Article

Species and bio-availability of inorganic and organic phosphorus in primary, secondary and digested sludge

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Abstract: The species and bio-availability of phosphorus (P) in primary, secondary and digested sludge were fractionated and further analyzed in this study. Results showed that inorganic P (IP) was the primary P fraction in the secondary sludge and digested sludge, in which non-apatite IP (NAIP) amounted to 91.6% and 69.3% of IP, respectively. Organic P (OP), accounting for about 71.7% of total P (TP), was the dominant P composition in primary sludge. The content of bio-available P was about 9.7, 43.4, 29.8 mg-P/g-TS in primary sludge, secondary sludge and digested sludge, respectively, suggesting secondary sludge is the optimal choice when land application of sewage sludge is taken into consideration, followed by digested sludge and primary sludge. Polyphosphate and orthophosphate, comprising approximately 54.3% and 89.2% of TP, was the dominant P species in the secondary sludge and digested sludge, respectively. Monoester-P (54.6% of TP in extract) and diester- P (24.1%) were identified as OP species in primary sludge by Phosphorus-31 nuclear magnetic resonance (^{31}PNMR). The present results would be helpful for P recovery and recycle from sewage sludge in wastewater treatment plant.

Keywords: Sewage sludge; P bio-availability; P species; SMT protocol

1. Introduction

Phosphorus (P) is one of the essential mineral elements for all living organisms, which accounts for around 2 - 4% of the dry weight of most cells [1]. The extensive application of P fertilizers is one of the main reasons that the current crop production has been able to meet the food demand and security associated with an ever-expanding world population. It is estimated that approximately 90% of the P derived from phosphate rock is used in agriculture as fertiliser [2]. However, on one hand, phosphate rock is a non-renewable resource which is estimated to be exhausted in 50 - 100 years with a peak in its production occurring in 2030s if the growth of demand for fertilizers remains at 3% per year [3-5]. On the other hand, according to the statistics of the US Geological Survey, eight countries or areas including United States, Algeria, China, Jordan, Morocco and Western Sahara, Russia, South Africa and Syria control 93.9% of global phosphate rock reserves [6]. In order to secure domestic raw materials, some major producer countries have designated phosphorous as a strategic resource and restricted export of the P rock or in the form of processed and chemical products with added value in recent years [3]. With these trends, the price of P rock has been increasing in the international trade market. Therefore, P resource protection and P recycling is prerequisite for a

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sustainable agriculture and society on a global scale, especially for the countries that lack of P resources.

Sewage sludge, a by-product of biological wastewater treatment process, is regards as a potential P reservoir due to its high production and P content [7]. Various P recovery technologies from sewage sludge have been developed, including incineration, alkaline/acid extraction, thermal treatment, phosphorous crystallization as HAp (hydroxylapatite) and MAP (magnesium ammonium phosphate) [3, 8, 9]. However, the costs of these projects are too high due to their complex processes for phosphorus recovery. Land application of sewage sludge as P fertilizer is now very attractive, because it not only solves the sludge disposal problem but also benefits to crop production [10]. In US and EU-15, land application of sludge now is the predominant choice for sludge management (41% in US and 53% in EU-15) [11, 12]. In China, about 45% and 3.5% of sludge is applied to agriculture and gardening, respectively. As well known, Japan is a P resource- poor country. While the percentage of treated sewage sludge for farmland and green areas has been stable at around 14% for many years in Japan, much lower than those in other countries. Thus land application of sewage sludge as P fertilizer has great development potential to alleviate P resource shortage to a certain extent in Japan.

Generally, P content in sewage sludge accounts for about 0.3-4.8% of total solid [13]. But it is well known that not all the forms of P exhibit similar mobility and bio-availability in the sludge, identification of the P fraction and species in the sludge is beneficial for both land application of sewage sludge as P fertilizer and understanding the characteristics and function of P in different sludge. Primary sludge, secondary sludge and digested sludge are the three main types of sludge produced from WWTP. Previous study mainly focus on investigate the bio-availability of P in secondary sludge and P species in secondary sludge and digested sludge. It was reported that 75%-88.7% of TP in secondary sludge possesses high mobility and bio-availability [14]. Poly-P and ortho-P was the major P in enhanced P removal activated sludge and digested sludge, respectively [15-17]. However, to date, little detailed and comprehensively information could be found about the P fractions and species in primary sludge, secondary and digested sludge.

This study aimed to investigate the P bio-availability and species in primary sludge, secondary and digested sludge. The Standards, Measurements and Testing (SMT) protocol was applied to analyze the fractionation of P in sewage sludge and to evaluate the mobility and bio-availability of P in various sludge. Solution 31P nuclear magnetic resonance (^{31}P NMR) spectroscopy is employed to characterize the inorganic and organic phosphorus species in sewage sludge. It is expected that this work will be useful for P utilization and recovery from sewage sludge, especially when agriculture utilization of sludge is taken into consideration.

2. Materials and Methods

2.1. Sewage sludge sample

Sludge samples were collected from a WWTP treating domestic wastewater in Shimodate, Ibaraki Prefecture, Japan. The process flow diagram of the WWTP is shown in Figure S1 (See Supplementary Material).The sludge samples were collected from the primary tank, secondary sedimentation tank and digestion tank, respectively. The collected sludge was kept in a refrigerator at 4°C and analyzed within 2 days.

2.2. Phosphorus fractionation in sludge

In this study, the SMT programme extraction protocol was applied to analyze P fractions in all sludge samples (Figure S2), which has been widely used in soil, sediment and sewage sludge samples [18-20]. After sequential extraction based on the SMT method, P in sludge was fractionated into the following 5 categories: (1) concentrated HCl-extractable P, namely total P (TP), (2) organic P (OP), (3) inorganic P (IP), (4) non-apatite inorganic P (NAIP, the P fraction associated with oxides and hydroxides of Al, Fe and Mn), and (5) apatite P (AP, the P fraction associated with Ca). In order to avoid the transformation of P species in sludge during preparation, the samples centrifuged at

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6000×g for 10 min at 4℃, and then the residue were frozen immediately at -80℃, lyophilized at -50℃ for 48 h and stored at -20℃ until analysis. The P concentration in the supernatant collected after extraction was determined with molybdenum blue method.

2.3. Extraction of P from sludge

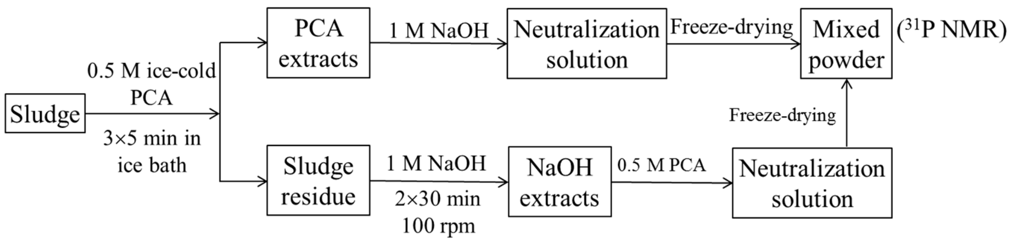

Figure 1. Schematic diagrams for the fractionation and characterization of various forms of P in sewage sludge.

PCA and NaOH extraction methods have been efficiently used for IP and OP extraction from sludge, soil and sediment samples [21-24]. In this study, the PCA-NaOH extraction procedure was applied to fractionate and characterize P in the sludge samples according to the schematic diagram shown in Figure 1. Before extraction, a certain amount of sludge was washed twice with 100 mM NaCl solution (4℃) with the supernatant being discharged. After extraction, neutralization was conducted immediately to minimize P transformation. 2 ml of the resultant supernatant was taken for TP, IP and OP analysis. The remaining extracts were freeze-dried at -50℃ for 48 h, and the dried PCA-NaOH extracts were uniformly mixed and them stored at -20℃ till ^{31}P NMR analysis.

2.4. Chemical analysis

Mixed liquor (volatile) suspended solids (ML(V)SS), chemical oxygen demand (COD), ammonia nitrogen (NH_4-N), and phosphorus (PO_4-P) were measured in accordance with the standard methods [25]. Total concentration of phosphorus in the liquid was determined with molybdenum blue method after digestion by potassium persulfate at 120℃. Metal ions in sludge samples were quantified after the sludge samples being digested and filtered through 0.22 μm cellulose nitrate membrane filters (Nalgene). 0.1 g of dried sludge was digested in a mixture of 3 ml hydrochloric acid (37%, Wako), 1 ml nitric acid (70%, Wako), and 1 ml perchloric acid (60%, Wako) on an electric heating plate for 10 min. The concentration of each metal was measured by inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, Perkin Elmer, USA).

2.5. ^{31}P NMR Analysis

To obtain the ^{31}P NMR spectrum, 200 mg of freeze-dried sludge extracts were re-dissolved in 0.8 ml of 1M NaOH and 0.2 ml D_2O and then 0.2 ml of 100 mM EDTA solution was added. The dose of EDTA and NaOH solutions was to minimize the interference of divalent/trivalent cations and to adjust pH above 12.0, respectively, to ensure consistent chemical shifts and optimal spectral resolution during the ^{31}P NMR measurement.

The ^{31}P NMR spectrum was obtained by using a Bruker Avance-600MHz NMR Spectrometer at 242.94 MHz. 90℃ of pulse width, 25℃ of regulated temperature, and acquisition time of 0.67 s (with relaxation delay of 2s) were applied in the experiments. To obtain accurate phosphorus forms, spectra were collected immediately after preparation and the process was finished within 2 h to minimize transformation of phosphorus species. Chemical shifts of signals were determined relatively to an external standard of 85% H_3PO_4 via signal lock. The peaks were assigned to P species according to the reports in literature with peak areas calculated by integration [26-29].

3. Results

3.1. Characterization of sludge

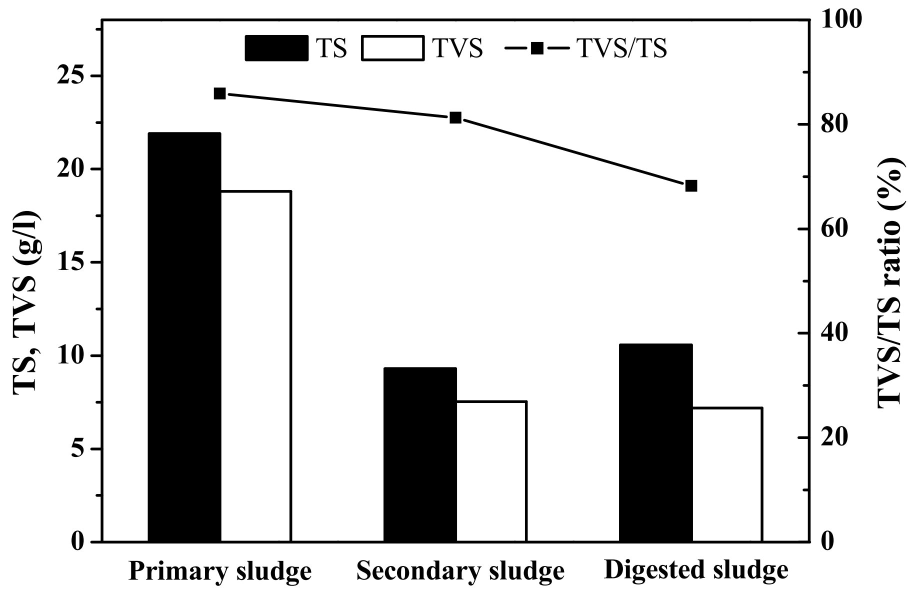

Figure 2. Changed in TS, TVS and TVS/TS ratio of the sludge sample.

Table 1. Average metal content in sewage sludge (Unit: mg/g-TS).

Sample	Na	K	Mg	Ca	Fe
Primary sludge	1.6	2.3	1.4	1.7	0.6
Secondary sludge	4.3	12.4	9.6	7.2	6.4
Digested sludge	6.2	22.7	17.1	13.1	11.5

TS, TVS and TVS/TS ratio of sludge were shown in Figure 2. The concentration of TS in primary sludge is about 21.9 g/L, which is approximately 2 times of that in secondary and digested sludge. The TVS/TS ratio of primary sludge, secondary sludge and digested sludge were 85.9%, 81.2% and 68.1%, respectively. In anaerobic digestion stage, biodegradable organic substances can be converted to biogas, leaving most inorganic and recalcitrant materials in the digested sludge. This can be explained the lower TVS/TS ratio of digested sludge than those of primary sludge and secondary sludge. Mineral element analysis showed that K, Mg, Fe and Ca were the major ions in all sludge samples (Table 1). In addition, the concentration of mineral elements in digested sludge was much higher than that in primary sludge and secondary sludge, proved previously explanation.

3.2. P fractionation in sludge by SMT protocol

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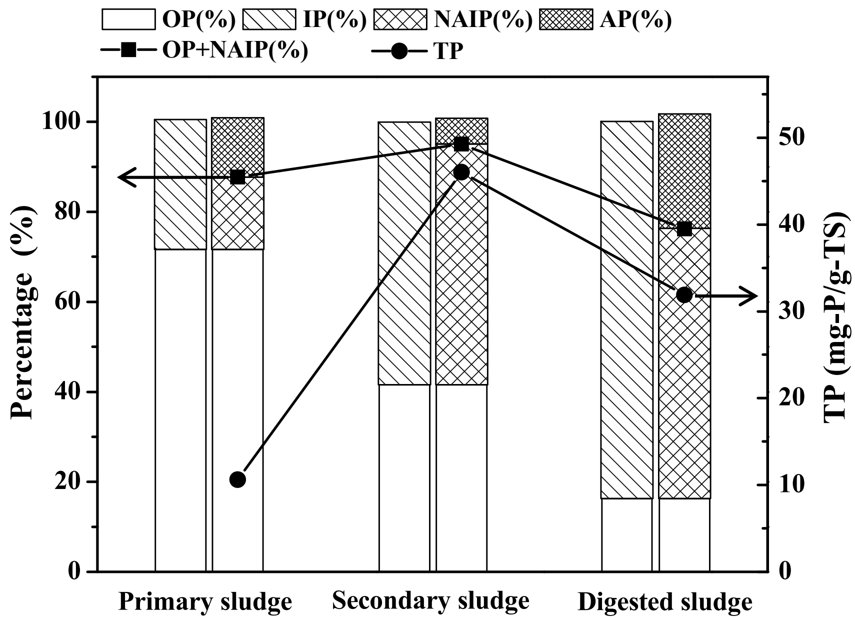

Figure 3. TP concentrations and percentage of each P fraction to TP in the sludges by using the SMT extraction protocol. TS, total solids; TP, total phosphorus; OP, organic phosphorus; IP, inorganic phosphorus; NAIP, non-apatite inorganic phosphorus; AP, apatite phosphorus.

Table 2. Average contents of each P fraction in sludge by using the SMT extraction protocol.

Sludge	TP (mg-P/g-TS)	OP (mg-P/g-TS)	IP (mg-P/g-TS)	NAIP (mg-P/g-TS)	AP (mg-P/g-TS)	OP+NAIP (mg-P/g-TS)	Bio-availability (%)
Primary sludge	10.6±3.2	7.6±2.4	3.1±1.6	1.7±0.8	1.4±0.6	9.3	87.7
Secondary sludge	46.1±5.6	19.1±3.5	26.9±2.8	24.6±3.1	2.6±1.1	43.7	94.8
Digested sludge	31.9±5.1	5.2±2.9	26.7±3.4	19.1±2.3	8.2±2.7	24.3	76.2

TS, total solids; TP, total P; OP, organic P; IP, inorganic P; NAIP, non-apatite P; AP, apatite P; Bio-availability, the percentage of OP+NAIP to TP.

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Table 2 summarized the P fractions of primary sludge, secondary sludge and digested sludge, respectively. The TP concentration in secondary sludge was about 46.1 mg/g-TS, which was around 4.3 times of that in primary sludge. This result may be brought about by the secondary sludge, which accumulated high concentration of P and responsible for P removal from wastewater. While the primary sludge directly settled from wastewater is mainly suspended solid contaminants. Compared to secondary sludge, lower concentration of TP in digested sludge was also detected, most probably due to parts of P in the secondary sludge was released to the effluent in digestion tank (Table S1). OP content in secondary sludge was about 19.1 mg/g-TS, much higher than that in primary sludge (7.6 mg/g-TS) and digested sludge (5.2 mg/g-TS). While the percentage of OP to TP was 71.7% in primary sludge, which was higher than that in secondary sludge (41.4% of TP) and digested sludge (16.3% of TP), indicating OP is the dominant P in primary sludge (Figure 3). It was reported that OP can be released from sediments and utilized by algae [30]. Moreover, OP in soil can be hydrolyzed by phosphatase and then used by the rhizosphere of plants [31, 32]. Thus high content of OP in sludge may play an important role in P recycle when land application of sludge as P fertilizer is taken into consideration.

On the other hand, IP was the major P fraction in secondary sludge and digested sludge, in which IP accounted for 58.4% and 77.5% of TP, respectively. Only about 3.1 mg/g IP (about 28.8% of TP) was detected in primary sludge (Table 2, Figure 3). NAIP was the dominant IP in primary sludge, secondary sludge and digested sludge, accounting for 55.8%, 91.6% and 69.3% of IP, respectively. In addition, NAIP was about 16.0%, 53.4% and 59.9% of TP in primary sludge, secondary sludge and digested sludge, respectively. Compared with OP and NAIP, AP content was relatively low, about 13.2% of TP in primary sludge and 5.6% of TP in secondary sludge. However, a high content and percentage of AP was founded in digested sludge (8.2 mg/g-TS and 25.7% of TP), most probably due to the formation of Ca-P precipitates in the digestion tank resulting from the saturation status of co-existing calcium phosphate.

OP and NAIP were considered to be releasable and bio-available P. In this study, the concentration of NAIP+OP were 9.3, 43.7 and 24.3 mg/g-TS, accounting for about 87.7%, 94.8% and 76.2% of TP in primary sludge, secondary sludge and digested sludge, respectively. It was obviously that secondary sludge not only has the highest NAIP+OP content but also has the highest percentage of NAIP+OP among the three sludge samples. Although digested sludge has the lowest percentage of NAIP+OP, its NAIP+OP contents are much higher than those in primary sludge. Thus digested sludge should be reused in preference to primary sludge when utilization of sewage as P fertilizer is taken into consideration. These indicated the content of bio-available P was a more intuitive and better parameter than TP concentration and proportion of bio-available P to assess the bio-availability of P in sewage sludge.

3.3. Identification of P species in sludge by ^{31}P NMR analysis

Table 3. Contents of different P fractions extracted by PCA + NaOH method and their relative proportions (%TP) identified by 31P NMR in the sludge samples.

Sample	TPExtract	IP			OP	
		Ortho-P	Pyro-P	Poly-P	Monoester-P	Diester-P
	(mg-P/g-TS)	(%)	(%)	(%)	(%)	(%)
Primary sludge	9.7±2.8	21.3±3.1	-	-	54.6±5.7	24.1±4.2
Secondary sludge	43.4±4.7	17.6±6.5	2.4±1.2	54.2±5.7	16.1±4.9	9.7±2.2
Digested sludge	29.8±4.1	89.2±5.8	-	-	6.6±3.3	4.3±3.6

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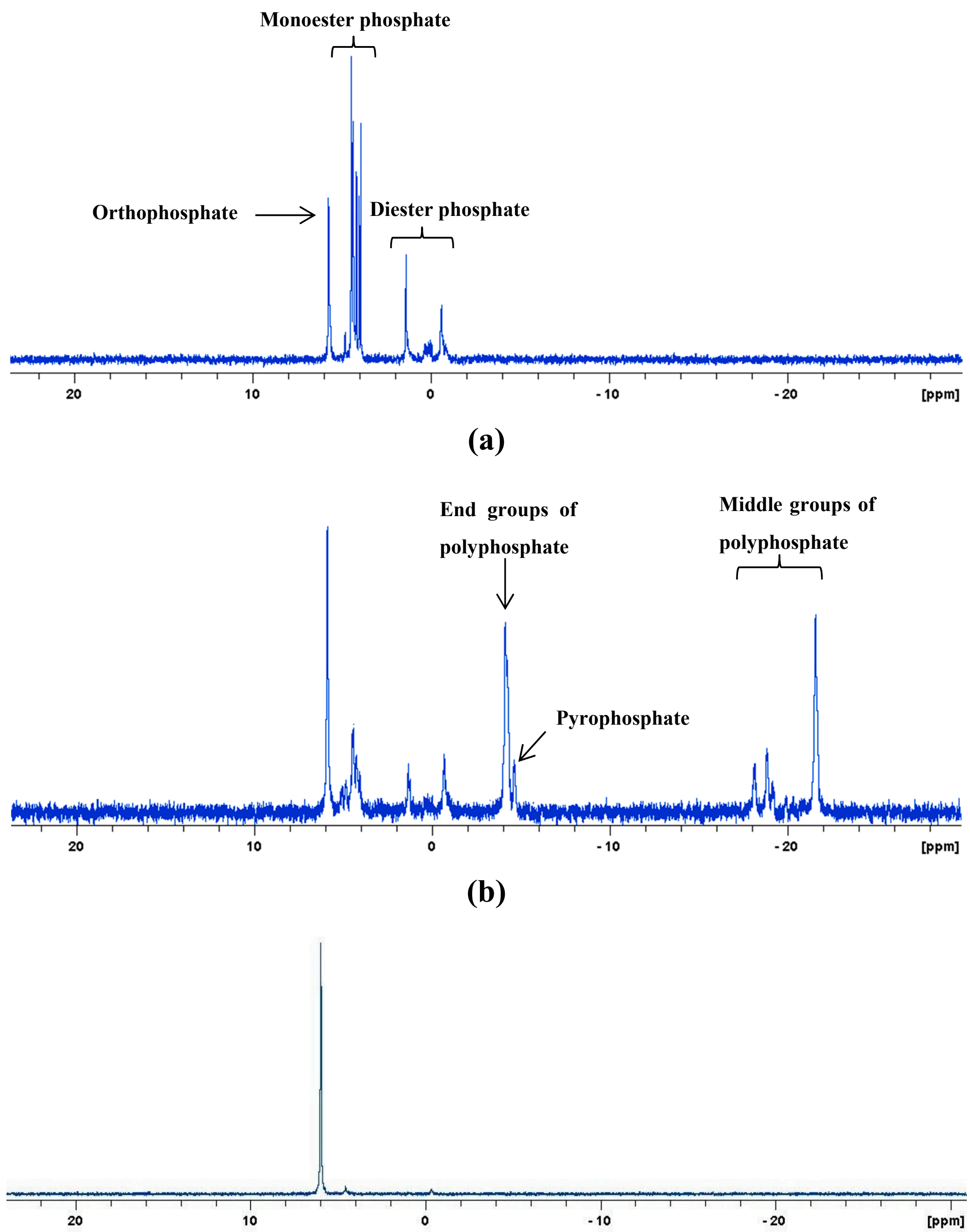
Quantification of various P fractions by integrating the peak areas in NMR spectra has been widely used to estimate the relative proportions of P fractions. All NMR-spectra show peaks in the areas for ortho-P, monoester-P, diester-P, pyro-P, poly-P (Figure 4). Table 3 summarizes the contents of these compounds and their relative proportions (% TP) in primary sludge, secondary sludge and digested sludge extracts identified by 31P NMR. The average TP contents in the PCA + NaOH extracts from primary sludge, secondary sludge and digested sludge were 9.7, 43.4 and 29.8 mg-P/g-TS with average extraction rate of approximately 91.5-94.1% of TP, respectively, indicating the high efficiency of PCA + NaOH procedure for P extraction from sludge samples.



(c)

Figure 4. Typical 31P NMR spectra of PCA+NaOH extracts from (a) primary sludge, (b) secondary sludge and (c) digested sludge.

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In primary sludge extracts, ortho-P, monoester-P and diester-P were identified as the major P species, accounting for approximately 21.3, 54.6 and 24.1% of TP, respectively. Obviously, the monoester-P and diester-P were the dominant OP species in primary sludge extracts. Generally, monoester-P and diester-P are regarded as potential bio-available P due to that they can be hydrolyzed and utilized by plants and algal in certain conditions. In the secondary sludge, poly-P was the major form of P species, comprising 54.2% of the extractable TP from sludge (Table 3). The high content of poly-P in secondary sludge signals the high amount and bioactivity of PAOs in activated sludge in the aeration tank during sampling period. Specifically, pyro-P was only identified in the secondary sludge, around 2.4% of extractable TP. The presence of pyro-P could reflect microbial activity in sludge due to it was directly related to adenosine triphosphate (ATP) hydrolysis in cells. Ortho-P, a main nutrient for living organisms, was about 89.2% of TP in the digested sludge and much higher than that in the primary sludge and the secondary sludge. These results indicated that most of poly-P, pyro-P, monoester-P and diester-P in primary sludge and secondary sludge were converted to ortho-P or reused by anaerobic bacteria during digestion process.

3.4. Implication of this study

The TP content in primary sludge, secondary sludge and digested sludge was about 10.6, 46.1 and 31.9 mg/g-TS, respectively. Obviously, secondary sludge has the highest concentration of P, followed by digested sludge and primary sludge. In primary sludge, OP was the dominant P fractions and accounting for about 71.7% of TP. IP was the major P fraction in secondary sludge and digested sludge, in which IP accounted for 58.4% and 77.5% of TP, respectively. Monoester-P, poly-P and ortho-P, accounting for 54.6%, 54.3% and 89.2% of TP, were the dominant P species in the primary sludge, secondary sludge and digested sludge, respectively. The concentration of NAIP+OP were 9.3, 43.7 and 24.3 mg/g-TS, accounting for about 87.7%, 94.8% and 76.2% of TP in primary sludge, secondary sludge and digested sludge, respectively, indicating the high mobile and bio-available P stored in those sludge samples.

Some studies have been conducted to investigate the P in sewage sludge, but most have only focused on the concentration of TP and ignoring the bio-available P in sludge. The content of bio-available P may provide a more intuitive and convenient parameter to evaluate the P in sewage sludge. The results of the present study indicated that secondary sludge not only contains the highest concentration and proportion of TP among different sludge samples, but also has the highest content of potential mobile and bio-available P. In this study, secondary sludge was the optimal choice for land application of sewage sludge as P fertilizer source. Although the proportion of bio-available P in primary sludge was slightly higher than that in digested sludge, digested sludge is more suitable for land application as it contains much more bio-available P content. This study proved that land application of sewage sludge as P fertilizer should be in the order secondary sludge>digested sludge>primary sludge.

4. Conclusions

In this study, the P species and bio-availability in primary sludge, secondary sludge and digested sludge were identified and evaluated. The following results can be obtained:

(1) IP was the primary P fraction in the secondary sludge and digested sludge, in which NAIP amounted to 91.6% and 69.3% of TP, respectively. OP content (about 7.6 mg/g-TS) was the dominant P in the primary sludge.

(2) Two OP fractions (monoester-P and diester-P) and three IP compounds (ortho-P, poly-P and pyro-P) were identified P species in the secondary sludge. Poly-P was the dominant P species in the secondary sludge, comprising approximately 54.3% of TP. Monoester-P and ortho-P, accounting for 54.6% and 89.2% of TP, were the major P species in the primary sludge and digested sludge, respectively.

(3) The content of bio-available P is a good parameter to evaluate the bio-availability of P in sewage sludge. About 9.3, 43.7 and 24.3 mg/g-TS bio-available P were stored in primary sludge and digested sludge, respectively.

These results revealed that P species and bio-availability were different in primary sludge, secondary sludge and digested sludge, which is much meaningful for P removal and recovery from wastewater and sludge in WWTPs.

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Conflicts of Interest: The authors declare no conflict of interest.

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