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Plant responses to nano and micro structured carbon allotropes: Water imbibition by maize seeds upon exposure to multiwalled carbon nanotubes and activated carbon

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Abstract. Multiwalled carbon-nanotubes (MWCNT) and micro-structured carbon, such as biochar or activated carbon (AC), have been seen to significantly increase the growth indices of certain plant species such as maize (*Zea* mays L.). Seed imbibition is the stage where environmental factors that affect water transport across the seed coat barrier, make a large impact. This work explores the effect on water imbibition by maize seeds when the aqueous environment surrounding the seed is diluted by small concentrations (10 and 20 mg/l) of pristine MWCNT (p-MWCNT), carboxylate functionalized MWCNT (COO-MWCNT) and AC. The degree of sensitivity of the process to (i) large structural changes is seen by utilizing the nano (the MWCNT) and the micro (the AC) allotropic forms of carbon; (ii) to small changes in the purity and morphology of the p-MWCNT by utilizing 95% pure and 99% pure p-MWCNTs of slightly differing morphologies; and (iii) to MWCNT functionalization by using highly pure (97%) COO-MWCNT. Water imbibition was monitored over a 15 hour period by Near Infrared Thermography (NIRT) and also by seed weighing. Seed surface topography was seen by SEM imaging. Analysis of the NIRT images suggests rapid seed surface topological changes with the quantity of water imbibed. While further work is necessary to arrive at a conclusive answer, this work shows that the imbibition phase of the maize seed is sensitive to the presence of MWCNT even to small differences in the purity of the p-MWCNT and to small differences in the physicochemical properties of the medium caused by the hydrophilic COO-MWCNT.

Keywords: MWCNT; maize; activated carbon; imbibitions; infrared thermography

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1. Introduction

Recent work has demonstrated the efficacy of carbon nanotubes (CNTs) for plant growth, especially during the early developmental period. Most work has been done with multi-walled CNTs (MWCNTs), with pristine MWCNTs (p-MWCNTs) apparently showing the greatest relative effect in the early stages (till 7days) of seedling growth (Villagarcia *et al*. 2012, Khodakovskaya *et al*. 2009, Tiwari *et al*. 2014). In other works, chiefly on the growth of plants in the field, biochar or activated carbon (AC) have been seen to improve plant health (Bamberg *et al*. 1986, Pacek-Bienieck *et al*. 2010, Brennan *et al*. 2014). In the case of MWCNT mediated plant growth, improved water transport to the seed has been suggested as the driver of the growth mechanism (*ibid*.). Certain experiments reported in the literature purport to show that the CNT penetrates the seed coat, of which one consequence is improved uptake of water by the seed (Khodakovskaya *et al*. 2009, Lahiani *et al*. 2013). Water entry into the embryo within the seed – a process termed imbibition - triggers germination and subsequent plant growth by activating enzymes and hormones involved in the metabolisis of the stored food energy in the seed. However, all such studies have been conducted on seeds that are at least 1 day old (*ibid*.) whose drawback is that the long duration makes it likely that the seed coat could have opened at the point of radicle emergence or the seed coat could have developed cracks due to turgor pressure. Thus the CNT could have been carried in by the water through such openings. Studies on seed imbibition in a short time period before such openings develop, would therefore help to shed light on the question of whether the CNT is able to penetrate the seed-coat or not. The question is intrinsically interesting because the seed coat is several times thicker than the length of the CNT and its orifices variable in diameter, often lower than the outer diameter (OD) of the CNT and most certainly lower than the OD of the other allotrope of carbon, the AC. The process of imbibition is water diffusion and is therefore sensitive to factors in the aqueous medium that affect water transport. In this work we examine the water uptake during 12 hours of imbibition of maize (*Zea* mays L.) seeds, when the de-ionized (DI) water is diluted by small concentrations (10-20 mg/l) of p-MWCNT, by soluble carboxylate functionalized (COO-MWCNT) MWCNT 97% pure and by 99% pure AC. Marked differences between the aforesaid treatments and the Control would indicate that the carbon allotropes significantly alter the environment near the seed coat of the seed to affect the water imbibition process. The degree of sensitivity of the interaction with the p-MWCNT is investigated by using 2 grades of the p-MWCNT that only slightly differ in purity. One is 99% and the other 95% pure. A simple method of near infrared thermography (NIRT) was developed to observe seed water content and concomitantly, seed surface modification, non-destructively. Additionally, water uptake was also measured by physical weighing of the seeds. Presented here are the preliminary results.

2. Materials and methods

The p-MWCNT (95% and 99% pure), 99% AC and Agarose were purchased from Sigma-Aldrich Co- LLC, Missouri, USA while the 97% pure COO-MWCNT was obtained from the MWCNT synthesis laboratory of the U.M.S.N.H (Lara-Romero *et al*. 2017). Maize (sweet corn) seeds were purchased from a local seed supply company (Hortaflor, Rancho Los Molinos SA de CV, Mexico). The make and model of the instruments used for the study are mentioned in context in the following.

(A) Imbibition studies: 200 maize seeds were dried in the incubator (KitLab™ model EK-25) at 310 C for 24 h and their dry weights taken. The NIRT of the dry seed was taken as described in B) below. Next, an appropriate mass of each carbon allotrope was added to DI water to make up a concentration of 20 mg/l but only 10 mg/l for 99% p-MWCNT. The mixture was stirred slowly magnetically (~30 rpm) at ambient temperature (~240 C) for 18 h to create a suspension (a solution in the case of COO-MWCNT). 20 seeds were imbibed in 200 ml of each medium including the control (pure DI water), in covered plastic containers for 12 h at 300 C in an incubator. After fixed periods of time, a seed from each medium was extracted, rinsed in DI water, patted dry in absorbent paper, its fresh weight taken and then imaged by NIRT.

(B) Near infrared thermography (NIRT) and analysis: The sample stage was covered with an Al foil that had previously been spray painted with black acrylic paint. The stage was mounted on a Peltier element (Multicomp MCTE1-19913L-S) that maintained a uniform temperature (T) of 300 C. The IR camera used was from Testo Inc. Model 870-2 with a spectral range of 7.5-14 μm and an IR resolution 160×120 pixels. The IR camera was fixed with its objective at a distance 10 cm above the sample stage. The whole assembly was mounted on a black Al optical bread-board in a room maintained at near constant T and relative humidity (RH). The temperature/emissivity read-out of the camera's software was calibrated using a IR thermocouple (Fluke Model 566-2) such that the emissivity of the background was fixed at 0.97. This provided sufficient contrast to the emissivity of the imbibed seed (Fig. 1(a)). The colour digital image was imported into the software Mathematica 9.0 (Wolfram Research Inc.), converted to grey scale and a defined rectangle of grey pixels 121×151 was chosen in the centre of the seed's image (Figs. 1(b) and (c)) . The IR images showing changes of the seed due to water absorption, showed up better in the grey scale. Given

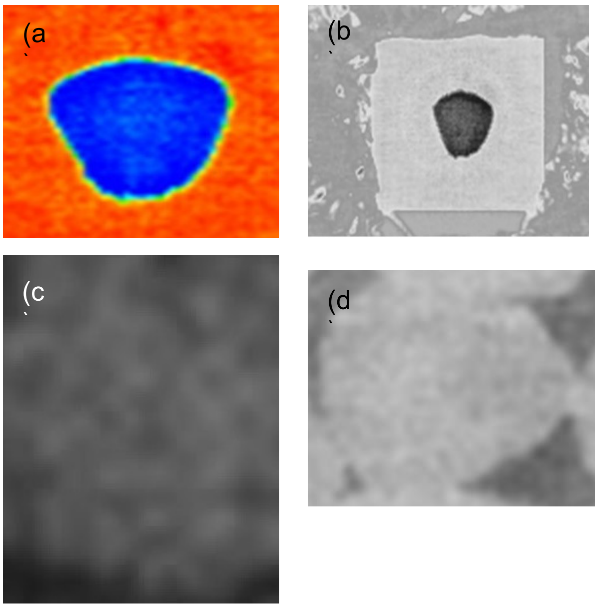


Fig. 1 (a) the NIRT photo of the maize seed imbibed in the presence of 20 mg/l of 95% pure p-MWCNT. The colors correspond to temperature, with blue being the coolest (on account of water absorption); (b) the same as (a) but converted to the grey scale; (c) the central portion of the seed that corresponds to the coolest region, magnified (see text for details). This section was analyzed by Mathematica 9; (d) the magnified NIRT image of the dry control maize seed at 30°C that each pixel contains a numerical value depending on its degree of grey and that this degree possesses a 1:1 correlation with the emissivity, the matrix of pixel counts in the rectangle were summed using Mathematica 9.0 to yield the measure of the emissivity of the seed surface as a function of time of imbibition.

3. Results and discussion

Fig. 2 concerns the water uptake by mass measurements during a 3 h imbibition period. It shows the beginning of a divergence between the treatments near the 3 h mark with 95% and 99%

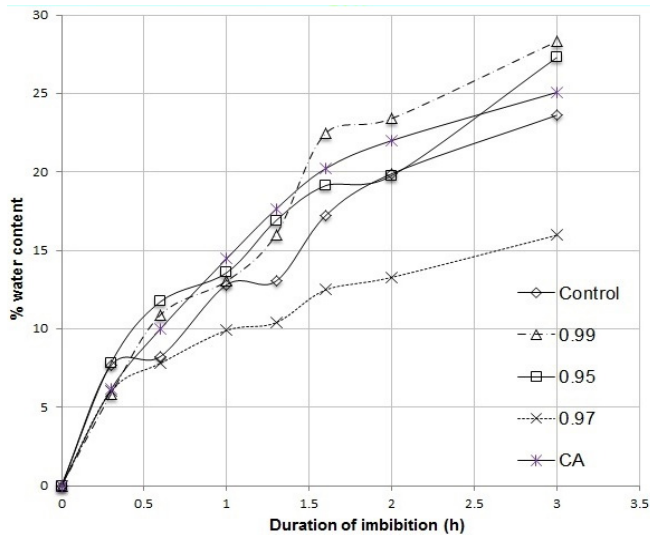


Fig. 2 The weight % of water absorbed by the maize seed with respect to duration of imbibitions as measured by mass. In the legend 0.99 corresponds to 99% pure p-MWCNT, 0.95 to 95% pure p-MWCNT, 0.97 to 97% pure COO-MWCNT, CA to Activated Carbon

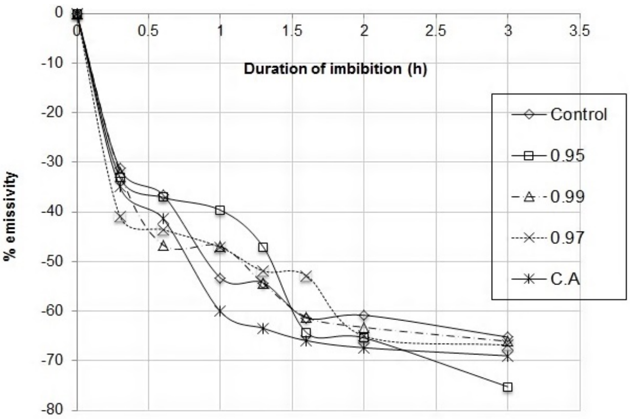
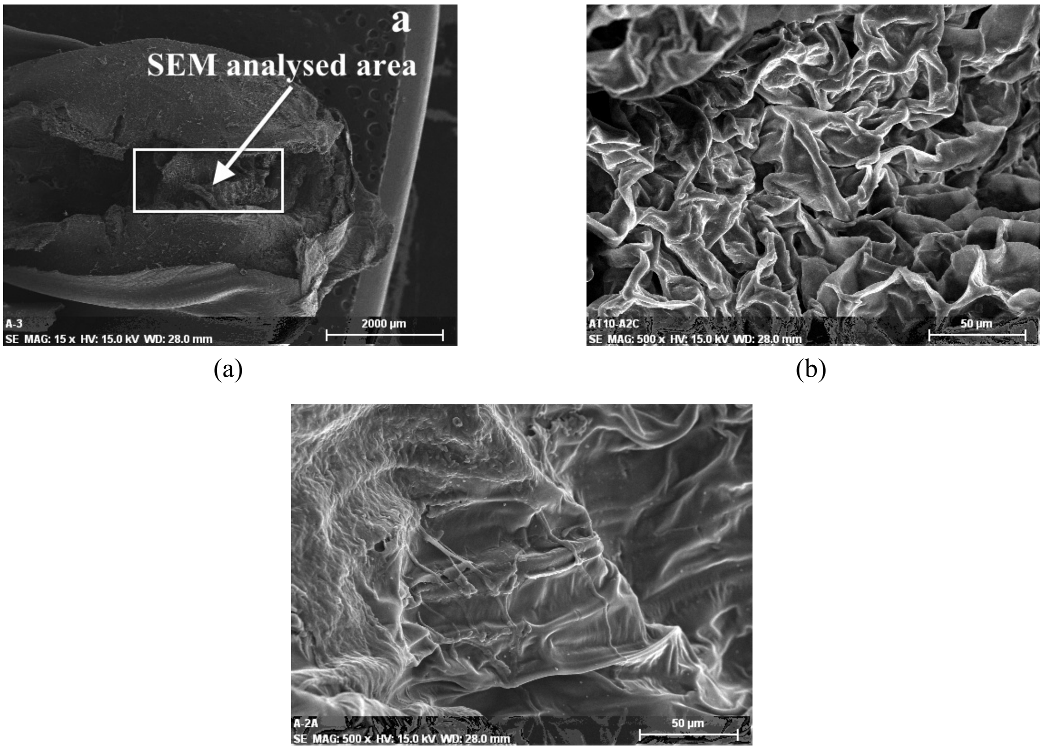


Fig. 3 The % NIRT emissivity of the maize seed as it imbibes water with respect the duration of imbibition

p-MWCNTs showing a tendency to cause more water imbibition and 97% COO-MWCNT the least. Passive diffusion drives the process. The pulsed variation is possibly due to volume changes of the expanding seed affecting the water concentration gradient. Figure 3 shows the NIRT of the same seeds. The surface emissivity drops with the expansion of seed volume upon the accumulation of water. It is also affected by the surface topography of the seeds and the emergence of exudates. Overall the profile of the NIRT mirrors the water content profile of Fig. 2, i.e., a progressively reducing emissivity signifies ~~a~~ progressively increasing water absorption. 95% p-MWCNT shows the least emissivity (largest water uptake) compared to the other treatments. The same pulsed variation at approximately the same points of time as in Fig. 2 show up in the NIRT but the fluctuations between the treatments are more pronounced. This shows that the NIRT is more sensitive to small changes in the seed's surface and volume characteristics than the gross weight measurements. The differences between the weight (Fig. 2) and NIRT (Fig. 3) measurements lie in the relative magnitudes of the water absorptions in the different treatments. Better resolution is achieved in the weight measurements than in the NIRT. Insofar as the current preliminary results are concerned, this indicates that for mass quantification, the conventional measurement by weight is better than the NIRT measurement. Fig. 2 shows that the water absorbed in the p-MWCNT treatments is the highest with the 99% showing a slightly higher level than the



(a) (b)

(c)

Fig. 4 SEM images of the seed body near the 'black layer of seeds germinated in agarose gel treated by 20 mg/l of p-MWCNT (95% pure). (a) Analyzed area; (b) Magnified image showing the corrugation of the testa and possible holes; (c) Magnified image of the black layer of the Control seed that does not show a similar corrugation

95%. The 97% COO-MWCNT shows the lowest water absorbance. It is as yet unclear why this should be so but the answer could lie in the lowering of the water activity by the solvated COO-MWCNT in contrast to the rest of the carbon allotropes that are all insoluble.

The question of the seed coat surface modification by the p-MWCNT (95%) was investigated by us using scanning electron microscope (SEM) imaging (field emission SEM, JSM 7401F, JEOL Ltd. Tokyo, Japan) of the region near the seed 'black layer' (Figs. 4(a)-(c)). The SEM images correspond to seeds germinated in pure agarose media (Control seeds) or agarose spiked with 20 mg/l of 95% p-MWCNT. Agarose is ~~an~~ inert water bound gel (Serwer 1983) with very low dissolved ions or nutrients and thus resembles water as the germination platform. The seed coat or testa clearly shows a surface roughening and possible perforations upon exposure to the MWCNT (Fig. 4(b)). The latter would certainly enhance water entry. The seed coat is of the order of several tens of micrometers in thickness and the orifices within it are tortuous with variable diameter of which a large fraction are smaller than the CNT diameter. Hence a priori, it would seem difficult for the CNT to penetrate the seed coat. The question of whether the CNT is able to penetrate the seed coat is still an open one with conflicting reports (Khodakovskaya *et al*. 2011, Ratnikova *et al*. 2015).

4. Conclusions

The imbibitions phase of the maize seed investigated in this work is sensitive to the presence of MWCNT even to small differences in the purity of the p-MWCNT and to small differences in the physicochemical properties of the medium caused by the hydrophilic COO-MWCNT. NIRT is a technique that captures variations in surface emissivity caused by the different extents of water imbibition and surface modifications caused by different types of nanocarbon (MWCNT) and microcarbon (AC). The highest purity p-MWCNT (99%) and the highly pure soluble MWCNT (COO-MWCNT) showed the highest and the lowest quantities of water absorbed. SEM images show seed surface modification in the presence of the 95% p-MWCNT but it is not clear whether these constitute perforations of the testa (seed-coat) caused by the MWCNT. Further work on the topic is warranted to answer these questions and conclusively establish patterns of imbibition under various conditions.

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