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Full Length Research Paper

Effect of ultrasonic specific energy on waste activated sludge solubilization and enzyme activity

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The effect of ultrasonic specific energy on waste activated sludge (WAS) solubilization and enzyme activity was investigated in this study. Experimental results showed that the increase of ultrasonic specific energy in the range of 0 - 90000 kJ/kg dried sludge (DS) benefited WAS particle size reduction and the solubilization of organic matter in WAS. The changes of ultrasonic specific energy also gave impact on the activities of hydrolytic enzymes (protease, α-glucosidase, acid and alkaline phosphatase) which were related to the hydrolysis of protein and carbohydrate in WAS. Further investigation revealed that the activities of four hydrolytic enzymes reached the highest at the specific energy of 30000 kJ/kg DS with the disintegration degree of 38.2%. Also, the releases of soluble ammonia and phosphorus was studied during the ultrasonic treatment of WAS.

Key word: Waste activated sludge (WAS), ultrasonic, solubilization, disintegration degree, enzyme activity.

INTRODUCTION

The activated sludge process is widely used during both domestic and industrial wastewater treatment. However, large amounts of waste activated sludge (WAS) is generated and the disposal of WAS has become a major economic problem in wastewater treatment plants (WWTPs) (Mikkelsen and Keiding, 2002). At present, many treatment techniques, such as anaerobic digestion, burning and land filling, have been carried out for the disposal of WAS, among which WAS anaerobic digestion sludge has the following advantages: generated methane can be utilized as fuel, digestion only has a low energy requirement, the pathogenic microorganisms in WAS are effectively killed, and in addition, the digested sludge is stable and harmless to dispose (Tiehm et al., 1997). However, extremely slow hydrolysis rate of WAS anaerobic digestion, induced by the cell wall and membrane of bacteria in WAS which inhibit the release of organic substances to the outside of the cell, results in long digestion time. In order to improve the rate of anaerobic hydrolysis, many pretreatment attempts, such as thermal energy, ozonation, acid and alkaline adjustments, high pressure, mechanical disintegration and ultrasound, have been made. Among these, the employment of ultrasonic technique exhibits the benefit of not being hazardous to the environment.

Several recent reports have demonstrated the efficacy of ultrasonic as a method for disintegrating sludge and thereby accelerating the rate of anaerobic digestion process and improving methane yield (Tiehm et al., 2001; El-Hadj et al., 2007) and dewaterability (Bien et al., 1997; Dewil et al., 2006). Various ultrasonication parameters, including frequency (Tiehm et al., 2001), duration and intensity (Chu et al., 2001; Wang et al., 2006), have been studied as well as their effects on the characteristics of sludge. These previous works indicated that long ultrasonication time (120 min) and low-frequency ultrasonic (20 kHz) were effective for sludge disintegration and the obtained results are attributed to the phenomenon of ultrasound-induced acoustic cavitation.

The phenomenon of acoustic cavitation can be described that when the power is turn on, gas bubbles are

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Abbreviations: WAS, Waste activated sludge; **DS**, dried sludge; **WWTP**s, wastewater treatment plants; **COD**, chemical oxygen demand; **BOD**, biochemical oxygen demand; **SCOD**, soluble chemical oxygen demand; **TCOD**, total chemical oxygen demand; **TSS**, total suspended solids; **VSS**, volatile suspended solids; Es, specific ultrasonic energy.

formed and grown by absorbing gas and vapor from the liquid (Laborde et al., 1998). As a result of alternating expansion and compression cycles, these bubbles can implode, locally resulting in very extreme conditions of temperature (5000 K) and pressure (500 bar) (Riesz et al., 1985), whereas the bulk solution remains near ambient conditions. This phenomenon is called cavitation and encountered only at the frequency less than 1 MHz (Dewil et al., 2006). Tiehm et al. (2001) confirmed that the rate of sludge disintegration increases proportionally with the bubble size.

In addition, the solubilization of organic matter during sludge anaerobic treatment usually drives some biochemical effects, such as the changes of enzymes activities. Some researchers reported that the hydrolytic enzymes, such as protease, α-glucosidase, acid and alkaline phosphatases, which were involved in the hydrolysis of organic matter, were observed in WAS anaerobic treatment system (Yuan et al., 2006; Feng et al., 2009). It has also been documented that the activities of hydrolytic enzymes usually depend on the substrate concentration, pH and temperature (Goel et al., 1998). It is well known that the hydrolysis of particulate organic matter is believed to be the rate-limiting step during WAS anaerobic digestion. If the activities of hydrolytic enzymes increased, the rate of WAS anaerobic digestion would be accelerated.

Previous studies have reported how ultrasonic energy, duration and intensity affect the sludge's physical and chemical characteristics of WAS, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD) and capillary suction time (Zhang et al., 2007; Na et al., 2007). However, very few reports have addressed how ultrasonic specific energy affects the solublization and microbiologic characteristics of WAS (enzymes activities). Specific energy was an important parameter observed in our investigation which influenced the efficiency of WAS ultrasonic disposal. The purpose of this work was therefore to investigate the effect of specific energy on the solubilization of organic matter and the variations of particle size, especially the activities of hydrolytic enzymes during the ultrasonic treatment on WAS. Nitrogen and phosphorus releases were also discussed.

MATERIALS AND METHODS

Source of WAS

The WAS used in this study was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant (WWTP) in Shanghai, China. This plant was operated with an anaerobic-aerobic process for biological nutrients removal. The WAS was concentrated by settling at 4°C for 24 h and its main characteristics were as follows: pH 6.8, total suspended solids (TSS) 10119 mg/l, volatile suspended solids (VSS) 6964 mg/l, soluble chemical oxygen demand (SCOD) 150 mg/l, total chemical oxygen demand (TCOD) 10004 mg/l, total carbohydrate 113.3 mg COD/g TSS, total protein 601.2 mg COD/g TSS, lipid and oil 9.7 mg COD/g TSS.

Ultrasonic experiment

Ultrasonic tests were conducted with a cell-breaker (US Sonics, VCX 105), which was equipped with an MS 73 titanium microtip probe working an operating frequency of 20 kHz and supplied a power of 100 W. Batch experiments without temperature regulation were carried out in triplicate in a cylindrical vessel with 5 cm inner diameter. The specific ultrasonic energy (Es) was defined as the product of the ultrasonic power (P) and the ultrasonic time (t) divided by the sample volume (V) and the initial total solids concentration (TSS) and ranged from 0 to 90000 kJ/ kg dried sludge (DS).

$$E_S = \frac{P \times t}{TSS \times V} \quad (1)$$

Analytical methods

The determinations of protein, carbohydrate, lipid, COD, ammonia nitrogen, soluble phosphorus, TSS and VSS were conducted according to the standard methods (APHA, AWWA and WPCF, 1998). According to Miron et al. (2000), 1 g protein, carbohydrate and lipid is equivalent to 1.5 g COD, 1.07 g COD and 2.91 g COD, respectively. The turbidity of the supernatant (water fraction) of the sludge was measured when the water phase was separated form the sludge by centrifugation at a speed of 3500 rpm.

The assays of four hydrolytic enzymes (protease, α-glucosidase, acidic phosphatase and alkaline phosphatase) were performed according to Goel et al. (1998). The substrate used in the assays of α-glucosidase, acidic phosphatase and alkaline phosphatase is ρ-nitrophenyl; hence, 1 enzyme unit was defined to produce 1 μM ρ-nitrophenyl in one hour. To quantify the amount of ρ-nitrophenyl released in enzyme reaction, a calibration curve using known amounts of ρ-nitrophenyl was prepared. In the case of protease, 1 enzyme unit was defined to hydrolyze 1 mg of azocasein per hour. The specific enzyme activity was defined as the unit of enzyme activity per gram of DS (DS = TSS×V).

The particle size distribution of sludge sample was determined by the laser light obscuration method based on the time-of-transition principle. The laser instrument (Coulter LS230) was equipped with a universal liquid module (ULM) which could detect particle size from 0.04 up to 2000 μm. Particle counts and size distribution were calculated and displayed automatically.

Disintegration degree

The degree of disintegration (DD_{COD}) was defined by Müller and Pelletier (1998) as the comparison between SCOD after ultrasonic pretreatment and the maximum SCOD obtained by alkaline hydrolysis ($SCOD_{NaOH}$), as presented in Equation 2. For alkaline hydrolysis, sludge was mixed with NaOH (0.5 mol/l) for 24 h at room temperature as described by Gonze et al. (2003).

$$DD_{COD} = (SCOD_{Ultrasonic} - SCOD_0)/(SCOD_{NaOH} - SCOD_0) \times 100\% \quad (2)$$

where $SCOD_{Ultrasonic}$ was the COD in the supernatant of the sonicated sample (mg/l), $SCOD_0$ was the COD in the supernatant of the untreated sample (mg/l), $SCOD_{NaOH}$ was the COD in the supernatant of the alkaline hydrolyzed sample (mg/l).

RESULTS AND DISCUSSION

Effect of specific energy on particle size reduction

The effect of specific energy on mean particle size

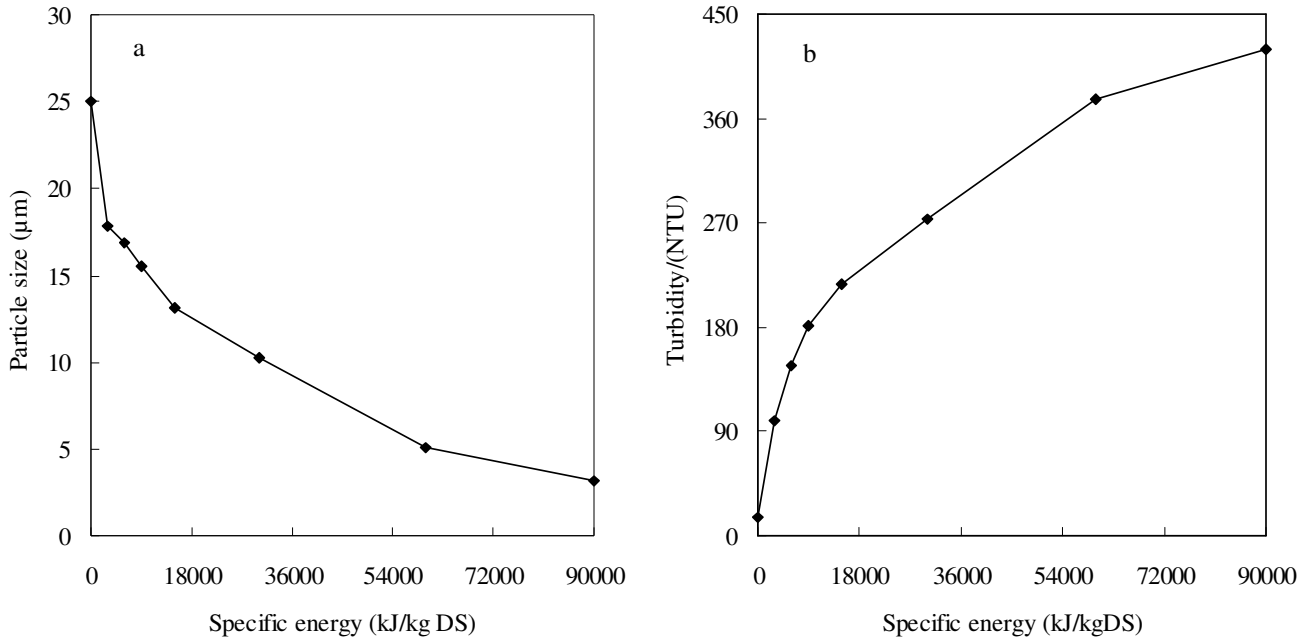

Figure 1. Effect of specific energy on particle size reduction and aqueous phase turbidity. Error bars represent standard deviations of triplicate tests.

reduction of WAS is shown in Figure 1a. It can be seen that the mean particle size of WAS decrease with the ultrasonic specific energy. In this study the original mean particle size of WAS was 25.0 μm. When WAS was treated by ultrasonics at different specific energies, the mean particle size changed dramatically. For example, at the specific energy of 15000 kJ/kg DS, the mean particle size was 13.1 μm, which was only one half of that of original WAS. Further increase of specific energy gave positive influence on the mean particle size reduction of WAS. When the ultrasonic specific energy was 90000 kJ/kg DS, the mean particle size decreased dramatically to 3.2 μm. These results showed good agreement with both El-Hadj et al. (2007) and Feng et al. (2009), who observed similar changes in particle size following ultrasonic treatment.

It has been stated in the literature that WAS treatment usually led to an increase of the released organic matter into the aqueous media, which resulted in the variations of WAS aqueous turbidity (Feng et al., 2009). In addition, the decrease in particle size due to ultrasonic treatment can also result in the increase of WAS turbidity. The effect of specific energy on WAS aqueous turbidity during WAS ultrasonic treatment is shown in Figure 1b. It was observed that the WAS aqueous turbidity increased with ultrasonic specific energy. The aqueous turbidity of original WAS was 15.3 NTU, whereas the turbidity increased to 420.0 NTU when the specific energy was 90000 kJ/kg DS. The reason for this might be attributed to that the ultrasonically treated sludge was broken down into smaller pieces and then more particulate matters were released to the aqueous medium of WAS. Feng et al. (2009) investigated the effect of ultrasound on supernatant turbidity when WAS was treated ultrasonically and found that the turbidity increased with specific energy while ultrasonic specific energy was higher than 5000 kJ/kg DS, which were consistent with the present results obtained in this study.

Effect of specific energy on the solubilization and disintegration of organic matter

During the ultrasonic treatment of WAS, the transfer of COD from the particulate matter to the soluble fraction always occurs. Figure 2 presents the effect of specific energy on the concentration and disintegration degree of particulate COD. It can be seen that the SCOD concentration increased with the specific energy. In this investigation the initial SCOD in WAS was 150 mg/l, whereas the SCOD dramatically increased to 3752.2 mg/l with the increase of the specific energy to 90000 kJ/kg DS. Further investigation revealed that the SCOD concentration increased linearly with the specific energy in the range of 0 - 90000 kJ/kg DS (y_{SCOD} = 495 E_s -376, R^2 = 0.97).

Similar to the results of COD concentration, the disintegration degree of particulate COD also increased linearly with specific energy and their relationship could be expressed by the Equation (y_{DD} = 8.3 E_s - 8.9, R^2 = 0.97). When the specific energy increased from 0 to 90000 kJ/kg DS, the disintegration degree of COD

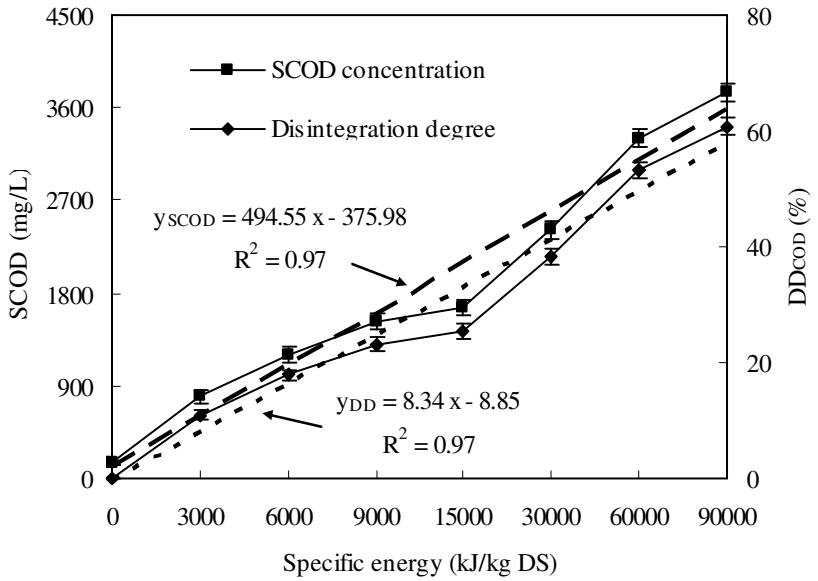

Figure 2. Effect of specific energy on the concentration and disintegration degree of COD. Error bars represent standard deviations of triplicate tests.

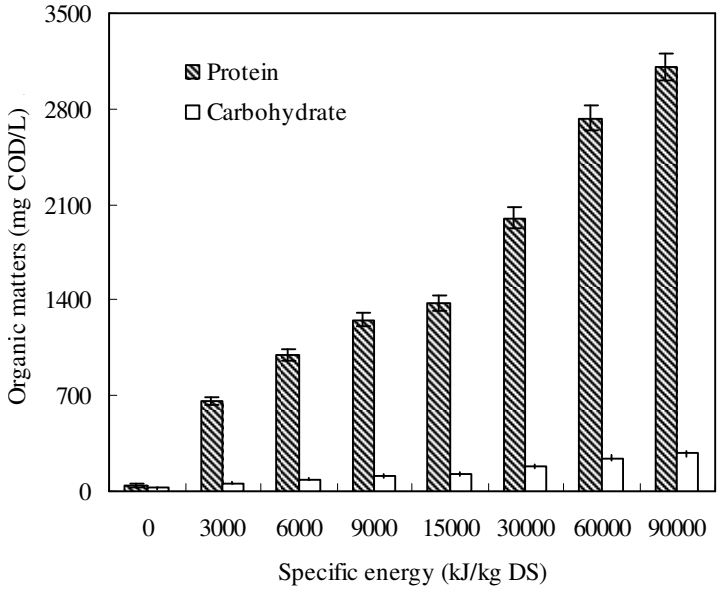

Figure 3. Effect of specific energy on the concentrations of soluble protein and carbohydrate. Error bars represent standard deviations of triplicate tests.

improved from 0 to 60.8%. It can be seen that the variation trend of COD disintegration degree was almost the same with that of SCOD concentration. The reason for this might be attributed to the fact that both the disintegration degree and SCOD concentration were correlated by Equation 2. In the literature, almost the same disintegration degree has been reported by other researchers (Lehne et al., 2001; Bougrier et al., 2005).

It is well known that protein and carbohydrate are the two predominant organic matters in WAS. Thus, the SCOD was mainly composed by the soluble protein and carbohydrate. Figure 3 shows the effect of specific energy on the concentrations of soluble protein and carbohydrate during the ultrasonic treatment of WAS. It was clearly observed that both the concentrations of soluble protein and carbohydrate increased with the specific energy. For example, the soluble protein in the ultrasonic-untreated WAS was only 44.1 mg COD/L,

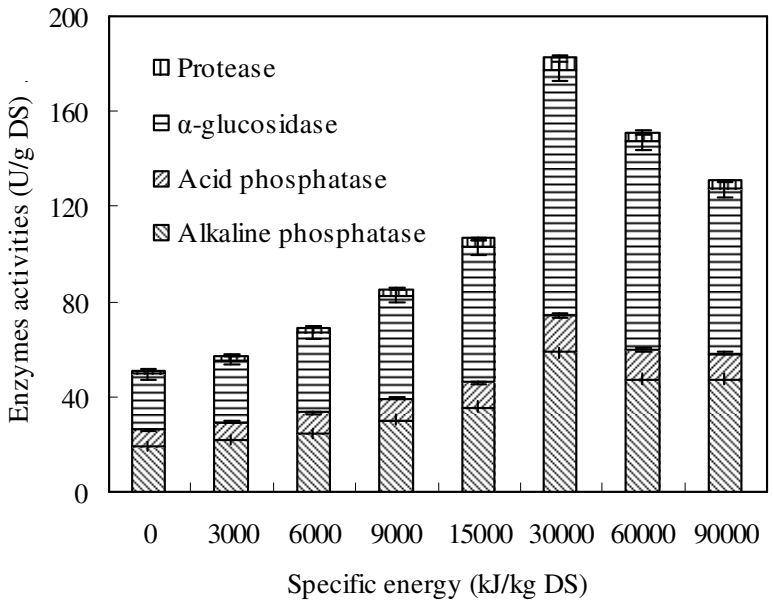

Figure 4. Effect of specific energy on the activities of hydrolytic enzymes. Error bars represent standard deviations of triplicate tests.

which increased to 3104.5 mg COD/L at the specific of 90000 kJ/kg DS. Similarly, the concentration of soluble carbohydrate increased from 23.8 to 274.8 mg COD/L in the specific energy range of 0 - 90000 kJ/kg DS. It should be noted that the concentrations of soluble protein at different specific energies were much higher than those of soluble carbohydrate. The reason for this was attributed to the fact that protein was the most predominant component of WAS.

The solubilizations of protein and carbohydrate during the ultrasonic treatment of WAS could also be expressed by the specific solubilization rate. The specific solubilization rate of protein or carbohydrate was defined as the concentration of solubilized protein or carbohydrate per minute. In this investigation the specific solubilization rates of protein at the specific energy of 30000, 60000 and 90000 kJ/kg DS, were respectively, 195.3, 73.5 and 37.2 mg COD/L $\cdot min^{-1}$, which clearly decreased with ultrasonic specific energy. The same trend of carbohydrate was observed with the solubilization rate of 15.3, 6.5 and 3.3 mg COD/L $\cdot min^{-1}$ at the specific energy of 30000, 60000 and 90000 kJ/kg DS.

Effect of specific energy on the activities of hydrolytic enzymes

It is well known that the solubilization of organic mater during the ultrasonic treatment of WAS usually leads to some biochemical effects, such as the variations of enzymes activities. Thus, in this study the activities of four hydrolytic enzymes (protease, α-glucosidase, acid phosphatase and alkaline phosphatase) were investigated at the different specific energies (Figure 4). These four hydrolytic enzymes can hydrolyze the protein and carbohydrate in WAS to the soluble substrates, such as amino acids and monosaccharide, for the following anaerobic acidification and methanogenesis.

It can be seen from Figure 4 that the ultrasonic treatment of WAS gave a positive influence on the activities of the hydrolytic enzymes in the specific energy range of 0 - 30000 U/g DS. For example, the activities of protease and α-glucosidase increased from 1.26 and 23.5 U/g DS in the original WAS to 5.98 and 102.8 U/g DS when WAS was treated at the ultrasonic specific energy of 30000 kJ/kg DS. This can be explained by the dependencies of the activities of protease and α-glucosidase on the concentrations of soluble protein and carbohydrate in WAS treatment system. However, further increase of specific energy had negative effects on the activities of hydrolytic enzymes. At the specific energy of 90000 kJ/kg DS, the activity of protease was only 4.13 U/g DS, which was much lower than that at 30000 kJ/kg DS. The activities of other three hydrolytic enzymes investigated in this study showed the same trend with that of protease. When the ultrasonic specific energy increased from 30000 to 90000 kJ/kg DS, the activities of α-glucosidase, acid and alkaline phosphatase decreased from 102.79, 15.87 and 58.39 to 69.25, 10.69 and 47.48 U/g DS, respectively. The reason for this might be attributed to the fact that more bacteria in WAS were disrupted and even damaged when the ultrasonic specific energy was higher than 30000 kJ/kg DS.

Based on the above analysis, the process of ultrasonic treatment on WAS could be divided into two stages: sludge floc were changed and disintegrated firstly, and

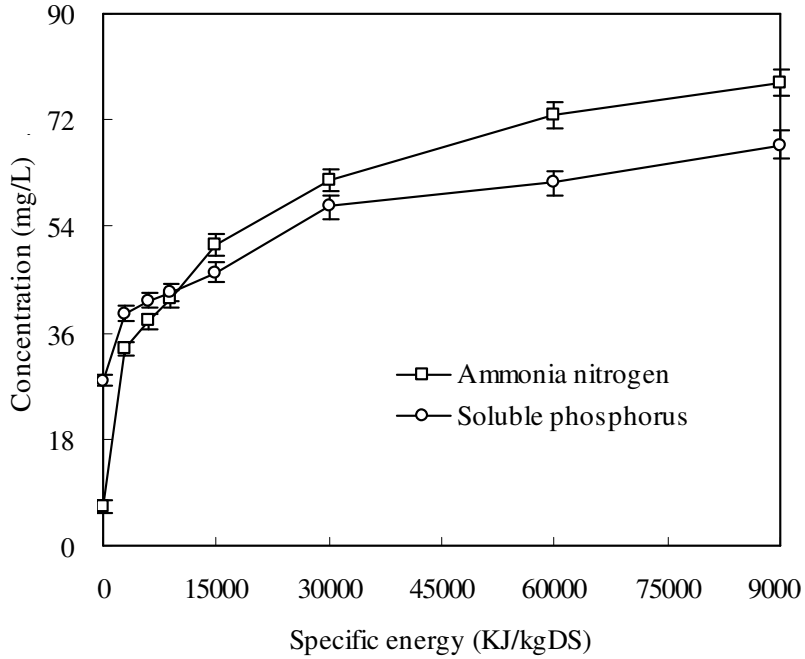

Figure 5. Effect of specific energy on ammonium and phosphorus releases during the ultrasonic treatment of WAS. Error bars represent standard deviations of triplicate tests.

then the exposed microbial cells were disrupted. During the first stage (specific energy ≤ 30000 kJ/kg DS), some organic matter contained in WAS were dissolved and the activities of hydrolytic enzymes, as a result increased. During the second stage (specific energy ≥ 30000 kJ/kg DS), some microbial cells were exposed and damaged by the ultrasonic treatment.

The releases of ammonium and phosphorus from WAS at different specific energies

Both ammonium and phosphorus released were observed when WAS was treated ultrasonically. Figure 5 shows the effect of specific energy on the concentrations of ammonia nitrogen and soluble phosphorus. The concentrations of ammonia nitrogen and soluble phosphorus both increased with ultrasonic specific energy. The initial concentrations of ammonia nitrogen and soluble phosphorus in the original WAS were 6.5 and 28.0 mg/l, respectively. When WAS was treated by ultrasonic at the specific energy of 3000, 15000, 30000, 60000 and 90000 kJ/kg DS, the concentrations of ammonia nitrogen were 33.3, 50.9, 61.7, 72.8 and 78.4 mg/l, respectively, and the corresponding soluble phosphorus were 39.3, 46.2, 57.3, 61.3 and 67.8 mg/l. Clearly, the increase of specific energy during the ultrasonic treatment of WAS induced the releases of ammonia nitrogen and soluble phosphorus. Comparing the release of ammonia nitrogen and soluble phosphorus, the former (78.4 - 6.5) was greater than the latter (67.8 - 28.0) in the ultrasonic specific energy range of 0 - 90000 kJ/kg DS. The reason for this might be attributed to the fact that as one of the main components of WAS in this study, the protein was readily released at different specific energies, whereas some of the released soluble phosphorus might co-precipitate with some compounds. In the literature almost the same phenomenon was found by Bougrier et al. (2005) and Feng et al. (2009).

Conclusion

WAS can be disintegrated and the organic matter in WAS can be released ultrasonically. During the ultrasonic treatment of WAS, the mean particle size of WAS deceased from 25.0 to 3.2 μm and the disintegration degree of WAS increased to 60.8% in the ultrasonic specific energy range of 0 - 90000 kJ/kg DS. The concentrations of soluble protein and carbohydrate both increased with ultrasonic specific energy during the ultrasonic treatment of WAS. The activities of hydrolytic enzymes related to the hydrolysis of organic matter in WAS were also affected by the ultrasonic treatment and reached the highest at the specific energy of 30000 kJ/kg DS. Furthermore, the release of ammonia nitrogen and soluble phosphorus were observed and their concentrations increased with the specific energy in the range of 0 - 90000 kJ/kg DS during the ultrasonic treatment of WAS.

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