

## Research Article

# Toxicity of Aqueous Fullerene nC<sub>60</sub> to Activated Sludge: Nitrification Inhibition and Microtox Test

Yongkui Yang,<sup>1</sup> Norihide Nakada,<sup>1</sup> Ryoji Nakajima,<sup>2</sup> Chao Wang,<sup>1</sup> and Hiroaki Tanaka<sup>1</sup>

<sup>1</sup> Research Center for Environmental Quality Management, Kyoto University, 1-2 Yumihama, Otsu 520-0811, Japan

<sup>2</sup> Testing and Analysis Division, Shimadzu Techno Research Inc., 1 Nishinokyo-Shimoaicho, Nakagyo-ku, Kyoto 604-8436, Japan

Correspondence should be addressed to Yongkui Yang, ykyang@biwa.eqc.kyoto-u.ac.jp

Received 3 April 2012; Accepted 11 June 2012

Academic Editor: Xiaoming Li

Copyright © 2012 Yongkui Yang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The increasing production and use of fullerene nanomaterials raised their exposure potential to the activated sludge during biological wastewater treatment process. In this study, the toxicity of aqueous nanoscaled C<sub>60</sub> (nC<sub>60</sub>) to activated sludge was investigated using nitrification inhibition and Microtox test. The test solutions of nC<sub>60</sub> were prepared using two methods: long stirring (Stir/nC<sub>60</sub>) and toluene exchange (Tol/nC<sub>60</sub>). The nC<sub>60</sub> aggregation in test medium was also evaluated for toxicity assessment. The results showed that the nC<sub>60</sub> aggregation behaved differently in two test mediums during the incubation periods. The nC<sub>60</sub> toxicity was greatly influenced by the preparation method. Stir/nC<sub>60</sub> presented no significant toxicity to both the nitrification sludge and bioluminescent bacteria at the maximum concentration studied. In contrast, the EC<sub>20</sub> of Tol/nC<sub>60</sub> was obtained to be 4.89 mg/L (3 h) for the nitrification inhibition and 3.44 mg/L (30 min) for Microtox test, respectively.

## 1. Introduction

Fullerenes, carbon-based nanomaterials, are demonstrating rapid increase in the commercial and scientific interest by their unique properties, such as, chemically and thermally stable, excellent electron acceptor and radical scavenger, and special optical properties [1, 2]. The production is expected up to 1500 t in 2007 compared to 400 kg in 2002 by the largest fullerene production company in the world [3]. In addition, until 2011 the class of fullerenes and other carbon-based nanomaterials is ranked as the second among all the nanomaterials used in consumer products available [4].

The C<sub>60</sub>, the main type of fullerenes, showed the toxicity on cell [5], bacterial [6, 7], and fish [8, 9]. Although the toxicity mechanism is still not clear, the most published is due to the oxidative stress via reactive oxygen species dependent [5] or independent [10]. The toxicities suggested the potential of adverse effect of C<sub>60</sub> on the activated sludge which is important for the removal of organic matters and nutrient compounds in wastewater. Recent studies pointed out the toxicity of metal nanoparticles (silver [11, 12], copper [13], zinc oxide, and titanium dioxide [14]), on the aerobic/anaerobic activated sludge. However, very limited

studies focused on fullerene C<sub>60</sub> toxicity on the activated sludge. Kang et al. [15] reported about 30% inactivation of microorganism in wastewater samples after 1 h exposure to nC<sub>60</sub>-coated filter. However, no significant change was identified to microbial community structure in the anaerobic sludge using the denaturing gradient gel electrophoresis analysis under 50 g/kg biomass [16].

The objective of this study is to assess the toxicity of aqueous nC<sub>60</sub> on the activated sludge. The effect of nC<sub>60</sub> on nitrification activities was investigated using the cultivated activated nitrifying sludge. The Microtox test was also conducted as a standardized screening test. To our best knowledge, this is the first study on the nC<sub>60</sub> toxicity on activated sludge by checking the nitrification inhibition. This work is expected to provide useful information to assess the effect of nC<sub>60</sub> on activated sludge and consequent treatment performance of biological wastewater treatment process.

## 2. Materials and Methods

**2.1. Preparation of Aqueous nC<sub>60</sub> Suspension.** Two types of aqueous nC<sub>60</sub> were prepared using the extended mixing of

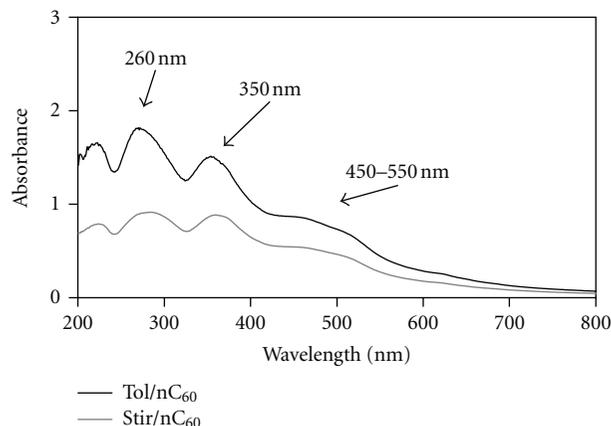


FIGURE 1: UV-visible absorption spectra of prepared  $nC_{60}$  (pH 5.6).

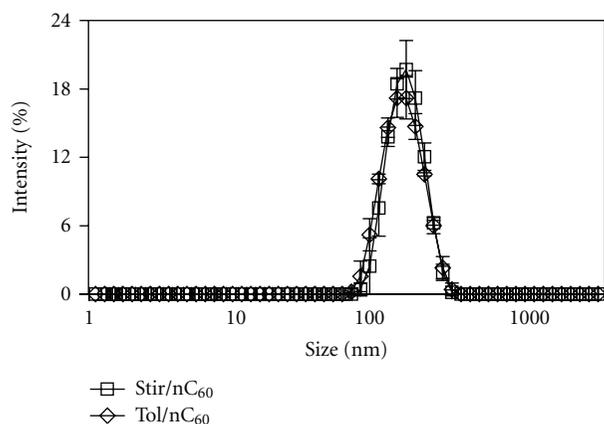


FIGURE 2: Size distribution of prepared  $nC_{60}$ . Error bars represent the standard error (pH 5.6,  $n = 2$ ).

powder  $C_{60}$  in water (Stir/ $nC_{60}$ ) and the toluene-involved solvent exchange method (Tol/ $nC_{60}$ ), both of which are widely used for the studies of the  $C_{60}$  toxicity [7, 9, 15] and its fate [17, 18]. Briefly, the Stir/ $nC_{60}$  was produced by adding 200 mg of powder  $C_{60}$  (purity: 99.9%, SES research, USA) to 300 mL of ultrapure water (Millipore, USA) and then mixing with a magnetic stirrer at 500 rpm for three weeks. And the Tol/ $nC_{60}$  was prepared by following the previously reported method with minor modifications [7]. Specifically, 1000 mg/L  $C_{60}$  solution was obtained by dissolving the powder  $C_{60}$  into HPLC grade toluene (stock  $C_{60}$  toluene solution). And then 40 mL of the solution was added to 100 mL ultrapure water. The toluene was removed by sonication at 40°C using an ultrasonic cleaner (AS ONE, Japan) followed by the purge with gentle stream of nitrogen gas at 0.5 L/min for 1 h. The prepared Stir/ $nC_{60}$  and Tol/ $nC_{60}$  suspensions were, both of yellow colour, sequentially filtered through a glass filter (pore size: 1  $\mu\text{m}$ , Pall Life Sciences, USA) and cellulose acetate filter (pore size: 0.45  $\mu\text{m}$ , Advantec, Japan). In addition, a blank sample for Tol/ $nC_{60}$  was also prepared by adding the same amount of toluene (without  $C_{60}$ ) in the pure water followed by the same procedures of

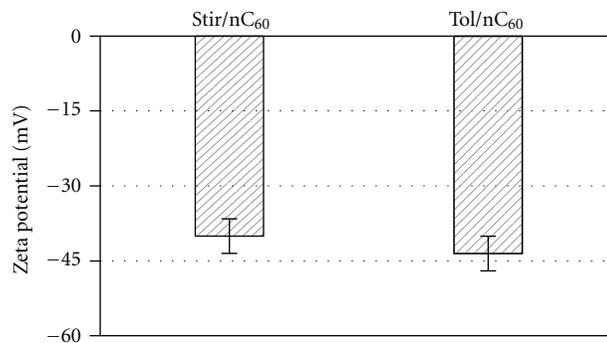


FIGURE 3: Zeta potential of prepared  $nC_{60}$ . Error bars represent the standard error (pH 5.6,  $n = 2$ ).

sonication and filtrations. The resulting suspensions were stored in the dark at 4°C until use. The  $nC_{60}$  concentration was determined by extracting  $nC_{60}$  into the toluene phase and quantifying it at 332 nm by the UV/vis spectrometer, described elsewhere [7, 19, 20]. The calibration curve was obtained by the stock  $C_{60}$  toluene solution at different concentrations ( $n = 5$ ).

**2.2. Cultivation of Nitrifying Activated Sludge.** The seed-activated sludge was collected from the nitrification tank of a biological wastewater treatment plant. The nitrifying activated sludge was obtained by cultivating the seed sludge at 30°C in a 3 L water-jacketed glass reactor. The reactor was operated at fill and draw mode at a hydraulic retention time of 12 h and sludge retention time of 20 d in the dark. Dissolved oxygen was kept at above 1.0 mg/L by introducing the filtered compressed air via diffusers, and the pH was maintained at  $7.5 \pm 0.1$  with the automatic addition of 1 M  $\text{Na}_2\text{CO}_3$ . The feed solution only consisted of the inorganic medium with the 25 mM  $(\text{NH}_4)_2\text{SO}_4$ . The other nutrient composition, data acquisition, and process control were described in the previous study [21]. After reaching the steady state by checking the  $\text{NH}_4^+$  removal efficiency, the mixed liquor in the reactor was collected and washed three times using 40 mM  $\text{KH}_2\text{PO}_4$  buffer (pH 7.8) by centrifugation ( $2000 \text{ g} \times 5 \text{ min}$ ).

**2.3. Nitrification Inhibition Experiment.** The nitrification inhibition studies were conducted in accordance with the ISO 9509 test guideline [22]. Batch experiments were carried out by agitating 100 mL Erlenmeyer flasks containing 50 mL medium solution (2 mM  $(\text{NH}_4)_2\text{SO}_4$  and 6 mM  $(\text{NaHCO}_3)$ ), determined nitrifying activated sludge and different amounts of  $nC_{60}$  at controlled room temperature (25°C). The experimental conditions, the incubating time of 3 h and MLSS of 40 mg/L, were determined to ensure about 50% of initial  $\text{NH}_4^+$  that was left at the end of incubating time to avoid rate limiting [22]. The dissolved oxygen was kept at above 4 mg/L by the shaking (150 rpm) on a rotary shaker. The nitrification inhibition (I) was calculated by the difference of oxidized N

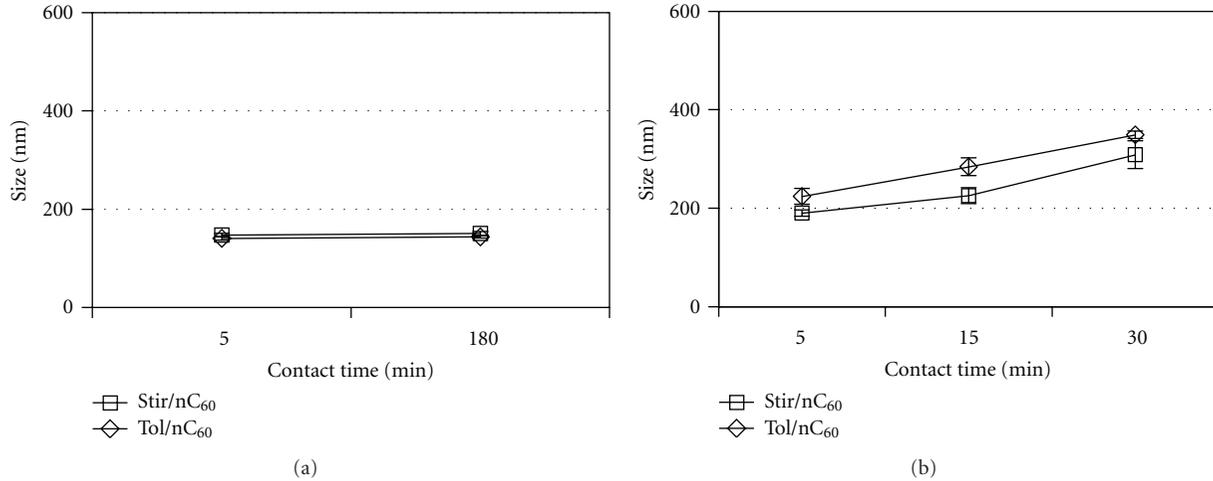


FIGURE 4: Change in nC<sub>60</sub> size in medium for nitrification inhibition (a) and Microtox test (b). Error bars represent the standard error ( $n = 2$ ).

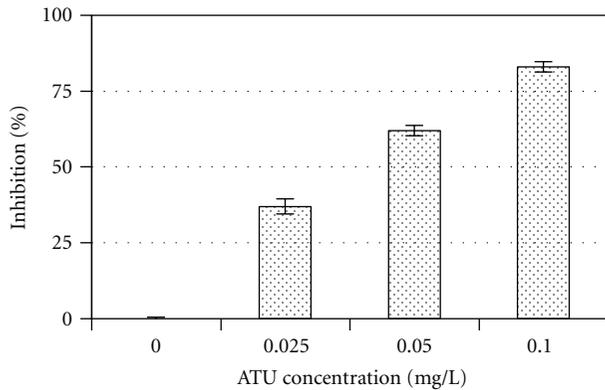


FIGURE 5: Nitrification inhibition as a function of ATU concentration. Error bars represent the standard error ( $n = 2$ ).

formation ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ) between the control and the nC<sub>60</sub> exposure test after 3 h. The equation is given as,

$$I (\%) = \frac{(N_c - N_f)}{(N_c - N_i)} \times 100, \quad (1)$$

where  $N_c$  (mg-N/L) is the concentration of oxidized N in the control flask after 3 h,  $N_f$  (mg-N/L) is the concentration of oxidized N in the flask containing nC<sub>60</sub> after 3 h,  $N_i$  (mg-N/L) is the concentration of oxidized N in the flask containing the reference inhibitor of N allylthiourea (ATU). The EC<sub>20</sub>, nC<sub>60</sub> concentration with a reduction of oxidized N formation by 20%, was calculated using the SPSS probit regression analysis (SPSS, USA).

**2.4. Microtox Test.** The *V. fischeri* bioassay is also used for assessing the toxicity of compounds on the activated sludge [23, 24] which is based on the decrease in bioluminescence from the bacterium due to the exposure to the toxicants. The experiment was carried out using a Model 500 luminometer (Azur Environmental, USA) in accordance with the Microtox

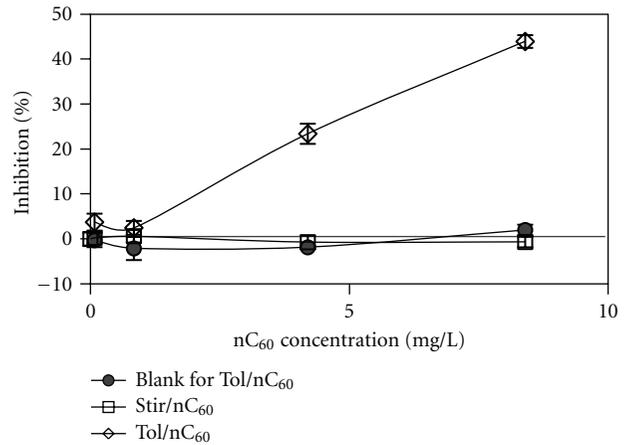


FIGURE 6: Nitrification inhibition of nC<sub>60</sub> as a function of preparation method and exposure concentration. Error bars represent the standard error ( $n = 2$ ).

acute toxicity procedure [25]. The reagent (freeze-dried *V. fischeri*) and the solutions (diluent, reconstitution, and osmotic adjusting solution) were purchased from Strategic Diagnostics Inc., USA. The EC<sub>20</sub>, nC<sub>60</sub> concentration with a reduction of bioluminescence by 20%, was calculated at each test with different exposure periods (5, 15, and 30 min) using the Microtox Omni Software (Strategic Diagnostics, USA). The phenol was used for the quality control of this test.

**2.5. nC<sub>60</sub> Aggregation in the Incubation Medium for the Toxicity Test.** The aggregation of nC<sub>60</sub> as a function of incubation time was determined using the same medium of the toxicity tests. For the nitrification inhibition test, the nC<sub>60</sub> size in medium (2 mM  $(\text{NH}_4)_2\text{SO}_4$ , 6 mM  $\text{NaHCO}_3$  and 2 mM  $\text{KH}_2\text{PO}_4$  buffer) was measured after 5 min (minimum time for one measurement) and 3 h. And for the Microtox

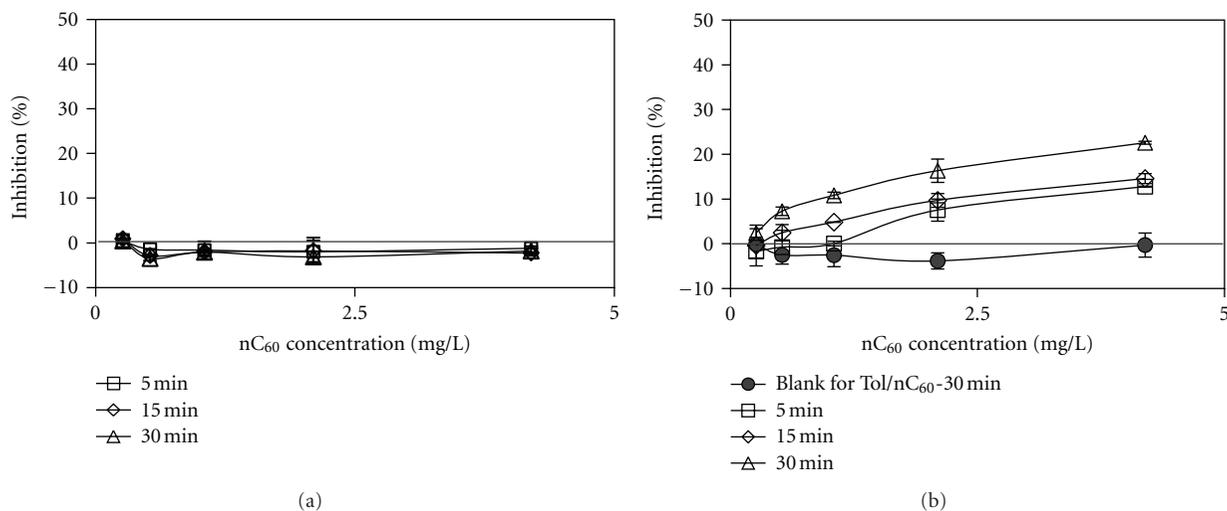


FIGURE 7: Bioluminescence inhibition of Stir/nC<sub>60</sub> (a) and Tol/nC<sub>60</sub> (b) as a function of exposure time and concentration. The Error bars represent the standard error ( $n = 2$ ).

test, the nC<sub>60</sub> size in 2% NaCl solution was measured after 5, 15, and 30 min.

**2.6. Analysis.** The NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> concentrations were determined based on the standard methods [26]. The UV absorbance of the stock C<sub>60</sub> toluene solution and nC<sub>60</sub> suspension in water was measured using a UV-2500 spectrophotometer (Shimadzu Scientific Instruments, Japan). nC<sub>60</sub> size and its distribution were determined by the dynamic light scattering using a Zetasizer Nano ZS equipped with a 633 nm laser source and a detection angle of 173° (Malvern Instruments, UK). The same instrument was used to measure the electrophoretic mobility which was subsequently calculated into Zeta potential using the Smoluchowski equation. All the tests in this study were conducted in duplicate.

### 3. Results and Discussion

**3.1. Characterization of Prepared nC<sub>60</sub>.** Figure 1 shows the UV-visible absorption spectra of Stir/nC<sub>60</sub> and Tol/nC<sub>60</sub>. Both nC<sub>60</sub> show the absorption peaks at 260 and 350 nm for the electronic structure of molecular C<sub>60</sub> cage [27] and a broad absorption band at 450–550 nm for the aggregated C<sub>60</sub>-C<sub>60</sub> interactions [6]. Similar findings have also been reported for nC<sub>60</sub> prepared via tetrahydrofuran (THF) [28]. However, the ratio of absorbance at 260, 350, and 450 nm varied with the nC<sub>60</sub> types suggesting the difference in the structure and composition of nC<sub>60</sub> aggregates [29].

Two types of nC<sub>60</sub> demonstrated very similar size distribution with an average size of 154 and 144 nm for Stir/nC<sub>60</sub> and Tol/nC<sub>60</sub>, respectively, as shown in Figure 2. Both nC<sub>60</sub> were negatively charged with no significant difference at pH 5.6 (Figure 3), which is in agreement with the reported results of nC<sub>60</sub> prepared using similar methodologies [29].

**3.2. Aggregation of nC<sub>60</sub> in Toxicity Test Medium.** The aggregate size is an essential information when assessing the toxicity of nanoparticles because of proved correlation [7, 30]. Figure 4(a) shows the nC<sub>60</sub> size with time in medium for nitrification inhibition test. After the incubation time of 3 h the aggregate remained stable with only several nm of increase in size for both nC<sub>60</sub>. It can be explained by the low ionic strength of this medium (~15.8 mM) which was much lower than the reported threshold destabilization concentration of ~120 mM for the nC<sub>60</sub> [31]. In contrast, the obvious increase in size was found in medium with high ionic strength (~342 mM) for the Microtox test (Figure 4(b)). Compared to the initial sizes in pure water, the size in medium increased by 31, 56, and 114% for Stir/nC<sub>60</sub> and 55, 97, and 142% for Tol/nC<sub>60</sub> after 5, 15, and 30 min. In addition, the aggregation rate of Stir/nC<sub>60</sub> was slower than that of Tol/nC<sub>60</sub> presumably due to the difference in the structure and chemistry of nC<sub>60</sub> aggregate. Brant et al. found the hydrophobicity of Stir/nC<sub>60</sub> was lower than the nC<sub>60</sub> prepared via the organic solvent, such as, toluene and THF [29].

**3.3. Effect of nC<sub>60</sub> on Nitrification Activity.** The sludge nitrification activity and test performance were confirmed using the ATU. The EC<sub>50</sub> was calculated to be 0.040 mg/L from the data (Figure 5) which was close to the published value of 0.025 mg/L [32]. Figure 6 shows the percent nitrification inhibition by two types of nC<sub>60</sub> at varying concentrations after 3 h, as well as the blank sample for Tol/nC<sub>60</sub>. For the Stir/nC<sub>60</sub>, no nitrification inhibition was observed up to 8.4 mg/L indicating its low toxicity on nitrifying activated sludge. Previous studies also presented similar results that no significant impact was identified on the microbial community structure in aerobic soil [33] and anaerobic sludge [16]. In the case of the Tol/nC<sub>60</sub>, no obvious effect was found in the blank sample (less than 2%).

But ~40% nitrification was inhibited at 8.40 mg/L, and the EC<sub>20</sub> was calculated to 4.89 mg/L for Tol/nC<sub>60</sub>. It clearly showed that the nC<sub>60</sub> toxicity depended on the preparation method. Zhu et al. [8] compared the toxicity of Stir/nC<sub>60</sub> and nC<sub>60</sub> produced via THF on *Daphnia magna* and founded EC<sub>50</sub> (48 h) for the latter was at least one order of magnitude (0.8 mg/L) less than that for Stir/nC<sub>60</sub> (>35 mg/L).

**3.4. Effect of nC<sub>60</sub> on Bioluminescent Bacteria.** Figure 7(a) shows the percent inhibition due to the exposure to Stir/nC<sub>60</sub> at different concentrations. No inhibition was observed at the concentration up to 4.2 mg/L for all the incubation time. In contrast, the Tol/nC<sub>60</sub> showed obvious inhibition at >1.05 mg/L, and the toxicity increased with the incubation time (Figure 7(b)). No inhibition was also observed for the blank sample up to 30 min. The EC<sub>20</sub> was calculated to 4.96, 4.98, and 3.44 mg/L for 5, 15, and 30 min, respectively. Both the facts above confirmed the low toxicity of Stir/nC<sub>60</sub> and the toxicity dependent on the preparation methods. The result is consistent with that obtained from the nitrification inhibition test.

## 4. Conclusions

The prepared Stir/nC<sub>60</sub> and Tol/nC<sub>60</sub> showed similar surface properties, such as, the size distribution, zeta potential, and UV-vis absorption spectra. However, two types of nC<sub>60</sub> presented different aggregation rates in test medium during the incubation periods. Both the nitrification inhibition and Microtox test showed that the nC<sub>60</sub> toxicity was greatly affected by the preparation method. Stir/nC<sub>60</sub> presented no significant toxicity to the nitrification sludge and bioluminescent bacteria at maximum concentration studied. In contrast, the EC<sub>20</sub> of Tol/nC<sub>60</sub> was obtained to be 4.89 mg/L (3 h) for the nitrification inhibition and 3.44 mg/L (30 min) for Microtox test, respectively.

## Conflict of Interests

The authors declare that there is no conflict of interests.

## Acknowledgments

This research was partly supported by Grant-in-Aid for Scientific Research (B), Japan Society for the Promotion of Science (JSPS) and CREST project, Japan Science and Technology (JST).

## References

- [1] S. J. Duclos, K. Brister, R. C. Haddon, A. R. Kortan, and F. A. Thiel, "Effects of pressure and stress on C<sub>60</sub> fullerite to 20 GPa," *Nature*, vol. 351, no. 6325, pp. 380–382, 1991.
- [2] B. S. Sherigara, W. Kutner, and F. D'Souza, "Electrocatalytic properties and sensor applications of fullerenes and carbon nanotubes," *Electroanalysis*, vol. 15, no. 9, pp. 753–772, 2003.
- [3] H. Murayama, S. Tomonoh, J. M. Alford, and M. E. Karpuk, "Fullerene production in tons and more: from science to industry," *Fullerenes Nanotubes and Carbon Nanostructures*, vol. 12, no. 1-2, pp. 1–9, 2004.
- [4] Woodrow Wilson, "The project on emerging technologies," 2012, <http://www.nanotechproject.org/consumerproducts>.
- [5] C. M. Sayes, J. D. Fortner, W. Guo et al., "The differential cytotoxicity of water-soluble fullerenes," *Nano Letters*, vol. 4, no. 10, pp. 1881–1887, 2004.
- [6] J. D. Fortner, D. Y. Lyon, C. M. Sayes et al., "C<sub>60</sub> in water: nanocrystal formation and microbial response," *Environmental Science and Technology*, vol. 39, no. 11, pp. 4307–4316, 2005.
- [7] D. Y. Lyon, L. K. Adams, J. C. Falkner, and P. J. J. Alvarez, "Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size," *Environmental Science and Technology*, vol. 40, no. 14, pp. 4360–4366, 2006.
- [8] S. Zhu, E. Oberdörster, and M. L. Haasch, "Toxicity of an engineered nanoparticle (fullerene, C<sub>60</sub>) in two aquatic species, *Daphnia* and fathead minnow," *Marine Environmental Research*, vol. 62, supplement 1, pp. S5–S9, 2006.
- [9] K. T. Kim, M. H. Jang, J. Y. Kim, and S. D. Kim, "Effect of preparation methods on toxicity of fullerene water suspensions to Japanese medaka embryos," *Science of the Total Environment*, vol. 408, no. 22, pp. 5606–5612, 2010.
- [10] D. Y. Lyon, L. Brunet, G. W. Hinkal, M. R. Wiesner, and P. J. J. Alvarez, "Antibacterial activity of fullerene water suspensions (nC<sub>60</sub>) is not due to ROS-mediated damage," *Nano Letters*, vol. 8, no. 5, pp. 1539–1543, 2008.
- [11] Z. Liang, A. Das, and Z. Hu, "Bacterial response to a shock load of nanosilver in an activated sludge treatment system," *Water Research*, vol. 44, no. 18, pp. 5432–5438, 2010.
- [12] T. S. Radniecki, D. P. Stankus, A. Neigh, J. A. Nason, and L. Semprini, "Influence of liberated silver from silver nanoparticles on nitrification inhibition of *Nitrosomonas europaea*," *Chemosphere*, vol. 85, no. 1, pp. 43–49, 2011.
- [13] R. Ganesh, J. Smeraldi, T. Hosseini, L. Khatib, B. H. Olson, and D. Rosso, "Evaluation of nanocopper removal and toxicity in municipal wastewaters," *Environmental Science and Technology*, vol. 44, no. 20, pp. 7808–7813, 2010.
- [14] H. Mu, Y. Chen, and N. Xiao, "Effects of metal oxide nanoparticles (TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> and ZnO) on waste activated sludge anaerobic digestion," *Bioresource Technology*, vol. 102, no. 22, pp. 10305–10311, 2011.
- [15] S. Kang, M. S. Mauter, and M. Elimelech, "Microbial cytotoxicity of carbon-based nanomaterials: implications for river water and wastewater effluent," *Environmental Science and Technology*, vol. 43, no. 7, pp. 2648–2653, 2009.
- [16] L. Nyberg, R. F. Turco, and L. Nies, "Assessing the impact of nanomaterials on anaerobic microbial communities," *Environmental Science and Technology*, vol. 42, no. 6, pp. 1938–1943, 2008.
- [17] Z. Chen, P. Westerhoff, and P. Herckes, "Quantification of C<sub>60</sub> fullerene concentrations in water," *Environmental Toxicology and Chemistry*, vol. 27, no. 9, pp. 1852–1859, 2008.
- [18] H. Hyung and J. H. Kim, "Dispersion of C<sub>60</sub> in natural water and removal by conventional drinking water treatment processes," *Water Research*, vol. 43, no. 9, pp. 2463–2470, 2009.
- [19] S. Deguchi, R. G. Alargova, and K. Tsujii, "Stable dispersions of fullerenes, C<sub>60</sub> and C<sub>70</sub>, in water. Preparation and characterization," *Langmuir*, vol. 17, no. 19, pp. 6013–6017, 2001.
- [20] C. Wang, C. Shang, and P. Westerhoff, "Quantification of fullerene aggregate nC<sub>60</sub> in wastewater by high-performance liquid chromatography with UV-vis spectroscopic and mass spectrometric detection," *Chemosphere*, vol. 80, no. 3, pp. 334–339, 2010.

- [21] G. C. Ghosh, T. Okuda, N. Yamashita, and H. Tanaka, "Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation," *Water Science and Technology*, vol. 59, no. 4, pp. 779–786, 2009.
- [22] ISO, 9509, "Water quality-toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms," International Organization for Standardization, 2006.
- [23] D. J. B. Dalzell, S. Alte, E. Aspichueta et al., "A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge," *Chemosphere*, vol. 47, no. 5, pp. 535–545, 2002.
- [24] S. Ren, "Assessing wastewater toxicity to activated sludge: recent research and developments," *Environment International*, vol. 30, no. 8, pp. 1151–1164, 2004.
- [25] Azur, *Microtox Acute Toxicity Basic Test Procedures*, Azur Environmental, Carlsbad, Calif, USA, 1995.
- [26] APHA, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 20th edition, 1999.
- [27] J. Lee, M. Cho, J. D. Fortner, J. B. Hughes, and J. H. Kim, "Transformation of aggregated  $C_{60}$  in the aqueous phase by UV irradiation," *Environmental Science and Technology*, vol. 43, no. 13, pp. 4878–4883, 2009.
- [28] J. D. Fortner, D. I. Kim, A. M. Boyd et al., "Reaction of water-stable  $C_{60}$  aggregates with ozone," *Environmental Science and Technology*, vol. 41, no. 21, pp. 7497–7502, 2007.
- [29] J. A. Brant, J. Labille, J. Y. Bottero, and M. R. Wiesner, "Characterizing the impact of preparation method on fullerene cluster structure and chemistry," *Langmuir*, vol. 22, no. 8, pp. 3878–3885, 2006.
- [30] M. Luna-delRisco, K. Orupöld, and H. C. Dubourguier, "Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion," *Journal of Hazardous Materials*, vol. 189, no. 1-2, pp. 603–608, 2011.
- [31] K. L. Chen and M. Elimelech, "Aggregation and deposition kinetics of fullerene ( $C_{60}$ ) nanoparticles," *Langmuir*, vol. 22, no. 26, pp. 10994–11001, 2006.
- [32] R. Cui, W. J. Chung, and D. Jahng, "A rapid and simple respirometric biosensor with immobilized cells of *Nitrosomonas europaea* for detecting inhibitors of ammonia oxidation," *Biosensors and Bioelectronics*, vol. 20, no. 9, pp. 1788–1795, 2005.
- [33] Z. Tong, M. Bischoff, L. Nies, B. Applegate, and R. F. Turco, "Impact of fullerene ( $C_{60}$ ) on a soil microbial community," *Environmental Science and Technology*, vol. 41, no. 8, pp. 2985–2991, 2007.



**Hindawi**

Submit your manuscripts at  
<http://www.hindawi.com>

