cell 1 at the beginning of operation. There was a little of residual aerobic P-Fe containing sludge in cell 4, which was transferred to anaerobic condition directly when aeration was stopped.

During 124 days of anaerobic operation under static condition, tap water was added to each cell to maintain its liquid level. At the end of operation, sludge from each cell was discharged from the bottom of the reactor and then centrifuged at the speed of 5000 r/min for 20 min. After discarding the supernatant, four sludge samples were dried in vacuum drying oven under 80 °C for three days. After that, the dried sludge samples were grinded and screened by griddle with 150 mesh. All samples were stored in sealed drying vessel before using.

2.2. Water quality analysis

After the refluxing, the water quality of supernatant from each cell was monitored every other day, which lasted for 7 days. Then the water quality parameters were tested after 120 days later as the lab was shut down due to the effect of COVID-19. Oxidation reduction potential (ORP) and pH were measured by ORP instrument (MTIC101, HACH, America) and pH meter (pH, HACH, America), respectively. The total organic carbon (TOC) was analyzed by total organic carbon analyzer (TTOC-LCPN, Japan) and the phosphate was determined by the national standard method after passing through 0.45 μm filter (APHA, 2005).

2.3. Sludge characteristic analysis

In order to determine the transformation of phosphorus fraction in sludge, the Hufner method was adopted to identify the labile-P, MCO₂-P, Fe/Al-P, Org-P, Ca-P and Residual-P (Uhlmann et al., 1990). The structure of wet aerobic sludge from cell 4 and four sludge samples after 124 days of anaerobic condition were observed by microscope (Nikon, 50i, Japan). At the same time, SS and VSS of those samples were measured by national standard method (APHA, 2005). FE-SEM (ZEISS sigma500) and Energy Dispersive Spectrometer (EDS) (Energy spectrum: BRUKER XFlash 6130) were used to analyze the microscopic morphology and element composition of the dry and grinded sludge samples from each cell. The phase and crystal structure of dry and grinded sludge samples were analyzed by XRD (BRUKER D8 ADVANCE).
XPS could provide information on the elemental composition, content, and valence state of the material. In this study, k-Alpha X-ray photo-electron spectrometer with Al target emission was used to characterize different dry and grinded samples. The whole spectrum was collected with a transmittance of 200 eV and a step length of 1.0 eV. The narrow spectra of different elements were collected with a transmittance of 50 eV and a step length of 0.1 eV. The spectra were analyzed by Avantage software.

2.4. Microbiological analysis

Before operation and after 124 days of anaerobic operation, the activated sludge was collected from the bottom of cell 1, cell 2, cell 3 and cell 4 respectively. And the samples were marked as sample 1, 2, 3, 5 and A1, A2, A3, A5, respectively, which were entrusted to SANGON Biotechnology (Shanghai, China) for sequencing and data analysis after being frozen overnight. The microbiological sequencing steps of the two sets of samples were the same. DNA extraction was carried out according to the instructions of the E.Z.N.ATM Mag-Bind-Soil-DNA Kit (OMEGA). After that, the Qubit 3.0 DNA detection kit was used to determine the amount of DNA added in the PCR reaction. The primer of bacterial 16S sequence was V3-V4 (341F/805R). The Archaea were amplified by V3-V4 primers and nested PCR. (Two primers: the first round was 340F/1000R; the second round was 349F/806R). Illumina-miseq platform was used to sequence the amplified library. After processing the sequencing data, the microbial community diversity and species classification were analyzed.

3. Results and discussion

3.1. Water quality of supernatant under anaerobic condition

When the refluxing was started, water quality parameters of the supernatant were tested. As the first three days, the aerobic P-Fe containing sludge (cell 4) was refluxed quickly into cell 1, the ORP and pH of cell 1–3 were about −270 mV and 6.5, respectively, ORP and pH of cell 4 were about 28 mV and 7.5 respectively (Fig. 1a, b). After that, the sludge was operated under anaerobic condition for 124 days. In the first four days of anaerobic condition, the pH of each cell increased to about 7.5, and the ORP of cell 1–3 decreased from −270 mV to −370 mV, the ORP of cell 4 decreased from 30 mV to −298 mV, mainly because the aerobic sludge contained in each cells was gradually converted into anaerobic sludge after the aeration was stopped (Fig. 1a, b). Finally, the ORP and pH of cell 1–4 stabilized at around at −350 mV and 7.5, respectively (Fig. 1a, b), which was exactly consistent with the formation conditions of vivianite (Wu et al., 2019a). Most of aerobic P-Fe containing sludge was entrapped by cell 1 because of the refluxing in the baffle reactor. Therefore, the TOC concentration was between 80 and 90 mg/L in cell 1, which was below 50 mg/L in cell 2–4 due to the relatively low sludge content (Fig. 1c). The long-term anaerobic process might lead to sludge disintegration, resulting in a slight increase in TOC concentration (Fig. 1c). When the refluxing was stopped after the 3rd day, the concentration of soluble PO$_4^{3-}$ in the reactor gradually decreased to almost 0 through chemical action (Fig. 1d) (Zhang et al., 2018a). It showed that almost all the soluble phosphorus in the solution was...
transferred to the sludge. During the 124 days of anaerobic process, the content of soluble \( \text{PO}_4^{3-} \) increased as microorganisms released part of \( \text{PO}_4^{3-} \) in the anaerobic environment (Ren et al., 2019). The \( \text{PO}_4^{3-} \) content in cell 2 and cell 3 were 12–14 mg/L (Fig. 1d), indicating that Fe\(^{3+}\) was reduced by microorganisms. Only a very small amount of soluble P was released in the whole anaerobic process, so it had little influence on P recovery.

The water quality change indicated that the ORP of each cell could be maintained at lower level as the anaerobic circumstance was formed with the help of special baffled structure in this study. Normally, the pH decreased during anaerobic digestion process (Chuenchart et al., 2020), but they were stable and even higher than 7 for each cell after 124 days of operation in this study (Fig. 1b). The stable pH was mainly due to the alkalinity from the matters of Fe(OH)\(_3\), Fe\(_2\)O\(_3\) in sludge and the buffering effect of zero valent iron (ZVI) (Baek et al., 2019). In this baffled reactor system, the ORP and pH was exactly consistent with the formation conditions of vivianite. In addition, the TOC and \( \text{PO}_4^{3-} \) release during long term standing process were very slight. All of those water quality change results were beneficial for \( \text{PO}_4^{3-} \) stabilization and transformation to the form of vivianite during the transition process of aerobic and anaerobic conditions.

### 3.2. P and Fe substances composition of P-Fe containing sludge samples

The total sludge solid content (SS) and total volatile sludge solid content (VSS) of aerobic sludge (sample 5) in MBR were 117.2 g and 57.1 g, respectively, before anaerobic process (Fig. 2a). Most of the P-Fe containing sludge was trapped in cell 1 through refluxing, so the highest SS and VSS of 132.2 g and 58.9 g were observed in cell 1 after operation (Fig. 2a). Compared with the cell 1, the SS and VSS content in cell 2–4 were less, and gradually decreased from cell 2 to cell 4 (Fig. 2a). According to Fig. 2b, before the anaerobic process, the inorganic solid in sample 5 accounted for 51.2% of the total sludge solids. The inorganic solids of sample 4 accounted for the highest proportion of 67% after 124 days of anaerobic condition (Fig. 2b). This was attributed that the inorganic matters in aerobic sample was high (51.2%) and part of organic matters in activated sludge was decomposed during long-term anaerobic standing, but inorganic solid could still be maintained, so its content was increased after anaerobic conditions further. Due to the structural characteristics of the baffled reactor, a lot of aerobic sludge was contained in cell 1 through sludge refluxing, so the inorganic solid in sample 1 accounted for 55.5% (Fig. 2b). Only a small amount of aerobic sludge was in cell 2 and pure anaerobic sludge was in cell 3, so less proportion of inorganic solid in sample 2 and sample 3 was.

![Fig. 2](image-url)
observed, which was 42.5% and 39%, respectively (Fig. 2b).

After that, Hupfer method was used to determine the specific components of phosphorus in dry sludge (DS). The proportion of Fe/Al-P (Org-P) in sludge was the highest, between 93% and 97% (Fig. 2c). There was no Al element found in EDS and XPS analysis (Supplementary Information). Meanwhile, the proportion of Org-P in XPS analysis was less, indicating that P was mainly existed in the form of Fe-P in sludge. Among them, 96% of P in aerobic sludge (sample 5) was mainly existed in the form of P-Fe through flocculation (Fig. 2c). A large amount of aerobic Fe-P containing sludge was trapped in cell 1 due to refluxing, so the highest TP content in sample 1 was 64.5 mg/gDS (Fig. 2d). Sample 4 was mainly composed of aerobic Fe-P containing sludge before anaerobic process, with TP content of 59.1 mg/gDS (Fig. 2d). Fe-P accounted for 96% in both sample 1 and sample 4 (Fig. 2c). There was a small amount of refluxing sludge in sample 2, and the TP content was 55.0 mg/gDS (Fig. 2d) and the proportion of Fe-P was 95% (Fig. 2c). The lowest TP content of 32.6 mg/gDS was observed in sample 3 (Fig. 2d), and the Fe-P ratio was at least 94% (Fig. 2d). This was mainly because that the sludge in cell 3 did not include aerobic Fe-P containing sludge (Fig. 2d). The sludge would generate CO₂ in the whole anaerobic process, and MCO₃ would be generated after dissolving in water with metal ions in the system, which had a certain adsorption effect on P (Puyol et al., 2018). As shown in the Fig. 2c, Carbonate adsorption phosphorus (MCO₃-P) accounted for about 3% of TP. In addition, the proportion of Ca-P in sludge was about 1%, which was mainly due to the presence of a small amount of Ca²⁺ in the wastewater. PO₄³⁻ could react with Ca²⁺ to produce hydroxyapatite (HAP) (Lin et al., 2019). However, no HAP was detected in XRD, which was possibly attributed that its content was below the detection limit. The change of SS and ratio of P in sludge samples indicated that part of organic matters from aerobic sludge would be digested during anaerobic process. Whether the conditions change or not, most of P substances could be combined with Fe substances, which could promote sludge reduction and enhance the P substances recycle efficiency in the form of vivianite.

3.3. Morphology analysis of wet and dry P-Fe containing sludge samples

According to the microscope observation, compared with the yellow aggregated aerobic sludge particles, free diamond blue crystals were found in sludge (sample 1–4) after anaerobic process (Supplementary Information), which was similar to the vivianite crystals found in the sediments (Egger et al., 2015). These particles were distributed in 10–50 μm, around which was sludge flocs with irregular shape (Supplementary Information). SEM image further indicated that most of the crystals from sample1-4 were plate-like and lamellar aggregates in regular morphology (Supplementary Information). But no similar structure was observed in sample 5. EDS spectra analysis of these crystals showed that the sludge was mainly composed of O, Fe and P elements and distributed uniformly in the dry sludge samples (Supplementary Information). The Fe/P mole ratios of each sample were 0.91, 1.04, 0.56, 1.28 and 1.06, respectively (Supplementary Information). It was previously reported that the molar ratio of Fe/P was 1.5 for the optimal formation of vivianite, and only about 0.2–0.4 of Fe/P ratio was needed before vivianite precipitation occurred (Cao et al., 2019; Prot et al., 2020). EDS results also indicated that the P-Fe mole ratio of each sample was similar and no obvious change was occurred during the aerobic and anaerobic transformation process, although the structure of P-Fe substances were changed totally. That stable elements structure and PO₄³⁻ concentration in supernatant also indicated that the releasing of PO₄³⁻ and ferric from P-Fe containing sludge during anaerobic process was slight.

3.4. XPS and XRD analysis of dry P-Fe containing sludge samples

The composition, chemical and electronic states of elements in different sludge powders were measured by XPS (Supplementary Information). The photoelectron peaks of Fe, P and O elements appeared at 712 eV (Fe2p), 133 eV (P2p), and 532 eV (O1s) were contained in sample 1–5, which were exactly consistent with EDS analysis results (Supplementary Information). The high-resolution XPS spectra of Fe2p and P2p were shown in supplementary information. The peak of Fe²⁺ was found at 707 eV (Marcus and Grimel, 1992), accounting for 1% of the total (Supplementary Information), which indicated that Fe²⁺ was almost completely oxidized. Previous studies indicated that the binding energy of FeO₄ was 711.4 eV (Wang et al., 2020a; Liu et al., 2019b) and the binding energy of FePO₄ and FeO₃ were about 712 eV (Zhang et al., 2019a; Liu et al., 2019b). Combined with XRD analysis, all the sludge contained FeO₄, FeO₃ and FePO₄ (Supplementary Information). The peaks of Fe²⁺ appeared at around 715 eV, 725 eV and 729 eV (Supplementary Information), which were consistent with the reference value of Fe²⁺ in Fe₅(PO₄)₉₂ (Dai et al., 2020). It indicated that Fe₅(PO₄)₉₂ was found in sample 1–5, accounting for 33%, 41%, 31%, 41% and 45%, respectively (Supplementary Information). According to the comprehensive analysis, the proportion of Fe²⁺ and Fe³⁺ in sample 3 was 36.7% and 60.8%, respectively (Supplementary Information). The highest content of Fe³⁺ in other sludge samples was 62.6% (sample 1), 48.1% (sample 2), 57.5% (sample 4) and 58.8% (sample 5) (Supplementary Information). It showed that more than half of the Fe³⁺ in sludge was reduced to Fe²⁺, which was a necessary condition for the formation of vivianite. In addition, the peak appeared at around 707.1 eV was corresponded to FeP/Fe₂P/Fe₃P, accounting for about 2% (Supplementary Information) (Yang et al., 2020), which indicated that amorphous iron phosphorus compounds and other by-products would be formed in the whole reaction process.

In the P2p XPS spectra, the peak of PO₄³⁻ was found at 133.2 eV in all sludge samples (Supplementary Information) (Zhang et al., 2019a; Dai et al., 2020; Xie et al., 2014). And the atomic mass percentage was around 80%, which indicated that P in all sludge samples was basically in the form of PO₄³⁻. Combined with XRD analysis, the phosphate was mostly in the form of Fe₅(PO₄)₉₂·8H₂O and also might be existed in few forms of Fe₅(PO₄)₉₂·3H₂O and Fe₅Fe₂(PO₄)₉ (Supplementary Information). The peak of 133.8 eV was attributed to HPO₄²⁻·3H₂O (Supplementary Information) (Zhang et al., 2019a; Xie et al., 2014), which was consistent with XRD, indicating that Fe₅(HPO₄)₉₂·4H₂O existed in all sludge samples, and the proportion of HPO₄²⁻ was 11.4% at most in sample 3 (Supplementary Information). The XRD spectrum also showed that the diffraction peak intensity of Fe₅(HPO₄)₉₂·4H₂O in sample 3 was the strongest (93%). It was speculated that sample 3 was pure anaerobic sludge before anaerobic process, and its iron ion content was less. The binding energy of 132.6 eV was corresponded to the peak of organic-P, which was mainly released from dead microbial cells and phosphorus accumulating organisms (PAOs) (Zhang et al., 2019a). Among them, samples 1, 4 and 5 had high content of organic phosphorus, which was 8.4%, 12.7% and 14.7%, respectively (Supplementary Information). As sample 1 from cell 1 contained a large number of aerobic sludge before long-term standing process, sample 5 and 4 from cell 5 before and after anaerobic standing, respectively, it could be speculated that the organic phosphorus in aerobic microorganisms could not be transformed into inorganic phosphorus under anaerobic condition. That kind of phenomenon against vivianite recovery and should be concerned further.

XRD analysis of dry sludge (sample 1–4) after 124 days of anaerobic process showed that the 20 had strong and sharp peaks at 11.20, 13.17, 18.17, 23.13 and 27.82 (Supplementary Information), which were similar to the standard vivianite spectrum (PDF #75-1186). The main diffraction peaks were corresponded to (1 0 0), (0 2 0), (2 0 0), (2 0 1) and (1 3 1) crystal faces of vivianite (PDF # 75-1186), respectively. The results indicated that the final product of P-Fe containing sludge in anaerobic process was mainly vivianite (Diffraction peak intensity: 83%–92%) (Supplementary Information). Compared with sample 1–4, the peak of 20 of aerobic sludge (sample 5) in the non-anaerobic process appeared very weak and wide at the same position (Supplementary Information), which indicated that there was a small amount of vivianite in aerobic sludge formed in the obscure corners of reactor.
research reported that the formation of vivianite occurred as long as anaerobic condition were present in the reactor before the anaerobic process (Wang et al., 2019). In the aerobic process, there would be some anaerobic areas such as corner of reactor, which might cause the formation of vivianite. That explained why a small amount of vivianite was found in the aerobic sludge before anaerobic condition. In addition, 20 of the sludge (sample 1–5) showed weak diffraction peaks at 35.76, 30.36 and 63.16 (Supplementary Information), corresponding to the crystal faces of (3 1 1), (2 2 0) and (4 4 0) of magnetite (Fe₃O₄) (PDF # 74-1910). Besides magnetite, the diffraction peaks of hematite (Fe₂O₃) (PDF # 89-7047) were also found (Supplementary Information). The diffraction peak intensity of magnetite (62%) and hematite (55%) in aerobic sludge (sample 5) were higher (Supplementary Information). In sample 1–4, the main phase was vivianite and followed by Fe₃(PO₄)₃⋅4H₂O and Fe₂(PO₄)₂⋅3H₂O, which indicated that vivianite could be effectively formed under anaerobic condition in mixed sludge (aerobic and anaerobic) (sample 1 and 2), pure anaerobic sludge (sample 3) or pure aerobic sludge (sample 4). The main phases in aerobic sludge (sample 5) before anaerobic process were Fe₃O₄ and Fe₂O₃ (Supplementary Information). It showed that the vivianite was not the main product in the aerobic sludge. The difference of XRD atlas between sample 1–4 and 5 also indicated that most of the P-Fe substances in aerobic sludge were amorphous, which could be transformed into crystals very well no matter in cell 1–3 or cell 4 with different sludge constitution. So, the vivianite formation via aerobic P-Fe containing sludge in this baffled reactor by the way of long term standing was effective and noneffective.

3.5. Microbiological structure of P-Fe containing sludge

Before anaerobic condition, Proteobacteria was the most abundant phylum in all sludge samples (Sample 1, 2, 3, 5: 45.6%, 42.2%, 24.4%, 73.8%, respectively), especially for aerobic sludge (sample 5), accounting for 73.8%. After 124 days of anaerobic condition, the proportion of Proteobacteria in sample A1, A2, A3 was increased to 53.7%, 64.6% and 59.2% respectively. Except for sample A5, it was decreased from 73.8% to 50.8% (Supplementary Information), which was due to the presence of many iron-reducing bacteria (IRB) in Proteobacteria (Zhang et al., 2018b). Secondly, the abundance of Bacteroides decreased from 20%–50% to 9%–18% after anaerobic condition (Supplementary Information). This might be related to the change of environment during anaerobic process and the weak alkali resistance of Bacteroides. Proteobacteria and Bacteroides had also been reported as important species during anaerobic process (Wu et al., 2019b). The richness of Planctomycetes was increased from about 1% to about 5% after anaerobic condition (Supplementary Information). One of them was anaerobic ammonia oxidizing bacteria (Anamox), which could maintain nitrate-dependent oxidation of Fe(II). In addition, studies have indicated that the increase of Fe(II) had a certain promoting effect on Anamox bacteria (Wang et al., 2020b).

The relative abundance of Rhodopseudomonas in all sludge samples was increased about 25–30% after anaerobic condition (Fig. 3). In particular, the relative abundance was increased from 2% to about 26% in sample 2 and 3 (Fig. 3). This fact might be due to the high versatility and flexibility of Rhodopseudomonas in the utilization of low fatty acids (Gonzalez et al., 2017), and Fe could promote the conversion of other substances to low fatty acids, especially acetate (Lu et al., 2020). The content of Ferruginibacter in the aerobic sludge (sample 5) was up to 16.6%, and the proportion in other sludge samples was between 0.03% and 1% (Fig. 3). Studies have shown that Ferruginibacter was related to the formation of sludge flocs (Han et al., 2018), which might promote the formation of P-Fe containing sludge in aerobic cell.

Before anaerobic condition, there was no Aeromonas in either aerobic or anaerobic sludge. After 124 days of anaerobic condition, the proportion of Aeromonas in sample A1, A2, A3 and A5 was 1.4%, 4.4%, 2.1% and 0.1%, respectively (Fig. 3). It had been reported that Aeromonas was closely related to iron reduction, which might be a key microorganism for the formation of vivianite (Wang et al., 2019). In addition, Dechloromonas and Desulfovibrio were also found to be related to dissimilated iron reduction in the sludge (Wang et al., 2019; Yang et al., 2019a). The proportion of Dechloromonas in aerobic sludge (sample 5) was up to 1.3% before anaerobic condition (Fig. 3), but the relative abundance of Dechloromonas in other sludge samples was less than 1% (Fig. 3). The proportion of Desulfovibrio in sample 1, 2 and 3 before anaerobic condition was 6.27%, 28.2% and 12.1% respectively, and the proportion was about 1% after 124 days of anaerobic condition (Fig. 3).

Before and after anaerobic condition, the dominant archaea were Methanosarcina, Methanospaerula, Methanosipilum, Methanoregula, Methanomassilicoccus and Methanobacterium, all of which belonged to Euryarchaeota. The relative abundance of Euryarchaeota in each sample was more than 99% (Table 1). The proportion of Methanospaerula and Methanosipilum decreased after anaerobic condition (Table 1). They could only use H₂ and CO₂ to produce methane (Lu et al., 2019). In comparison, Methanosarcina had more advantages in the anaerobic process. It could generate methane by using acetic acid, methanol, H₂, CO₂ and CO, etc (Lu et al., 2019; Zhang et al., 2019b). Previous studies had pointed out that Fe²⁺ could promote the conversion of other substances into acetic acid (Lu et al., 2020). Therefore, the richness of Methanosarcina was increased after anaerobic condition, especially for
sample A1, A2 and A3, which was increased to 45%, 44% and 32%, respectively (Table 1). Meanwhile, Methanosarcina maintained the neutral pH value of the reactor by continuously degrading acetic acid (Yang et al., 2019b). After 124 days of anaerobic condition, the relative abundance of Methanobacterium was increased, especially for the sample A5, which was increased from 1% to 50% (Table 1). Previous research reported that Methanobacterium was associated with anaerobic oxidation methane (AOM), which could be coupled with iron oxide reduction to produce Fe^{2+} (He et al., 2019). The increase in the abundance of Methanobacterium suggested that Methanobacterium might play a major role in AOM-dependent reduction of iron hydride. At the same time, Methanobacterium could maintain the partial pressure balance of hydrogen, providing a better environment for anaerobic condition (Zhang et al., 2020b).

### Table 1
The relative abundance of Archaea community at the genus level in different samples (sample 1, 2, 3, 5: before anaerobic condition; sample A1, A2, A3, A5: after anaerobic condition).

| Dominant archaea     | 1 (%) | 2 (%) | 3 (%) | 5 (%) | A1 (%) | A2 (%) | A3 (%) | A5 (%) |
|----------------------|-------|-------|-------|-------|--------|--------|--------|--------|
| Methanosarcina       | 2     | 32    | 14    | 21    | 45     | 44     | 32     | 13     |
| Methanospaerula      | 54    | 37    | 40    | 24    | 3      | 5      | 27     | 5      |
| Methanospirillum     | 34    | 25    | 27    | 31    | 14     | 14     | 11     | 6      |
| Methanoregula        | 9     | 5     | 18    | 16    | 7      | 18     | 20     | 5      |
| Methanomassiliicoccus | 1     | 0     | 2     | 7     | 21     | 19     | 14     | 22     |
| Methanobacterium     | 1     | 1     | 1     | 1     | 10     | 4      | 2      | 50     |

During the transition process of aerobic and anaerobic conditions, the hydroxy iron (Fe(OH)_3, HFO) adsorbed PO_4^{3-} to form P-Fe precipitated sludge in aerobic condition. Then, in anaerobic process, iron reducing bacteria (Dechloromonas, Desulfovibrio, Aeromonas and Methanobacterium) reduced Fe^{3+} to Fe^{2+} in P-Fe containing sludge, and finally Fe^{2+} reacted with PO_4^{3-} to form vivianite (Fe_3(PO_4)_2·8H_2O) under suitable conditions (ORP and pH).

### 3.6. Phosphate substances transformation and vivianite formation mechanism during the transition process of aerobic and anaerobic conditions

The iron anode was dissolved in the electrolysis process to produce Fe^{2+} in anaerobic unit (Fe - 2e\ = \ Fe^{2+}), which could react with a small
amount of PO$_4^{3-}$ to generate Fe$_3$(PO$_4$)$_2$·8H$_2$O directly (Fig. 4). Most of the rest Fe$^{2+}$ was oxidized to Fe$^{3+}$ in the MBR, which was mainly hydrolyzed and formed flocculent hydroxyl iron (OH)$_4$ through reaction of 4Fe (OH)$_2$ + O$_2$ + 2H$_2$O = 4Fe(OH)$_3$ (Owomie et al., 2018). Hydroxyl iron showed strong adsorption performance and could adsorb PO$_4^{3-}$ to form precipitation due to its large specific surface area (Fig. 4). The hydrolyzed Fe$^{3+}$ would directly generate FePO$_4$ precipitate with PO$_4^{3-}$ (Cheng et al., 2017; Owomie et al., 2018), so it would produce large amounts of the aerobic P-Fe containing sludge in MBR. At the same time, the rearrangement of iron and oxygen atoms in the iron hydride resulted in the formation of hematite (Fe$_2$O$_3$) (2Fe(OH)$_3$ = Fe$_2$O$_3$ + 3H$_2$O (Fig. 4). According to the analysis of microorganisms, the iron reducing bacteria (IRB) were found in the reactor before anaerobic condition, including Desulfovibrio and Dechloromonas (Wang et al., 2019; Yang et al., 2019a) (Fig. 3). Therefore, there would be a small part of P-Fe was reduced by IRB to produce vivianite. And then the aerobic P-Fe containing sludge was refluxed to anaerobic unit. During long term anaerobic standing, Fe(III) would be reduced by IRB (Aeromonas, Dechloromonas and Desulfovibrio) and AOM (Methanobacterium) in the presence of anaerobic and organic matters (Fe(OH)$_3$ + 3H$^+$ = 3H$_2$O + Fe$^{2+}$) (Wang et al., 2019; He et al., 2019) (Fig. 3 and Table 1). This reduction process would make Fe-P precipitate dissolve and release of PO$_4^{3-}$ and Fe$^{3+}$ when the concentration of PO$_4^{3-}$ and Fe$^{3+}$ in the solution reached saturation. The vivianite would be formed and separated from the solution (3Fe$^{2+}$ + 2PO$_4^{3-}$ + 8H$_2$O = Fe$_3$(PO$_4$)$_2$·8H$_2$O; 3Fe$^{3+}$ + 2HPO$_4^{2-}$ + 8H$_2$O = Fe$_3$(PO$_4$)$_2$·8H$_2$O + 2H$^+$) (Fig. 4). In addition, the reduced Fe (II) might also react directly with the nearby hydrated iron oxide to form magnetite (Fe$_3$O$_4$) through recrystallization (Wei et al., 2018) (Fig. 4). In this process, some amorphous ferrophosphorus compounds, such as Fe$_5$(PO$_4$)$_2$H$_2$O, Fe$_6$(HPO$_4$)$_2$·4H$_2$O, Fe$_2$(PO$_4$)$_2$·3H$_2$O would also be produced (Fig. 4). Combined with water quality and microscope analysis, as there was small part of PO$_4^{3-}$ in supernatant and all crystals were wrapped by sludge flocs, it could be speculated that the amorphous P-Fe substances transformation and vivianite generation were occurred inside the sludge flocs. That process was beneficial to the P element stabilization and recovery from wastewater. But the challenge of separation of vivianite from sludge was shown and it should be studied further.

4. Conclusion

Phosphate substances transformation and vivianite formation in P-Fe containing sludge during the transition process of aerobic and anaerobic conditions was proposed. The conclusions were as follows: 1) The P-Fe compounds accounted for 93%–97% of TP in all P-Fe containing sludge, among which Fe$_3$(PO$_4$)$_2$ accounted for 30%–45%. 2) In the anaerobic process, ORP and pH were around ~350 mV and 7.5, which exactly met the conditions for the formation of vivianite. 3) Vivianite was the main product of P-Fe substance under anaerobic conditions. 4) There were IRB in the sludge, including Dechloromonas, Desulfovibrio, Aeromonas and Methanobacterium.

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Appendix A. Supplementary data

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