RSC Advances




ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

# The effects of carbon nanotubes on the nitrogen and phosphorus removal from real wastewater in the activated sludge system

Xiong Zheng, Yinglong Su, Yinguang Chen*, Yuanyuan Wei, Mu Li, Haining Huang

*(State Key Laboratory of Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China)*

*Corresponding author

Phone: +86 21 65981263

Fax: +86 21 65986313

E-mail: yg2chen@yahoo.com

## Abstract

The wide use of carbon nanotubes (CNTs), such as single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs), inevitably causes their release into the environment. Previous studies pointed out that the released CNTs would cause the negative effects on model animals, plants, or microorganisms. Nevertheless, to date, it is unclear whether the presence of CNTs in wastewater treatment plant (WWTP) could affect biological nitrogen and phosphorus removal. In this paper, the potential effects of CNTs (SWNTs and MWNTs) on nitrogen and phosphorus removal from real wastewater in an activated sludge system were investigated. It was found that the presence of CNTs had no significant impacts on nitrogen and phosphorus removal even at the exposure concentration of 100 mg/L. Mechanism studies indicated that the sludge membrane integrity, viability and the respiration of both heterotrophic and autotrophic microorganisms were not affected by CNTs. Further experiments revealed that the presence of CNTs also did not change the transformations of intracellular metabolites (mainly glycogen and polyhydroxyalkanoates) and activities of key enzymes (mainly ammonia monooxygenase, nitrite oxidoreductase, nitrate reductase, nitrite reductase, exopolyphosphatase, and polyphosphate kinase), which was consistent with no observed influences on nitrogen and phosphorus removal.


## 1. Introduction

The discovery and subsequent applications of numerous nanomaterials have accelerated the development of nanotechnology. In recent years, nanomaterials have been used in a wide range of fields, such as catalysts, semiconductors, microelectronics,

and drug carriers.[1] Among these nanomaterials, carbon nanotubes (CNTs), a group of carbon-based nanomaterials, have become prevailing since their discovery in 1991.[2] The outstanding structures of CNTs determine the exceptional electrical, chemical, mechanical, and thermal characters, which lead to their growing applications in the fields of electronics, optics, material science, and biomedicine.[3-5]

Recently, numerous studies have been conducted to investigate the potential risks of CNTs. Nevertheless, these studies mainly focused on the toxicity of CNTs to human cells,[6-9] animals,[10-13] and model bacteria.[14-17] With the increasing production and applications, CNTs have been found to release into the environment inevitably and finally enter wastewater treatment plant (WWTP).[18, 19] Usually, activated sludge process is widely used to achieve biological nitrogen and phosphorus removal in WWTP. Previous studies have reported the impacts of CNTs on the removal of chemical oxygen demand (COD) and physical property of activated sludge fed with synthetic wastewater.[20, 21] However, whether the release of CNTs into activated sludge systems affects the performance of biological nutrient removal from real wastewater is still unknown. Therefore, it is necessary to investigate the potential influence of CNTs on biological nitrogen and phosphorus removal, especially when the real wastewater was used as feed.

It is well-known that large numbers of microorganisms and intracellular metabolites are involved in biological nitrogen and phosphorus removal in activated sludge. For example, autotrophic and heterotrophic microorganisms play important roles in nitrification, denitrification and phosphorus release and uptake processes, and their respiration rates are closely linked with the nutrient removal efficiency. Besides, it was

reported that some pollutants such as heavy metal ions, organic pollutants and metal oxides nanoparticles can affect the nitrogen removal via inhibiting microbial respiration, enzymes activities, and intermediate metabolites transformations in activated sludge.[22-26] Hence, to understand the mechanisms of CNTs-induced effects on biological nutrient removal, bacterial respiration, key enzyme activity, and intermediate metabolites of activated sludge should be investigated.

The aim of this study was to evaluate whether the CNTs including SWNTs and MWNTs could affect biological nitrogen and phosphorus removal from real wastewater in an activated sludge system. The removal efficiencies of nitrogen and phosphorus were measured to examine the possible effects of CNTs on activated sludge. Then, oxygen and ammonium uptake rates were assayed to study the responses of heterotrophic and autotrophic microbes to SWNTs and MWNTs. Finally, sludge activity, surface integrity, enzymes activities, and metabolites (glycogen and polyhydroxyalkanoates) transformations were determined to reveal the reasons for nutrient removal performance in the absence and presence of CNTs.

## 2. Experimental

### 2.1. SWNTs and MWNTs preparation

The powders of SWNTs and MWNTs were obtained from Shenzhen Nanotech Port Co. Ltd, China. The main diameters of SWNTs and MWNTs are less than 2 nm and less than 10 nm, respectively. Their lengths are both 5-15 μm and the specific surface areas (SSA) of SWNTs and MWNTs are 500-700 and 250-300 $m^2/g$, respectively. The nanotubes were placed into a 12 M HCl solution for 8 h to remove residual metal catalysts,

washed with copious amounts of Milli-Q water until neutral pH, and then dried in an oven (60 $^{o}$C) overnight to get powder SWNTs and MWNTs. Before the exposure experiment, 1000 mg/L CNTs stock suspension was prepared in Milli-Q water following by ultrasonication (25 $^{o}$C, 250 W, 40 kHz) for 1 h.

**2.2. Parent sequencing batch reactor operation**

The anaerobic-low dissolved oxygen (DO: 0.15-0.50 mg/L)) sequencing batch reactor (SBR) was used to culture activated sludge, and this process was proved to achieve efficient nitrogen and phosphorus removal.[25, 26] Activated sludge was obtained from a wastewater treatment plant (WWTP), and then cultured in an anaerobic-low DO SBR with 16 L working volume. The SBR was worked at 21 ± 1 $^{o}$C with 8 h operation cycle, and each cycle consisted of 1.5 h anaerobic and 3 h low DO periods, followed by 1 h settling, 10 min decanting and 140 min idle periods. In the low DO period, air was provided using an on/off control system with an on-line DO detector to maintain the DO level between 0.15-0.50 mg/L. The real wastewater was obtained from a WWTP, and the characteristics were listed as follows: chemical oxygen demand (COD) 170-215 mg COD/L, ammonia-nitrogen ($NH_4^+$-N) 19-29 mg/L, and soluble ortho-phosphorus (SOP) 3.2-5.5 mg/L. Before being pumped into the reactor, the raw wastewater was adjusted to get average initial $NH_4^+$-N, SOP and COD of 35, 10 and 300 mg/L by $NH_4Cl$, $KH_2PO_4$ and $CH_3COONa$ supplementation, respectively. Sludge was wasted at regular intervals to keep the solids retention time (SRT) at approximately 20 d. After cultivation for 100 d, the stable removal efficiencies of nitrogen and phosphorus were observed in the parent SBR, and then the following exposure experiments were conducted.

99 **2.3. Exposure of activated sludge to SWNTs and MWNTs**

100 In this study, the exposure concentrations of SWNTs and MWNTs were chosen to be
101 10 and 100 mg/L according to the literature.[27, 28] To conduct the experiments, activated
102 sludge was withdrawn from the parent SBR at the end of cycle and washed with 0.9%
103 NaCl solution to remove residual substances. Then, stock suspensions of SWNTs and
104 MWNTs were added to prepare 10 and 100 mg/L CNTs in the batch reactors of 400 mL
105 working volume, and all reactors were covered with aluminum foil to avoid light. The
106 suspended activated sludge and raw wastewater were fed into each reactor following by
107 pH adjustment. Prior to the start of cycle, nitrogen was purged into the reactors to assure
108 anaerobic circumstance. The reactors were operated anaerobically stirred for 1.5 h and
109 aerobically stirred under low DO (0.15-0.50 mg/L) condition for 3 h.

110 **2.4. Sludge viability assay**

111 Cell Counting Kit-8 (CCK-8) (Dojindo Co., Kumamoto, Japan), was used for sludge
112 viability assessment after 4.5 h of exposure to CNTs in this study. Compared to the
113 conventional assay, CCK-8 was a convenient, efficient and sensitive assay to test cell
114 viability in cytotoxicity tests.[29] Briefly, 5 min before the end of cycle, 100 μL sludge
115 suspension was gotten for viability measurement following by 10 μL CCK-8 being added
116 to wells in a 96 well microplate. Then, the microplate was placed in a $CO_2$ incubator at
117 constant 30 $^{o}$C for 1 h of reaction. The last procedure was to take a colorimetric reading
118 on a microplate reader at 450 nm.

119 **2.5. Specific oxygen uptake rate (SOUR) and ammonium uptake rate (AUR) assays**

120 In this study, SOUR assay was used to assess the impacts of SWNTs and MWNTs on

sludge respiration. The sludge mixture was obtained from the parent SBR at the idle period, pre-aerated and then placed into biochemical oxygen demand (BOD) bottles, in which sodium acetate and thiourea ($CH_4N_2S$) were added to ensure sufficient carbon source for respiration and to inhibit autotrophic nitrifying microbial respiration respectively.[30] A certain volume of stock SWNTs and MWNTs were taken into the bottles, and Milli-Q water was supplied to keep equivalent volume and 10 and 100 mg/L CNTs in the BOD bottles. After equipping an online dissolved oxygen electrode and magnetic stirrer for bottles, rubber stopper was used to seal the BOD bottle, and the dissolved oxygen concentration was recorded verse time until the oxygen depletion in all bottles. After the completion in tests, the concentrations of mixture liquor volatile suspended solids (MLVSS) were measured, and the SOUR data were obtained from the DO concentration gradient verse time being divided by MLVSS.

For the AUR measurement, activated sludge was transferred into batch reactors with continuous aeration in the dark. An appropriate amount of ammonium chloride solution, CNTs stock suspensions and phosphorus buffer were added into reactors to yield final concentration of 40 mg/L $NH_4^+$-N and 10 or 100 mg/L SWNTs and MWNTs, following by adding Milli-Q water to 400 mL. Homogenized sludge mixture was taken periodically for $NH_4^+$-N analysis. The slope of $NH_4^+$-N concentration verse time was divided by MLVSS to calculate AUR.

**2.6. Enzyme activity assays related to biological nitrogen and phosphorus removal**

For the activities of several key enzymes measurements, the activated sludge samples were taken from batch reactors and washed by corresponding buffer for 3 times. In detail,

143 0.01 M phosphate buffer (pH 7.4) was for ammonia monooxygenase (AMO), nitrite
144 oxidoreductase (NOR), nitrate reductase (NAR) and nitrite reductase (NIR); and 1.5 M
145 NaCl buffer (pH 7.4, including 0.01 M EDTA and 1 mM NaF) was used in the
146 measurements of exopolyphosphatase (PPX) and polyphosphate kinase (PPK). Then, the
147 resuspended sludge was sonicated (4 $^{o}$C, 20 kHz) for 5 min to break down bacterial cell
148 structure in sludge. After centrifugation at 12000*g* for 10 min (4 $^{o}$C), the supernatant was
149 used for the specific enzyme activity measurement. The detailed procedure and
150 constituent of assays mixture were according to the literature.[25] The activities of
151 enzymes were calculated as the basis of protein content, which was determined with
152 bovine serum albumin as a standard.[31]

153 **2.7. Other analytical methods**

154 The liquid samples were immediately centrifuged at 12000 rpm, and the supernatant
155 was for testing the concentrations of $NH_4^+$, $NO_3^-$, $NO_2^-$ and $PO_4^{3-}$ by a spectrophotometer
156 according to the Standard Methods.[32] The analyses methods of glycogen and PHA were
157 the same as that described previously.[33]

158 The structural integrity of activated sludge was measured by the lactic dehydrogenase
159 (LDH) release assay and the detection kit for LDH activity was purchased from Roche
160 Applied Science. At the end of batch exposure experiment, the mixture was centrifuged
161 at 12000 rpm for 5 min and then the supernatant was seeded on a 96-well plate. After the
162 addition of 50 μL of substrate mix in wells, the microplate was placed in an incubator at
163 30 $^{o}$C in dark for 30 min. Finally, 50 μL of stop solution was added to each well, and the
164 absorbance was recorded at 490 nm using a microplate reader (BioTek, USA).

Scanning electron microscope (SEM) images were used to analyze the surface morphology of activated sludge after exposure to CNTs. Activated sludge was gotten from the batch reactor at the end of cycle and washed with 0.1 M phosphate buffer for 3 times followed by fixing in 0.1 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde at 4 $^{o}$C for 4 h. After being washed twice with 0.1 M phosphate buffer (pH 7.4), the sludge pellets were dehydrated in a gradient ethanol serials (50%, 70%, 80%, 90% and 100%), and then dried in air for the imaging by the FEI Quanta 200 SEM. To conduct the transmission electron microscope (TEM) analysis, CNTs were sonicated and dispersed in water, and a drop of mixture liquid was placed on Cu grids. After the samples were dried, TEM images of CNTs were taken using a FEI Tecnai F20 200 kV microscope (Philips).

### 2.8. Statistical analysis

All tests were performed in triplicate and the results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was used to test the significance of results, and $p < 0.05$ was considered to be statistically significant.

## 3. Results and Discussion

### 3.1. Impacts of SWNTs and MWNTs on nitrogen and phosphorus removal

Figure 1 shows the biological nutrient removal performance of activated sludge in one cycle. In the control reactor, the $NH_4^+$-N concentration was not significantly varied in the anaerobic stage, but the SOP was increased to the maximum (91.2 mg/L) due to the anaerobic phosphorus release. In the low DO stage, the $NH_4^+$-N and phosphorus can be removed thoroughly at the end of this stage, and partial $NH_4^+$-N was transferred to nitrate.

At the end of reaction, the removal efficiencies of TN and SOP were 70.8% and >99%, respectively. According to the data, when the MWNTs dosage increased from 0 mg/L (the control) to 100 mg/L, it did not make significant difference in the $NH_4^+$-N, $NO_3^-$-N and $NO_2^-$-N concentration variations at any period ($p$>0.05). Similarly, the addition of SWNTs did not cause any negative effects on the transformation or removal process, and the final TN removal efficiency were 69.0%, 68.6%, 68.0% and 67.6% after exposure to 10 mg/L SWNTs, 100 mg/L SWNTs, 10 mg/L MWNTs and 100 mg/L MWNTs ($p$ > 0.05). Besides, neither SWNTs nor MWNTs caused residual SOP in all reaction systems at last, therefore the presence of SWNTs and MWNTs did not affect the nitrogen and phosphorus removal in activated sludge system even at a high concentration of 100 mg/L.

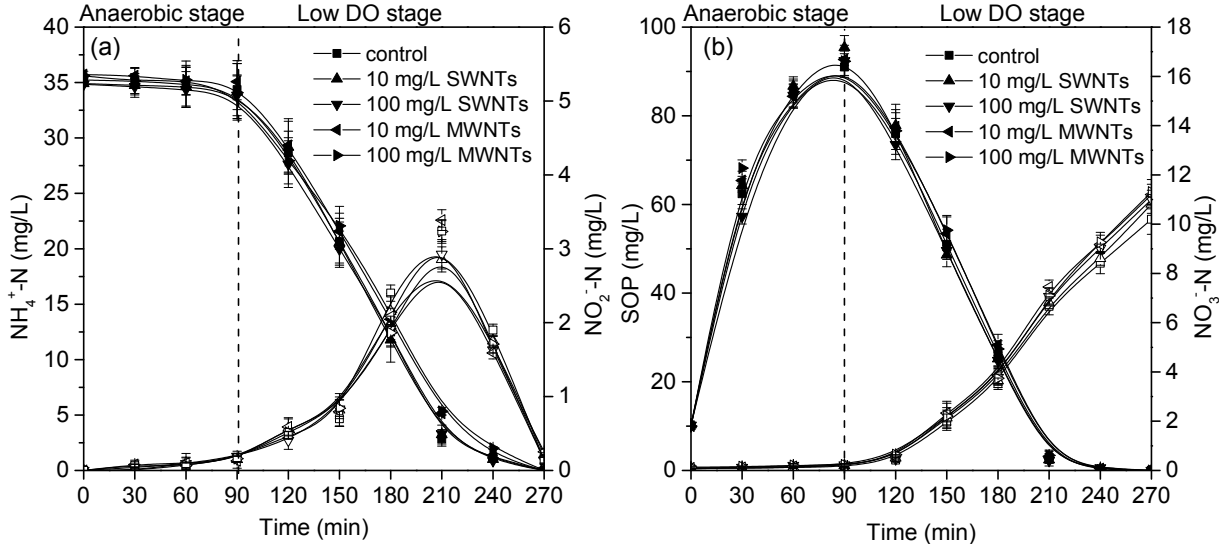



**FIGURE 1. Effects of SWNTs and MWNTs on the variations of $NH_4^+$-N (solid), $NO_2^-$-N (white) in (a), SOP (solid) and $NO_3^-$-N (empty) in (b).**

### 3.2. Effects of SWNTs and MWNTs on structural integrity and viability of activated sludge

Since the unaffected nitrogen and phosphorus removal was shown in

above-mentioned observation, the underlying mechanisms should be revealed according to CNTs cytotoxocity. Most studies proved that the SWNTs and MWNTs might exhibit strong cytotoxicity due to their special physico-chemical properties.[9, 15-17, 34, 35] About the inhibitory mechanism of CNTs, the cell membrane damage and the subsequent cytoplasm leakage were thought to be the main reasons.[1, 14, 15] Hence, in this study, the structural integrity of activated sludge was investigated. Figure 2 shows the SEM images of activated sludge in the presence of 100 mg/L of SWNTs or MWNTs. Previous studies showed that CNTs could adsorb microorganisms such as *Escherichia coli* and *Bacillus subtilis*.[36, 37] It can be seen that SWNTs and MWNTs were attached onto the surface of sludge flocs, but no significant surface structural damages were observed. The LDH release analysis has been used to study the influence of nanomaterial on cell structural integrality. It can be found in this study that the LDH releases in the 10 and 100 mg/L of SWNTs or MWNTs exposure tests were almost the same as that in the control (Figure 3a), suggesting that the presence of SWNTs or MWNTs did not cause the damage of activated sludge and the leakage of cytoplasm. Similar results were achieved by Worle-Knirsch *et al*., in whose study that the LDH assay data indicating no acute toxicity for CNTs.[38]

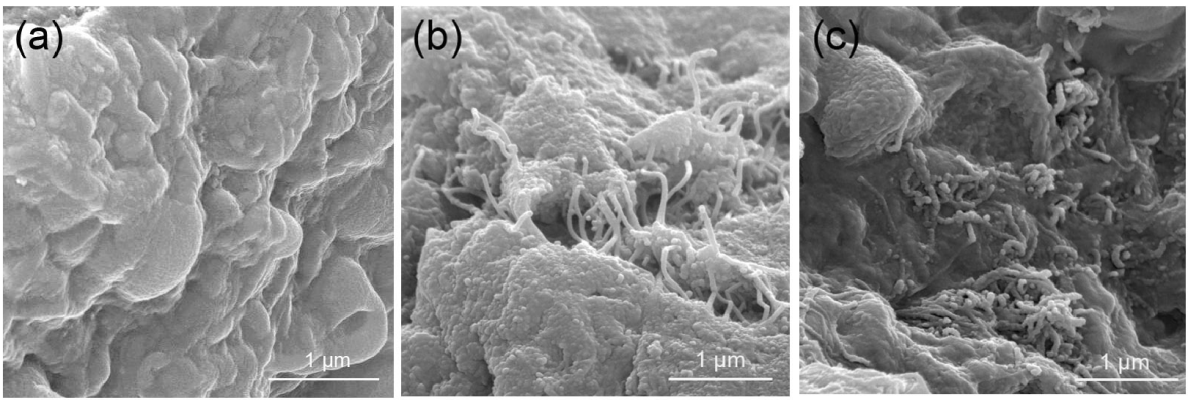
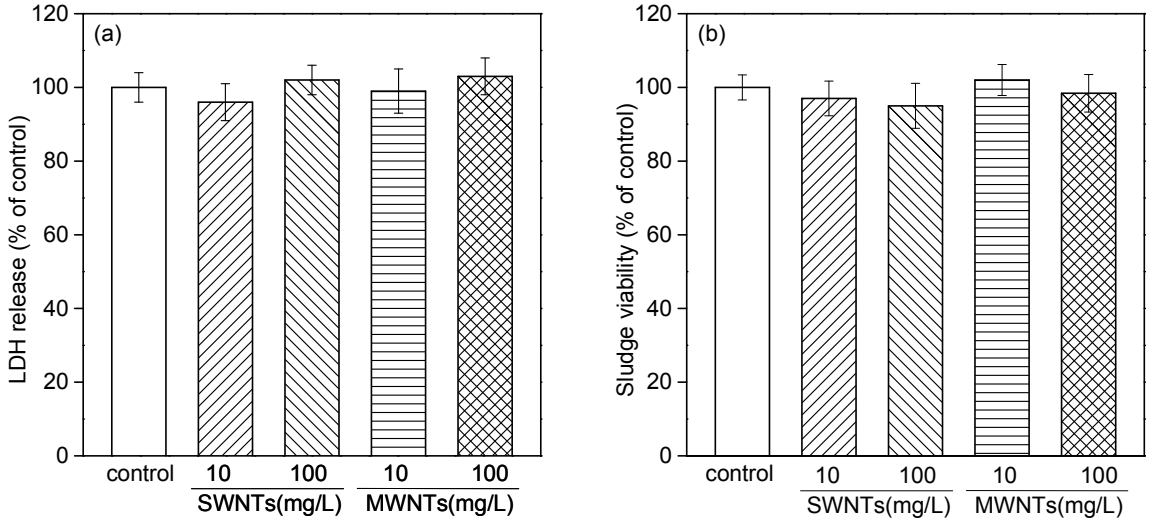



**FIGURE 2. SEM images of activated sludge exposed to CNTs. Control (a), 100**

221 **mg/L SWNTs (b) and MWNTs (c)**




222

223 **FIGURE 3. The LDH release (a) and viability (b) of activated sludge after contact**

224 **with SWNTs and MWNTs.**

225 Besides the structural integrity, cell viability was also an effective method to show
226 the toxic effect in the test of cytotoxicity research. For example, the viabilities of
227 bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* were found to be affected
228 by the exposure to Ag nanoparticles (NPs), CuO NPs and SWNTs.[35, 39, 40] In this study,
229 the viability of activated sludge exposed 10 and 100 mg/L SWNTs or MWNTs was shown
230 in Figure 3b, and the results indicated that activated sludge viability would not decrease
231 with the MWNTs concentration increasing from 0 to 100 mg/L. Kang *et al*. concluded
232 that the SWNTs can show stronger antibacterial properties than MWNTs due to the
233 smaller size,[15] but SWNTs did not cause significantly effects on the viability of activated
234 sludge ($p > 0.05$) in this study. Although CNTs were often thought to be toxic in many
235 studies, some publications reported that CNTs showed no sign of acute toxicity.[34, 41]
236 Worle-Knirsch *et al*. also found that the cell viability was not influenced by CNTs unless
237 inappropriate assay was taken for viability measurement.[38] Besides, this results were

consistent with our previous publication, which indicated that $SiO_2$ NPs did not induce acute influence on activated sludge viability,[26] and therefore the pure culture bacteria and cells were likely more susceptible than mixed culture such as activated sludge.

### 3.3. Effects of SWNTs and MWNTs on oxygen and ammonia utilization of activated sludge

The well structural integrity and viability are the basis for microbial metabolism, and wastewater pollutant removal by activated sludge depends on the efficient cellular respiration. Many results showed that the toxin might inhibit the microbial respiration in activated sludge and further affect the function.[22, 24, 42] SOUR and AUR were often taken as important indicators to measure the toxic effects towards oxygen and ammonium uptake rate of microbe in activated sludge, and the results were associated with the pollutants removal capacity closely. As Figure 4 illustrated, activated sludge exposed to 10 and 100 mg/L SWNTs did not exhibit significant difference on the respiration compared to control. Similarly, the MWNTs caused no significant inhibition to the cellular respiration ($p > 0.05$). Then, the AUR data showed that SWNTs and MWNTs did not produce inhibitory effects on ammonia uptake. Therefore, these results indicated that the presence of SWNTs or MWNTs slight lowered the microbial metabolism and pollutants removal capacity of activated sludge.

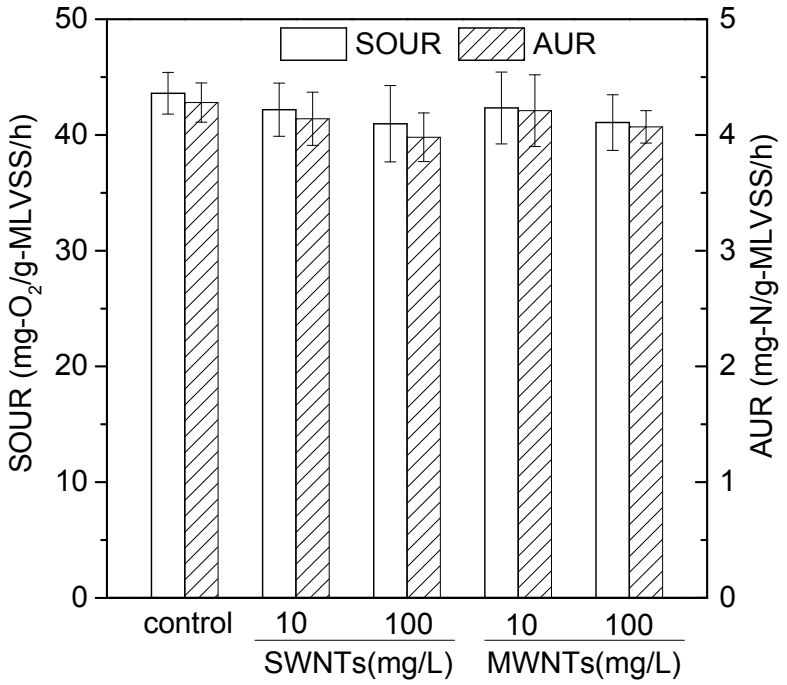



**FIGURE 4. The SOUR (a) and AUR (b) of activated sludge after exposure to 10 and 100 mg/L SWNTs and MWNTs**

In literature, Pulskamp *et al*. investigated the cytotoxicity of SWNTs and MWNTs and concluded that it's the metal traces associated with the commercial nanotubes were responsible for the toxic effects rather than the purified CNTs.[28] Moreover, other than the unremarkable effects of SWNTs and MWNTs on activated sludge, the heavy metal ions were found to inhibit the growth, viability and metabolism significantly due to their negative effects on SOUR and AUR.[22] Also, Zheng *et al*. proved that ZnO NPs exposure towards activated sludge caused adverse impacts on biological nitrogen and phosphorus removal and the release $Zn^{2+}$ was the main reason responsible for low removal efficiency.[25] Like ZnO NPs, the toxicity of silver NPs, copper oxide NPs mostly accounted for the dissolution of metal ions.[43, 44] Therefore, in this study, the SWNTs and MWNTs did not contain any metal elements after residual removal by HCl, so it was reasonable that the CNTs did not show obvious toxicity compared to heavy metal ions,

271 metal NPs and metal oxides NPs.

272 **3.4. Effects of SWNTs and MWNTs on enzymes activities and metabolic**
273 **intermediates related to nitrogen and phosphorus removal**

274 Apart from the cellular respiration, the efficient nitrogen and phosphorus removal
275 process depends on a series of biomacromolecule, such as enzymes, glycogen and PHA.
276 Enzymes are a series of special substances which often play vital roles in biological
277 metabolic reactions. In biological nitrogen and phosphorus removal, several enzymes
278 such as AMO, NOR, NAR NIR, PPX, and PPK, catalyze the vital steps. Previous
279 literature reported that nanomaterials such as $Al_2O_3$ and ZnO NPs decreased the activities
280 of key enzymes involved in denitrification and phosphorus removal process.[25, 45] Our
281 further investigation showed the activities of key enzymes after activated sludge exposure
282 to SWNTs and MWNTs (Figure 5). The data showed that the presence of SWNTs and
283 MWNTs did not decrease the specific activities ($p > 0.05$), indicating that the presence of
284 CNTs would not affect the biological transformation process of nitrogen and phosphorus.
285 This result was consistent with the above results of unaffected nitrogen and phosphorus
286 removal efficiencies.

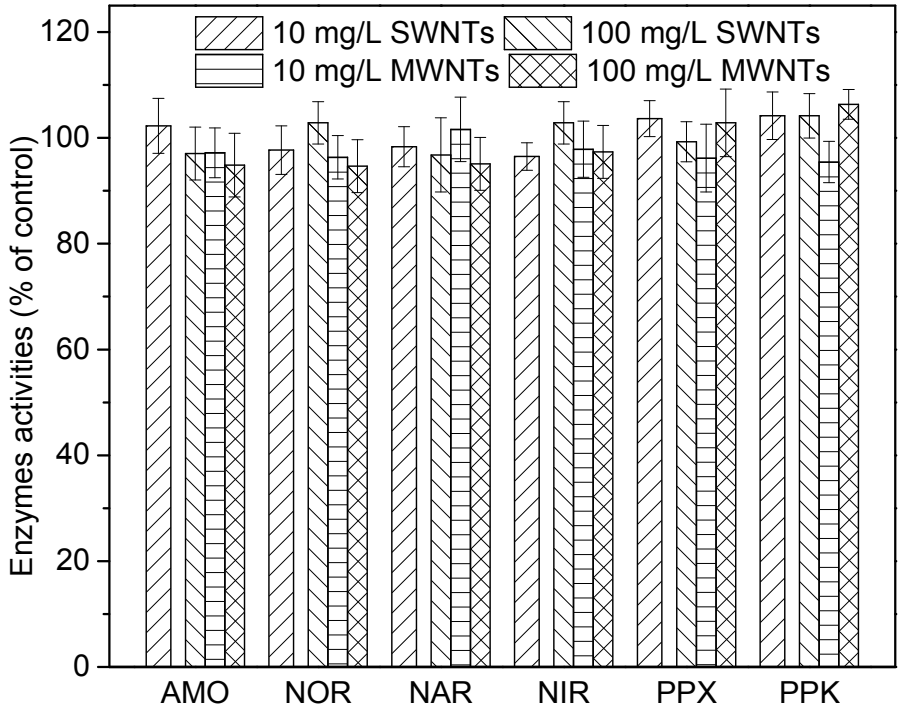



**FIGURE 5. The key enzymes activities involving the transformation of nitrogen and phosphorus in activated sludge after exposure to 10 and 100 mg/L SWNTs and MWNTs.**

In biological phosphorus removal process, the synthesis and consumption of glycogen and PHA are connected with the SOP release and uptake. Specifically, in anaerobic stage, intracellular glycogen was utilized and PHA was synthesized, which were accompanied with the SOP release. Then in low DO stage, the consumption of PHA, synthesis of glycogen and uptake of SOP were observed. As seen in Figure 6a, all the activated sludge systems kept the same levels of glycogen consumption in anaerobic stage ($p > 0.05$). Then, the maximum synthesis amount of PHA was measured at the end of anaerobic phase (Figure 6b). It can be found that 10 or 100 mg/L SWNTs and MWNTs did not influence the synthesis of PHA. It is well-known that two groups of microbe, polyphosphate-accumulating organisms (PAO) and glycogen-accumulating organisms

(GAO), were involved in the metabolism of glycogen and the synthesis of PHA.[46] In this study, the glycogen variation and PHA accumulation were similar between the control and CNTs groups. Hence, it can be inferred that the metabolisms of both GAO and PAO were not disturbed by the presence of SWNTs and MWNTs.

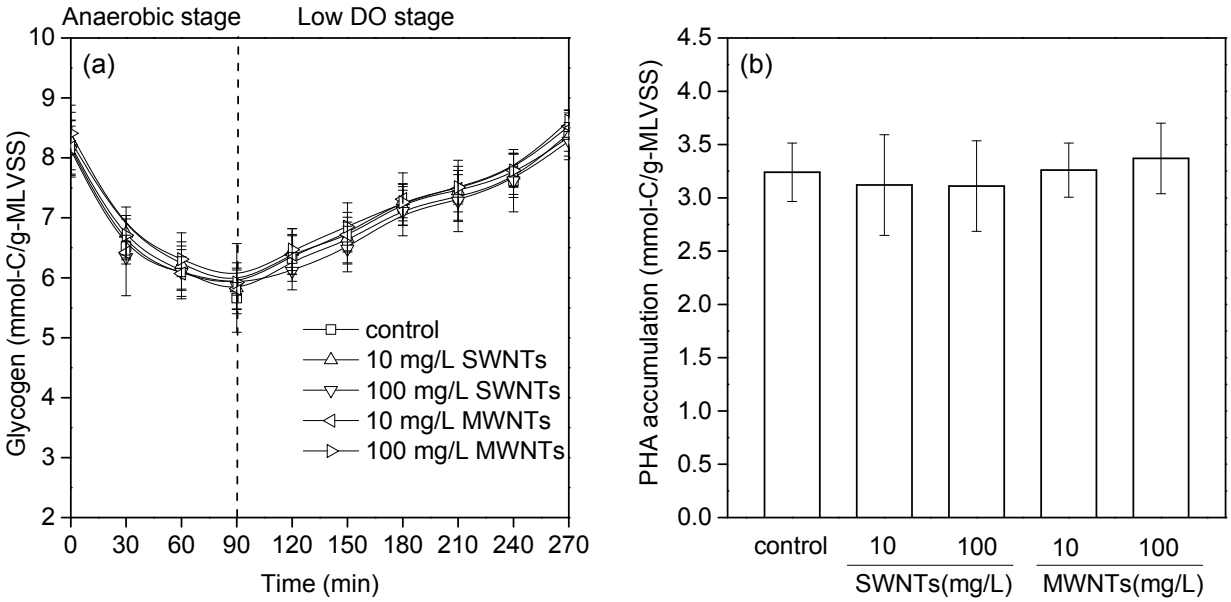
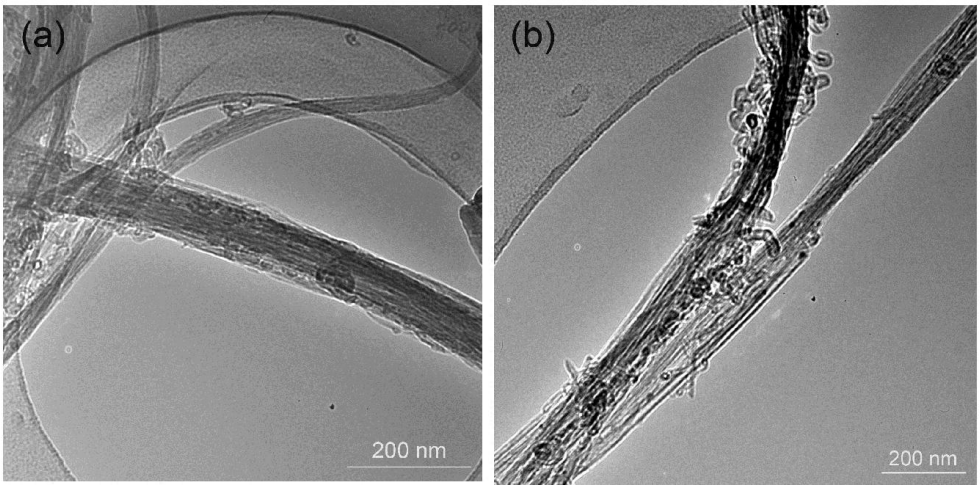



**FIGURE 6. Effects of SWNTs and MWNTs on the transformations of glycogen (a) during one cycle and the PHA synthesis amount (b).**

From the above results, activated sludge showed high tolerance to SWNTs and MWNTs from the aspects of structural integrity, cell viability, microbial respiration, ammonium uptake and enzymes activities. Compared to other CNTs toxic studies on pure culture, the complexity in activated sludge system might be a primary reason for the unobservable toxicity. In aqueous solution, the CNTs appear to buddle together forming aggregation easily due to the strong π-π interaction (Figure 7), and the dispersion status is a key factor to the toxic expression of CNTs. Kang *et al*. had proved that partially debundled MWNTs of 4.1 μm have shown higher toxicity than MWNTs bundles of 77 μm in diameter.[16] Besides the poor dispersity, the extracellular polymeric substances (EPS)

317 excreted by activated sludge could obstruct the direct contact and further protect the
318 microorganisms from the toxicity of CNTs.[47] Finally, the acute exposure of SWNTs and
319 MWNTs towards activated sludge would not disturb metabolism and function.




320

321 **FIGURE 7. TEM images of SWNTs (a) and MWNTs (b) used in this study.**

322 **4. Conclusion**

323 In this study, the effects of SWNTs and MWNTs on wastewater nutrient removal
324 were investigated. It was found that both SWNTs and MWNTs showed no significant
325 inhibition to the nitrogen and phosphorus removal at the dose of 10 and 100 mg/L. Then,
326 the further analysis showed that the structural integrity, cell viability, respiration rate and
327 key enzymes activities were not influenced by the presence of CNTs. All these results
328 were in accordance with the former observation of unaffected removal efficiencies of
329 nitrogen and phosphorus. Although CNTs were reported to show antibacterial properties
330 in many studies, our investigation demonstrated that the presence of SWNTs and MWNTs
331 would not cause adverse effects on microorganism in activated sludge at a dosage as high
332 as 100 mg/L. The neglectable toxicity of CNTs might be due to the poor dispersity, EPS
333 protection and CNTs metal residual removal. Overall, the nitrogen and phosphorus

334 removal process of activated sludge would not be affected when CNTs were released into
335 WWTP in a short-term exposure period.

336 **Acknowledgements**

337 This work was financially supported by the National Hi-Tech Research and
338 Development Program of China (863 Program) (2011AA060903), National Natural
339 Science Foundation of China (41301558 and 51278354), and China Postdoctoral Science
340 Foundation (2013M530210 and 2014T70432).

341 **References**

342 1. A. Nel, T. Xia, L. Madler and N. Li, *Science*, 2006, **311**, 622-627.
343 2. S. Iijima, *Nature*, 1991, **354**, 56-58.
344 3. D. Tasis, N. Tagmatarchis, A. Bianco and M. Prato, *Chem. Rev.*, 2006, **106**, 1105-1136.
345 4. M. F. L. De Volder, S. H. Tawfick, R. H. Baughman and A. J. Hart, *Science*, 2013, **339**,
346 535-539.
347 5. S. Maity, S. Goswami and K. K. Chattopadhyay, *RSC Adv.*, 2013, **3**, 26321-26327.
348 6. L. Ding, J. Stilwell, T. Zhang, O. Elboudwarej, H. Jiang, J. P. Selegue, P. A. Cooke, J. W. Gray
349 and F. F. Chen, *Nano Lett*, 2005, **5**, 2448-2464.
350 7. G. Jia, H. Wang, L. Yan, X. Wang, R. Pei, T. Yan, Y. Zhao and X. Guo, *Environ. Sci. Technol.*,
351 2005, **39**, 1378-1383.
352 8. S. K. Manna, S. Sarkar, J. Barr, K. Wise, E. V. Barrera, O. Jejelowo, A. C. Rice-Ficht and G. T.
353 Ramesh, *Nano Lett*, 2005, **5**, 1676-1684.
354 9. A. Magrez, S. Kasas, V. Salicio, N. Pasquier, J. W. Seo, M. Celio, S. Catsicas, B. Schwaller
355 and L. Forro, *Nano Lett*, 2006, **6**, 1121-1125.

10. A. Baun, N. B. Hartmann, K. Grieger and K. O. Kusk, *Ecotoxicology*, 2008, **17**, 387-395.
11. S. Koyama, Y. A. Kim, T. Hayashi, K. Takeuchi, C. Fujii, N. Kuroiwa, H. Koyama, T. Tsukahara and M. Endo, *Carbon*, 2009, **47**, 1365-1372.
12. A. R. Murray, E. Kisin, S. S. Leonard, S. H. Young, C. Kommineni, V. E. Kagan, V. Castranova and A. A. Shvedova, *Toxicology*, 2009, **257**, 161-171.
13. C. M. Goodwin, G. G. Lewis, A. Fiorella, M. D. Ellison and R. Kohn, *RSC Adv.*, 2014, **4**, 5893-5900.
14. S. Kang, M. Pinault, L. D. Pfefferle and M. Elimelech, *Langmuir*, 2007, **23**, 8670-8673.
15. S. Kang, M. Herzberg, D. F. Rodrigues and M. Elimelech, *Langmuir*, 2008, **24**, 6409-6413.
16. S. Kang, M. S. Mauter and M. Elimelech, *Environ. Sci. Technol.*, 2008, **42**, 7528-7534.
17. D. F. Rodrigues and M. Elimelech, *Environ. Sci. Technol.*, 2010, **44**, 4583-4589.
18. F. Gottschalk, T. Sonderer, R. W. Scholz and B. Nowack, *Environ. Sci. Technol.*, 2009, **43**, 9216-9222.
19. F. Gottschalk, T. Y. Sun and B. Nowack, *Environ. Pollut.*, 2013, **181**, 287-300.
20. Y. Yin, X. J. Zhang, J. Graham and L. Luongo, *Proceedings of the Water Environment Federation*, 2007, **15**, 3196-3205.
21. Y. Yin and X. Zhang, *Water Sci Technol*, 2008, **58**, 623-628.
22. P. Madoni, D. Davoli and L. Guglielmi, *Water Res.*, 1999, **33**, 2459-2464.
23. M. D. C. Oviedo, D. S. Marquez and J. M. Q. Alonso, *Chem. Biochem. Eng. Q.*, 2002, **16**, 139-144.
24. A. S. Stasinakis, D. Mainais, N. S. Thomaidis, E. Danika, G. Gatidou and T. D. Lekkas, *Ecotox. Environ. Safe.*, 2008, **70**, 199-206.

25. X. Zheng, R. Wu and Y. Chen, *Environ. Sci. Technol.*, 2011, **45**, 2826-2832.
26. X. Zheng, Y. Su and Y. Chen, *Environ. Sci. Technol.*, 2012, **46**, 7182-7188.
27. C. N. Yang, J. Mamouni, Y. A. Tang and L. J. Yang, *Langmuir*, 2010, **26**, 16013-16019.
28. K. Pulskamp, S. Diabate and H. F. Krug, *Toxicol. Lett.*, 2007, **168**, 58-74.
29. M. Ishiyama, H. Tominaga, M. Shiga, K. Sasamoto, Y. Ohkura and K. Ueno, *Biol. Pharm. Bull.*, 1996, **19**, 1518-1520.
30. P. C. Pollard, M. A. Steffens, C. A. Biggs and P. A. Lant, *Water Res.*, 1998, **32**, 587-596.
31. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 1951, **193**, 265-275.
32. APHA, *Standard methods for the examination of water and wastewater, 20 th ed*, 1998.
33. Y. Jiang, Y. Chen and X. Zheng, *Environ. Sci. Technol.*, 2009, **43**, 7734-7741.
34. M. Pacurari, X. Yin, J. Zhao, M. Ding, S. S. Leonard, D. Schwegier-Berry, B. S. Ducatman, D. Sbarra, M. D. Hoover, V. Castranova and V. Vallyathan, *Environ Health Persp*, 2008, **116**, 1211-1217.
35. S. Liu, L. Wei, L. Hao, N. Fang, M. W. Chang, R. Xu, Y. Yang and Y. Chen, *Acs Nano*, 2009, **3**, 3891-3902.
36. S. Deng, V. K. K. Upadhyayula, G. B. Smith and M. C. Mitchell, *Ieee Sens J*, 2008, **8**, 954-962.
37. V. K. K. Upadhyayula, S. Deng, G. B. Smith and M. C. Mitchell, *Water Res*, 2009, **43**, 148-156.
38. J. M. Worle-Knirsch, K. Pulskamp and H. F. Krug, *Nano Lett*, 2006, **6**, 1261-1268.
39. P. Das, M. A. Xenopoulos, C. J. Williams, M. E. Hoque and C. D. Metcalfe, *Environ. Toxicol.*

*Chem.*, 2012, **31**, 122-130.

40. H. L. Karlsson, J. Gustafsson, P. Cronholm and L. Moller, *Toxicol. Lett.*, 2009, **188**, 112-118.
41. A. L. Alpatova, W. Shan, P. Babica, B. L. Upham, A. R. Rogensues, S. J. Masten, E. Drown, A. K. Mohanty, E. C. Alocilja and V. V. Tarabara, *Water Res.*, 2010, **44**, 505-520.
42. I. D. S. Henriques and N. G. Love, *Water Res.*, 2007, **41**, 4177-4185.
43. P. V. AshaRani, G. L. K. Mun, M. P. Hande and S. Valiyaveettil, *Acs Nano*, 2009, **3**, 279-290.
44. M. Heinlaan, A. Ivask, I. Blinova, H. C. Dubourguier and A. Kahru, *Chemosphere*, 2008, **71**, 1308-1316.
45. Y. Chen, Y. Su, X. Zheng, H. Chen and H. Yang, *Water Res.*, 2012, **46**, 4379-4386.
46. T. Mino, M. C. M. Van Loosdrecht and J. J. Heijnen, *Water Res.*, 1998, **32**, 3193-3207.
47. L. A. Luongo and X. Q. Zhang, *J. Hazard. Mater.*, 2010, **178**, 356-362.