

## *Supporting information for*

# Fullerol as a Potential Pathway for Mineralization of Fullerene Nanoparticles in Biosolid-Amended Soils

By Divina A. Navarro<sup>1,2\*</sup>, Rai S. Kookana<sup>1,2</sup>, Mike J. McLaughlin<sup>1,2</sup> and Jason K. Kirby<sup>1</sup>

<sup>1</sup> CSIRO Land and Water, PMB 2, Glen Osmond 5064, Australia.

<sup>2</sup> Soil Science, School of Agriculture, Food and Wine, University of Adelaide, PMB 1, Glen Osmond 5064, Australia.

\*Corresponding Author, E-mail: [divina.navarro@csiro.au](mailto:divina.navarro@csiro.au), Phone: +618 8303 8587

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## Materials and Methods

### **Preparation of hydroxylated $^{14}\text{C}$ - $\text{C}_{60}$ using the method described by Li et al.**

A 500  $\mu\text{L}$  aliquot of  $^{14}\text{C}$ - $\text{C}_{60}$  was taken from the stock solution (1.53 MBq/mL in toluene) and transferred into a 50-mL conical flask, after which, the solution was diluted with 2 mL of toluene. The solution was then vigorously mixed with 0.1  $\mu\text{L}$  of tetrabutyl ammonium hydroxide (TBAH 40% solution from Sigma). After five minutes, 2 mL of sodium hydroxide (2g in 2 mL) was added to the mixture. The purple colour of the  $\text{C}_{60}$  solution instantaneously disappeared with mixing, revealing a brown-coloured sludge in the aqueous phase. Ten millilitres of water was subsequently added to the mixture to further solubilise the material that is in the aqueous phase. Stirring was continued for 5 days under ambient conditions to ensure  $\text{C}_{60}$  hydroxylation. After 3 days, compressed air was used to facilitate evaporation of toluene that may still be present. The aqueous solution containing the product was then washed using methanol (30 mL per 5 mL of extract). Addition of methanol results in precipitation of the product, while solubilising TBAH. The mixture was centrifuged for 10 minutes at 1500 rpm to separate the product. Methanol was decanted then replaced with fresh solvent. The washing step was done thrice. The recovered solids were reconstituted with 8 mL of water producing a brown coloured solution. To reduce the amount of salt in the recovered product, the reconstituted solution was filtered through a 3kDa ultracentrifugation filter. This procedure lets the salt pass through the membrane while keeping most of the desired hydroxylated product in the retentate. The process was done thrice, each time, reconstituting the volume of solution to 8mL. The amount of  $^{14}\text{C}$  recovered in the aqueous phase is ~64%. Most of the activity lost is present in the washings which suggests the formation of less hydroxylated  $\text{C}_{60}$  that would be soluble in methanol. As a control for the experiments, a solution constituting all the reagents from the experiment except for  $\text{C}_{60}$  was also prepared. This was used in the experiment to evaluate the effect of the synthesis matrix on microbial activity.

## C<sub>60</sub>-OH characterisation

Pristine <sup>14</sup>C-C<sub>60</sub> was successfully converted to a more water-soluble, hydroxylated form using the procedure followed.<sup>24</sup> The product was significantly soluble in water and had negligible partitioning in toluene. C<sub>60</sub>-OH synthesised exhibit broad UV absorption, unlike C<sub>60</sub> which has three prominent peaks in the 200-400 nm region (Figure S1).<sup>25, 26</sup> The presence of oxygenated functional groups on C<sub>60</sub>-OH was confirmed by Fourier Transform Infrared spectroscopy (Figure S2). The spectra correlates very well with commercially purchased fullerol (MER, Arizona). IR band positions at 1050, 1400, 1600-1700 cm<sup>-1</sup>, could be assigned to C-O stretches, C-O-H in plane bending and/or O=C-O symmetric stretches, O=C-O asymmetric stretch and/or C=C stretches, respectively. The exact formula for the synthesised material was not determined but can be assumed to be polyhydroxylated based on the procedure followed: <sup>14</sup>C-C<sub>60</sub>(OH)<sub>x</sub>(ONa)<sub>y</sub> (where x+y = 24 to 26).<sup>24</sup>

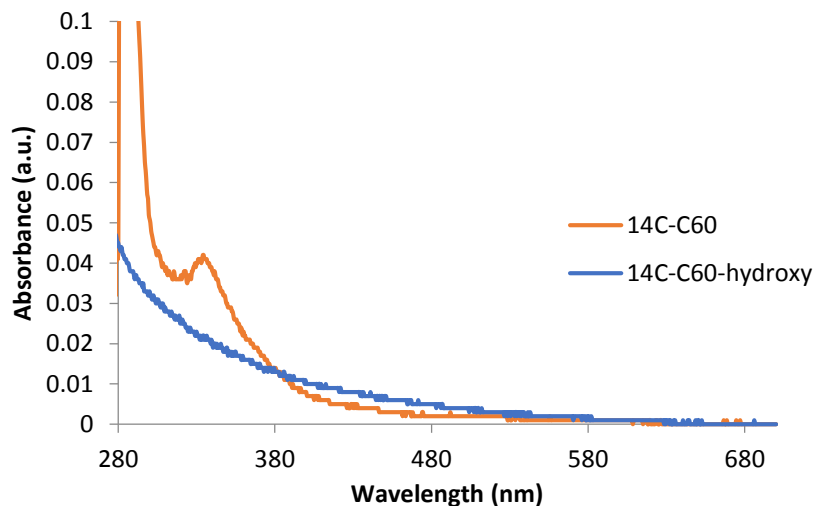


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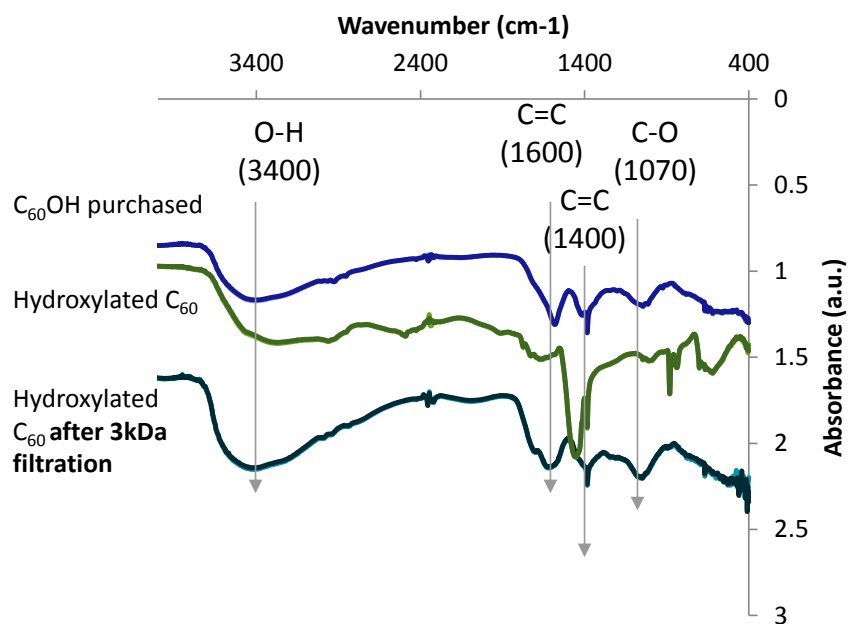


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## Calculations

### C<sub>60</sub> spiking rates

- Calculation demonstrates that at low spiking concentrations, 0.25% decomposition of C<sub>60</sub> (in 28 days) could be detected; at high spiking concentrations, 0.025% decomposition of C<sub>60</sub> (in 28 days) could be detected.

Factor	Equation	Value	
		LOW C <sub>60</sub>	HIGH C <sub>60</sub>
Soil per incubator flask (g)		50	50
<b>Soil fullerene concentration (µg/kg)</b>		<b>200</b>	<b>2000</b>
Fullerene per incubator flask (µg)	$g\ soil * \mu g\ C_{60}/kg\ soil / 1000$	10	100
<b>Decomposition assumed (%)</b>		<b>0.25</b>	<b>0.025</b>
Fullerene mineralised to CO <sub>2</sub> (µg)	$\%decomposition * \mu g\ fullerene$	0.025	0.025
Fullerene mineralised to CO <sub>2</sub> (nmol)	$\mu g\ fullerene\ mineralised/720 * 1000$	0.035	0.035
fullerene 14C mineralised to CO <sub>2</sub> (Bq)	$nmol\ mineralised * 425.5\ Bq/nmol$	14.77	14.77
Trapped in 5 ml NaOH soln (Bq/ml)	$Bq\ mineralised/ volume\ NaOH\ trap$	2.95	2.95
<b>Expected dpm/vial</b>	$Bq * volume\ aliquot * 60s/min$	<b>354.6</b>	<b>354.6</b>

- Limit of Detection = 27 dpm; Calculated as: *Average background dpm + 10x standard deviation of background dpm*
- Reporting Limit = 270 dpm; Calculated as: *10xLimit of Detection*

## Estimating [<sup>14</sup>C]C<sub>60</sub>-OH concentration

Assumptions:

- specific activity is maintained (425.5 MBq/mmol)
- theoretical composition of C<sub>60</sub>(OH)<sub>x</sub>(ONa)<sub>y</sub> where x+y=24 to 26

Calculation:

$$118.2 \text{ Bq/g soil} * (1 \text{ mmol} / 425.5 \times 10^6 \text{ Bq}) * (1260 \text{ mg} / \text{mmol}) * (1000 \text{ } \mu\text{g} / 1 \text{ mg}) \\ = 0.350 \text{ } \mu\text{g/g} \text{ or } 350 \text{ } \mu\text{g/kg}$$

Estimated Concentrations using Theoretical Molecular Weights

C <sub>60</sub> (OH) <sub>x</sub> (ONa) <sub>y</sub>	x+y=24		Concentration
		MW	in soil (μg/kg)
x	y		
18	6	1260	350
17	7	1282	356
16	8	1304	362
15	9	1326	368
14	10	1348	374

## Amount of NaOH required for each test system

### Glucose

Factor	Equation	Value
Rate ( $\mu\text{gC/gsoil/hr}^*$ )		12.3
Rate ( $\mu\text{gC/gsoil/day}$ )	$\mu\text{gC/gsoil/hr} * 24$	295.2
Amount of soil (g)		50.0
Rate ( $\mu\text{gC/day}$ )	$\mu\text{gC/gsoil/day} * \text{g soil}$	14760.0
$\mu\text{moles of NaOH required per day based on CO}_2$ production	$\mu\text{gC/day} / 12\text{g/mol} * 2\text{moles NaOH/1mole C}$	2460.0
mmol NaOH needed/day	$\mu\text{moles}/1000$	2.5
Concentration NaOH (M)		1.0
Volume 1M NaOH needed per day (mL)	$\text{mmoles NaOH} / \text{mmoles NaOH/mL}$	2.5
Volume 1M NaOH needed per 4 days (mL)		9.84

\*rate is determined from previously collected SIR data



Basal

Factor	Equation	Value
Rate ( $\mu\text{gCO}_2/\text{gsoil}/\text{hr}^*$ )		2
Rate ( $\mu\text{gCO}_2/\text{gsoil}/\text{day}$ )	$\mu\text{gCO}_2/\text{gsoil}/\text{hr} * 24$	48
Amount of soil (g)		50.0
Rate ( $\mu\text{gCO}_2/\text{day}$ )	$\mu\text{gCO}_2/\text{gsoil}/\text{day} * \text{g soil}$	2400
$\mu\text{moles}$ of NaOH required per day based on $\text{CO}_2$ production	$\mu\text{gCO}_2/\text{day} / 12\text{g}/\text{mol} * 2\text{moles NaOH}/1\text{mole C}$	109.1
mmol NaOH needed/day	$\mu\text{moles}/1000$	0.1
Concentration NaOH (M)		1.0
Volume 1M NaOH needed per day (mL)	$\text{mmoles NaOH} / \text{mmoles NaOH}/\text{mL}$	0.1
Volume 1M NaOH needed per 4 days (mL)		0.4

C<sub>60</sub>

- Calculated assuming 5% decomposition of C<sub>60</sub>

Factor	Equation	Values	
Amount of soil (g)		50	50
Concentration NaOH (M)		1.0	1.0
C <sub>60</sub> spiking concentration (µgC <sub>60</sub> /g soil)		0.2	2
Rate of C addition µgC/g soil	$\mu\text{gC}/\text{gsoil} * \text{gsoil}$	0.2	2
mass of C available (µg)	$\text{mass C}/12\text{g/mol}$	10.0	100.0
µmoles C	$\mu\text{moles C} * 0.05$	0.8333	0.1389
µmoles C mineralised (assume 5% in 28 day test)	$\mu\text{moles C} * 2\mu\text{moles NaOH}/1\mu\text{moleC}$	0.04167	0.00694
µmoles of NaOH required if 5% C is mineralised in 28 days		0.08333	0.01389
µmoles of NaOH required if 100% C is mineralised in 28 days	$\mu\text{moles NaOH} / \mu\text{moles NaOH}/\mu\text{L} / 1000$	1.66667	0.27778
total volume of 1M NaOH needed (5% mineralisation) (mL)		0.0000833	0.0000139
total volume of 1M NaOH needed (100% mineralisation) (mL)		0.0016667	0.0002778

### **Amount of C respired per gram of soil per day**

- a. Slope of the plot, percentage of added substrate respired versus time, was determined by linear regression analysis.
  - Slope is equivalent to the rate of respiration, expressed as a change in the percentage of added substrate respired over time
  
- b. Convert slope to obtain  $\mu\text{g}$  Carbon respired as  $\text{CO}_2$  per gram soil per day
  - $\text{Slope} / 100 * 250000 \mu\text{g C introduced} / \text{amount of soil}$

Table S 1. Soil properties

Soil	Location	Type	EC* μS/cm	pH* 2mM KNO <sub>3</sub>	MWHC %	Organic C %	CEC (NH <sub>4</sub> <sup>+</sup> ) cmol <sup>+</sup> /kg	Oxalate Extractable			Particle Size Analysis			
								Al mg/kg	Fe mg/kg	Mn mg/kg	Clay %	Silt %	Fine Sand %	Coarse Sand %
S1	Kalangadoo	Palixeralf	24.1	7.42	25.4	0.48	14	381	356	<50	12	2.5	32	19
S2	Mintaro	Rhodoxeralf	515	5.72	40.4	0.54	6.9	400	901	395	16	17	49	16
S3	Mt. Shank	Haploxerand	194.6	6.32	75.4	8.94	47	26300	15700	476	14	23	21	23
Biosolids **	Bolivar			8.5		11.1	48	10700	5860	171	25.1	14.8	21.4	11.1

\*For pH and EC, samples were analysed on a 1:5 soil:solution ratio in duplicate. Reported values are based on the average.

*Abbreviations:* EC = electrical conductivity; MWHC = maximum water holding capacity; CEC = cation exchange capacity

\*\*Biosolids from the Bolivar wastewater treatment plant were produced through a two-step feed plug flow design with mesophilic anaerobic digestion (average temperature of 34°C and a sludge retention time of 7 days) and sludge stabilisation lagoons.

Table S 2. Rates of C (glucose) consumption in the different soils and treatments.

Rate of C consumption ( $\mu\text{g C}$ respired per g of soil per hour) <sup>a</sup>			
		Average from the slope of the line <sup>b</sup>	Standard error
Glucose	S1 G	12.0	0.4
	S2 G	16.8	0.9
	S3 G	14.7	1.0
Toluene + glucose	S1 TG	11.8	0.6
	S2 TG	16.1	0.5
	S3 TG	15.2	0.9
Synthesis matrix + glucose	S1 SG	7.3*	1.1
	S2 SG	13.6	1.2
	S3 SG	10.8	1.2
C <sub>60</sub> + glucose <sup>c</sup>	S1 C60G	10.3	0.7
	S2 C60G	13.0	0.6
	S3 C60G	15.0	1.3
Unspiked (aged) <sup>e</sup> + glucose <sup>c</sup>	S1 UAG	10.4	0.3
	S2 UAG	14.2	1.0
	S3 UAG	11.7	0.4
C <sub>60</sub> OH + glucose <sup>c,d</sup>	S1 C60OHG	7.9	0.4
	S2 C60OHG	8.2	0.6
	S3 C60OHG	11.4	0.8
Synthesis matrix (aged) <sup>e</sup> + glucose <sup>c</sup>	S1 SAG	7.9	0.3
	S2 SAG	11.2	0.9
	S3 SAG	9.9	0.7

<sup>a</sup>Rates are reported from the average of n=4.

<sup>b</sup>The slope was taken from the data up to 7 days (168 h). See page S8 for calculations. \*linearity of this measurement was 0.89 when using data upto 7 days.

<sup>c</sup>from post SIR tests

<sup>d</sup>calculations corrected for potential contribution of hydrophilic C<sub>60</sub> following a rate of conversion of 1.5-2.1  $\times 10^{-5}$   $\mu\text{g C}$  per hour (per g of soil) (or 0.02-0.03 percent respiration per day)

<sup>e</sup> aged treatments refer to soils where synthesis matrix have been incubating for 28 days (the duration of the SIR test)