Oxidation of Graphitic Carbon in Certain Soils

Abstract. *Artificial graphitic carbon-14 was oxidized to carbon-14 dioxide in the presence of certain nonsterile soils. Treatment of these soils for the inhibition of biologic activity, by several methods including 5 megarads of electron-beam irradiation, yielded much-less-reactive systems in the oxidation of carbon. Intervention of a biologic agent in some of these oxidative processes is suggested.*

The gradual disappearance of carbonized residues from forest fires is usually explained as by erosion of this friable material into the surrounding soils. Another possibility is that erosion is supplemented by substantial oxidation of the carbon to carbon dioxide, since the thermodynamic equilibrium strongly favors this product (*1*).

$$C(s) + O_2(g) = CO_2(g)$$
$$\Delta F_{298^\circ} = -94{,}260; \Delta H_{298^\circ} = -94{,}052$$

This process, however, is extremely slow under usual conditions. The evidence of several early investigators suggests that the oxidation rate is considerably increased by the intervention of microorganisms (*2*). Most such evidence was obtained by microcalorimetry and by collecting CO_2 evolved from inoculated and sterilized systems containing "amorphous" carbon; in fact there is no evidence of the existence of such carbon. Gas carbon, charcoals, coke, kish graphite, lampblack, and soot are all microcrystalline forms of graphite; their differences primarily reflect the size of and irregularities in the rhombohedral lattice structure of the carbon network, such as layer-stacking defects, carbon bond isomerism defects, and edge defects (*3*).

In graphite, the carbon atoms are arranged in layers of fused hexagons, whose carbon-to-carbon bond distance is 1.415 Å. Graphite is therefore likely to exhibit more of the properties of a quinonoid than of an aromatic structure (*4*). The layers are separated by an interplanar distance, which, for the near-ideal case of Ceylon natural graphite, is 3.3544 Å; this spacing increases with increasing disorder of stacking, which is characteristic of most of the other forms of microcrystalline graphitic carbon (*5*).

The greater the extent of defects in the lattice structure of graphitic carbon, the more carbon atoms are exposed that have unsatisfied valence electrons, and thus the more reactive they can be expected to be; formation of carbon suboxides by these structures supports the validity of this conclusion (*6*). Thus, ample opportunity exists for the interaction of graphitic carbon with outside agencies, including microorganisms.

I now report on the oxidation of graphitic carbon to CO_2 in certain soils. I have demonstrated a substantially higher rate of oxidation in nonsterile soils than in treated controls. Microcrystalline carbon-14, prepared by the reduction of $^{14}CO_2$ with magnesium, specific activity of 0.026 mc/mmole, was obtained from New England Nuclear Company. After 3 hours of heating under highly reduced pressure, no radioactive volatiles could be detected in the liquid nitrogen trap. The carbon was then sealed in ampules under sterile conditions for storage under argon gas. This carbon contained 1.1 ± 0.3 percent impurities, composed mostly of silicon residues.

The following soils were used—two of them from forest-fire areas on the assumption that they would be enriched by the desired microflora: (i) soil from a timbered-area fire (Lowe Creek fire, 27 July 1962), Mt. Hood National Forest, 1067-m elevation; collected 4 December 1963 (*7*) and designated LCF; (ii) soil from a timbered-area fire caused by eruption and flow of lava on 5 October 1963, Napau Crater, Hawaii National Park; collected 5 December 1963 (*8*) and designated NCE; and (iii) (the control) Lane Library soil, Stanford University Medical Center; Yolo clay loam, 1- to 3-percent slope; collected 11 July 1963 and designated LLS; the most active of the three soils, possibly because of exposure to the carbon-laden atmosphere of an urban area (*9*).

Each sample was sieved to remove rocks and other grossly heterogeneous material, air-dried over filter paper, ground with mortar and pestle, sealed in glass ampules under aseptic conditions, and stored at 4°C.

Soils are notoriously difficult to sterilize and there is no fully reliable method of determining the extent of sterilization. Samples were treated by one of several methods, and, because no significant differences were observed among the methods, dry heating at 200°C for 72 hours in an argon atmosphere was used for most of the experiments I report.

Soils mixed with finely divided ^{14}C were incubated by inserting 1.0 g of soil plus carbon into the peripheral wall of a sterilized Pyrex Conway microdiffusion dish to which 1.0 ml of sterile distilled water was added; the resultant slurry was less than 1 mm thick at the bottom of the dish. Then 0.5 ml of 3*N* KOH solution was pipetted into the center well of the dish, which was sealed with a silicone-greased ground-glass cover.

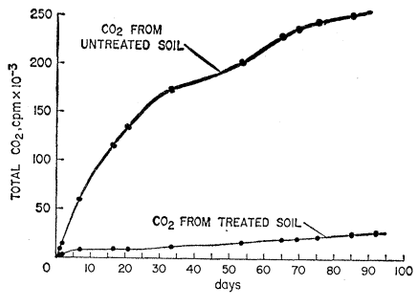
After incubation, KOH solution was transferred to a disposable plastic microdiffusion dish (*10*) having a partition which, when tilted a few degrees from the horizontal, permits the placement of two separate solutions in the outer well. The KOH solution was pipetted to one side, while 0.5 ml of 6*N* HCl was pipetted on the other side. Hydroxide of Hyamine 10-X solution (*11*) (0.5 ml) was inserted in the center well and the dish was sealed with a silicone-greased plastic top. The dish was then tilted away from the partition to release CO_2 from the neutralization of the contents in the outer well; the CO_2 was trapped in the Hyamine 10-X solution in a few seconds. After 1 minute, the dish was carefully opened and the Hyamine solution was quantitatively transferred to a 20-ml scintillation flask containing 9.5 ml of scintillation solution No. 1

Table 1. Carbon-oxidatic activity of soils. Carbon-14 added: 5 mg, 0.026 mc/mole (5×10^6 dpm/g). Treatment was by heating at 200°C for 72 hours under dry argon.

Radioactive CO_2 evolved (count/min)			Untreated: treated
Soil			
Nature	Untreated	Treated	
None		130	
LCF	1561	330	4.7
LCF	1584	358	4.4
NCE	20,505	4051	5.0
NCE	17,955	3530	5.1
LLS	15,190	4600	3.3
LLS	15,054	4628	3.2

Table 2. Effects on LLS soil of various treatments for inhibition of biologic activity; 24-hour incubation followed each treatment.

Treatment method	$^{14}CO_2$ Evolved per 24 hours (cpm)
None, no soils	320
None	19,250
200°C, dry, 72 hours	4820
135°C, steam, 1 hour	6440
Electron beam, 5 Mrad	4100
Chloroform, 24 hours	5060
Toluene, 24 hours	5210



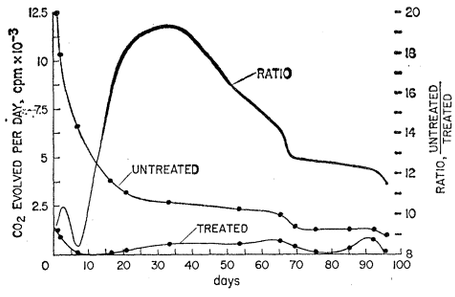


Fig. 1. (left). Total $^{14}CO_2$ evolved by LLS soil, treated and untreated. Fig. 2 (right). Specific activity per day of $^{14}CO_2$ evolved by LLS soil, treated and untreated.

(*12*). The solution was then assayed under standard conditions in a Packard Tricarb liquid-scintillation counter, model 3000; statistical error, within 5 percent.

The relative carbon-oxidatic activities of the three soils were determined by incubation for 24 hours at 22° ± 2°C, and the CO_2 was trapped and assayed by the technique described above; the results appear in Table 1. In other typical experiments, the ratio of a radioactive CO_2 has varied from 2.0 to 10.0, depending on the soil and the conditions of incubation.

LLS soil being easily available, it was selected for a long-term experiment. The contents of the KOH traps were removed at various intervals and assayed (Fig. 1); conditions were the same as those for the results in Table 1.

After 96 days, the total radioactivity trapped in KOH above the treated control was 2 percent of the total ^{14}C added as graphite. It seems reasonable to suggest that the substantial increase in the untreated : treated ratios of Fig. 2 is characteristic of soil enrichment by microorganisms capable of utilizing graphitic carbon.

It was essential to rule out nonbiologic catalysis of the observed oxidation of graphite in soils. Treatment of soils by each of the widely differing methods described in Table 2 should have had different effects on nonbiologic catalysts, yet should be equally able to destroy biologic activity at the cellular level; this proved to be so.

McLaren, Luse, and Skujins have shown that the bacterial population of a soil approaches zero when the soil is treated with a 2-Mrad dose of electron irradiation, and that 4 Mrad are required to insure complete sterility (*13*). Under such irradiation, however, phosphatase and urease activity is still manifest in the presence of suitable substrates. Table 2 shows that oxidation of graphite by soils was inhibited by all the methods of sterilization; apart from steam, all procedures yielded essentially the same results. This fact suggests that a biologic process was implicated, because a nonbiologic catalyst would not be expected to respond identically to the widely differing procedures used for sterilization.

The spontaneous formation of graphitic suboxides (*6*), especially at lattice defects, might well be carried through to complete oxidation to CO_2 by microorganisms. This process would expose additional carbon atoms to partial oxidation—again followed by complete oxidation to CO_2 by such organisms. Since this oxidative erosion of the graphitic macromolecule would selectively first remove the highly accessible lattice defects, the remaining graphite crystal should become progressively less reactive to these oxidations. An alternative possibility, based on the remarkably uniform activity ratio observed for any given soil, might be as follows: An agent, perhaps eventually of biologic origin, liberates or dislocates carbon atoms from the graphite matrix, which are then oxidized by what is terminally a nonbiologic process. Postgate has shown that such processes may occur in the reduction of sulfur by *Desulfovibrio desulfuricans* (*14*).

The expected progressive decrease in the rate of graphitic oxidation as a function of time is clearly demonstrated in Fig. 2.

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9. I am indebted to C. B. Van Niel for this suggestion.
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Polyribosome Disaggregation during Metaphase

Abstract. *Polyribosomes as examined by both sucrose-gradient analysis and electron microscopy disaggregate during metaphase in both normal HeLa cells and those arrested in metaphase by treatment with colchicine.*

Autoradiographic studies of cultured animal cells show that during mitosis there is a rapid decrease in the rate of protein synthesis (*1, 2*). In the case of HeLa cells, this depression is most pronounced during metaphase and anaphase (*1*) which together last 20 to 30 minutes (*3*). Since aggregates of ribosomes (polyribosomes) associated with messenger-RNA molecules are the site of nascent protein synthesis in many cell systems (*4, 5*), ribosomal organization, as determined by sucrose-gradient analysis and electron microscopy, has been investigated in metaphase cells.