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Proceeding Paper

Green Synthesis of Luminescent Carbon Nanomaterials from *Porphyridium cruentum* Microalgae †

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Abstract: Microalgae (μAlgae) biomass was employed as a sustainable source for the synthesis of fluorescent carbon dots (μAlgae-CNDs) using a hydrothermal carbonization method and ethylenediamine (ED) as a nitrogen additive. The μAlgae-CNDs synthesized with a ratio of 0.64 of ED did not show cytotoxicity against non-tumor NIH 3T3 cells and sarcoma S180 cells, revealing some potential attractive properties for bioimaging.

Keywords: carbon dots; microalgae; eco-friendly; fluorescent; cytotoxicity; bioimaging

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1. Introduction

Over the past few years, microalgae (μAlgae) have emerged as a valuable source of renewable raw materials rich in high-value products suitable for a wide range of applications, such as animal nutrition, the production of dietary supplements for human consumption, etc [1,2]. The efficient photosynthetic capacity of μAlgae, along with their high growth rate and vast biodiversity, endow this biomass source with tremendous potential for the synthesis of carbon materials with highly diverse physicochemical and morphological characteristics [3,4].

In recent decades, the production of sustainable carbon dots (C-dots) from biomass of various origins, such as agricultural, industrial, and urban waste, has been extensively explored in the field of nanomaterials [5–7]. The recognized properties of C-dots, particularly their high luminescence, photostability, water solubility, and small size (diameters < 10 nm), coupled with their low toxicity and ease of surface functionalization, enabling the customization of their characteristics for various applications (e.g., nanomedicine, chemo/biosensing, photocatalysis, optoelectronics devices), have encouraged the development of diverse approaches for their synthesis [8].

In this communication, we report the primary results concerning the sustainable synthesis of carbon nanodots (μAlgae-CNDs) from industrially produced *Porphyridium cruentum* biomass using one-pot hydrothermal carbonization (HTC) methods. A preliminary evaluation of in vitro cytotoxicity in different cell lines will also be presented.

2. Materials and Methods

2.1. Materials

The biomass of *P. cruentum* microalgae was produced in Allmicroalgae's industrial facilities and was kept refrigerated at 5 °C until use. All reagents were used as received. Analytical-grade solvents were used and purified and/or dried by standard methods. In all experiments, we used ultrapure water (Milli-Q, Millipore, Burlington, MA, USA).

2.2. Methods

The hydrothermal carbonization of microalgae biomass was performed in a high-pressure reactor (Parr model 4560) equipped with pressure, temperature, and stirring sensors/controllers (Parr, model 4843) using an inox vessel.

FTIR spectra were taken on a Bruker Vertex 70 as KBr pellets. Band assignments were made by tentatively indicating the nature of the vibration [stretching (str) and bending (ben)].

Absorption measurements of the aqueous dispersions of CNDs were taken on a Jasco UV V-750 spectrophotometer using 1 cm quartz cells.

Excitation and emission fluorescence spectra were obtained using a Perkin Elmer LS45 fluorimeter with a 1 cm quartz cuvette in right angle (RA) geometry at 25 °C and air-equilibrated conditions. Fluorescence quantum yields (Φ_F) of aqueous solutions at 25 °C were measured using quinine sulphate in 0.01 M H_2SO_4 (Φ_F = 0.54) as a reference standard [9,10]. To prevent homo-inner filter effects, the optical densities of the samples and reference standard were kept below 0.05 at the excitation wavelength.

Microscopy fluorescence images were obtained using a fluorescence microscope (Olympus BX51, Feasterville, PA, USA).

2.2.1. Synthesis of μAlgae-Carbon Nanodots

The μAlgae-CNDs were synthesized from μAlgae biomass using ED as additive in different ratio ED/biomass, at 250 °C during 6 h, under air-equilibrated conditions. After cooling at room temperature, the reaction mixture was filtered through 0.2 µm cellulose membrane, generating light brown filtrate. The filtrate was extracted with organic solvents.

2.2.2. Cytotoxicity Assays

The highest fluorescent μAlgae-CNDs were selected for cell toxicity studies against normal and tumor mouse cells. NIH/3T3 (ATCC® CRL-1658) and Sarcoma 180 (ATCC® TIB-66) cells were treated with different concentrations of the μAlgae-CNDs and later incubated with tetrazolium salt (MTT) [11]. The blue formazan crystals produced by living cells were quantified in a microtiter plate reader at 570 nm and 650 nm, and cell viability (%) was expressed as a percentage relative to the untreated control cells.

3. Results and Discussion

3.1. Synthesis and Structural Characterization

Eco-friendly methods were applied to produce fluorescent μAlgae-CNDs. The effect of the additive (ED)/biomass ratio on the properties of the C-dots (e.g., fluorescence quantum yield) was explored, maintaining the reaction temperature (250 °C) and the dwell time (6 h) (Table 1).

Table 1. Effect of ED/biomass ratio on fluorescence quantum yield [1].

Entry	ED/Biomass Ratio	Φ_F (λ = 340 nm)
1	—	0.058
2	0.16	0.124
3	0.32	0.152
4	0.64	0.171

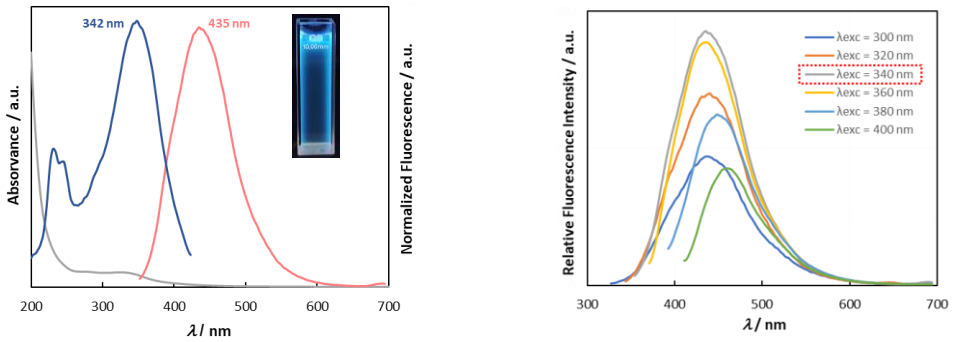
[1] Typical reaction conditions: μAlgae biomass (37 mgmL^{-1}), 40 bar, stirring, under air-equilibrated conditions.

The presence of ED as an additive proved to have an important impact on the optical properties of μAlgae-CNDs, progressively increasing its quantum yield (ca. a three-fold increase for 0.64 of ED compared to no additive presence; Table 1, entry 1 vs. 4).

The FTIR spectrum was used to assign the functional groups of CNDs, showing a broad band at 3415 cm^{-1} (O-H str), along with a N-H str shoulder at 3263 cm^{-1} and weak bands at 2958 and 2938 cm^{-1} (C-H, str), 1655 cm^{-1} (C=O, str and C=C, str, overlapped), 1458 cm^{-1} (CH_2, ben), and 1375 cm^{-1} (CH_3, ben).

3.2. Optical Properties

Ground-state absorption and steady-state fluorescence spectra are shown in Figure 1a. The excitation spectrum shows that the main chromophores responsible for the emission appear at around 232, 245, and 342 nm. When excited at 340 nm, the emission spectrum revealed a band with a maximum at 435 nm. Figure 1b shows that μAlgae-CNDs display variable emission maxima which are dependent on excitation wavelength.

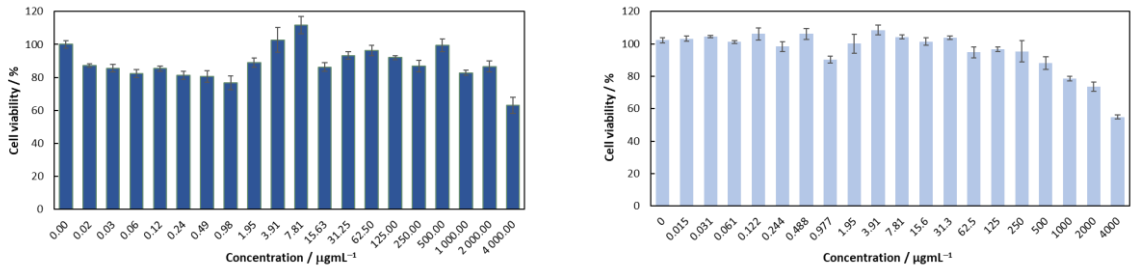


(**a**) (**b**)

Figure 1. (**a**) UV-Vis spectra (gray), excitation (blue, monitored at 435 nm), and emission (orange; λ_{exc} = 340 nm) of μAlgae-CNDs in aqueous dispersion (0.1 $mgmL^{-1}$) (inset: UV light at 366 nm); (**b**) Fluorescence emission spectra of μAlgae-CNDs in aqueous dispersion (0.1 mg mL^{-1}) with different excitation wavelengths. The red dashed line emphasize the excitation wavelength that has the highest intensity.

3.3. Cell Viability—Citotoxicity Evaluation

The in vitro cytotoxicity of the μAlgae-CNDs was studied using MTT assays against non-tumor NIH-3T3 cells and sarcoma S180 cells (Figure 2).



(**a**) (**b**)

Figure 2. In vitro cytotoxicity assay of μAlgae-CNDs against NIH-3T3 cells (**a**) and mouse sarcoma cell line S180 (**b**) (expressed by the decrease in cell viability).

The μAlgae-CNDs exhibited no cytotoxicity for the cell lines studied, and for the sarcoma S180 cell lines, the cell viability remained at 80–100%, decreasing only for concentrations above 2 × 10^3 μgmL^{-1}.

To explore interactions between μAlgae-CNDs and cells, after incubation for 18 h at 37 °C, fluorescence microscopy was also carried out with sarcoma S180 cells (Figure 3).

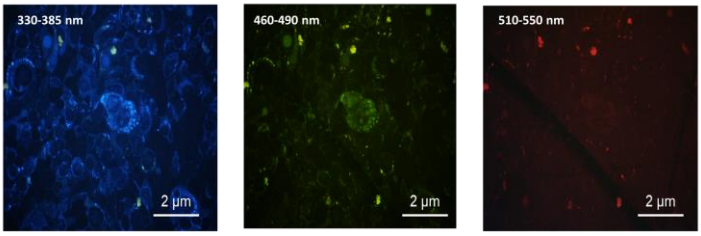


Figure 3. Fluorescence microscopy images of mouse sarcoma cell line S180 with μAlgae-CNDs (400×).

4. Conclusions

The biomass from *P. cruentum* microalgae was a suitable carbon source for obtaining fluorescent CNDs by sustainable one-pot methods. Higher photostability was attained, and fluorescent quantum yield was observed to be dependent on the additive/biomass ratio used in its synthesis. The as-prepared μAlgae-CNDs did not exhibit cytotoxicity in the animal cell lines tested, and the preliminary results reveal good interaction between the μAlgae-CNDs and the cell plasma membrane, as ascertained via fluorescence microscopy.

Author Contributions: Conceptualization, A.I.C., J.V.P. and P.D.B.; methodology, J.V.P., F.M.H.C. and M.L.L.; investigation, I.C., F.M.H.C. and M.L.L.; resources, A.I.C.; data curation, A.I.C., I.C., M.C.S., P.D.B. and S.M.; writing—original draft preparation, A.I.C., P.D.B. and S.M.; writing—review and editing, A.I.C., M.C.S., P.D.B. and S.M. All authors have read and agreed to the published version of the manuscript.

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