Microorganism mediated synthesis of reduced graphene oxide films

Y Tanizawa¹, Y Okamoto², K Tsuzuki², Y Nagao³, N Yoshida³,⁴, R Tero³,⁴, S Iwasa⁴, A Hiraishi⁴, Y Suda⁴, H Takikawa⁴, R Numano⁴, H Okada¹,³,⁴, R Ishikawa⁵ and A Sandhu¹,³,⁴,⁵

¹Dept. of Electrical and Electronic Information Engineering, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
²Dept. of Environmental and Life Sciences, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
³Electronics-Inspired Interdisciplinary Research Institute (EIIRIS), Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
⁴Toyohashi Tech Graphene Research Group, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
⁵Electrical and Electronic Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, 152-8550

E-mail: tanizawa-y@eiiris.tut.ac.jp

Abstract. The wide-ranging industrial application of graphene and related compounds has led researchers to devise methods for the synthesis of high quality graphene. We recently reported on the chemical synthesis, patterning, and doping of graphene films by the chemical exfoliation of graphite into graphene oxide (GO) with subsequent chemical reduction into graphene films [1, 2]. Here, we describe a hybrid approach for the synthesis of reduced graphene sheets, where chemically derived GO was reduced by microorganisms extracted from a riverside near the University. Our procedure enabled the production of ~100 µm sized reduced graphene sheets, which showed excellent Raman spectra associated with high quality reduced graphene. We give a detailed account of the relationship between the type of microorganisms and the properties of the resulting reduced graphene.

1. Introduction
Chemically-derived graphene, composed of a single layer of carbon, now has an available route for bulk production. GO seems to be a much more efficient, low-cost, and bulk production pathway to incorporate graphene sheets into hybrids [3].

Currently, the most intensely researched approach for large-scale graphene production is through the chemical reduction of chemically derived GO, which can be obtained from natural graphite powder. GO may be reduced to graphene, either by exposure to pure hydrazine or by means of a thermal treatment. However, these methods have some drawbacks. First, hydrazine vapors are highly toxic. Second, thermal reduction is a very complex phenomenon because of the thermal-energy-induced multi-step removal processes of intercalated H₂O molecules and oxide groups of –COOH...
(carboxyl group), -OH(hydroxyl group), and >O(epoxy group) [3]. These approaches may cause environmental or economic concerns when large-scale production is considered.

Here, inspired by a recent report describing how graphene oxide behaves as a terminal electron acceptor for bacteria [4], GO is reduced by microbial action (e.g. bacteria) in the process of breathing or electron transport. In this study, we describe a hybrid approach for the synthesis of reduced graphene sheets, where chemically derived graphene oxide was reduced by microorganisms extracted from a river bank near the Tempaku Campus of Toyohashi University of Technology, in Aichi Prefecture.

2. Experimental

2.1. Synthesis of graphene oxide

GO was synthesized by the modified Hummer’s method [5, 6] using natural graphite powder (Ito Kokuen Co., Ltd, Japan) cleaned using K₂S₂O₈, P₂O₅ and H₂SO₄, and oxidized in KMnO₄ and H₂SO₄. After overnight sedimentation and washing with 3 wt% HCl aq. and MilliQ water, the resulting graphite oxide was exfoliated into GO without ultra-sonication. The resulting graphite oxide was then exfoliated into GO using centrifugation (10000rpm, 45min) instead of ultra-sonication, because if we use ultra-sonication, the size of GO sheets become smaller and smaller. Finally, a GO aqueous dispersion was purified by dialysis for one week to remove residual metal ions, acids and to adjust pH to neutral.

2.2. Microorganism-mediated reduction of graphene oxide

A microbial culture was extracted for the reduction of GO, which was obtained by repeating several iterations of transfer-cultivation of river sediments with a medium containing various mineral salts and organic substrates under anaerobic conditions. Silicon substrates with GO sheets attached to their surfaces were put into the microbial cultures in serum bottles. The silicon-GOs were incubated in the microbial culture at 28°C for 3 days in the dark. After incubation, they were fully washed with MilliQ and dried.

The GO was reduced when electrons from a sodium acetate solution were passed to the GO by anaerobic breathing of the environmental microorganisms. These microbes have the ability to use a large array of organic and inorganic compounds as terminal electron acceptors in their respiratory pathway [4, 7].

3. Results and Discussion

The GO sheets and the reduced GO sheets were characterized by drop-casting a solution containing GO sheets onto a thermally oxidized SiO₂/Si substrate. Then the SiO₂/Si substrate was slightly tipped at a shallow angle to prevent agglomeration of GO sheets in the liquid suspension those attached to the SiO₂/Si substrate during drying. The GO sheets dropped onto the substrates were examined under an optical microscope, Raman spectroscopy (excited by 532 nm ×10 objective lens, NRS-1000 JASCO CO., Ltd, Japan) and atomic force microscopy (in tapping mode using Si tips). The thickness of SiO₂ was about 90 nm in order to obtain high contrast images of the GO sheets under visible light [1, 8, 9].

Figure 1 shows optical images of chemically derived GO sheets. There are a few layers of GO sheets, which were several hundreds of micrometers in size, with signs of overlapped layers.

Figure 2 shows optical images of the surface of GO sheets on a SiO₂/Si substrate after bacterial respiration when a reduction medium was added to the solution. The bright dots are believed to be bacteria or metallic precipitates from the reduction medium. The reduction of GO was visibly apparent in the solutions due to the formation of black precipitates, and in the case of GO fixed to silicon substrates, as a sharp increase in contrast under optical microscopic observations.

Figure 3 shows an AFM image of reduced GO via bacterial respiration. If we can make perfectly reduced GO, the thickness of the reduced GO sheet would be the same as that of graphene, but we did
not observe this effect. We observed some bacteria, which have flagella and which appear as white dots of about 2µm in size.

Figure 4 shows the Raman spectra of reduced GO produced without the use of a reduction agent. The GO exhibited two peaks centered at 1347 and 1610 cm\(^{-1}\), corresponding to the D and G bands, the latter corresponding to the in-phase vibration of the graphite lattice [10]. The prominent D peak is the disorder band from the structural imperfections created by the attachment of hydroxyl and epoxide groups on the carbon basal plane or edge [11]. The reduced GO, where the D band peak shifts from 1347 to 1320 cm\(^{-1}\), and the G band became a broad peak. The intensity ratio of D/G was greater than that observed before reduction, due to reduction of the GO. These results indicate that the number of sp\(^2\) domains increased due to the reduction process [1]. In the former paper, C.Tung V et al observed the same phenomenon. However, fully reduced samples would exhibit small D/G ratios if large-scale aromaticity were re-established [12].

We give a detailed account of the relationship between the type of bacteria and the properties of the resulting reduced graphene.

![Figure 1](image1.png)

**Figure 1.** Bright field images of GO sheets with differing layer thicknesses on a SiO\(_2\)/Si substrate. (a) Optical microscope image and (b) close up of the white box area.

![Figure 2](image2.png)

**Figure 2.** Image of reduced GO sheets on a SiO\(_2\)/Si substrate. (a) Optical microscope image and (b) high magnification.
Figure 3. AFM image of reduced GO sheets on a SiO$_2$/Si substrate. The white dots that are than 2µm are bacteria.

Figure 4. Raman spectra of (a) chemically synthesized GO sheets, (b) reduced GO sheets after bacterial reduction without the addition of reducing agent and (c) the surface of SiO$_2$.

4. Conclusions
Bacteria extracted from a riverside in Toyohashi city were used to reduce graphene oxide sheets without the addition of a reducing agent, opening up the possibility of a facile means of reducing graphene oxide dispersed in a bacterial suspension or immobilized on solid substrates.

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