ChemPlusChem

A Multidisciplinary Journal Centering on Chemistry

Chemistry Europe

European Chemical Societies Publishing

Accepted Article

Title: Nitrogen Fixation and Biological Behavior of Nanodiamond Colloidal Solutions

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To be cited as: *ChemPlusChem* 10.1002/cplu.202000437

Link to VoR: https://doi.org/10.1002/cplu.202000437

WILEY-VCH

Nitrogen Fixation and Biological Behavior of Nanodiamond Colloidal Solutions

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Abstract

Detonation-produced nanodiamond, both as a powder (with adsorbed water) and especially when suspended in an aqueous colloid, can support the growth (both aerobic and anaerobic) of bacteria and fungi, which were isolated and identified by microbiological methods, optical and electron microscopy, as species of *Penicillium, Purpureocillium, Beaveria, Trichoderma* and *Aspergillus* genera. The C:N molar ratio of the developing fibers (comprising fungal mycelia with attached bacteria and entrapped nanodiamond) decreased from 25 to 11 between the 1st and 10th week of incubation (cf. 40 in initial nanodiamond, 4.6 typical for bacteria and 8.3 for fungi), and from 4 to <1 after 12th week, as the lysis of microorganisms releases carbon as CO_2 and nitrogen as NH_4^+ or NO_3^-. The nitrogen content of the colloid increased by an order of magnitude and more, due to fixation of N_2 by nanodiamond under ambient conditions, the process which requires water but not necessarily oxygen present.

Introduction

Nanodiamond produced by detonation of oxygen-deficient explosives (DND),[1] has many actual and prospective biomedical applications.[1-4] However, DND does not possess the extreme chemical, and near-absolute biological, inertness of bulk diamond. Nanomaterials generally can differ very substantially from the corresponding bulk solids, both in physical and chemical properties.[5] This applies *a fortiori* to DND particles, as these are not pure diamond, nor even pure carbon: the diamond core is surrounded by a shell of non-diamond carbon (the nature of which is still debated) and surface-terminating atoms or functional groups[1,2,6] the nature of which – and hence the biological properties of DND – strongly depend on the history of its preparation and purification.[7] A better understanding of the biochemistry of DND itself is therefore of crucial importance.

The surface of as-produced DND is terminated by oxygen-containing groups and is highly hygroscopic: even 'air-dry' DND usually contains a few per cent of water and under moist conditions can easily absorb up to its own weight of water,[8] full dehydration requires temperature well above 100°C.[9] The adsorbed water has peculiar structure and properties,[10] it is responsible, *inter alia*, for the remarkable electrophysical behavior of DND.[8] Such interfacial water is of immense biological importance in a variety of ways.[11,12] It is well known that adsorbed water can provide a suitable environment for microorganisms – although this requires thicker coats than a few molecular layers properly described as interfacial.

Fig. 1. 'Nanodiamond fibers' (NDF) formed in nanodiamond colloids (DW) due to growth of fungal mycelium

Recently we found[13] that the so-called 'nanodiamond fibres' (NDF, Fig. 1) forming spontaneously on storage in highly diluted aqueous nanodiamond colloids ('diamond water', DW),[14] are in fact biological entities (probably fungi) containing ND particles inside them,[15-17] as was demonstrated (indirectly) by various physico-chemical properties and by cell membrane staining, although the organisms involved were not identified. Besides obvious implications that these results may have for biomedical safety of DND (see above), they pose an intriguing question: as these organisms were thriving in the absence of any apparent source of nutrients other than DND itself, where did they obtain bioaccessible nitrogen? Could DND, with its so many other fascinating properties, also achieve what has been the target of so many catalytic chemists for a century – fixation of atmospheric N_2 under ambient conditions?[18] Advancing on the results of our previous work,[18] presently we systematically studied the microbiology of DND colloids, isolated and identified the species of bacteria and fungi participating in the development of NDF, followed the corresponding evolution of the chemical composition of NDF through the development and decay of microorganisms, explored the role of atmospheric gases and impurities in the process and demonstrated the development of NDF under anaerobic and oligotrophic conditions.

Results and Discussion

The visually clear DW with DND concentration of ca. 0.015 wt% was prepared by the standard technique we described earlier.[8] An alloy (1:1) of TNT-RDX was detonated inside an ice shell. The resulting soot was boiled with concentrated perchloric acid to dissolve the graphitic phase. To remove the excess of the acid, the solid residue (crude DND) was washed with boiling distilled water five times, to pH = 6.5-7, then dried at 160°C. 2 g of DND was sonicated in 1 L of double-distilled water for 1 h, left for several days until precipitation of a solid residue stopped and the solution became clear, then the clear layer of the liquid was syringed off and further cleared by centrifugation (at 15,000 rpm); all operations were carried out in an air-tight vessel.

DW was left to evaporate spontaneously at ambient temperature from open vials. Clusters of entangled fibers (NDF) resembling cotton wool, began to form after several days. For comparison, identical vials of distilled water were kept alongside; in these, no fibers appeared, thus confirming that the presence of DND was crucial for the development of microorganisms. Note that in crude DND, primary particles (*ca*. 5 nm) particles are usually connected through their vertices into larger (up to several μm) agglomerates of porous (framework) structure.[19] As no attempt was made to break up agglomerates into primary particles, the low DND concentration in DW was obviously limited by the amount of small particles naturally present in the crude product and segregation and deposition of larger particles due to Stokes' law.[20] To increase this concentration and thus to encourage NDF growth, we prepared DW from a slurry of crude DND ball-milled in water. Unexpectedly, although the starting DND concentration was thus increased to 1%, no NDF formed during evaporation: DW remained clear until dried down to 5-10% of the original volume, after that fine crystalline colorless sediment began to form, but still no fibers. The inhibition, similar to that produced by annealing DND powder at 300°C before mixing it with water or by adding an antibiotic to DW, has two possible explanations: ball-milling may either sterilize the DND powder through destruction of microbial cells and spores, or modify the surfaces (or surface-terminating functional groups) of DND particles in such a way as to prevent them from supplying nutrients to these microorganisms (see below).

To identify the microorganisms present in NDF, nutrient media (glucose-peptone broth, malt, oat meal, Czapek and aqueous agar) were inoculated with (i) as-prepared DND powder, (ii) DND after exposure to humid air for 24 h, (iii) DND sterilized in an autoclave (1 h at 1 atm), (iv) NDF from DW, (v) clear DW above NDF, (vi) sterilized DND dispersed in sterilized water, (vii) sterilized DND dispersed in distilled water and, as blank tests, (viii) distilled water kept in a flask plugged with cotton-wool, (ix) distilled water kept in an open flask for 2 h, and (x) freshly boiled water. All assays were incubated at 25ºC for 3 weeks and regularly monitored evolving colonies of fungi were isolated as pure cultures and identified by cultural-morphological characteristics, using standard compendia (keys).[21]

Investigation of NDF by optical and scanning electron microscopy, revealed entangled fungal mycelia (Fig. 2) entrapping dark particles (presumably DND) between them, as well as fungal spores (Fig. 3) identifiable by the diameter characteristic of the mycelium hypha (1.5-18.0 μm), and bacteria-like cells (Fig. 4) with the dimensions of 0.2 to 2.0 μm. Fungi growing in DW formed conidiophores with singular spores (Fig. 5a). Bacterial cells and fungal spores were observed in DW in free state. Hyphae formed bundles with lateral ties (Fig. 5b), which were visible to naked eye as fluffy flakes and slimy fibres up to 0.5 – 1.0 cm long. Thin rod-like and coccus-shaped structures were observed in NDF-containing DW, both on the surfaces of fungal mycelia (Fig. 6) and in loose state.

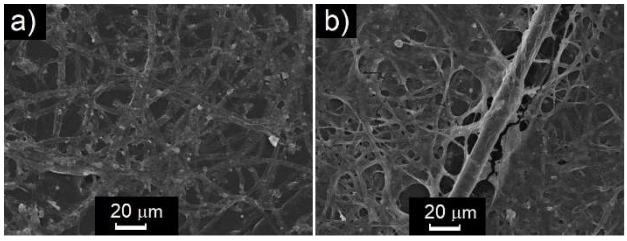

Fig. 2. Fungal mycelia and spores in the slime in NDF (observation by scanning electron microscopy of NDF samples)

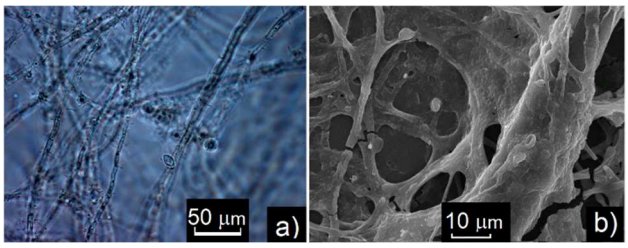

Fig. 3. Fungal hyphae and spores in NDF (a: light microscopy, b: scanning electron microscopy). The diameters of hyphae of various fungal species are 3,5 to 15 μm, spore sizes 2.5 × 4.5 μm

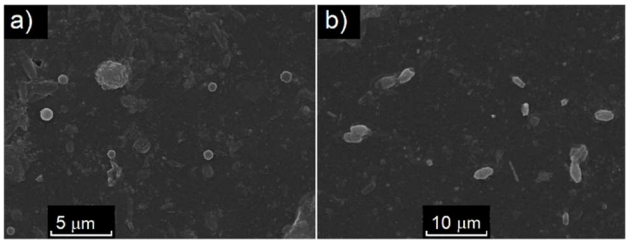

Fig. 4. Bacterial cells in NDF, by scanning electron microscopy: (a) cocci, diameters 0.25 to 0.75 μm; (b) rods, width 1 to 2 μm, length 2 to 4 μm

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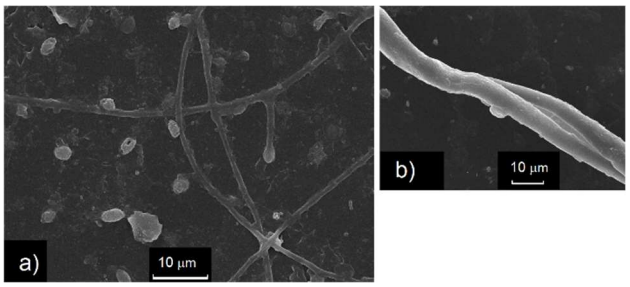

Fig. 5. (a) Fungal spores (2 × 5 µm) and hyphae (diameter 2.0 – 2.5 µm) with condiophores with forming spores; (b) associated growth of hyphae (diameter 7–8 µm) in NDF, from scanning electron microscopy

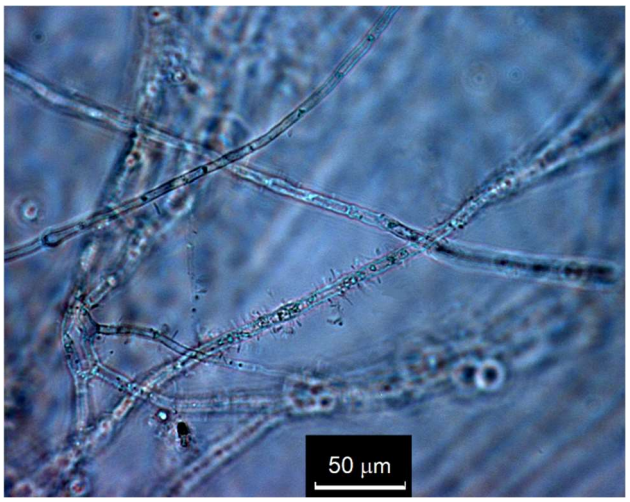

Fig. 6. Rod- and coccus-like cells on the surfaces of hyphae (most abundantly located on the hypha in the centre of the photograph); optical microscopy of an NDF sample

Culturing on nutrient media confirmed that NDF contained both fungi and bacteria. The assays inoculated with fragments of fibres, already after a few days showed robust growth and formation of various colonies of microorganisms on the surface of the nutrient. Several species of fungi were isolated, viz. *Penicillium cyclopium*, *P. aurantiogriseum*, *P. chrysogenum*, *Purpureocillium lilacinum* (Fig. 7a,b), *Beaveria bassiana, Trichoderma* sp., and some species characterized by dark-coloured mycelium with chlamidospores and light-coloured mycelium, as well as several types of bacterial mucous colonies (white, pinkish and light yellow), and colonies characteristic of *Bacillus mycoides*. Samples of visually clear DW also contained microorganisms, of which the most abundantly isolated were representatives of the genus *Penicillium* (Fig. 7c).

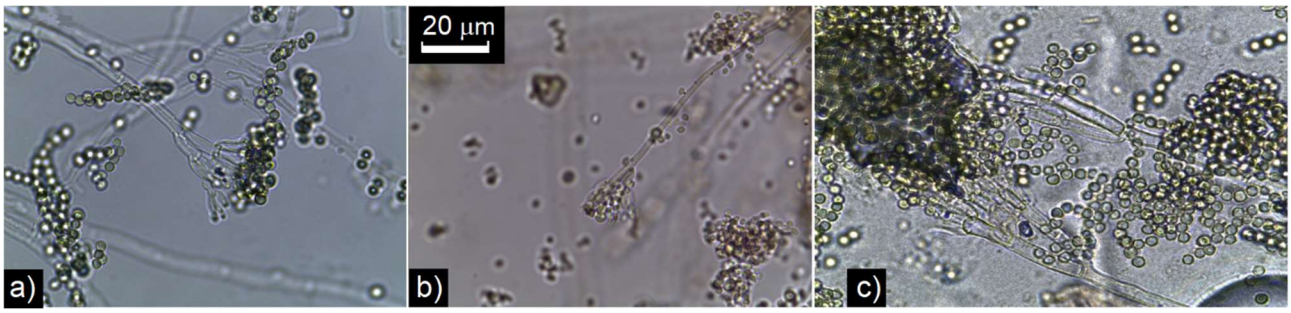

Fig. 7. Conidiophores and conidia of *Penicillium* sp. (a) and *Purpureocillium lilacinum* (b), isolated on nutrient medium from NDF; optical microscopy of pure cultures of *Penicillium* sp. and *Purpureocillium lilacinum*, respectively; (c) conidiophores and conidia of representatives of genus *Penicillium*, isolated from clear DW (optical microscopy of pure cultures cultured on malt agar)

Inoculation with freshly prepared DND (assay i) did not produce any growth and formation of colonies within 10 days, although separate bacterial cells and fragments of fungal mycelium were detected around powder particles; after 1 month of incubation, both microscopic fungi and bacteria were observed. DND powder which had been briefly exposed to humid air (assay ii), already after one week of incubation produced isolated colonies of representatives of the genus *Penicillium* (*P. cyclopium*), sterile white mycelium, *B. mycoides*, while during the third week, there evolved fungal colonies conspicuous for dark-colored sterile mycelium (Fig. 8). The sample of boiled water (assay x) yielded no microorganisms in the first two weeks of cultivation and a light-pink colony of bacteria in the third week. With distilled water pre-exposed to air (assay ix), microscopic fungus *Aspergillus niger* was noticed as early as the fourth day. Distilled water from a cotton-wool plugged flask (assay viii), yielded no microorganisms within the same period, but a longer incubation revealed both fungi and bacteria.

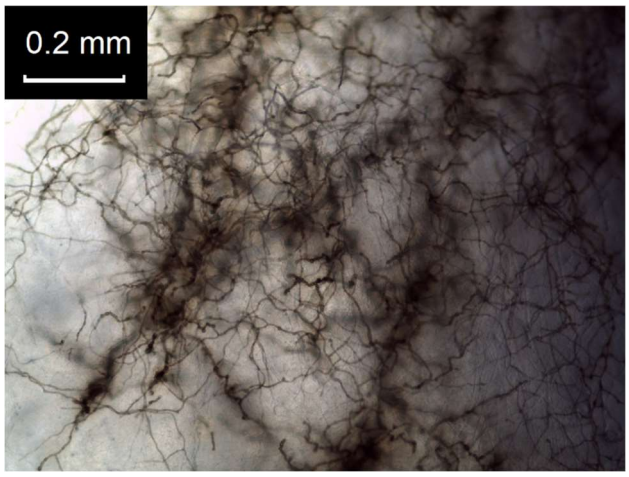

Fig. 8. Dark-colored mycelia of fungal species isolated from DND exposed to air

From the autoclave-sterilized samples of DND powder (assay iii) or the dispersions of the latter in sterilized (assay vi) or distilled (assay vii) water, neither fungi nor bacteria could be isolated,

even after prolonged incubation (1 month). On the other hand, when a non-sterilized DND powder was placed into sterile distilled water or a sterile sample was exposed to atmosphere for 1 to 3 h, a month later growing fungal mycelium and bacterial cells could be observed. On the surface of the mycelia, accumulation of DND particles was often found.

The above results agree with the assumption that the growth of NDF is initiated either by infestation of slowly evaporating DW by microscopic fungi and bacteria from the air, or by development of microorganisms which had previously infested DND powder during prolonged storage in non-sterile conditions. It is evident that 'air-dry' DND can host viable microorganisms for considerable time. This necessarily rises the question about the sources of nutrients for these organisms, and of the NDF stoichiometry generally.

The composition of starting DND presently used is shown in Table 1. The carbon content of 86.5% (which can vary up to ± 2% between different detonation experiments and purification methods) is mostly nanodiamond, with some contributions from amorphous sp^3-C and graphitic or ketonic sp^2-C on the surface.[8] Detonation-produced diamond particles contain ubiquitous admixtures of nitrogen (*ca*. 2 %), originating from nitro-groups of the explosives, and of hydrogen (*ca*. 0.5%). The balance is mainly oxygen (introduced mostly during oxidative purification), with small admixtures (yielding non-combustible residue) of iron (≤0.2%) and silicon (≤0.6%) which are always extracted by shock waves from the steel walls of the explosion chamber during detonation synthesis. Heavier impurities mostly concentrate in the sediment and are removed during purification of DW (see above), thus for NDF the balance is entirely oxygen.

Table 1. Composition of dried NDF, depending on their age

Age, weeks	sample	Elemental analysis, %				molar C/N ratio
		C	N	H	O	
0[a]	DND	86.5	2.15	0.5	*ca.* 10	40.2
2-3	1	40.1	1.6	1.95	56.35	25.1
	2	67.4	2.9	3.45	26.25	23.2
	3	27.2	1.3	1.8	69.7	20.9
	4	55.0	2.8	3.2	39.0	19.6
4-5	5	34.4	2.0	1.9	61.7	17.2
	6	69.6	4.2	1.2	25.0	16.6
	7	35.0	2.4	1.5	61.1	14.6
	8	52.1	3.6	1.3	43.0	14.5
6-9	9	42.6	3.4	3.0	51.0	12.5
	10	51.2	4.8	1.3	42.7	10.7

10-12	11	10.6	2.5	2.7	84.2	4.24
	12	4.7	2.2	2.1	91.0	2.14
13-16	13	15.7	14.9	3.0	66.4	1.05
	14	2.9	6.85	2.95	87.3	0.42
	15	3.6	13.5	4.3	78.6	0.27
	16	2.65	17.8	5.3	74.25	0.15

[a] Original DND, dried at 160ºC

As shown in Table 1, NDF (dried at 60°C) have substantially lower carbon content and higher nitrogen and oxygen content than the parent DND, shifting towards the compositions typical for fungal mycelium and spores, or for bacteria.[22-24] Generally, these changes increased with the age of the sample, albeit erratically, different samples progressing at widely different rates. This is not surprising, since infestation of the samples occurring accidentally from air, could involve different bacteria and /or fungi. In the mycelia of micromycetes, the nitrogen content can vary from 1.3% to 11% (depending on the abundance of nitrogen in the environment) and that of carbon from 44 to 57%.[22] The nitrogen content of bacteria is generally higher (4 to 15%) and less variable than in fungi.[23] The C:N molar ratio in biological matter (Redfield ratio) varies in the range 3 to 10 for bacteria and 4.5 to 43 for fungi,[22-24] a recent survey giving weighted averages of 4.6 and 8.3, respectively.[25] Thus, the compositions of 2- to 9-week old NDF (with C:N ratio from 25 to 11) are fit for a mixture of DND particles with such organisms, as argued above. This confirms our earlier conclusion that NDF are fungi occluding DND particles, in agreement with the refractive index of NDF (1.64-1.67)[13] being intermediate between those of DND (2.04)[13] and living cells (1.35-1.37),[26] and with TEM experiments where NDF were destroyed under the electron beam but left behind 'skeletons' of DND particles.[13] The latter result is remarkably similar to earlier observations[16] of fibers shrinking in volume under laser ablation to yield chains consisting of DND particles alone (and which probably led to subsequent misconception that these fibers were pure DND from the start).

However, after week 12, the carbon content of NDF dropped much below the 'biological' level, while the nitrogen content increased, ultimately giving the C:N ratio of <1. Probably, this reveals the decay of fungi and bacteria and the release of their organic carbon and nitrogen compounds as CO_2, NH_4^+ or NO_3^-, resembling that which occurs on certain stages of natural nitrogen cycles, viz. ammonification (mineralization) and nitrification.[27] This topic requires further investigation, which is currently in progress.

This build-up of nitrogen – without which no organisms can prosper – is the key to understanding the entire process. Four possible explanations can be considered:

1) NDF merely concentrates nitrogen impurities already present in DND (see above), without any overall accumulation of nitrogen. This can be rejected, as we found[13] that NDF can also grow in DW made with micro-powders ground from ordinary synthetic diamond prepared by high (static) pressure-high temperature treatment of C/Ni/Mn alloys (HPHT diamond), which does not contain nitrogen.

2) Trace amounts of ammonia and nitrogen-containing organic compounds, as well as proteins from airborne microorganisms, which are always present in the air (especially in inhabited buildings), can gradually build-up nutrient (including bioaccessible nitrogen) content in inorganic media to a level supporting the growth of bacteria,[28] which in turn can support fungi – notoriously capable to grow under oligotrophic conditions.[29] Could the same happen in DW during its long evaporation from open vials? To rule out this possibility, each vial of DW was accompanied by a control vial of pure (de-ionised) water, kept alongside the former under identical conditions and with equal exposure to air. Although some bacteria and/or fungi were microscopically observed in the control vials, and could be cultured (see above, assay), none of the control vials showed the *intense* fungal growth, visible to naked eye: the water remained apparently clear. Furthermore, even well-developed NDF transferred from DW into pure water, did not thrive. Thus, airborne nutrients were not sufficient. Indeed, we proved they were not even necessary, by allowing DW to evaporate without access of atmospheric air (Fig. 9). An air-tight vial with 20 ml of DW (sterilized by boiling for 1 h, or alternatively by γ-irradiation from a 1.3 MeV ^{60}Co source for 50 min) was placed into a quartz vessel containing annealed (at 400°C to constant weight) silica gel. The vessel was thoroughly purged of air by 5 cycles of vacuuming/refilling with high-purity argon, then filled with high-purity N_2 (maintained at 1.1 atm to keep out atmospheric air) and the vial was opened remotely. After two weeks, as the volume of DW in the vial decreased by half, NDF began to form. Thus, airborne nutrients, as well as oxygen, are not indispensable for their growth. It is noteworthy that some mycelial fungi are known to develop in anaerobic *and* oligotrophic conditions.[29, 30]

Repeating the same procedure under argon atmosphere instead of N_2, we also observed the formation of NDF, but in much smaller amounts (which precluded their detailed investigation) which in this case was probably limited by the small amount of nitrogen present in ND as surface-terminating groups.

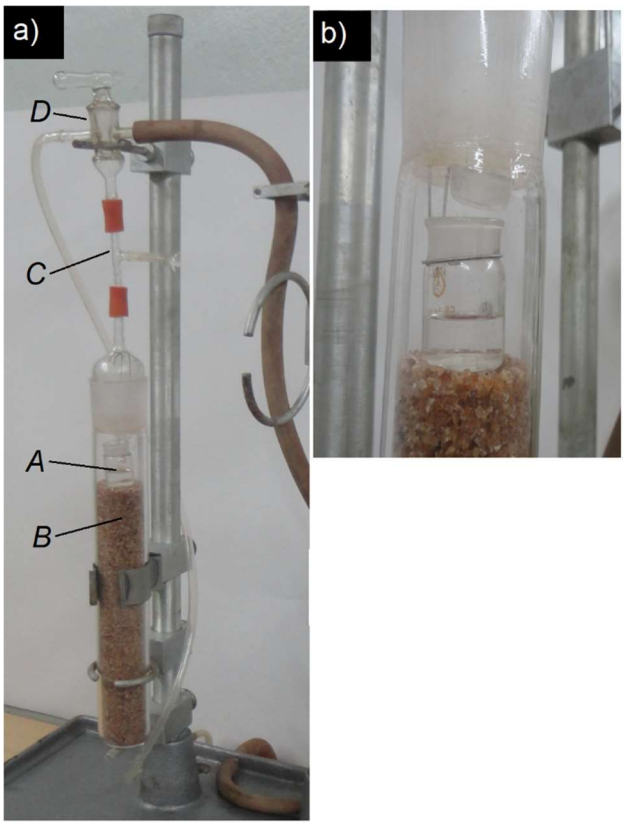

Fig. 10. Device for evaporation of DW under inert atmosphere. (a) General view: *A* – vial with DW, *B* – silica gel; (b) lid of the vial is lifted by a wire passing through the pipe *C* and vacuum valve *D*.

3) Presence in DW of N_2-fixing bacteria. Today it is known that nitrogen fixation is not an exclusive property of a few bacterial species, e.g. those symbiotic with leguminous plants,[31] but is much more widespread.[32] However, none of the microorganisms isolated from DW in the present study, has been tested so far for such ability. In any case, biological nitrogen-fixing is an extremely energy-consuming process, requiring an abundance of readily accessible organic compounds and monomeric substrates.[24,33] Thus, reduction of 1 mol of N_2 to ammonia requires (stoichiometrically) 16 mols of ATP,[32] actually *Azobacter* needs 50 g of hydrocarbons to reduce 1 g of N_2, and *Clostridium* needs up to 170 g.[33]

4) This leaves us with the only possible explanation: fixation of atmospheric N_2 by, or with the help of, DND. Indeed, as we have shown earlier,[18] bubbling pure N_2 gas through DW facilitates the growth of NDF; the fixation can proceed through the formation of dicyan from nanocarbon and N_2 (thermodynamically unfavorable with ordinary solid carbon, but possible with highly reactive nanocarbon), with the subsequent hydrolysis and/or oxidation of the latter to yield ammonia or nitrogen oxides, which were detected by mass spectroscopy.[18] While various reaction paths can be envisaged, it is important that water is always indispensable, whereas atmospheric O_2 is not, in agreement with the present observations. The process cannot be described as catalytic in

the narrow sense, as DND is not (fully) recycled. However, it is noteworthy that DND do catalyze certain reactions of organic compounds,[34] e.g. reforming ethanol[35] or azo-coupling,[36] while a hybrid system of nanodiamond cores and graphene shells showed high catalytic activity and selectivity over a long period of time for dehydrogenation of ethylbenzene.[37] The catalytic properties of DND are still relatively little explored and may be very unusual.

It must be emphasized that this effect is very specific to diamond nanoparticles (whether DND or HPHT): analogous experiments with nanoparticles of sulfur, boron and even graphite gave no fungal growth.[18] Generally, studies of biological effects of nanomaterials usually focus on their cytotoxicity, either as a health hazard or a possible application (e.g. silver nanoparticles inhibiting both bacteria and fungi[38]). Biological studies of DND, while very extensive,[1-4] are mainly concerned with its biomedical applications (drug delivery, intracellar probes, etc.) and therefore with biocompatibility. Not surprisingly, the *reported* effects of nano-solids generally, and DND particularly, on living cells range from indifferent to detrimental. But even allowing for this bias, the ability of DND to *facilitate* bacterial and fungal growth is quite unusual and is obviously related to the widely variable and still imperfectly understood structure of the particle surface and its immediate environment. For example, technical-grade DND from Plasmachem GmbH acquired bactericidal properties after high-temperature oxidative treatment, whereas the high-purity variety of the same product did not.[39] It may be hypothesized that in the former case, oxidation established on the DND surface a nanoscopic layer of reactive oxygen species (ROS), i.e. free radicals, peroxides, etc., known to be highly destructive to living cells.[40] The principal difference between these experiments and ours is that we use DND suspensions under equilibrium conditions which take a long time to establish, during which the DND particles covalently bind water and N_2 molecules with the formation of N–O, N–H or C–N bonds in the outer layer, thus neutralizing or blocking the ROS. The abovementioned fact that ball-milled DND ceases to stimulate microbial growth, may also be due to incidental surface modification. An important corollary is that more attention must be to *spontaneous*, rather than intentional, modification of DND surface (and even subsurface) layers during biomedical studies/applications. Indeed, it is amazing how often the elemental composition of DND is not monitored in such studies, on the over-confident assumption that 'diamonds are forever'.

The above results are relevant to Szent-Györgyi's[11] heuristic idea that solid surfaces with their layers of interfacial water could function as catalytic platforms for the synthesis of prebiotic compounds, and thus to the fascinating problem of the origin of life,[13,41-44] e.g. whether the nanodiamond occurring naturally in meteorites (and probably generated by shock waves, i.e. broadly similar to DND in origin) could be such a platform. Potentially, man-made DND can be used as a testing model for this hypothesis.

Conclusions and outlook

We have definitely confirmed the earlier hypothesis about biological nature of nanodiamond fibers (NDF) growing in aqueous colloids of nanodiamond ('diamond water'). Obviously, the colloids can be contaminated by airborne microorganisms during their preparation, which was carried out in non-sterile conditions. Contamination can come both from distilled water and from nanodiamond itself: the apparently air-dry, but hygroscopic, DND can be easily colonized by bacteria and fungi, for which the adsorbed water creates a propitious environment, and which can then develop in aqueous colloids prepared from such DND. Scarcity of bio-accessible organic compounds in these colloids cannot be an unsurmountable obstacle to the growth of fungi, as the latter can develop under oligotrophic conditions.[29] Fungal growth and the formation of flakes/fibres, was observed even with restricted supply of oxygen, when diamond water was purged with nitrogen gas – but micelial fungi able to develop in anaerobic, as well as oligotrophic, conditions, are also known.[30]

Nevertheless, it is significant that the presence of nano- and micro-particles of diamond in water greatly *facilitates* the growth of fungal mycelium and multiplication of bacteria. Prolonged incubation of nanodiamond powder in water results in the formation of fibrous, cotton-wool-like structures, comprising entanglements of separate or associated fungal gifs (mycelia) with bacterial cells attached to their surfaces, as demonstrated by direct observations by optical and scanning electron microscopy, as well as by isolating microscopic fungi and bacteria and culturing them in nutrient media. The fungi and bacteria discovered in the cotton-wool structures, were identified by the shapes and dimensions of these organisms, which correspond to the sizes of bacteria (from a fraction of μm to several μm) or fungi (from 2–3 μm to 10 μm for the gif diameter and spore size).

Isolation from NDF of microorganisms, particularly various species of microscopic fungi and bacteria, into pure cultures and their attribution to certain taxa according to cultural-morphological features, also proves that it is these organisms that form the cotton-wool-like structure. The microbial growth occurs simultaneously with, and probably because of, a build-up of the nitrogen content in the suspension from the surface of DND. Then, in the course of incubation the chemical composition of NDF evolves from that of DND towards that of biological matter, as far as the carbon/nitrogen ratio is concerned. At the end of incubation (after 10 weeks), as the sources of nutrients for fungi and bacteria are exhausted, their lysis causes the loss of carbon in the form of CO_2 and further reduction of the C/N ratio. Ultimately, the fibers yield unidentified product rich in nitrogen and oxygen but poor in carbon, characterization of which requires further work.

We can conclude that micro- and nanoparticles of diamond initiate fungal growth in aqueous media. The effects of nanoparticles on microorganisms are diverse, but most reported and researched are the *inhibition* of fungal and bacterial growth, e.g. by nanoparticles of silver and other metals.[38b] Diamond particles, on the contrary, apparently have stimulating effect or create conditions favouring microbial growth; this requires close attention and further investigation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Dr. T. Hawkins (Durham University) for helpful discussions.

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[15] It is probable that similar fibers were incidentally observed before but never characterized properly. The observations, originally reported in an unpublished DSc thesis (P.I. Belobrov, Physical Models of Supramolecular Self-Organisation, Krasnoyarsk, 1996, in Russian) and in a non-peer-reviewed journal, were brought to light through citations in influential reviews.[6, 16] In the absence of analytical data, these fibers were believed to be 1D chains of directly interacting DND particles, which prompted much theoretical work aiming to explain such interactions.[17]

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10.1002/cplu.202000437

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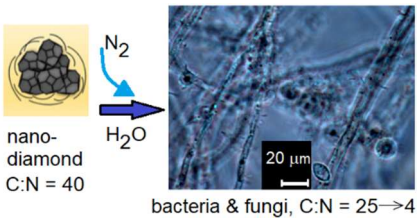

Nanodiamond in low-concentration aqueous colloids facilitates oligotrophic growth of bacteria and microscopic fungi (which does not occur in pure water or other nano-solid suspensions) through fixation of nitrogen, both from atmospheric air and pure N_2. The microorganisms were isolated and identified; the relative abundance of nitrogen vs carbon in the colloid steadily increases through their growth and lysis.