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Metagenomic and metabolomic analysis reveals the effects of chemical phosphorus recovery on biological nutrient removal system

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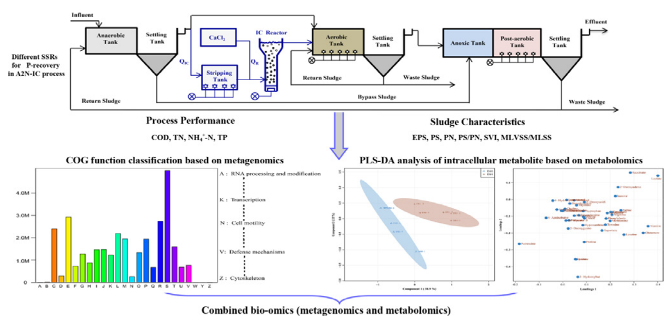
HIGHLIGHTS

- The effects of side-stream P recovery on biological nutrient removal were explored.
- Both nutrients removal and P recovery were achieved at the optimal SSR of 0.3.
- Higher SSRs (>0.3) disrupted the phosphorus balance of A2N-IC process.
- Microbial community and metabolism were changed by the introduced P recovery unit.
- Combined bio-omics approach was valuable for revealing the influencing mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

Side-stream phosphorus recovery coupled with biological nutrient removal can achieve the dual goals of resource recycling and meeting effluent discharge standards in wastewater treatment processes. However, suboptimal side-stream ratios (SSRs) for phosphorus recovery exert a negative effect on the performance of the combined process. The aim of this study was to reveal the comprehensive effect of chemical phosphorus recovery on the biological nutrient removal process using combined bio-omics (metagenomics and metabolomics) analysis. The optimal SSR was 0.3, at which point the effluent concentrations of COD, TN, NH_4^+-N and TP were approximately 32.56, 11.35, 3.71 and 0.23 mg/L, respectively. Initially increasing the SSR favored resource recovery; however, SSRs > 0.3 disrupted the phosphorus balance of the system and inhibited the metabolic activity of microorganisms related to the phosphorus removal process. Variations of activated sludge characteristics at different SRR indicated that the polysaccharide were more important than protein in resisting the toxicity of the imported Ca^{2+} on microorganisms. Relevant changes in the microbial community, functional potential, key enzymes and intracellular metabolites were illustrated in the bio-omics analysis, which revealed the influencing mechanism of chemical phosphorus recovery on activated sludge system at the micro level. The results of this study provide a novel perspective on the interactions between resource recovery and pollutant removal in a combined process using a bio-omics approach.

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1. Introduction

Phosphorus is a limited and non-renewable mineral resource that has contributed substantially to the development of agriculture and various industries [1]. However, excessive phosphorus discharged into natural water causes eutrophication, which seriously threatens the ecological health of water bodies and the safety of drinking water [2]. Phosphorus recovery from domestic wastewater can not only improve the regeneration of non-renewable mineral resources but also alleviate the environmental impacts of excess phosphorus on the receiving waters [1,3]. Therefore, wastewater treatment processes that combine phosphorus recovery and nutrient removal are essential for promoting sustainable development [4,5].

Several integrated processes combining biological nutrient removal and phosphorus recovery have been reported [6–8]. In these combined processes, phosphorus is usually recovered in the form of valuable products, such as hydroxyapatite (HAP, $Ca_5(PO_4)_3OH$) and struvite (magnesium ammonium phosphate (MAP), $MgNH_4PO_4$). Thereinto, chemical phosphorus recovery from the anaerobic digester effluent through precipitation has the disadvantages of higher operation costs, impure products formation, and complicated product separation [6]. Contrarily, recovering phosphorus *via* the induced crystallization (IC) approach to form HAP from anaerobic supernatant can overcome these drawbacks, and the crystallized products can be used directly in agricultural or industrial activities [9,10]. Based on this information, IC phosphorus recovery in the form of HAP coupled with an anaerobic-anoxic/nitrifying (A2N) two-sludge process (hereafter referred to as A2N-IC) was proposed by our research group, and this yielded effective phosphorus recovery and nutrient removal from domestic wastewater [5]. In the A2N-IC process, the side-stream chemical phosphorus recovery in IC reactor is the main operating mode, which can flexibly adjust phosphorus loading on biological system to improve the stability of the process. However, the higher side-stream ratios (SSRs) for phosphorus recovery negatively affect the nutrient removal performance and phosphorus balance of the system. Moreover, the role of Ca^{2+} in the enhanced biological phosphorus removal (EBPR) process and its possible impacts for microbial metabolic pathway have been studied recently [11–13]. Zhang et al. [13] demonstrated that Ca^{2+} had a negative effect on the EBPR process, resulting in a shift of the metabolic pathway of phosphate-accumulating organisms (PAOs) from a phosphate-accumulating metabolism to a glycogen-accumulating one. Therefore, the introduction of chemical agent (e.g., Ca^{2+}) in A2N-IC process may also have a major impact on the community structure and physiological characteristics of microorganisms in the activated sludge system.

To clarify the comprehensive effects of chemical phosphorus recovery on the biological nutrient removal system, it is essential to obtain the accurate information on the community structure, functional potentials and metabolic characteristics of microorganisms in the system. However, the traditional methods, such as cultivation-based, fluorescence *in situ* hybridization, denaturing gradient gel electrophoresis, microarray, and amplification of 16S rRNA partial genes, are limited when used to analyze the microbial community structure and gene diversities due to the microbe cultures properties, PCR bias, and lower sequencing data [14]. In contrast, high throughput metagenomic sequencing, based on the direct sequencing of genomic DNA extracted from environmental samples, allows for a comprehensive analysis for the taxonomic and functional diversity of microbial community without prior enrichment and PCR bias [14]. Several metagenomic studies have reported on the microbial community and gene function profiles of activated sludge collected from lab- and full-scale wastewater treatment systems [14–20]. However, metagenomic analysis can only show the potential possibility of microbial metabolic activities by revealing the genetic information of microbes, and they cannot explain the results of metabolic activities [21].

Metabolomics, which provides detailed metabolites profiling information, offers a better understanding of the microbial responses to perturbations or environmental variations [22]. Microbial metabolomics studies have received much attention in recent years because they offer detailed pictures of the metabolic pathways and reveal the mechanisms underpinning the interplay between microbial communities and the environment [23]. The rapid development of metabolomics is due to improvements in the detection methods, such as nuclear magnetic resonance (NMR) and mass spectrometry (MS), and this approach has been successfully applied to different fields [24–26]. However, the combined bio-omics (metagenomic and metabolomics) investigative approach has not been applied to studying the effects of side-stream phosphorus recovery on the microbial communities, functional potentials, and metabolic characteristics of activated sludge systems.

In this study, different SSRs for phosphorus recovery were conducted to investigate the variation of process performance and activated sludge characteristics in the A2N-IC process. Metagenomics, based on high-throughput sequencing, and metabolomics, based on NMR, were employed in profiling mode to reveal the changes of microbial community structure, functional potentials, key enzymes, and intracellular metabolites at the optimal SSR in comparison with those of the A2N process. The findings in this study provide a novel insight into the comprehensive effects of chemical phosphorus recovery on biological nutrient removal system and lay a foundation for the subsequent industrial scale operations.

2. Material and methods

2.1. Experimental apparatus and wastewater composition

A laboratory-scale continuous flow A2N-IC process was employed to remove nutrients and recover phosphorus from wastewater (Fig. 1). The solids retention time (SRT) of the denitrifying phosphorus removal sludge was 15 days and its mixed liquid suspended solid (MLSS) was maintained at 3500–4100 mg/L, whereas the SRT and MLSS of the nitrifying sludge were 25 days and 3300–3800 mg/L, respectively. In the IC system, the seed dosage was 30 g/L, and the aeration rate was 300 L/h. Two stock solutions, $CaCl_2$ in the reagent tank and anaerobic supernatant in the stripping tank, were continuously pumped into the IC reactor at a volume ratio of 0.1, and the final molar ratio of Ca^{2+} to PO_4^{3-} was 2.5. The synthetic wastewater used as influent contained (per liter): COD (CH_3COONa) 250 mg; TN (NH_4Cl) 45 mg; TP (KH_2PO_4) 5.0 mg; Mg^{2+} ($MgSO_4{\cdot}7H_2O$) 3.5 mg; Ca^{2+} ($CaCl_2$) 10.0 mg; and 0.3 mL of trace solution. The composition of trace element solution referred to the study of Shi et al. [5].

2.2. Experimental design

Six stage experiments (total 180 d) with different SSRs (0, 0.1, 0.2, 0.3, 0.4, and 0.5) were conducted to investigate the effects of chemical phosphorus recovery on the performance of the A2N-IC process. At the end of every stage, 100 ml of both nitrifying sludge and denitrifying phosphorus removal sludge were collected to investigate the variations of extracellular polymeric substances (EPS, including protein (PN) and polysaccharide (PS)) and the