Chemosphere 77 (2009) 269–272

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Technical Note

Changes in biomass activity and characteristics of activated sludge exposed to low ozone dose

Libing Chu [a,*], Jianlong Wang [a], Bo Wang [a], Xin-Hui Xing [b,*], Sangtian Yan [b], Xulin Sun [c], Benjamin Jurcik [c]

[a] Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing 100084, China
[b] Department of Chemical Engineering, Tsinghua University, Beijing 100084, China
[c] Air Liquide Laboratories, Tsukuba-shi, Ibaraki-Pref. 300-4247, Japan

ARTICLE INFO

Article history:
Received 30 March 2009
Received in revised form 21 July 2009
Accepted 22 July 2009
Available online 20 August 2009

Keywords:
Ozonation
Sludge activity
Sludge reduction
Wastewater treatment

ABSTRACT

In this paper, the response mechanism of activated sludge exposed to low-dose ozone at less than 20 mg O_3 g^{-1} total suspended solids (TSS) was studied by analyzing the changes in sludge activity and the evolution of C, N, P and metals from sludge following ozonation. The intracellular ATP concentration was not affected at less than 5 mg O_3 g^{-1} TSS and thereafter decreased rapidly to around 60% when the ozone dose increased to 20 mg O_3 g^{-1} TSS. Similarly, the efficiency of sludge solubilization initially changed a little and then increased rapidly to around 30% at an ozone dose of 20 mg O_3 g^{-1} TSS. However, the activities of superoxide dismutase and protease decreased immediately upon exposure to ozone. These findings indicate that ozone firstly destroys the floc, leading to the disruption of the compact aggregates, which does not affect cells viability but induces a decrease in enzyme activities. Ozone then attacks the bacterial cells of the sludge, causing a decrease in cells viability. During ozonation, the content of carbon, nitrogen and phosphorus in the sludge matrix decreased, while the content of these elements in the micro-solids and supernatant gradually increased. Most of the released metals from the sludge matrix were found in the micro-solids.

1. Introduction

The treatment and disposal of excess sludge represents a bottleneck in wastewater treatment plants (WWTP) worldwide, due to environmental, economic, social and legal factors. Reduction of excess sludge is becoming one of the biggest challenges in biological wastewater treatment (Wei et al., 2003; Perez-Elvira et al., 2006; Chu et al., 2008). One promising technique is the sludge ozonation process. Up to now, ozonation processes have been employed to reduce excess sludge directly in the biological wastewater treatment processes or as pretreatment techniques prior to anaerobic sludge digestion (Yasui and Shibata, 1994; Weemaes et al., 2000; Goel et al., 2004; Lee et al., 2005; Dytczak et al., 2007). Producing ozone for sludge treatment is costly and remains as a major constraint to its application in full-scale plants. Optimization of the ozonation process must be pursued to achieve cost-effective performance (Dytczak and Oleszkiewicz, 2008; Chu et al., 2009).

For economic reasons and to inhibit filamentous bulking by ozonation, low-ozone doses are applied (Dziurla et al., 2005; Caravelli et al., 2006; Vergine et al., 2007). However, there have been few studies aimed at analyzing the reaction mechanism of sludge exposed to low-ozone doses. Caravelli et al. (2006) reported that at an ozone dose of 18 mg O_3 g^{-1} volatile suspended solids (VSS), the respiratory activity in the whole flocs was inhibited by 54–60%, and by 87% for filamentous bacteria. Such ozonation conditions were adequate to control filamentous bulking. The study by van Leeuwen (1988) demonstrated that continuous dosing of ozone at 1, 2 and 4 mg O_3 g^{-1} mixed liquid suspended solids (MLSS) d^{-1} resulted in a decreased sludge volume index of about 50 mL g^{-1} less than the control. Nitrification–denitrification was not affected, even at dose of 30 mg O_3 g^{-1} MLSS d^{-1}. According to Dziurla et al. (2005), the critical ozone dose leading to a decrease in the maximum oxygen uptake rate was estimated to range between 1 and 16 mg O_3 g^{-1} TSS (recalculated assuming the conversion biomass to COD as 1.2 g COD g^{-1} TSS), depending on the sludge tested. Our previous studies (Yan et al., 2009) have shown that when the ozone dose was less than 20 mg O_3 g^{-1} total suspended solids (TSS), a large variation in the denaturing gradient gel electrophoresis fingerprint was not observed, indicating that the total DNA of the sludge had not been attacked by ozone. Studies performed on *Escherichia coli* K12 showed that short-term exposure of this organism to ozone compromised the membrane permeability but did not affect cell viability which progressively

* Corresponding authors. Tel.: +86 10 62783765; fax: +86 10 62771150 (L. Chu), tel./fax: +86 10 62794771 (X.-H. Xing).
E-mail addresses: chulibing@tsinghua.edu.cn, chulibing@yahoo.com.cn (L. Chu), xhxing@tsinghua.edu.cn (X.-H. Xing).

0045-6535/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2009.07.047

decreased with longer exposure (Komanapalli and Lau, 1996). Oxidation of lipids and sulfhydryl compounds and a strong inhibition of glyceraldehyde-3-phosphate dehydrogenase activity (which contains an –SH group at its active group) within 3–5 min of exposure of the cells to ozone were observed, whereas the decrease in culturability started after 10 min of exposure and leakage of nucleic acids from the cells was followed later, after 15 min of exposure (Komanapalli et al., 1997). A systematic study of the changes in sludge characteristics and activities when exposed to low-dose ozone would be helpful in the efforts to design and optimize an economic sludge reduction process.

In this study, the ATP content and some typical enzymatic activities were used as indicators to evaluate the effects of low-dose ozone on sludge microbial activity. All living organisms contain and use ATP as their main energy source. When cells die, most of their ATP is damaged. ATP concentration has been used as an indicator of viable biomass (Gikas and Livingston, 1993). Considering the protein content in activated sludge is reported to be as high as 20–60% (w/w) (Shier and Purwono, 1994), the protease of the sludge was chosen as the enzymatic indicator to assess the sludge ozonation process. Since ozonation is an oxidation process, the superoxide dismutase (SOD) activity was used to represent the anti-oxidization activity.

The objective of the present paper was to study the response mechanism of activated sludge to low-dose ozone by assessing the changes in sludge characteristics and activities. Moreover, the fate of some heavy metals was also examined since heavy metals are of great concern in sludge treatment due to their health impacts.

2. Materials and methods

2.1. Ozonation of sludge

The sludge was ozonated in a 1.5-L bubble column operated in batch and at room temperature. Ozone was generated by passing pure gaseous oxygen through a Sorbios ozone generator (Berlin, Germany) at a flow rate of 0.37 L min^{-1} and introduced into the reactor continuously. The inlet ozone concentration was around 30 mg L^{-1} and the contact time was about 15 min. The ozonated sludge was periodically collected as samples. The ozone concentrations in the gas phase (before and after the reaction with sludge) were determined every 30 s using an UV BMT ozone analyzer (Messtechnik, Berlin, Germany). The ozone dose transferred to the sludge was calculated from the difference between the amounts of ozone at the inlet and outlet of the ozonation reactor per amount of initial TSS. All the experiments were carried out at least three times with independent preparation. A representative experiment was presented in the paper.

2.2. Sludge source

The sludge used in this study was obtained from a municipal WWTP in Beijing, China. The plant adopts an A^2/O (anoxic-anaerobic-oxic) process. The tested sludge was taken from the oxic tank. The initial TSS concentration was 4600 ± 200 mg L^{-1}, with 63–72% of VSS.

2.3. Analytical methods

Following ozonation, the characteristics of the sludge are greatly changed. Floc disintegration and solubilization generates a large number of micro-solids dispersed in the supernatant in addition to soluble organic substances (Chu et al., 2008). The changes in carbon, nitrogen, phosphorus, and metals content in the total sludge mixture, the micro-particles and the supernatant following ozonation were evaluated. The ozonated sludge mixture was allowed to settle. The unsettled micro-particles were defined as the micro-solids. The sludge mixture was centrifuged at 5000 rpm for 15 min. The pellets were heated at 105 and 550 °C to measure TSS and VSS, respectively. The supernatant was analyzed for SCOD, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), protein, polysaccharides and pH. Since the micro-solids were also been precipitated when the sludge mixture was centrifuged, the pellets obtained from centrifugation include micro-solids and residuals after ozonation of sludge. The efficiency of sludge solubilization was defined by the following equation (TSS_0 indicates the initial TSS): Solubilization efficiency = $(TSS_0 - TSS)/TSS_0$.

The carbon and nitrogen content in solids was determined using an element analyzer (Elementar Vario EL, Germany). The content of COD, TN in the supernatant were determined according to Chinese SEPA Standard Methods (SEPA, 2002). The content of TP in both solids and supernatant was examined by using ammonium molybdenum spectrophotometric method (SEPA, 2002). The samples were digested by potassium persulfate oxidation before measurement. TOC in the supernatant was evaluated by a TOC analyzer (TOC-V_{CPH}, SHIMADZU). Protein was detected by using the Protein Assay Kit based on the protocol of Bradford (Bio-Rad, USA) with bovine serum albumin as the standard protein. Polysaccharides were measured using the phenol sulfuric acid method (Dubois et al., 1956). For analyzing metals in solids, the sludge pellet and micro-solids were submitted to acid digestion. The metal concentrations in the acid-digested samples were quantified using an atomic absorption spectrometry (TAS-990, China).

The level of ATP was determined by an ATP assay kit (Beyotime Institute of Biotechnology, China) based on a bioluminescence technique. The SOD activity was measured using a SOD enzyme activity kit (Jiancheng Technology, China) based on the cytochrome *c* reduction method (Fisher et al., 2000). One unit of SOD activity was defined as the amount required to inhibit the rate of cytochrome *c* reduction by 50%. Protease activity of sludge suspensions and supernatants was assessed using the chromogenic substrate azocasein (0.5%) (Kim et al., 2002). One unit of enzyme activity was defined as the amount of enzyme that degraded 1 mg of azocasein in 90 min at 37 °C.

3. Results and discussion

3.1. Alteration of microbial activity in sludge exposed to low-dose ozone

Fig. 1a shows the changes in ATP concentration, protease and SOD activities following ozonation. After exposure to ozone, protease and SOD activities in the sludge decreased immediately. The total protease activity was inhibited by 37% at an ozone dose of 10 mg O_3 g^{-1} TSS and 72% at 20 mg O_3 g^{-1} TSS, whereas the extracellular protease activity in the supernatant and in the micro-solids increased as the ozone dose increased from 0 to 20 mg O_3 g^{-1} TSS (Fig. 1b). The decreasing SOD activity was lower than that of protease activity probably due to the fact that SOD is a kind of antioxidant enzymes. Approximately 18% of SOD was inactivated at an ozone dose of 10 mg O_3 g^{-1} TSS and 50% at 20 mg O_3 g^{-1} TSS. However, the intracellular ATP concentration was not affected by ozone treatment up to 5 mg O_3 g^{-1} TSS, and thereafter decreased rapidly. ATP decreased by around 15% with an ozone dose of 10 mg O_3 g^{-1} TSS and 60% at 20 mg O_3 g^{-1} TSS.

Following ozonation, sludge solubilization occurred slowly at initial stage. The efficiency of sludge solubilization was only around 2.5% at an ozone dose of 5 mg O_3 g^{-1} TSS. Thereafter, the

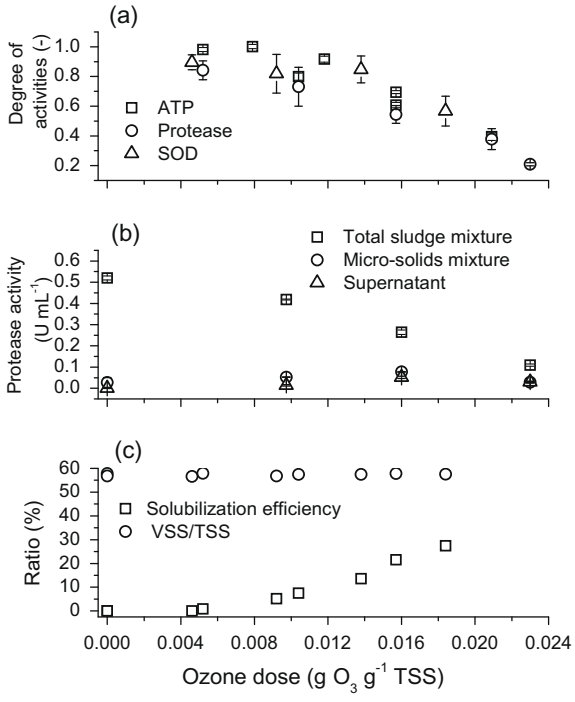

Fig. 1. Effect of low-dose ozone exposure on biomass activity and solubilization: (a) ATP and enzyme activities of the total sludge mixture ($n = 3$); (b) Changes in protease activities in the total sludge mixture, micro-solids mixture and the supernatant ($n = 3$) and (c) Efficiency of sludge solubilization and VSS/TSS.

efficiency of sludge solubilization increased rapidly to around 30% at an ozone dose of 20 mg O_3 g^{-1} TSS (Fig. 1c). Little change in the ratio of VSS/TSS was observed implying that the primary effect of ozonation at this dosage is sludge disintegrations and solubilization (Yan et al., 2009). The pH value decreased from 7.82 to 7.28 with the same ozone doses.

Various studies have suggested that ozone attacks the bacterial cell surface, alters the permeability of the cell membrane and ultimately results in the leakage of cell contents (Christensen and Giese, 1951; Komanapalli and Lau, 1996). The interactions of ozone with activated sludge are more complicated because the microbes in the sludge form a complex matrix. In activated sludge, microorganisms (mainly bacteria) are present in the form of floc embedded in the organo-mineral matrix. Bacterial cells living in aggregates are physically better protected against ozone attack. The process of sludge ozonation is generally described by the sequential decomposition reactions of floc disintegration, solubilization, and the subsequent oxidation of the released organics into carbon dioxide (mineralization) (Ahn et al., 2002; Lee et al., 2005). From this study, short-term exposure to ozone did not affect the intracellular ATP concentration in the activated sludge, which was decreased rapidly thereafter at concentrations higher than 5 mg O_3 g^{-1} TSS. The efficiency of sludge solubilization also increased significantly after the ozone dose was increased above 5 mg O_3 g^{-1} TSS. However, the activities of SOD and protease decreased immediately upon exposure to ozone. Our previous studies also showed that the maximum oxygen uptake rate decreased slightly by 9% when the ozone dose was lower than 5 mg O_3 g^{-1} TSS, and significantly decreased by around 33% at 10 mg O_3 g^{-1} TSS and by around 59% at 20 mg O_3 g^{-1} TSS (Chu et al., 2007). These findings suggest that when sludge is exposed to ozone, ozone firstly destroys the flocs. The compact aggregates are dispersed, which does not affect cells viability but induces a decrease in enzyme activities. It has been reported that enzymes may be attached to the surface of cells or imbedded in the extracellular polymeric substances in the matrix of the floc (Gessesse et al., 2003). Following that, ozone attacks the bacterial cells in the sludge, causing a decrease in cells activity.

3.2. Effects of ozone on the characteristics of activated sludge

The initial activated sludge consists mainly of residuals with a negligible soluble fraction. Following ozonation, the amount of the micro-solids increased. The fraction of the micro-solids amount in the sludge pellets increased to around 12% at an ozone dose of 10 mg O_3 g^{-1} TSS and 19% at 20 mg O_3 g^{-1} TSS. In order to study the fate of sludge after ozonation, we calculated the variation of carbon, nitrogen and phosphorus of the sludge following ozonation (Fig. 2). As ozonation continued, the carbon, nitrogen and phosphorus content in the sludge matrix decreased, while the content of

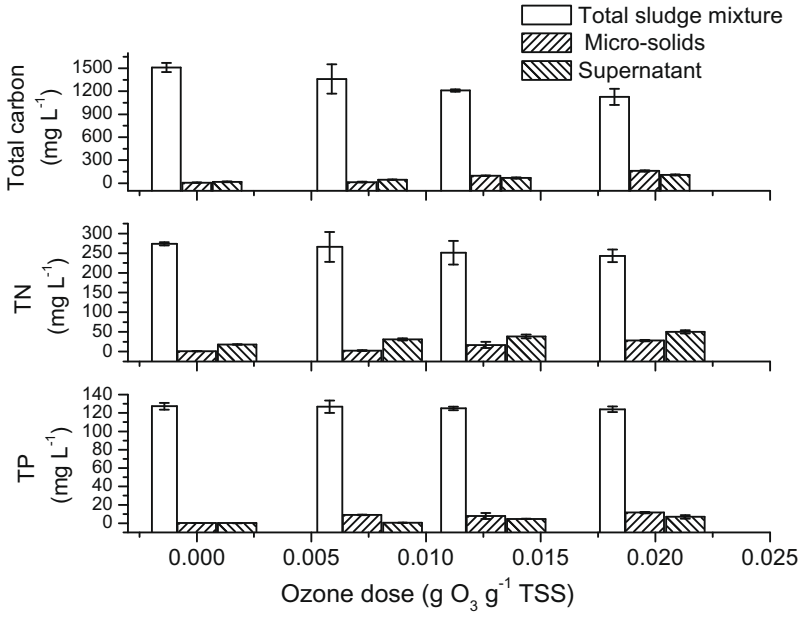

Fig. 2. Changes in carbon, nitrogen and phosphorus content in sludge following ozonation ($n = 3$).

Table 1
Changes in the content of some metals and S during ozonation (mg L^{-1}).

Ozone dose	Sludge mixture			Micro-solids			Supernatant		
	0	7	20	0	7	20	0	7	20
Fe	45.6	40.8	41.4	0.3	1.2	6.2	BDL	BDL	BDL
Zn	3.5	3.0	3.4	0.04	0.15	0.79	BDL	BDL	0.04
Mn	0.50	0.45	0.45	BDL	BDL	0.08	BDL	BDL	BDL
Ti	0.49	0.45	0.67	BDL	BDL	0.09	BDL	BDL	BDL
Cu	0.38	0.32	0.34	BDL	BDL	0.07	BDL	BDL	BDL
S	32.5	33.2	33.0	0.3	0.8	2.9	15.5	19.0	22.8

The unit of ozone dose is mg O_3 g^{-1} TSS.
BDL: below detection limit.

these elements in the micro-solids and supernatant gradually increased. At an ozone dose of 20 mg O_3 g^{-1} TSS, 25% of carbon and 11% of nitrogen were lost, respectively. The phosphorus content in the total sludge mixture changed very little due to the fact that phosphorus does not form a gas. The fractions of carbon, nitrogen and phosphorus in the micro-solids and supernatant increased to 14 and 8%, 12 and 16%, 9 and 5%, respectively. A large part of the released substances from the sludge were in the form of micro-solids. The ratio of C/N in the fresh sludge was 6.0 ± 0.1, while it was 5.7 ± 0.1 in the micro-solids, indicating its higher nitrogen concentration.

With an ozone dose of 0–20 mg O_3 g^{-1} TSS, protein was not detected and the polysaccharide content increased significantly from 5.0 to 38.0 mg C L^{-1} in the supernatant.

In the fresh sludge, the elements Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ti, Zn, P and S were detected. We selected metals Cu, Fe, Mn, Zn, Ti, and S to examine the changes in these elements during sludge ozonation (Table 1). Sludge showed a higher content of Cu, Zn, S of the elements analyzed. Ozonation released metals from sludge floc to the micro-solids and supernatant. The released metals increased as the ozonation progressed. Moreover, most of the released metals were enriched in the micro-solids. With regard to S, the content in the supernatant increased gradually by about 7.3 mg L^{-1} at an ozone dose of 20 mg O_3 g^{-1} TSS. The gradual increased concentration of sulfur in the supernatant might be due to oxidation of sulfhydryl compounds. It was proposed that sulfhydryl groups in the membrane were the primary targets of ozone attack (Komanapalli et al., 1997).

Following ozonation it was found that a large number of micro-particles were dispersed in the supernatant in addition to soluble organic substances due to floc disintegration and solubilization. A large part of the released C, N, P and most of the released metals from the sludge matrix were in the micro-solids. Some researchers have reported that the suspended micro-particles are suitable for denitrification as a good carbon source (Bohler and Siegrist, 2004; Cui and Jahng, 2004). These micro-solids may be hydrolyzed at first to soluble substances and then utilized by microorganisms or directly devoured by microfauna. The mechanism of degradation and utilization of the micro-solids by microorganisms should be studied further and is currently ongoing.

Acknowledgments

We thank the project 20777045 supported by the National Natural Science Foundation of China, the Hi-Tech Research and Development Program of China (863 Plan) (No. 2007AA06Z347) and the National Key Basic Research Program (973 plan) (No. 2007CB109203).

References

Ahn, K.H., Park, K.Y., Maeng, S.K., Hwang, J.H., Lee, J.W., Song, K.G., Choi, S., 2002. Ozonation of wastewater sludge for reduction and recycling. Water Sci. Technol. 46 (10), 71–77.
Bohler, M., Siegrist, H., 2004. Partial ozonation of activated sludge to reduce excess sludge, improve denitrification and control scumming and bulking. Water Sci. Technol. 49 (10), 41–49.
Caravelli, A., Giannuzzi, L., Zaritzky, N., 2006. Effect of ozone on filamentous bulking in a laboratory scale activated sludge reactor using respirometry and INT-dehydrogenase activity. J. Environ. Eng.-ASCE 132, 1001–1010.
Christensen, E., Giese, A.C., 1951. Changes in absorption spectra of nucleic acids and their derivatives following exposure to ozone and ultraviolet radiations. Arch. Biochem. Biophys. 51, 208–216.
Chu, L.B., Xing, X.H., Yu, A.F., Zhou, Y.N., Sun, X.L., Jurcik, B., 2007. Enhanced ozonation of simulated dyestuff wastewater by microbubbles. Chemosphere 68, 1854–1860.
Chu, L.B., Yan, S.T., Xing, X.H., Yu, A.F., Sun, X.L., Jurcik, B., 2008. Enhanced sludge solubilization by microbubble ozonation. Chemosphere 72, 205–212.
Chu, L.B., Yan, S.T., Xing, X.H., Sun, X.L., Jurcik, B., 2009. Progress and perspectives of sludge ozonation as a powerful pretreatment method for minimization of excess sludge production. Water Res. 43, 1811–1822.
Cui, R., Jahng, D., 2004. Nitrogen control in AO process with recirculation of solubilized excess sludge. Water Res. 38, 1159–1172.
Dubois, M., Gilles, K.A., Hamilton, J.K., Reders, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Dytczak, M.A., Oleszkiewicz, J.A., 2008. Performance change during long-term ozonation aimed at augmenting denitrification and decreasing waste activated sludge. Chemosphere 73, 1529–1532.
Dytczak, M.A., Londry, K.L., Siegrist, H., Oleszkiewicz, J.A., 2007. Ozonation reduces sludge production and improves denitrification. Water Res. 41, 543–550.
Dziurla, M.A., Salhi, M., Leroy, P., Paul, E., Ginestet, P., Block, J.C., 2005. Variations of respiratory activity and glutathione in activated sludges exposed to low ozone doses. Water Res. 39, 2591–2598.
Fisher, C.W., Lee, D., Dodge, B.A., Hamman, K.M., Robbins, J.B., Martin, S.E., 2000. Influence of catalase and superoxide dismutase on ozone inactivation of *Listeria monocytogenes*. Appl. Environ. Microb. 66, 1405–1409.
Gessesse, A., Dueholm, T., Petersen, S.B., Nielsen, P.H., 2003. Lipase and protease extraction from activated sludge. Water Res. 37, 3652–3657.
Gikas, P., Livingston, A.G., 1993. Use of ATP to characterize biomass viability in freely suspended and immobilized cell bioreactors. Biotechnol. Bioeng. 42, 1337–1351.
Goel, R., Komatsu, K., Yasui, H., Harada, H., 2004. Process performance and change in sludge characteristics during anaerobic digestion of sewage sludge with ozonation. Water Sci. Technol. 49 (10), 105–113.
Kim, Y.K., Bae, J.H., Oh, B.K., Lee, W.H., Choi, J.W., 2002. Enhancement of proteolytic enzyme activity excreted from *Bacillus stearothermophilus* for a thermophilic aerobic digestion process. Bioresource Technol. 82, 157–164.
Komanapalli, I.R., Lau, B.H.S., 1996. Ozone-induced damage of *Escherichia coli* K-12. Appl. Microbiol. Biotechnol. 46, 610–614.
Komanapalli, I.R., Mudd, J.B., Lau, B.H.S., 1997. Effect of ozone on metabolic activities of *Escherichia coli* K-12. Toxicol. Lett. 90, 61–66.
Lee, J.W., Cha, H.Y., Park, K.Y., Song, K.G., Ahn, K.H., 2005. Operational strategies for an activated sludge process in conjunction with ozone oxidation for zero excess sludge production during winter season. Water Res. 39, 1199–1204.
Perez-Elvira, S.I., Nieto Diez, P., Fdz-Polanco, F., 2006. Sludge minimisation technologies. Rev. Environ. Sci. Biotechnol. 5, 375–398.
SEPA, 2002. Water and Wastewater Monitoring Methods. Chinese Environmental Science Publishing House, Beijing. 4 pp.
Shier, W.T., Purwono, S.K., 1994. Extraction of single-cell protein from activated sewage sludge: thermal solubilization of protein. Bioresource Technol. 49, 157–162.
van Leeuwen, J., 1988. Bulking control with ozonation in a nutrient removal activated sludge system. Water SA 14, 119–124.
Vergine, P., Menin, G., Canziani, R., Ficara, E., Fabiyi, M., Novak, R., Sandon, A., Bianchi, A., Bergna, G., 2007. Partial ozonation of activated sludge to reduce excess sludge production: evaluation of effects on biomass activity in a full scale demonstration test. International Water Association Specialist Conference, Moncton, Canada.
Weemaes, M., Grootaerd, H., Simoens, F., Verstraete, W., 2000. Anaerobic digestion of ozonized biosolids. Water Res. 34, 2330–2336.
Wei, Y.S., Van Houten, R.T., Borger, A.R., Eikelboom, D.H., Fan, Y.B., 2003. Minimization of excess sludge production for biological wastewater treatment. Water Res. 37, 4453–4467.
Yan, S.T., Chu, L.B., Xing, X.H., Yu, A.F., Sun, X.L., Jurcik, B., 2009. Analysis of the mechanism of sludge ozonation by a combination of biological and chemical approaches. Water Res. 43, 195–203.
Yasui, H., Shibata, M., 1994. An innovative approach to reduce excess sludge production in the activated-sludge process. Water Sci. Technol. 30 (9), 11–20.