

## Implications of Fullerene-60 upon *in-vitro* LDPE Biodegradation

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Received: October 20, 2009 / Revised: December 23, 2009 / Accepted: January 8, 2010

**Fullerene-60 nanoparticles were used for studying their effect on the low-density polyethylene (LDPE) biodegradation efficiency of two potential polymer-degrading consortia comprising three bacterial strains each. At a concentration of 0.01% (w/v) in minimal broth lacking dextrose, fullerene did not have any negative influence upon the consortia growth. However, fullerene was found to be detrimental for bacterial growth at higher concentrations (viz., 0.25%, 0.5%, and 1%). Although addition of 0.01% fullerene into the biodegradation assays containing 5 mg/ml LDPE subsided growth curves significantly, subsequent analysis of the degraded products revealed an enhanced biodegradation. Fourier transform infrared spectroscopy (FT-IR) revealed breakage and formation of chemical bonds along with the introduction of  $\nu$  C-O frequencies into the hydrocarbon backbone of LDPE. Moreover, simultaneous thermogravimetric–differential thermogravimetry–differential thermal analysis (TG–DTG–DTA) revealed a higher number of decomposition steps along with a 1,000-fold decrease in the heat of reactions ( $\Delta H$ ) in fullerene-assisted biodegraded LDPE, suggesting the probable formation of multiple macromolecular by-products. This is the first report whereby fullerene-60, which is otherwise considered toxic, has helped to accelerate the polymer biodegradation process of bacterial consortia.**

**Keywords:** Fullerene-60, LDPE, consortium, biodegradation, FT-IR, simultaneous TG–DTG–DTA

In recent times, different kinds of nanoparticles made from pure metal (like nanoparticles of gold, silver, silicon, cobalt, etc.), metal oxide (silica, zinc oxide, iron oxide, alumina, titania, etc.), and carbon nanoparticles (fullerenes, etc.) are used in numerous applications such as in ceramics, polymer composites, filler materials, pigments, electronics, catalysts, and many others [21]. However, nanoparticles

have been shown to affect bacterial growth kinetics in both adverse and favorable ways, but the influence has been more profound against the bacterial growth. Barium nanoparticles have been shown to inhibit the growth of bacteria, fungi, mosses, and algae [9]. Titanium dioxide, silver, and gold nanoparticles are reported to have antimicrobial activities [3, 5], whereas cobalt-ferrite nanoparticles have been reported to increase the growth of *E. coli* and *C. xerosis* [4].

Carbon-based nanoparticles have potentially found widespread use in products such as cosmetics, drug delivery vectors, and semiconductors. In this perspective, fullerenes are known to exhibit a variety of pharmacological activities and also have industrial and synthetic applications. The most common and stable fullerene is Buckminster fullerene, having 60 carbon atoms. Fullerenes are mainly proposed to be useful in fullerene–polymer combinations, in electro-optical devices, and in biological applications [1, 17].

Plastic waste management is the area where this study tends to find a solution. Low-density polyethylene (LDPE) is widely used for manufacturing various containers, dispensing bottles, wash bottles, tubing, plastic bags, and various molded laboratory equipments. It possesses excellent resistance against dilute and concentrated acids, alcohols, bases, and esters, and accumulates at the rate of 25 million tons per year [15]. Thus, to deal with this environmental problem, biodegradation with the help of microorganisms appears to be the best choice, as land filling, recycling, and incineration have various environmental constraints. In this context, two efficient bacterial consortia capable of LDPE degradation has been reported earlier [10, 12, 18]. Moreover, one of these consortia has shown enhanced LDPE biodegradation in the presence of nanobarium titanate particles of size 38.0 nm [10]. The aim of this study was to determine the feasibility and extent of LDPE biodegradation in the presence of fullerene-60 nanoparticles, which otherwise have a toxic effect on bacteria. The present investigation would therefore help to increase the efficacy of plastic-waste biodegradation and prove to be an important step in easing out the problem of white pollution.

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## MATERIALS AND METHODS

### Polyethylene

LDPE beads were procured from Sigma-Aldrich Chemical Corporation, U.S.A. and converted into powdered form through boiling with xylene followed by solvent evaporation at room temperature (28°C). The powdered LDPE was successively washed with 70% ethanol, dried, and used for biodegradation studies as a primary carbon source. The above processing was done in order to increase the surface area for better degradation by the consortia.

### Fullerene-60 Nanoparticles

Fullerene-C60 (99.5%) was purchased from SES Research, Houston, TX, U.S.A.

### Bacterial Consortia

A total of six bacterial cultures [*viz.*, *Microbacterium* sp. strain MK3 (DQ318884), *Pseudomonas putida* strain MK4 (DQ318885), *Bacterium* Te68R strain PN12 (DQ423487), *P. aeruginosa* strain PS1 (EU741797), *P. putida* strain PW1 (EU741798), and *P. aeruginosa* strain C1 (EU753182)] were provided by the departmental culture collection of Microbiology, CBSH, G. B. Pant University of Agriculture and Technology, Pantnagar, India. These were originally isolated from different plastic waste disposal sites and artificial soil beds [10, 12]. The cultures were selected based on their pre-identified potential to degrade a variety of polymers like LDPE [10, 18, 19], HDPE [18], epoxy, and epoxy silicone blends [12]. These were characterized and developed into two different consortia in groups of three: consortium 1 comprising MK3, MK4, and PN12 strains, and consortium 2 comprising PS1, PW1, and C1 strains [10, 12]. The cultures were revived by inoculating into 10 ml of nutrient broth (HiMedia, India) and maintained on nutrient agar (HiMedia, India) at optimum pH (7.0±0.2) and temperature (37°C). A single colony from each culture was inoculated into 20-ml test tubes containing 5 ml of nutrient broth (pH 7.0±0.02) and active cultures were prepared by incubating the tubes at 37°C for 16 h with continuous shaking at 120 rpm [19]. The calculated amount (CFU/ml) of each strain was mixed for the development of consortium. However, the exact amount is not disclosed owing to the patent issues.

### Determination of Optimum Tolerance for Fullerene-60

The optimum concentration of fullerene tolerated by the respective consortia was determined by using Nutrient Broth (5 ml) incubated at optimum pH (7.0±0.02) and temperature (37°C) with continuous shaking (120 rpm) for 16 h. A 10% stock solution of fullerene was prepared by dissolving in xylene and added in minimal broth Davis w/o dextrose to make up the increasing concentrations from 0.01%, 0.25%, 0.5%, and 1% w/v, respectively. The solvent was allowed to evaporate and absorbance was recorded at 600 nm wavelength using a UV Visible Spectrophotometer after filtration of the suspended fullerene particles. The experiment was performed in triplicates and the values were expressed as their means.

### Comparative Growth Profiling in the Presence of Fullerene-60

The effect of fullerene-60 on the growth of both the consortia as well as their respective constituting bacterial strains was monitored in minimal broth Davis w/o dextrose. Fullerene-60 was sonicated for 2.5 min with 0.3 s repeating duty cycles (Labsonic U B. Brown, U.S.A.) to remove the cluster formation during storage. To study the

growth profiling, 300 µl of active log-phase cultures of individual strains and consortia were added separately to 100 ml of minimal broth in 250-ml Erlenmeyer flasks containing sonicated fullerene-60 at a fixed concentration of 0.01%. The flasks were incubated at 37°C with continuous shaking at 150 rpm. Samples were collected at regular intervals of 12 h and bacterial growth was determined by measuring the OD at 600 nm and colony forming units per milliliter.

### Comparative *In Vitro* LDPE Biodegradation Assay Using Fullerene-60

For the biodegradation assay, 100 ml of minimal broth (pH 7.0±0.02) was added to 250-ml Erlenmeyer flasks containing powdered LDPE at a concentration of 5 mg/ml [18]. The flasks were inoculated with 300 µl of active consortium. The assay was performed with respective positive (minimal broth+consortia) and negative (minimal broth+LDPE) controls in the presence and absence of fullerene-60 particles, respectively. The flasks were incubated at 37°C with continuous shaking (150 rpm). The assays were monitored for bacterial growth by measuring the OD at 600 nm after regular intervals of 1 day. The  $\lambda_{\text{max}}$  was also determined for suggestive changes in the broth due to polymer dissolution. Degraded samples were recovered from the broth after the consortium had reached the stationary growth phase.

### Recovery of Degraded Products

Degraded compounds were recovered from the broth after filtration and subsequent evaporation of the filtrate. The residue left after filtration was collected, and centrifugation of the filtrate was done at 2,348 ×g (5,000 rpm) for 15 min to remove bacterial biomass. The supernatant was kept in an oven at 60°C for overnight to evaporate water and the residual sample was recovered and analyzed by FT-IR and TG-DTG-DTA, taking pure LDPE as the control.

### Fourier Transform Infrared (FT-IR) Spectroscopy

The biodegraded samples obtained from the supernatant after the assay were analyzed by FT-IR and different peaks relative to CH<sub>2</sub> deformation, CH<sub>2</sub> bending (symmetrical), CH<sub>2</sub> bending (asymmetrical), CH<sub>2</sub> stretching (asymmetrical and symmetrical), CH<sub>2</sub> rocking, CH stretching, and C-O bond were compared, taking pure LDPE as a reference. Bending, stretching, and rocking vibrations have been depicted by  $\delta$ ,  $\nu$ , and  $\rho$ , with asymmetrical and symmetrical absorptions represented by subscripts "as" and "s", respectively. The spectra were recorded on a Perkin Elmer FTIR Spectrophotometer in potassium bromide (KBr).

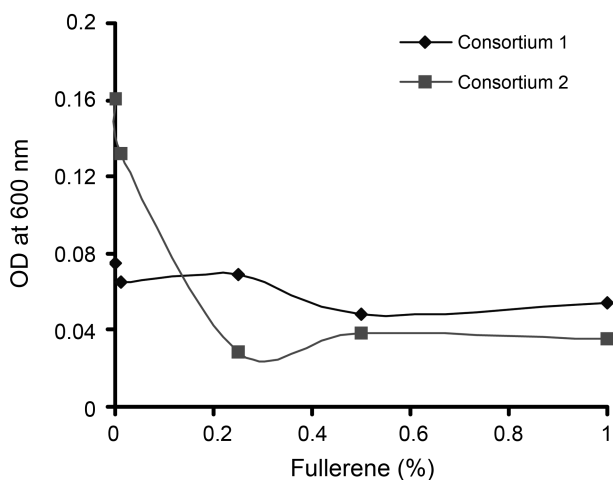
### Simultaneous TG-DTG-DTA

Simultaneous thermogravimetric-derivative thermogravimetry-differential thermal analysis (TG-DTG-DTA) was performed over a Perkin Elmer (Pyris Diamond) thermal analyzer under nitrogen atmosphere (200 ml/min) by subjecting the samples to a temperature range of 20°C to 550°C with a heating rate of 5°C/min on a platinum pan.

## RESULTS AND DISCUSSION

### Optimum Tolerance Level for Fullerene-60

Determination of the optimum concentration level for fullerene that could allow the growth of consortia was required in order to study its effect upon LDPE biodegradation. Of



**Fig. 1.** Tolerance level of the polymer-degrading consortia against fullerene-60 concentration.

the four concentrations 0.01%, 0.25%, 0.5%, and 1% (w/v), both the consortia exhibited a progressive decrease in bacterial OD (at 600 nm) with the addition of nanoparticles at increasing concentrations (Fig. 1). However, the addition of 0.01% fullerene-60 was found to moderately affect the bacterial growth and hence was selected as the optimum concentration for further experimentations. An optimum concentration of 0.01% nanobarium titanate has previously been reported to enhance LDPE biodegradation by using consortium 1 [10].

### Comparative Growth Profiling in the Presence/Absence of Fullerene-60

The bacterial growth curve was more or less subsided in the presence of 0.01% fullerene-60, irrespective of the bacterial culture used (Fig. 2). However, there was no significant influence on the lag phase, log phase, stationary phase, and death phase of the cultures, except for MK4, where the lag phase was found to be increased.

The CFU counts also showed a reduction when the cultures were grown in the presence of fullerene (Table 1). However, consortium 2 showed a similar pattern of decrease, but no negative influence of fullerene-60 was found on the growth (CFU/ml) of consortium 1. The mean generation time at the respective mid-log phases of the bacterial cultures showed an increase in the presence of fullerene as compared with control, except for PN12 (Table 1). However, when the consortia were grown in the presence of fullerene, only consortium 1 showed a slight increase in its generation time, whereas the rates were unaffected in the case of consortium 2. Nanoparticles have earlier been shown to affect bacterial growth kinetics. Fullerene-60 has been reported to have antibacterial activity [2, 11]. Silver nanoparticles have shown antibacterial and antiviral properties [13, 14], whereas cobalt-ferrite nanoparticles have been reported to increase

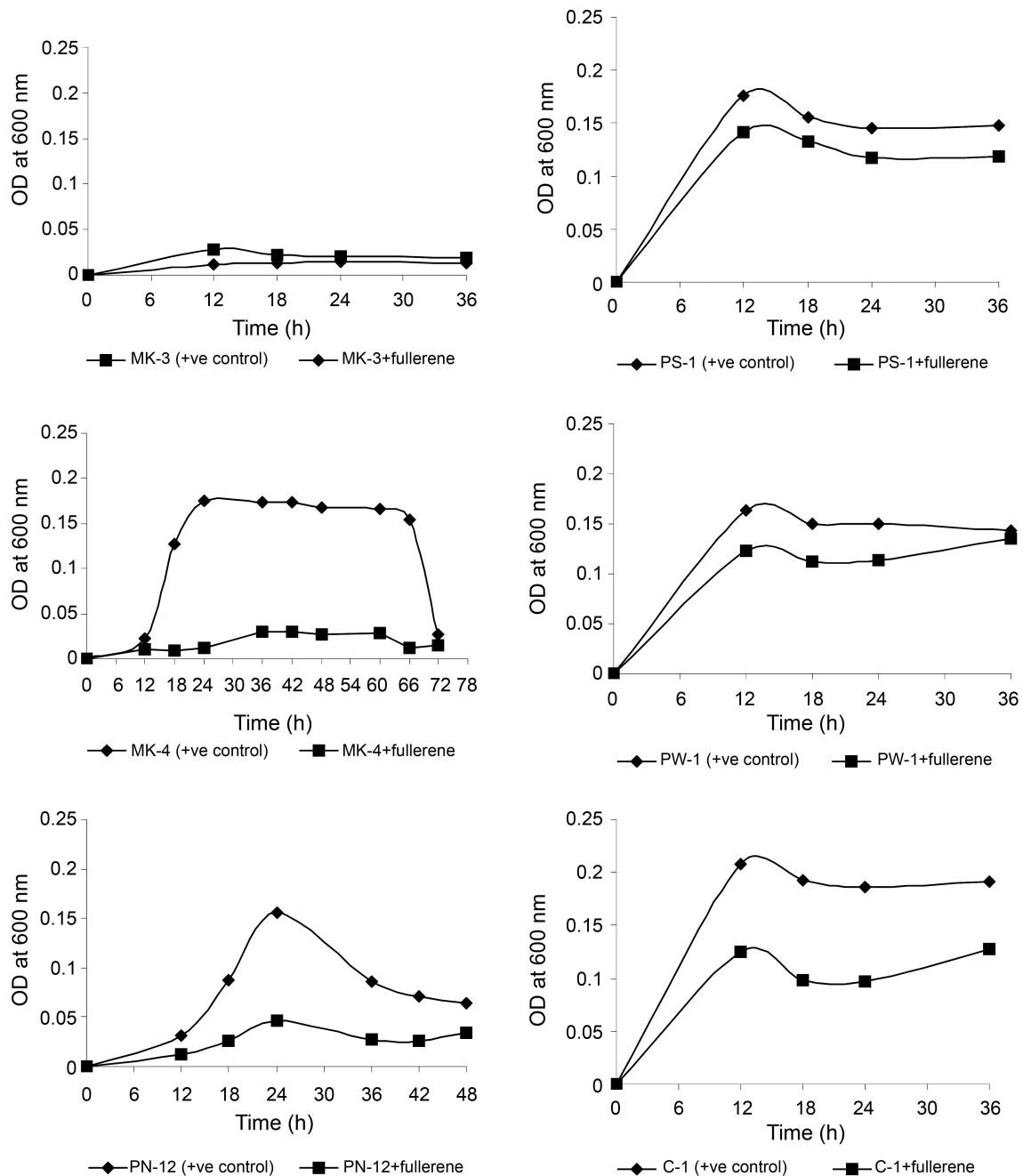
the growth of *Escherichia coli* and *Corynebacterium xerosis* [4]. Nanometric silicon particles have also been shown to alter the growth profiles of bacteria [16]. Several inorganic nanoparticles including silica, silica/iron oxide, and gold have been documented to exhibit no negative influence on the growth and activity of *E. coli* [22].

### Comparative LDPE Biodegradation Assay in the Presence of Fullerene-60

In the presence of fullerene-60 (w/ F-60), a significant decrease in the growth of both the consortia was exhibited in comparison with the positive control without fullerene-60 (w/o F-60) (Fig. 3). In the case of the LDPE biodegradation assays, a moderate increase in the growth of both the consortia was illustrated with the addition of LDPE in the absence of fullerene-60. However, in the presence of fullerene, addition of LDPE was found to insignificantly affect the growth of consortium 1, whereas consortium 2 illustrated a subsided growth curve. Interestingly, the duration of growth phases remained unaffected by the addition of either LDPE or fullerene into the medium. Comparatively, consortium 2 had a shorter lag phase and reached to the metabolically active log phase earlier than consortium 1. Moreover, the biomass of consortium 2 was found to be greater in the presence of LDPE and fullerene as compared with consortium 1.

The composite CFU/ml was further determined to measure the viability of the consortia during the assays. In case of consortium 1, the composite CFU was initially found to be  $230 \times 10^5$  CFU/ml and  $198 \times 10^5$  CFU/ml after 1 day of incubation in the absence and presence of F-60, respectively. The population was sustained throughout the assay, with  $212 \times 10^5$  CFU/ml and  $179 \times 10^5$  CFU/ml recorded after 5 days of incubation in the absence and presence of F-60, respectively. Similarly, in the case of consortium 2, the viable bacterial population showed sustained growth, recording counts of  $182 \times 10^7$  CFU/ml and  $123 \times 10^7$  CFU/ml after 1 day, and  $168 \times 10^7$  CFU/ml and  $112 \times 10^7$  CFU/ml after 7 days of incubation, in the absence and presence of F-60, respectively. The CFU determination revealed that the presence of LDPE has supported the growth of consortium, suggesting the assimilation of the polymer as a carbon source.

In the case of consortium 1, the  $\lambda_{\max}$  of LDPE was found to be constant at 209 nm for the first 2 days, which thereafter shifted to 220 nm after 3 days, finally attaining a constant value of 233 nm after 4 days. The shift in  $\lambda_{\max}$  suggests changes taking place in the polymer backbone between 2 to 4 days of incubation as a result of microbial action. However, in the presence of fullerene-60,  $\lambda_{\max}$  shifted from 209 nm to 207 nm after 3 days of incubation. On the other hand, in the case of consortium 2, a  $\lambda_{\max}$  shift from 209 nm to 224.97 nm was observed within 1 day, suggesting rapid changes occurring in the polymer backbone



**Fig. 2.** Comparative growth profiling of different bacterial strains in the absence and presence of fullerene-60.

during the log phase. The value of  $\lambda_{\max}$  became constant during the stationary phase, suggesting no significant changes in the chemical structure of LDPE during this period. However,  $\lambda_{\max}$  shifted from 209 nm to 212 nm in the presence of fullerene within 1 day.

The above findings suggested the differential influence of fullerene-60 upon the growth of two different polymer-degrading consortia. However, based upon an earlier study from our group, it was confirmed that nanoparticles

(nanobarium titanate) have an additive influence upon the polymer biodegradation process, which is attributed to their size, shape and physicochemical properties [10]. Therefore, fullerene-assisted LDPE biodegradation assays were further confirmed using FT-IR and simultaneous TG-DTG-DTA. Moreover, supplementation of polymers like LDPE, HDPE, polycarbonates, and epoxies have earlier been reported to increase microbial biomass as a result of their assimilation for cell-growth and multiplication [6, 12, 18–20].

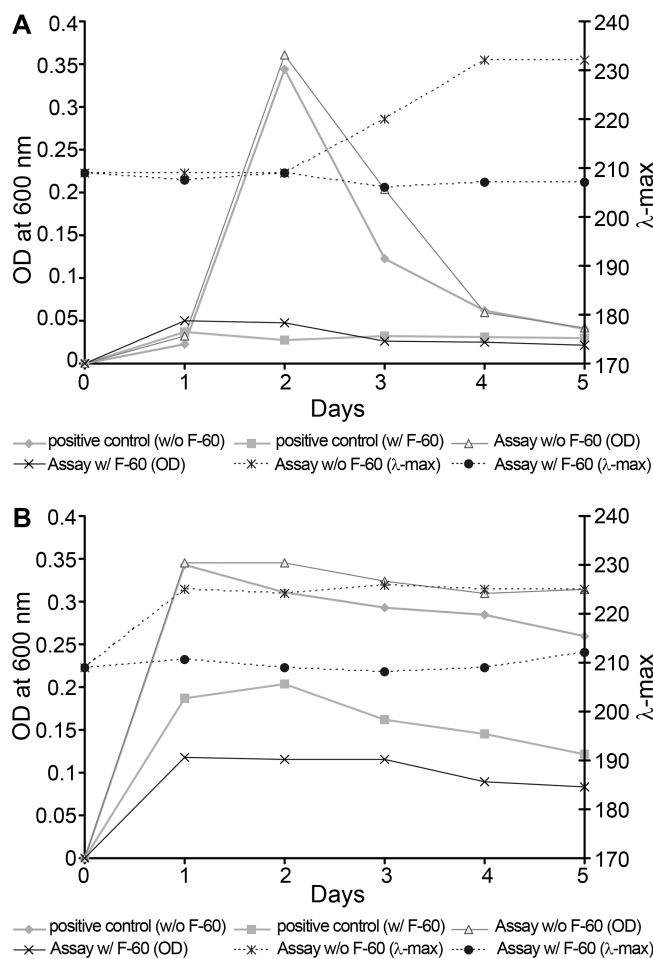
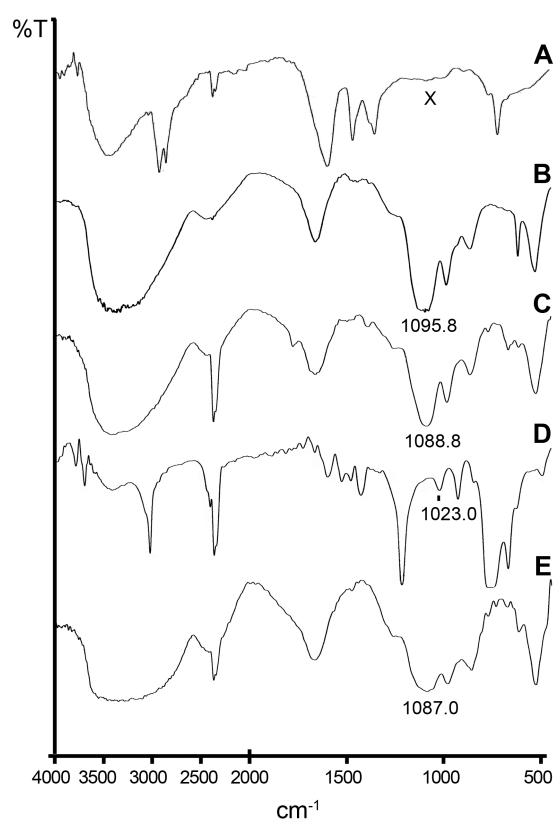
**Table 1.** Comparative *in vitro* growth statistics of the six bacterial strains and their respective consortia during mid-log phase in the absence and presence of fullerene-60.

S.No.	Bacteria/ Consortia	Absence of F-60		Presence of F-60	
		CFU/ml	Generation time (g)	CFU/ml	Generation time (g)
1	PN12	222×10 <sup>5</sup>	2.2	191×10 <sup>5</sup>	1.4
2	MK4	97×10 <sup>5</sup>	2.7	79×10 <sup>5</sup>	2.9
3	MK3	108×10 <sup>5</sup>	2.2	46×10 <sup>5</sup>	3.9
4	<b>Con-1</b>	<b>208×10<sup>5</sup></b>	<b>1.9</b>	<b>209×10<sup>5</sup></b>	<b>2.2</b>
5	PS1	180×10 <sup>7</sup>	0.9	78×10 <sup>7</sup>	1.4
6	PW1	177×10 <sup>7</sup>	2.6	162×10 <sup>7</sup>	3.2
7	C1	84×10 <sup>7</sup>	0.9	69×10 <sup>7</sup>	1.3
8	<b>Con-2</b>	<b>160×10<sup>7</sup></b>	<b>2.2</b>	<b>109×10<sup>7</sup></b>	<b>2.2</b>

**FT-IR Analysis of Biodegraded LDPE**

Pure LDPE has shown FT-IR absorptions (KBr, cm<sup>-1</sup>) corresponding to  $\rho$  CH<sub>2</sub> (720.2),  $\delta$  CH<sub>2</sub> (1,465.2),  $\delta$  CH<sub>3</sub> (symmetrical, 1,352.6), CH<sub>2</sub> deformation (1,595.1),  $\nu_s$  CH<sub>2</sub> (2,850.6),  $\nu_{as}$  CH<sub>2</sub> (2,919.9),  $\nu$  CH (3,426.0), and  $\nu_{as}$  CH<sub>3</sub>

(3,030.8) along with a pair of combination bands due to  $\delta$  CH<sub>2</sub> and  $\rho$  CH<sub>2</sub> at 2,151.8 and 2,368.0 respectively (Fig. 4A) [10, 18]. Biodegradation with consortium 1 in the absence of nanoparticles rendered LDPE with absorptions corresponding to  $\delta$  CH<sub>2</sub> (1,443.5), CH<sub>2</sub> deformation (1,658.9), and a pair of combination bands due to  $\delta$  CH<sub>2</sub> and  $\rho$  CH<sub>2</sub> at 2,370.3, respectively (Fig. 4B). This sample also showed an absence of various absorptions corresponding to methyl and methylene groups observed in pure LDPE.

**Fig. 3.** Comparative *in vitro* growth profiling and LDPE biodegradation assays in the absence and presence of fullerene-60 (F-60) by using consortia 1 (A) and 2 (B).**Fig. 4.** Comparative FT-IR spectra of LDPE degraded under *in vitro* conditions by consortia 1 and 2 in the absence (B and D) and presence (C and E) of fullerene-60, with reference to pure LDPE as control (A).

Furthermore, introduction of  $\nu$  C-O frequencies (1,095.8) was observed in the biodegraded samples owing to inclusion of O atoms into the hydrocarbon polymer backbone. Pure LDPE did not show  $\nu$  C-O frequencies.

Biodegradation with consortium 2 in the absence of nanoparticles also brought about significant shifts in the FT-IR absorption frequencies of LDPE as compared with the pure LDPE control. The degraded sample recorded newer absorptions ( $\text{cm}^{-1}$ ) corresponding to  $\delta_s$  CH<sub>3</sub> (1,215.9),  $\delta$  CH<sub>2</sub> (1,426.9), and CH<sub>2</sub> deformation (1,663.9) along with a pair of combination bands due to  $\delta$  CH<sub>2</sub> and  $\rho$  CH<sub>2</sub> at 2,361.0 and 2,401.8, respectively (Fig. 4D). Furthermore, inclusion of O atoms into LDPE, due to microbial action, introduced  $\nu$  C-O frequencies corresponding to 1,023.0  $\text{cm}^{-1}$ . In addition to this, deletion of frequencies corresponding to  $\rho$  CH<sub>2</sub>,  $\delta_s$  CH<sub>3</sub>,  $\nu_s$  CH<sub>2</sub>, and  $\nu_{as}$  CH<sub>2</sub> were also observed.

Similarly, LDPE degraded by each consortium in the presence of fullerene-60 in the minimal medium illustrated the absence of group frequencies corresponding to  $\nu$  CH<sub>3</sub> (asymmetrical),  $\nu$  CH<sub>2</sub> (symmetrical),  $\nu$  CH<sub>2</sub> (asymmetrical),  $\delta$  CH<sub>2</sub>, and CH<sub>2</sub> deformation as compared with pure LDPE control (Fig. 4C and 4E). Both the consortia were also found to significantly affect  $\delta$  CH<sub>3</sub> and  $\rho$  CH<sub>2</sub> group frequencies. FT-IR assignments revealed the presence of C60 in these assays from the absorption at 1,658.6 and 1,662.9  $\text{cm}^{-1}$ . Furthermore, the biodegradation of LDPE was ascertained by the presence of  $\nu$  C-O group frequencies at 1,088.8 and 1,087.0  $\text{cm}^{-1}$ , which were inflicted by consortia 1 and 2, respectively. The  $\nu$  C-O frequencies appear as a result of the introduction of O atoms into the hydrocarbon backbone of the polymers by the action of mono- and dioxygenases [8]. These bonds are more susceptible to attack by the microbial enzyme systems and might be responsible for the dissolution of polymers. Significant shifts in the FT-IR absorption frequencies of biodegraded LDPE and inclusion of hydroxyl (-OH) residues into the polymer backbone have also been reported in the presence of nanobarium titanate in the minimal medium by consortium 1 [10]. Changes in the FT-IR absorption frequencies have also been reported in the case of various polymers like LDPE, HDPE, and epoxy by different bacterial consortia [10, 12, 18–20]. Moreover, shifts in the CH and C=O stretching frequencies in polycarbonate spectra have been reported to be due to biodegradation inflicted by *Arthrobacter* and *Enterobacter* species [6]. Deviations in CH<sub>2</sub> frequencies along with a reduction in carbonyl index (*ca*) have also been reported in the FT-IR spectra of biodegraded LDPE by thermophilic bacterium *Brevibacillus borstelensis* [7].

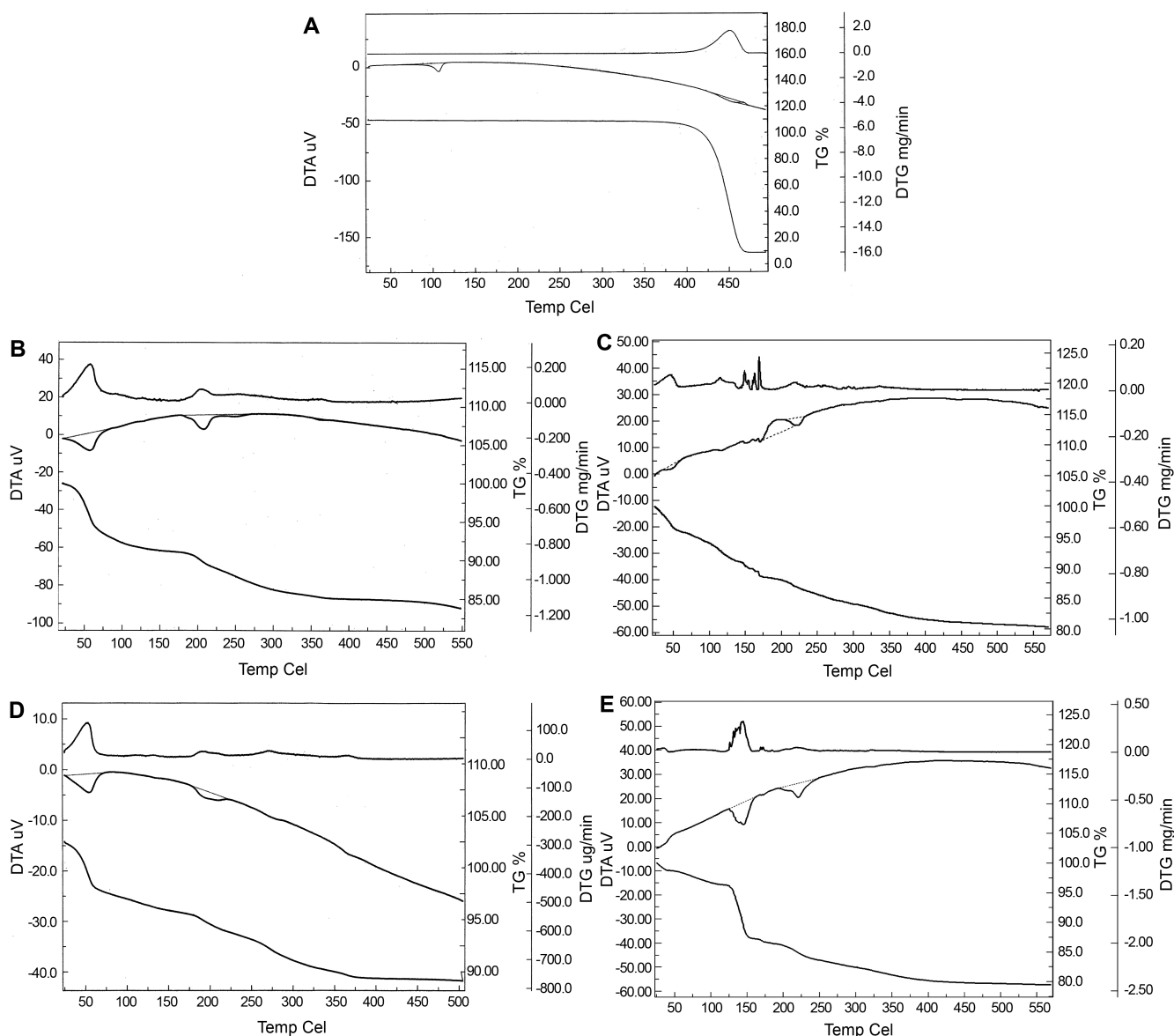
With the addition of fullerene-60 nanoparticles, the FT-IR profiles of degraded LDPE exhibited greater deformation along with inclusion of  $\nu$  C-O frequencies. However, in order to further verify the extent of biodegradation, thermal analysis was performed.

### Simultaneous TG-DTG-DTA Analysis

Decomposition of pure LDPE was observed in one step with a steep weight loss in temperature ranging from 400 to 466°C (Fig. 5). The related thermal data have been reproduced in Table 2. However, prior to this temperature, LDPE showed a DTA endotherm at 107°C with heat of decomposition ( $\Delta H$ )=129 mJ/mg. The steep weight loss range of LDPE was supported by a DTA endotherm at 457°C with  $\Delta H$ =-14.6 mJ/mg and a DTG at 451°C with rate of decomposition 1.79 mg/min. LDPE degraded by consortium 1 in the absence of nanoparticles has shown two-step decomposition. The first-step decomposition of LDPE was observed at 61°C with weight loss of 5.33%. This was supported by a DTA endotherm at 58°C with  $\Delta H$ =143 mJ/mg, and a DTG peak at 56°C with a rate of decomposition of 211.6  $\mu\text{g}/\text{min}$ . The second-step decomposition of LDPE was observed at 200°C with a weight loss of 9.74%, supported by a DTA endotherm at 207°C with  $\Delta H$ =120 mJ/mg and a DTG peak at 205°C with rate of decomposition of 71.4  $\mu\text{g}/\text{min}$ . In addition to this, a very weak and undetected DTG profile was illustrated corresponding to a DTA endotherm at 362°C with  $\Delta H$ = 5.03 mJ/mg.

In the presence of fullerene-60 with consortium 1, LDPE was found to degrade to a significant extent, as revealed by its five-step decomposition. The first step was recorded at 54°C with a weight loss of 3.8%, supported by a DTA endotherm at 50°C (2.30  $\mu\text{V}$ ) with  $\Delta H$ =34.7  $\mu\text{J}/\text{mg}$  and DTG peak at 45°C with rate of decomposition, of 70  $\mu\text{g}/\text{min}$ . This initial step was regressed to a lower temperature (54°C) as compared with the thermogram generated for biodegraded LDPE in the absence of fullerene-60 (61°C). The second and third steps were recorded at 100°C and 150°C, respectively, which were supported by DTG doublets at 114°C (57  $\mu\text{g}/\text{min}$ ) and 149°C (84  $\mu\text{g}/\text{min}$ ), corresponding to a pair of undetected, weak DTA endotherms. The fourth step occurred at 200°C (wt. loss, 12.1%), reported by a DTA endotherm at 195°C (20.42  $\mu\text{V}$ ;  $\Delta H$ =203  $\mu\text{J}/\text{mg}$ ) and a DTG peak at 169°C (rate of decomposition, 150  $\mu\text{g}/\text{min}$ ). The final (fifth) step was recorded at 251°C (wt. loss, 14.4%), supported by a DTA endotherm at 222°C ( $\Delta H$ =61.0  $\mu\text{J}/\text{mg}$ ) and a DTG peak at 221°C (rate of decomposition, 35  $\mu\text{g}/\text{min}$ ).

On the other hand, with reference to the single-step, steep weight loss in the temperature range of 400 to 466°C in the case of pure LDPE, the biodegraded sample recovered from the control assay (in the absence of nanoparticles) having consortium 2 exhibited two-step decomposition (Fig. 5, Table 2). The first-step was observed at 60°C with a weight loss of 4.33%, supported by a DTA endotherm at 55°C ( $\Delta H$ =79.5 mJ/mg) and DTG peak at 52°C with rate of decomposition of 125.7  $\mu\text{g}/\text{min}$ . The second step was observed at 178°C (7.06%), supported by a combination of DTA endotherms at 191°C and 209°C (combined  $\Delta H$ =



**Fig. 5.** Simultaneous TG–DTG–DTA of LDPE degraded under *in vitro* conditions by consortia 1 and 2 in the absence (**B** and **D**) and presence (**C** and **E**) of fullerene-60, with reference to pure LDPE as control (**A**).

43.2 mJ/mg) and a weak, undetected DTG profile. In the presence of fullerene-60, the resultant degraded LDPE exhibited a well-resolved two-step decomposition. A rapid first step was detected from 126°C to 155°C with a final wt. loss of 12.5%, supported by a DTA endotherm at 146°C (9.23  $\mu$ V) with  $\Delta H=167$   $\mu$ J/mg and a DTG peak at 144°C (322  $\mu$ g/min). A steady second step was recorded at 230°C (wt. loss, 15.7%), supported by a DTA endotherm at 221°C (20.52  $\mu$ V) with  $\Delta H=115$   $\mu$ J/mg and a DTG peak at 220°C (50  $\mu$ g/min).

Multiple-step decompositions have also been reported in the case of LDPE degraded by consortium 1 in the presence of nanobarium titanate [10]. Similarly, significant

changes in the thermal profiles of biodegraded samples of LDPE, HDPE [18], polycarbonate [6], non-poritized and poritized LDPEs [19], and epoxies and their silicone blends [12] have been reported earlier from our group.

Conclusively, the supplementation of fullerene-60 into the minimal medium rendered more efficient biodegradation of LDPE by both the consortia, as revealed by the higher number of decomposition steps, suggesting probable formation of multiple macromolecular by-products. Moreover, a nearly 1,000-fold decrease in the heat of reactions ( $\Delta H$ ) involved in the decomposition steps suggests significant weakening of the hydrocarbon polymer backbone. Comparatively, consortium 1 fared better than consortium 2 in LDPE

**Table 2.** Comparative thermal analysis of LDPE degraded under *in vitro* conditions in the absence/presence of 0.01% fullerene-60 by the two consortia with reference to undegraded LDPE control.

Consortium	0.01% Fullerene-60	DTG peak temp		DTA exotherm		DTA endotherm	
		°C	Rate (µg/min)	°C	ΔH (µJ/mg)	°C	ΔH (µJ/mg)
- (Pure LDPE control)	-	451	1,790.0	467	14.6	107 457	129×10 <sup>3</sup> 53×10 <sup>3</sup>
I	-	56	211.6	-	-	58	143×10 <sup>3</sup>
		205	71.4			207, 248	120×10 <sup>3</sup>
I	+	45	70	195	203	362	5.03×10 <sup>3</sup>
		114	57			50	34.7
		149	84			222	61.0
		169	150				
		221	35				
II	-	52	125.7	-	-	55	79.5×10 <sup>3</sup>
						191, 209	43.2×10 <sup>3</sup>
II	+	144	322	-	-	146	167
		220	50			221	115

biodegradation prowess with fullerene in the medium. This was clear from the higher number of well-resolved degradation steps in the thermal profile of LDPE degraded by the former (five) than the latter (two).

## Acknowledgments

This work is supported by a DBT grant to R.G. We are also thankful to CDRI (SAIF), Lucknow, India and Institute Instrumentation Centre, IIT Roorkee, India for the FT-IR and TG-DTG-DTA analyses, respectively.

## REFERENCES

- Bosi, S., T. Da Ros, G. Spalluto, and M. Prato. 2003. Fullerene derivatives: An attractive tool for biological applications. *Eur. J. Med. Chem.* **38**: 913–923.
- Chiron, J. P., J. Lamandé, F. Moussa, F. Trivin, and R. Céolin. 2000. Effect of micronized C60 fullerene on the microbial growth *in vitro*. *Ann. Pharm. Fr.* **58**: 170–175.
- Duran, N., P. D. Marcato, G. I. H. De Souza, O. L. Alves, and E. Esposito. 2007. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. *J. Biomed. Nanotech.* **3**: 203–208.
- Flores, M., N. Colón, O. Rivera, N. Villalba, Y. Baez, D. Quispitupa, J. Avalos, and O. Perales. 2004. A study of the growth curves of *C. xerosis* and *E. coli* bacteria in mediums containing cobalt ferrite nanoparticles. In: *Materials Research Society Symposium Proceedings, Vol. 820*. Materials Research Society, PA, U.S.A.
- Fu, G. F., P. S. Vary, and C. T. Lin. 2005. Anatase TiO<sub>2</sub> nanocomposites for antimicrobial coatings. *J. Phys. Chem.* **109**: 8889–8898.
- Goel, R., M. G. H. Zaidi, R. Soni, K. Lata, and Y. S. Shouche. 2008. Implication of *Arthrobacter* and *Enterobacter* species for polycarbonate degradation. *Int. Biodeter. Biodegrad.* **61**: 167–172.
- Hadad, D., S. Geresh, and A. Sivan. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol.* **98**: 1093–1100.
- Hayaishi, O. 2005. An odyssey with oxygen. *Biochem. Biophys. Res. Commun.* **338**: 2–6. Harwood Academics, Amsterdam.
- IPCS. 1990. In general, barium has been shown to inhibit the growth of bacteria, fungi, mosses, and algae. In J. Kost and D. M. Wiseman (eds.). *Handbook of Biodegradable Polymers*. Harwood Academics, Amsterdam.
- Kapri, A., M. G. H. Zaidi, and R. Goel. 2009. Nanobarium titanate as supplement to accelerate plastic waste biodegradation by indigenous bacterial consortia. *AIP Conf. Proc.* **1147**: 469–474.
- Lyon, D. Y., D. A. Brown, and P. J. J. Alvarez. 2008. Implications and potential applications of bactericidal fullerene water suspensions: Effect of nC60 concentration, exposure conditions and shelf life. *Water Sci. Technol.* **57.10**: 1533–1538.
- Negi, H., A. Kapri, M. G. H. Zaidi, A. Satlewal, and R. Goel. 2009. Comparative *in-vitro* biodegradation studies of epoxy and its silicone blend by selected microbial consortia. *Int. Biodeter. Biodegrad.* **63**: 553–558.
- Oka, M., T. Tomioka, K. Tomita, A. Nishino, and S. Ueda. 1994. Inactivation of enveloped viruses by a silver–thiosulfate complex. *Met. Based Drugs* **1**: 511.
- Oloffs, A., C. Crosse-Siestrup, S. Bisson, M. Rinck, R. Rudolph, and U. Gross. 1994. Biocompatibility of silver-coated polyurethane catheters and silver-coated Dacron® material. *Biomaterials* **15**: 753–758.
- Orhan, Y. and H. Buyukgungor. 2000. Enhancement of biodegradability of disposable polyethylene in controlled biological soil. *Int. Biodeter. Biodegrad.* **45**: 49–55.
- Perez, L., M. Flores, J. Avalos, L. S. Miguel, L. Fonseca, and O. Resto. 2003. Comparative study of the growth curves of *B.*



- subtilis*, *K. pneumoniae*, *C. xerosis* and *E. coli* bacteria in medium containing nanometric silicon particles. In: *Materials Research Society Symposium Proceedings, Vol. 737*. Materials Research Society, PA, U.S.A.
17. Prato, M. 1999. Fullerene materials. *Top. Curr. Chem.* **199**: 173–187.
  18. Sandlewal, A., R. Soni, M. G. H. Zaidi, Y. Shouche, and R. Goel. 2008. Comparative biodegradation of HDPE and LDPE using an indigenously developed microbial consortium. *J. Microbiol. Biotechnol.* **18**: 477–482.
  19. Soni, R., A. Kapri, M. G. H. Zaidi, and R. Goel. 2009. Comparative biodegradation studies of non-poritized and poritized LDPE using indigenous microbial consortium. *J. Polym. Environ.* **17**: 233–239.
  20. Soni, R., S. Kumari, M. G. H. Zaidi, Y. Shouche, and R. Goel. 2008. Practical applications of rhizospheric bacteria in biodegradation of polymers from plastic wastes, pp. 235–243. In I. Ahmad, J. Pichtel, and S. Hayat (eds.). *Plant Bacteria Interactions: Strategies and Techniques to Promote Plant Growth*. Wiley-VCH, Weinheim, Germany.
  21. Willert, M., R. Rothe, K. Landfester, and M. Antonietti. 2001. Synthesis of inorganic and metallic nanoparticles by miniemulsification of molten salts and metals. *Chem. Mater.* **13**: 4681–4685.
  22. Williams, D. N., S. H. Ehrman, and T. R. P. Holoman. 2006. Evaluation of the microbial growth response to inorganic nanoparticles. *J. Nanobiotechnol.* **4**: 3.