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Using the method of wastewater acidification to improve the efficiency of carbon utilization and nutrient removal in A_2N process from a lab-scale operation

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ABSTRACT

Many wastewater treatment processes were developed in order to avoid excessive discharge of nitrogen (N) and phosphorous (P) into the downstream water bodies to mitigate their eutrophication problems. However, one of common problems for these processes is that there is no sufficient carbon source for denitrification. In this research, a continuous anaerobic–anoxic–nitrification process, which was based on reaction of denitrifying phosphorous removing bacteria, was tested to improve carbon utilization and nutrient removal from sewage by employing the method of wastewater acidification. The results have presented that different acidification times could cause remarkable changes in the carbon transformation and utilization. The volatile fatty acids concentration has increased by 56.26 ± 2.46 mg/L during the period of 24-h acidification. Accordingly, the efficiency of carbon source utilization in the anaerobic and anoxic reactors increased by 21.21% and 25.03%, leading to the final removing efficiency of 80.53% total nitrogen and 92.37% total phosphorus, respectively. The effluent water quality could meet the Chinese Discharging Standards for Urban Wastewater Treatment Plants (GB18918–2002) grade A after 8-h acidification. It seemed that acidification had no significant influence on the internal transformation between Glycogen and Poly-β-hydroxybutyrate. Particulate organic carbon was found to be an important potential carbon source, and the removal efficiency of chemical oxygen demand showed no obvious relationship with acidification time as well.

Keywords: Carbon source; Acidification; Volatile fatty acids; Poly-β-hydroxybutyrate; Glycogen; Anaerobic–anoxic–nitrification; Denitrifying phosphorous removing bacteria

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1. Introduction

Intensive anthropogenic activities caused numerously environmental problems [1] accruing in different media such as air, water, and soil. Discharge of excessive nitrogen (N) and phosphorous (P) from municipal wastewater of cities or towns is one of the main problems that water environment is encountering, which could cause aquatic ecosystem deterioration [2], eutrophication [3,4], algal blooms, and drinking water pollutions [5]. Over the last few decades, many biological nutrient removal processes (BNR)were developed, such as anaerobic–anoxic–oxic (A^2O) [6,7], anaerobic oxicnoxic-Oxic (AOAO) [8], University of Cape Town [9,10], and biological–chemical phosphorus and nitrogen removal [11].

However, carbon/nitrogen (C/N) ratio less than 4:1 in raw domestic wastewater is usually considered to be a critical parameter in limiting nutrient treatment efficiency, which is too low to meet carbon source demand for the biological processes of N and P removal [12–15]. Whereas, nitrification and denitrification efficiencies could reach their maximum potential at the higher C/N ratio ranging from 8:1 to 9:1 according to previous researches [16].

In order to attain better nitrogen removal effect to meet the criteria of effluent's water quality standard on nitrogen concentration, additional carbon sources (e.g. methanol and sodium acetate) have to be added [17] in many wastewater treatment plants (WWTPs). Methanol has been widely used because of its rather low cost and smaller sludge production compared with other carbon sources used in WWTPs [18]. Leachate and plant carbon sources (e.g. Straws, leaves, and sawdust) are another two kinds of major alternatives that have been gradually applied in this field [13] for resources recycling. However, they also have some drawbacks, such as increased operating costs, management complexity, and seasonal variation of effluent chemical oxygen demand concentration, limiting their application in engineering.

Therefore, it is of great interest to find some better solutions to solve the carbon source insufficiency in municipal wastewater treatment. The facts that particulate organic carbon (POC) and some macromolecular organic matters (OM) can supply biodegradable substrates through anaerobic treatment [19–21] have recently received much attention. It is concerned with complex processes in effectiveness of OM transformation, usually including three different steps such as hydrolysis, acidification, and methanogenesis [22]. Hydrolysis mainly converts starch, lignocellulosic materials (i.e. cellulose and lignin), fats, and proteins into micromolecules (i.e. sugars, short-chain fatty acids, and amino acids) [23]. These compounds are further broken down to volatile fatty acids (VFAs) as major final products by acidogenic bacteria, and this is called acidification, and methanogenesis convert the VFAs into methane (CH_4) and carbon dioxide (CO_2) by strictly anaerobic bacteria [24].

In the anaerobic–anoxic–nitrification (A_2N) process, VFAs in the influent are usually taken up and stored as poly-β-hydroxybutyrate (PHB) by phosphate accumulating organisms (PAOs) in anaerobic reactor. Denitrifying polyphosphate accumulating organisms (DNPAOs) can use nitrate/nitrite as electron acceptors to degrade PHB under sequent anoxic conditions, which means that PHB of internal carbon source can be used for denitrification. Compared with the conventional BNR processes, A_2N can effectively use PHB as energy source and electron donor, while nitrate as electron acceptor instead of oxygen for removing N and P simultaneously under anoxic conditions [25]. As VFAs is a precursor of the PHB synthesis, much attention has been paid to acidification for elevating products of VFAs [26,27], which have been demonstrated to be very suitable carbon source for denitrification [28], avoiding costly external carbon addition [29].

Thus, how to obtain higher VFAs product is worthy to be further researched. Propionic acid or their salts produced, using typical extra carbon source and acetic in sludge acidification [25] in WWTPs, has been extensively studied. But the researches addressed to improve the utilization and transformation efficiency for external/internal carbon source by wastewater acidification have rarely been reported on the basis of the A_2N process. As a testing trial, our objectives were to (1) improve the concentration of VFAs in raw wastewater by acidification; (2) study the responses of internal (PHB, GLY) /external carbon source utilization to acidification time in anaerobic–anoxic reactor; and (3) analyze the transformation between POC and DOC in different acidification times. This research result could have important implication on improvement of performance of nutrient removal and proper operational conditions in A_2N process.

2. Materials and methods

2.1. Description of the A_2N process

The A_2N is a process characterized by continuous-flow, two-sludge, and typical denitrifying phosphorous removal process, in which poly-P bacteria are capable of P-uptake under anoxic conditions using nitrate as an electron acceptor (Fig. 1) [5,30].

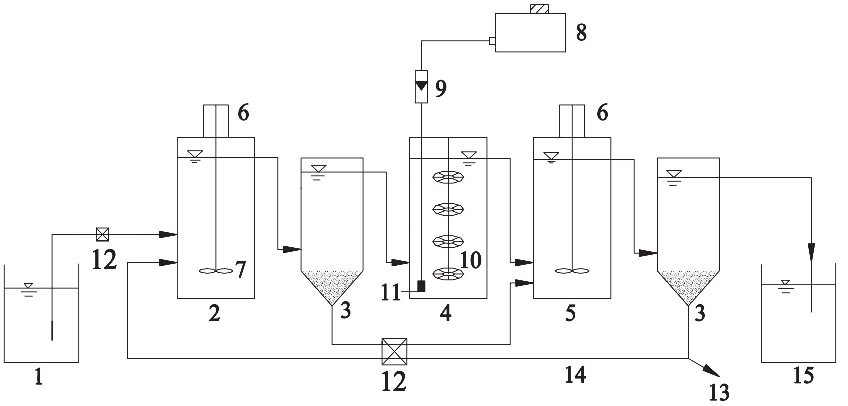

Fig. 1. Schematic diagram of the lab-scale A_2N process.
Note: 1. Influent tank, 2. anaerobic reactor, 3. settler, 4. aerobic reactor, 5. anoxic reactor, 6. small adjustable speed motor, 7. impeller, 8. air pump, 9. air flow meter, 10. combination packing, 11. aerator, 12. peristaltic pump, 13. sludge, 14. return sludge, and 15. effluent tank.

It consists of an anaerobic reactor (2) where DPB takes up VFAs, stores them as PHB in their cells and releases P, a nitrification reactor (4) with fixed biofilm for degradation of OM and nitrification, an anoxic reactor (5) for denitrifying phosphorous removal and two settlers (3). The first settler mainly separates phosphorus and ammonia-rich supernatant off PHB-rich anaerobic sludge. The supernatant flows into the aerobic reactor, where nitrification occurs and the sludge is circulated to the anoxic reactor. The second settler serves as a solid–liquid separator to guarantee clear effluent.

Compared with the conventional BNR processes, it can effectively use PHB as energy source and electron donor, and the nitrate as electron acceptor instead of oxygen for removing N and P simultaneously under anoxic conditions [25], this A_2N process has the following advantages:

(1) DPB and common PAOs coexist, but the DPB sludge and the aerobic biofilm are relatively separated in the process.
(2) The outlet sludge from the anaerobic reactor is divided into two parts: one enters the anoxic reactor, where the DPB dominates, and the other flows into the aerobic reactor, where the aerobic PAOs dominate.
(3) All the ammonium in the influent has a complete nitrification due to the aerobic biofilm with a relatively long sludge age and the abundance of microbial population.
(4) The most important of it can make carbon and energy saving.

2.2. Dimensions of lab-scale apparatus and operation parameters for A_2N

The dimensions of the lab-scale apparatus and its operational conditions of A_2N process are listed in Table 1. Effective volumes of the anaerobic, nitrification, and anoxic reactors were 5.6, 15, and 13.8 L, respectively. The hydraulic retention time (HRT) was 3 h in anaerobic reactor, 6 h in aerobic reactor, and 6 h with anoxic reactor, respectively. The dissolved oxygen (DO) concentration was controlled at levels less than 0.2 mg/L in the anaerobic reactor, around 3–4 mg/L in the nitrification reactor, and about 0.2–0.4 mg/L in the anoxic reactor.

2.3. Wastewater acidification

After taking 50 L of wastewater in a plastic bucket, 5–6 mL of 2.5% Na_2SO_3 were added to remove the original DO of wastewater. Some hydrolysis–acidification biological membranes have been domesticated and fixed on combination fillers in the bucket. Water samples were sampled at each set acidification time for analysis of chemical parameters, and then pumped into the anaerobic reactor as influent for lab-scale operation.

2.4. Operation and analytical methods for wastewater and sludge

The raw wastewater was collected from the septic tank effluent in a dormitory area of a university. It was characterized by water quality containing 178–

Table 1
Dimensions of lab-scale apparatus and its operational conditions for A_2N process

Reactors	HRT (h)	Volume (L)	DO (mg/L)	MLSS (mg/L)
Anaerobic reactor	3	5.6	<0.2	4,000–4,500
Aerobic reactor	6	15	3–4	
Anoxic reactor	6	13.8	0.2–0.4	3,500–4,000
Influent flow rate (L/h)	2.5			
Sludge bypass and return ratio (%)	40			
Settler volume (L)	4			
Total HRT (h)	15			
SRT (d)	15			
Operational temperature (℃)	25–28			

246 mg/L COD, 42.12–56.66 mg/L TN, and 3.98–5.86 mg/L TP with neutral pH.

Seed sludge was obtained from an urban wastewater treatment plant with function of enhancing nutrient removal, where nutrient removal organisms such as DNPAOs and nitrifying bacteria were expected to exist because this plant was operated with an A^2O process configuration. Concentration of mixed liquor suspended solid (MLSS) was maintained at levels of 4,000–4,500 mg/L in anaerobic reactor and 3,500–4,000 mg/L in anoxic reactor after 30 d operation.

Samples were taken from each reactor to analyze COD, TN, and TP according to standard methods [31]. DO was measured with HACH HQ30d (USA) and pH with glass electrode (Leici PHS-3E, Shanghai, China). Total organic carbon (TOC) was measured using the TOC analyzer (TOC-VCSN, Japan). VFAs was assayed according to the methods described previously [32,33], while PHB was analyzed by employing high-performance liquid chromatography (HPLC, Waters, USA) with an UV index detector and a ZorbaxEclipse SB-C18 column (Agilent, USA) [34,35]. Glycogen (GLY) was determined via digestion and hydrolysis to glucose [36,37], and then glucose was analyzed using an enzymatic method (Saint Louis, Missouri, USA) with a Shimadzu UV2401PC spectrophotometer at 425 nm wavelength.

2.5. Calculation of carbon source utilization and transformation

The relation of transformation from PHB and GLY to COD is explained in the schematic diagram of Fig. 2. PHB and GLY concentration are unified as COD unit by conversion coefficients of 1.674 and 1.185 according to Eqs. (1) and (2). Then following Eqs. (3–5) were employed for calculation of PHB, GLY, and OM utilization in the anaerobic reactor while Eqs. (6) and (7) were used for the anoxic reactor [38,39].

$$\mathrm{CH_{3/2}O_{1/2}(PHB)} + 9/8\mathrm{O_2} \rightarrow \mathrm{CO_2} + 3/4\mathrm{H_2O} \quad \gamma\ (\mathrm{PHB/COD}) = 1.674 \tag{1}$$

$$\mathrm{CH_{10/6}O_{5/6}(GLY)} + \mathrm{O_2} \rightarrow \mathrm{CO_2} + 5/6\mathrm{H_2O} \quad \gamma\ (\mathrm{GLY/COD}) = 1.185 \tag{2}$$

$$\mathrm{M_{OM1}} + \mathrm{M_{GLY1}} = \mathrm{M_{PHB}} \tag{3}$$

$$\mathrm{M_{OM1}} + \mathrm{M_{OM2}} + \mathrm{M_{OM3}} = \mathrm{M_{TOM}} \tag{4}$$

$$\eta = \mathrm{M_{OM1}}/\mathrm{M_{TOM}} \tag{5}$$

$$\mathrm{M_{PHB}} = \mathrm{M_{N,P}} + \mathrm{M_{GLY2}} \tag{6}$$

$$\eta = \mathrm{M_{N,P}}/\ \mathrm{M_{PHB}} \tag{7}$$

In Eqs. (3)–(7), $\mathrm{M_{OM1}}$ is the quantity of transformation from OM to PHB, $\mathrm{M_{OM2}}$ is the quantity of microbial metabolism, and $\mathrm{M_{OM3}}$ denotes the quantity of sludge adsorption. $\mathrm{M_{GLY1}}$ and $\mathrm{M_{GLY2}}$ indicate the quantity of transformation from GLY to PHB and from PHB to GLY, respectively. $\mathrm{M_{PHB}}$ is the synthetic quantity of PHB, $\mathrm{M_{N,P}}$ is the utilization quantity of N and P removal, and $\mathrm{M_{TOM}}$ is the total reduced quantity of OM in anaerobic reactor.

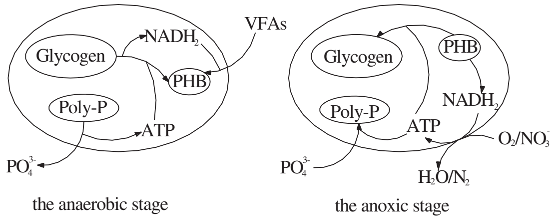

Fig. 2. Schematic diagram of PHB/GLY utilization and transformation.

3. Results and discussion

3.1. The concentration of VFAs in different acidification times

Fig. 3 shows the variation of VFAs concentration along the different acidification times, which is the preferred carbon source for wastewater BNR. The VFAs concentration increased along with longer acidification time from 0 to 24 h, but decreased along with acidification time from 24 to 72 h (Fig. 3). Although the maximum value of VFAs concentration occurred at 56.26 ± 2.46 mg/L at 24 h, the proper acidification time for wastewater was suggested to be set in 8 h (48.47 ± 1.62 mg/L) since it can obtain rather high VFAs concentration at rather shorter time.

The main reason for improved VFAs production by acidification from 0 to 24 was that hydrolysis–acidification micro-organisms can convert some particulate and macro-molecular OM into VFAs through metabolism. However, VFAs could be used by methanogens to produce CH_4 after longer acidification time of 24 h. Also, some obvious small bubbles in the liquid level during experiment were observed.

3.2. Effect of acidification time on carbon/GLY utilization and PHB synthesis in the anaerobic reactor

In the anaerobic reactor, the principal biochemical reactions were that DPB absorbed the small molecular weight and easily degradable organic matters, and then transformed them into PHB stored in cells accompanying by P-released at the same time. In addition to this, the intracellular GLY were widely transformed into PHB both as energy source and carbon source. The effect of P-release directly determines P-uptake in the sequent anoxic reactor. However, many factors could impact the P-releasing processes, including the concentrations of DO and VFAs. The denitrification also can produce indirect influence for it because the rest of the nitrate reflow can destroy anaerobic conditions [25,36,38].

The transformation and utilization of external/internal carbon sources along with acidification time were analyzed (Fig. 4). The synthetic quantity of PHB using VFAs in the anaerobic reactor increased with acidification time from 318.43 to 368.39 mg COD/h. But the rate of transformation from GLY to PHB was always kept around 186 mg COD/h, indicating that the increased PHB form from 133.22 mg COD/h at 0 h to 180.37 mg COD/h at 24 h by acidification time mainly had been taken by DPB sludge using the carbon source of raw wastewater. As a result, the efficiency of carbon source utilization rate increased from 59.92% at 0 h to 81.13% at 24 h, showing faster increasing efficiency before 8 h acidification time.

This phenomenon could be interpreted from two aspects. One side wastewater acidification could effectively promote the transformation from the POC and some macromolecular OM to micromolecular OM (i.e. VFAs). In another side, VFAs is a precursor of the PHB synthesis and VFAs concentration determines the amount of PHB synthesis in anaerobic reactor. An increase in PHB content resulted in a rapid P-releasing, making maximum bioavailability of carbon source for PHB synthesis. Above processes avoid excess carbon flowing into the aerobic reactor for degradation, which would lead to carbon source waste and incomplete nitrification.

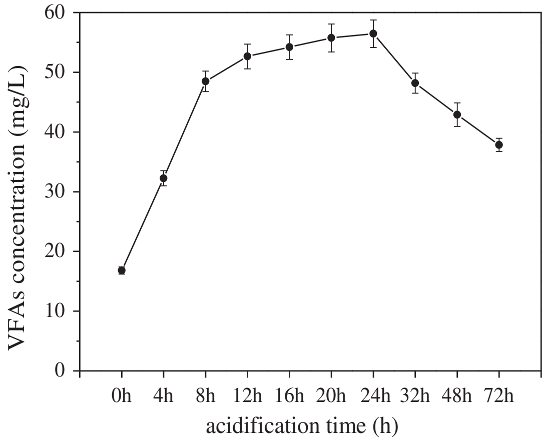

Fig. 3. Variations of VFAs in different acidification times.

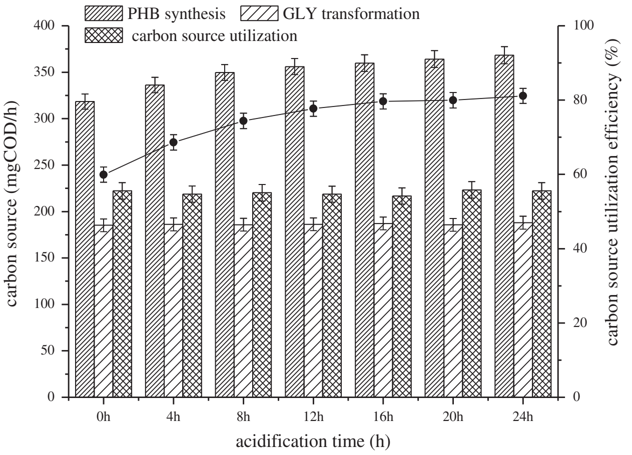

Fig. 4. Rates of PHB synthesis, GLY transformation, and external carbon utilization efficiency with acidification time in anaerobic reactor.

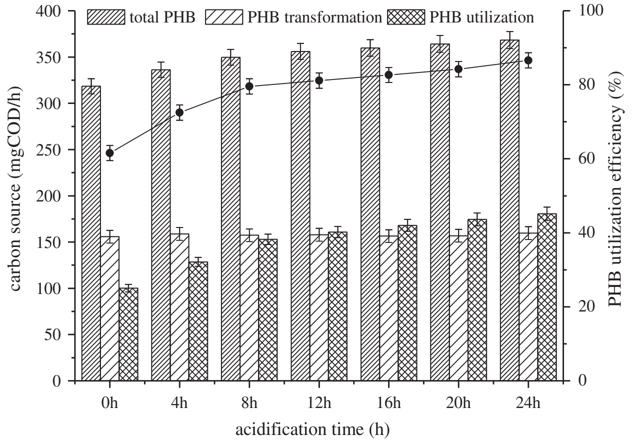

Fig. 5. Efficiencies of PHB utilization and transformation with the acidification time in anoxic reactor.

3.3. *Effect of acidification time on PHB/GLY utilization and transformation in the anoxic reactor*

In the anoxic reactor, the principal biochemical reactions were that DPB sludge used intracellular PHB as electron donor and nitrates as electron acceptor for denitrification and excessive P-uptake as well. Part of the intracellular PHB transformed into GLY stored in cells at the same time. Hence, substantial storage of PHB in anaerobic reactor is greatly favorable as internal carbon source in the anoxic reactor.

As shown in Fig. 5, PHB was the primary endogenous carbon source for N and P removal in terms of carbon utilization, and the transformation rate from PHB to GLY maintained at 157 mg COD/h. However, available PHB obviously improved from 100.13 at 0 h to 180.64 mg COD/h at 24 h, resulting in an increased efficiency in the range of 61.56–86.59%, respectively. PHB consumption in the anoxic reactor is as same as PHB synthesis and utilization in the prior anaerobic process, where 8-h acidification is the optimal time for producing the favorable carbon source.

N and P removal simultaneously are achieved in anoxic reactor regarding GLY synthesis and PHB utilization [38] by DNPAOs. However, intracellular carbon cycling patterns and quantity could impact the post-anoxic denitrification. Enhanced biological phosphorus removal (EPBR) theory emphasizes that VFAs are metabolized into PHB anaerobically, which is necessarily reduced in equivalents partly derived from GLY degradation.

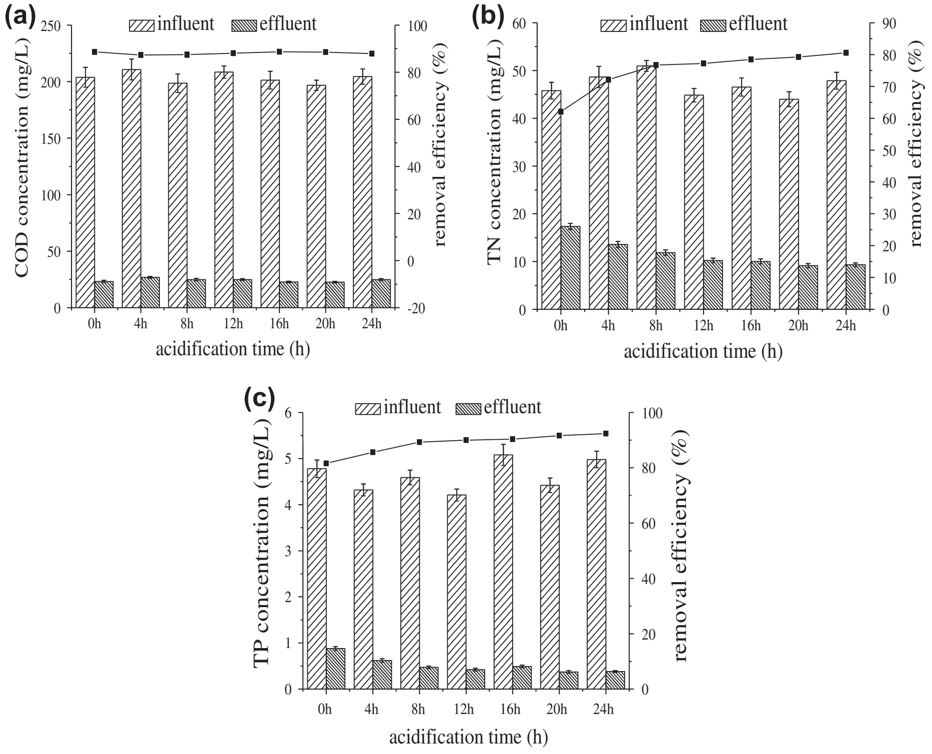

Fig. 6. Performance of the A_2N process regarding COD (a), TN (b), and TP (c) removal in what, respectively.

3.4. Effect of acidification time on the treatment performance

It is natural that acidification time could impact on the performance of wastewater treatment as a comprehensive consideration of all above-related processes, in which the biological nitrogen removal (BNR) is achieved by aerobic nitrification and anoxic denitrification while phosphorus removal is accomplished through P-release and excessive P-uptake under anaerobic–aerobic conditions.

It seemed that wastewater acidification had hardly impact on the COD removal but it played an important role for TN and TP removal (Fig. 6). The removal efficiency of TN increased from 62.08 to 80.53%, and from 81.59 to 92.37% along with acidification time, respectively. The effluent concentrations of COD, TN, and TP with 8-h acidification were 24.9, 11.9, and 0.47 mg/L, which all could meet the Chinese Discharging Standards for Urban Wastewater Treatment Plants (GB18918-2002) grade A. There is fast improved treatment performance with longer acidification time before 8h but no obvious changes after that. Furthermore, the effect of pre-acidification on TN removal was better than TP removal. Therefore, an 8-h acidification time was suggested to be reliable or reasonable to target high efficiency utilization of carbon source for the sewage with low C/N ratio.

3.5. The change of carbon forms of TOC, POC, and DOC

The changes of TOC, POC, and DOC in different acidification times were shown in Fig. 7. Significantly transformation from POC into DOC was observed, while TOC concentration shows smaller decrease caused by metabolism activities of acidification bacteria. An 8-h acidification has made DOC concentration improve to 33.48 mg/L from 20.40 mg/L in the raw wastewater without acidification while POC concentration has been decreased from 38.05 to 20.27 mg/L during the same lasting period of acidification.

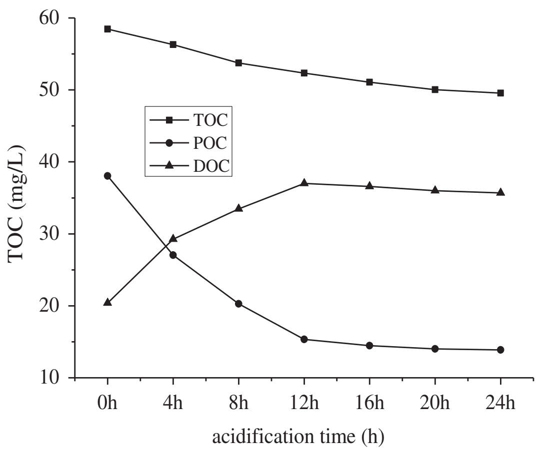

Fig. 7. Variations of TOC, POC, and DOC in different acidification times.

Therefore, it indicated that POC was a kind of potential carbon source for N and P removal from another aspect through not all of POC existed in the wastewater would be transformed into DOC by acidification method.

4. Conclusions

Wastewater acidification, which was based on the operational practice on lab-scale A_2N process, was proved to be an effective method to promote the POC transformation, enhance the VFAs production, improve the external/internal carbon source utilization, and nutrient removal. Conclusions in this research can be summarized as follows:

(1) Maximum VFAs concentration was achieved at 24 h with values of 56.26 ± 2.46 mg/L, concerning of a series of acidification time setting, which is greatly higher than its initiated concentration of 16.80 ± 0.83 mg/L without acidification. POC has decreased from 38.05 mg/L at beginning to 20.27 mg/L at 8 h acidification, and then balanced in 14 mg/L at 12 h. It is necessary to control the acidification time in a proper range.

(2) In anaerobic reactor, the efficiency of carbon source utilization had increased from initiated 59.92% in raw wastewater to 81.13% at 24-h acidification. In anoxic reactor, the efficiency of PHB utilization had improved from 61.56 to 86.59% as same as that in anaerobic reactor. However, acidification time has no obvious effect on interconversion between PHB and GLY.

(3) The performance of A_2N had been significantly improved after an 8 h pre-acidification. The effluent concentrations of COD, TN, and TP were 24.88, 11.87, and 0.47 mg/L with averaged removal efficiency of 87.41, 76.71, and 89.29% for them, respectively. However, acidification has no effect on of COD removal.

This research results have important implication on improvement of nutrient removal performance in A_2N process. An acidification tank with HRT of 8 h could be suggested to install in order to produce higher concentration of VFAs in raw wastewater for the subsequent A_2N operation in the practical engineering. It is probably considered to be a substitute to the method of long-term carbon source addition for low C/N ratio sewage, finally resulting in the reduction of operation costs.

Acknowledgments

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