

# Synthetic Routes to Biomass-Derived Carbon Dots: A Mini-Review.

Vo Chau Ngoc Anh<sup>a</sup>, Do Mai Nguyen<sup>b\*</sup>

**Abstract:**

Biomass-derived carbon dots (BDCDs) have emerged as sustainable photoluminescent nanomaterials for sensing, bioimaging, photocatalysis, and drug delivery, offering advantages of low cost, low toxicity, and waste valorization. BDCD properties - including particle size, quantum yield, surface chemistry, and emission wavelength - are critically influenced by the synthetic route and processing conditions, rather than the type of precursor alone. This mini-review systematically compares bottom-up methods (microwave-assisted, pyrolysis, hydrothermal/solvothermal) and top-down approaches (arc discharge, laser ablation, chemical oxidation), evaluating their influence on material characteristics and functional performance. Representative examples with quantitative data illustrate how reaction parameters control structural and photophysical properties. Critical analysis reveals persistent challenges, including batch-to-batch variability, structural ambiguity (between carbon dots and carbonized polymers), energy consumption ranging from 0.5 to 50 kWh/g, and inconsistent reporting standards. The review extracts empirical design rules that link synthesis conditions to target properties, compares BDCDs with conventional fluorophores, and assesses their environmental credentials. Directions for advancing the field include the development of standardized protocols, integration with renewable energy, comprehensive toxicological evaluation, and application-driven optimization to enable the transition from laboratory proof-of-concept to scalable, reproducible BDCD technologies.

**Keywords:** *biomass-derived carbon dots, synthetic routes, bottom-up methods, top-down methods, green synthesis*

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<sup>a</sup> University of Medicine and Pharmacy, Hue University; 06 Ngo Quyen Street, Thuan Hoa Ward, Hue City. e-mail: vcnanh@hueuni.edu.vn

<sup>b</sup> University of Sciences, Hue University; 77 Nguyen Hue Street, Thuan Hoa Ward, Hue City. e-mail: nguyendomai97@gmail.com

\* Corresponding author.

## Introduction

CDs are zero-dimensional carbon-based nanoparticles, typically measuring less than 10 nm in size, with near-spherical shapes and abundant surface functionalities (Yang et al., 2022). The material was first observed as a fluorescent fraction during the purification of carbon nanostructures and has since been produced deliberately as photoluminescent particles (Singh et al., 2025). CDs have been valued because they can be synthesized by relatively simple routes, usually disperse well in water, and exhibit low cytotoxicity in many *in vitro* and *in vivo* studies (Farshbaf et al., 2018). Compared to organic dyes, their emission is often more resistant to photobleaching, and, unlike many semiconductor quantum dots, they avoid concerns associated with toxic metal cores (Holzinger et al., 2014; Zheng et al., 2025).

A wide range of carbon-containing precursors has been used to prepare CDs (Ng et al., 2021; Nguyen et al., 2020). In recent years, biomass feedstocks - such as agricultural residues, food waste, and plant-derived materials- have attracted particular attention. These sources are abundant and inexpensive, and their conversion into functional nanomaterials supports more sustainable use of resources (Šafranko et al., 2021). Natural heteroatoms present in biomass (e.g., nitrogen, sulfur, phosphorus) can be partially retained during carbonization, generating surface functional groups without the need for separate passivation steps. Such groups enhance water compatibility and introduce potential coordination sites for metal ions, which are beneficial for sensing (Cui et al., 2021).

This mini-review is organized to provide such an overview for biomass-derived carbon dots. First, the key structural and photoluminescent features of CDs that are most sensitive to synthetic control are briefly outlined, with an emphasis on size, emission behavior, chemical stability, and ease of functionalization. The main synthetic routes from biomass are then discussed in detail, with particular focus on bottom-up methods such as microwave-assisted treatment, thermal pyrolysis, and hydrothermal or solvothermal processing. Top-down approaches, including arc discharge, laser ablation, and chemical oxidation of carbonized biomass, are described more concisely to highlight their principles, advantages, and limitations when applied to biomass-derived precursors. Throughout the review, representative applications-such as fluorescence sensing, photocatalysis, and bioimaging-are used primarily as case studies to illustrate how synthetic parameters influence the performance of BDCDs. The discussion concludes with a summary of current challenges and prospects for improving synthetic control, reproducibility, and “greenness” in the preparation of biomass-derived carbon dots.

## Properties of the CDs

The photoluminescence and physical traits of carbon dots (CDs) have been used for fluorescence sensing. In biological media, the small size of CDs allows for cell entry and influences their behavior. A report by Sun demonstrated a size-dependent antibacterial effect against *E. coli* (Sun et al., 2021). Larger CDs displayed weaker activity, which was attributed to changes in cellular uptake and membrane distribution. Good biocompatibility and low toxicity have also been documented, supporting its repeated use in biological studies.

The fluorescence emission from CDs can be tuned, which enhances sensitivity and selectivity. Diao and co-workers prepared CDs from Syringa obata precursors under different conditions (Diao et al., 2018). Particles made without NaOH emitted blue light (quantum yield 12.4%; emission 425 nm at 340 nm excitation). Particles made with NaOH emitted green light (quantum yield 6.5%; emission 520 nm at 450 nm excitation). Both materials were applied to  $\text{Fe}^{3+}$  sensing. The blue CDs provided higher selectivity and lower detection limits for  $\text{Fe}^{3+}$ , whereas the green CDs showed no response to  $\text{Fe}^{3+}$  but were quenched by  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ . These outcomes indicated that synthesis choices can tailor CDs for specific analytes.

Stability has further supported the use of CDs as sensors. Wang prepared glucose-derived CDs and enhanced their stability through glutathione passivation using a hydrothermal route (C. Wang et al., 2015). The fluorescence intensity remained unchanged in the presence of many cations. Under xenon irradiation for 2 h, only an 8% decrease in intensity was recorded. Long-term storage caused no detectable loss of signal. In contrast, pH and temperature affected the emission, with higher values producing quenching and red shifts. These responses suggested possible sensing of pH and temperature. The same work also demonstrated that surface functionalization can be easily carried out to adjust properties.

Taken together, size control, tunable emission, chemical stability, and ready functionalization have supported the suitability of CDs for fluorescence sensing in environmental and biological systems.

## *Comparative Performance with Conventional Materials*

To contextualize the properties and potential of biomass-derived carbon dots, it is instructive to compare their performance with conventional fluorescent materials used in sensing, bioimaging, and photocatalysis. Three primary classes of comparison materials

are considered: metal-based quantum dots (e.g., CdSe, PbS), organic fluorophores (e.g., rhodamine, fluorescein), and metal nanoclusters (e.g., Ag, Cu).

### *Quantum Yield and Photostability*

Metal-based semiconductor quantum dots such as CdSe exhibit quantum yields in the range of 40-90% when optimally passivated with shells (e.g., CdSe/ZnS core-shell structures). These values exceed those of most biomass-derived carbon dots, which typically report quantum yields between 5% and 45%, with the majority falling in the 10-25% range. Organic fluorophores, such as rhodamine B and fluorescein, can achieve quantum yields of 30-95%, surpassing those of most BDCDs. However, BDCDs offer significant advantages in photostability. Organic dyes are prone to photobleaching under prolonged exposure to UV or visible light, with fluorescence intensity often declining by 50% or more within minutes to hours. In contrast, well-synthesized BDCDs can retain >90% of their fluorescence intensity after continuous UV exposure for several hours, and some reports document stable emission over days (Shandilya et al., 2021).

Metal nanoclusters (Ag, Cu) represent an intermediate case. These ultrasmall clusters (typically fewer than 20 atoms) exhibit quantum yields in the range of 1-15%, generally lower than the best BDCDs. While metal nanoclusters benefit from straightforward synthesis and size-dependent emission tunability, their photostability can be limited, particularly in oxidative or acidic environments. BDCDs synthesized via hydrothermal or pyrolytic routes have demonstrated comparable or superior photostability under similar conditions (Atchudan et al., 2023; Šafranko et al., 2021).

A critical distinction lies in the emission characteristics. Metal-based quantum dots exhibit narrow, size-tunable emission bands with full-width-at-half-maximum (FWHM) values ranging from 20 to 40 nm, enabling precise color selection and multiplexing. Organic dyes also display narrow emission peaks. In contrast, many BDCDs - particularly those synthesized at lower temperatures or via microwave routes-exhibit broad, excitation-dependent emission with FWHM values exceeding 100 nm. This excitation-dependent behavior, often attributed to a distribution of surface states and emissive trap sites, has been viewed as a limitation when color purity is required. However, it also represents a unique advantage: a single BDCD sample can generate multiple emission colors by varying the excitation wavelength, enabling multicolor applications from a single material without the need for physical separation or complex synthesis of multiple dot populations (Atchudan et al., 2023; Šafranko et al., 2021).

## Synthesis of biomass-derived carbon dots

Biomass has been utilized as a renewable energy source. It has also been adopted as a precursor for carbon dots because it is abundant, inexpensive, and considered more benign to the environment. Biomass waste has served as a low-cost source of carbon. Its use supports waste reduction because underused residues can be converted into materials with practical value. For these reasons, a strong interest in biomass-derived carbon dots has been maintained (Xia et al., 2019).

Since the first reports in 2004, several preparation routes for biomass carbon dots have been described. Methods are commonly categorized into two main groups: bottom-up and top-down strategies. In the top-down route, bulk carbon materials, such as graphite or activated carbon, are broken down into nanoscale particles. Techniques such as laser ablation, arc discharge, chemical oxidation, and electrochemical oxidation have been applied to achieve this size reduction (Arole & Munde, 2014). In the bottom-up route, small molecules or biopolymers are converted into nanoparticles by carbonization. Microwave heating, thermal pyrolysis, and hydrothermal or solvothermal treatments are commonly applied (Chandrasekaran et al., 2021). These methods have been used widely for preparing biomass-derived carbon dots. Procedures are usually simple and low-cost. Reaction conditions are milder and are viewed as more environmentally friendly. Surface functionalization can be carried out with relative ease. Top-down routes depend on bulk carbon sources, which limit their use with biomass precursors. Even so, several top-down techniques are noted in brief for completeness (Crista et al., 2020).

### *Bottom-up pathway*

**Table 1. Comparison of Bottom-Up Synthetic Routes for Biomass-Derived Carbon Dots.**

Parameter	Microwave-Assisted	Pyrolysis	Hydrothermal/Solvothermal
Typical Temperature	80-200°C	300-800 °C	120-250 °C
Reaction Time	1-10 mins	30 minutes - 3 hours	2-24 hours
Pressure	Atmospheric	Atmospheric/Controlled	Elevated (autogenous)
Representative Biomass	Palm kernel shell, sesame seeds, eggshell membrane	Bamboo leaves, mangosteen peel, turtle shells	Banana peels, sugarcane bagasse, jackfruit peel

Parameter	Microwave-Assisted	Pyrolysis	Hydrothermal/Solvothermal
Typical Particle Size	3-10 nm	2-7 nm	3-6 nm
Quantum Yield Range	8-44%	8-45%	6-25%
Surface Functionalities	-OH, -COOH, C=O, limited -NH <sub>2</sub>	-OH, -COOH, -NH <sub>2</sub> , C=O	-OH, -COOH, -NH <sub>2</sub> , C=O
Advantages	Short reaction time, simple equipment, tunable by medium	Solvent-free, high temperature enables doping, and crystalline products are possible	High QY, mild conditions, good reproducibility, aqueous processing
Limitations	Variable QY, equipment-dependent, amorphous products	High energy consumption requires high temperature, limited biomass applicability	Long reaction time, requires autoclave, energy intensive
Ref	(Ang et al., 2020; Roshni & Divya, 2017)	(Aji et al., 2017; Fahmi et al., 2018)	(Atchudan et al., 2020; H. Liu et al., 2021)

Microwave energy has been utilized to produce carbon dots, making it a favored approach among bottom-up routes due to its short reaction times (Ng et al., 2021). In 2018, Jusuf et al. prepared eggshell-membrane-derived carbon dots through a one-step microwave treatment of charred membrane for the degradation of methylene blue (Jusuf et al., 2018). Particles obtained in NaOH solution showed an average diameter of  $3.88 \pm 0.56$  nm, while particles formed in water averaged  $4.46 \pm 0.77$  nm. The NaOH sample was mainly spherical, whereas the water sample was irregular in shape. Similar surface functional groups were observed in both products. The NaOH-derived dots displayed pH-dependent photoluminescence and photocatalytic activity. Under sunlight, low concentrations of methylene blue and other pollutants were degraded.

A one-step microwave route was reported for the preparation of blue-emissive carbon dots from sesame seeds by Roshni and Divya (Roshni & Divya, 2017). A quantum yield of 8.07% was measured, and particle sizes of 3-10 nm were obtained. Photostability

was confirmed by the retention of approximately 95% fluorescence intensity. Good water dispersibility and strong photoluminescence were also observed. Based on this, optical sensing of  $\text{Fe}^{3+}$  was performed. Selective detection was indicated by fluorescence quenching, and a limit of detection of 2.56  $\mu\text{M}$  was determined.

A microwave-assisted route was reported by Ang in which palm kernel shell (PKS) was converted to blue-emitting carbon dots to examine the role of the reaction medium (Ang et al., 2020). Carbonization was carried out for 1–5 min using three media: ultrapure water (UPW), UPW with chitosan, and diethylene glycol (DEG). The products in all media were amorphous, with sizes of approximately 6.00–7.00 nm, and bore similar surface groups, such as hydroxyls and alkenes; amino groups were not detected. On comparison, the DEG system delivered overall stronger optical performance than the aqueous systems. It should be noted that the quantum yields reported for microwave-synthesized BDCDs vary dramatically across studies (8.07% to 44.0%), even when using similar biomass precursors and comparable reaction times. This variability suggests that additional, often unreported parameters—such as microwave power density, cavity mode, and vessel geometry—may exert significant influence on product quality. Furthermore, the amorphous nature observed in many microwave-derived samples raises questions about whether these materials represent true carbon dots with defined crystalline cores or carbonized polymer dots with primarily surface-state emission. The distinction between these two classes has significant implications for photoluminescence mechanisms and long-term stability, yet it is rarely addressed in the literature. Systematic studies correlating microwave parameters with structural features are needed to establish reproducible synthetic protocols.

CDs formed from PKS in DEG showed a quantum yield of 44.0%, an average size of 7.00 nm, brighter emission under UV light, and higher absorbance and photoluminescence intensities. The DEG medium also enabled shorter carbonization times and yielded more fully formed particles. In contrast, UPW and UPW-chitosan produced weaker fluorescence, with identical quantum yields of 26.3%. No meaningful difference was observed between these two aqueous media, indicating that PKS and chitosan did not generate amino-rich dots and underscoring the effect of medium choice. All materials were successfully applied to cellular imaging for the detection and removal of metal ions.

Pyrolysis is used to decompose organic matter at high temperature under controlled pressure. Crystalline carbon dots were produced by Fahmi through the pyrolysis of cellulose from bamboo leaves (Fahmi et al., 2018). The particles were modified with 4-carboxybenzylboronic acid and then used for tumor imaging and therapy. Good biocompatibility, multicolor emission, and low toxicity were reported.

Aji and co-workers prepared nitrogen-doped carbon dots by a simple pyrolysis of mangosteen peel with urea (Aji et al., 2017). The effects of urea amount and temperature were examined. Amine, ketone, and methyl groups were identified on the surface, which indicated passivation by urea. The number of particles formed was increased by higher urea concentrations. Absorbance and photoluminescence remained unchanged with increasing amounts of urea. Higher temperatures led to stronger photoluminescence.

A green route was reported by Guo using turtle shells as the precursor. Carbon dots with an average diameter of 2.62 nm were obtained (Guo et al., 2019). Amino, hydroxyl, and carboxyl groups were identified on the surface, resulting in good water dispersibility. The dots showed stable fluorescence, excitation-dependent emission, a quantum yield of 45%, and low toxicity. These features were linked to the collagen content of turtle shells. During carbonization, amino acids in collagen introduced oxygen-and nitrogen-containing groups that supported photoluminescence. The product was applied to multi-signal anti-counterfeiting and to optoelectronic patterns by inkjet printing.

Agricultural and industrial residues have been converted to carbon dots by pyrolysis. Yerba mate, avocado seeds, and orange peels were used as separate precursors, yielding products with distinct physicochemical profiles (Monje et al., 2021). Differences in morphology, fluorescence behavior, and related properties were examined for use as emulsion stabilizers and as photocatalysts.

Waste peels of *Carica papaya* were also carbonized to prepare carbon dots for chromium sensing in water (Pooja et al., 2019). The peel extract was heated in a hot-air oven, and the carbonized residue was dispersed in deionized water. Blue emission was observed under UV light, confirming the presence of photoluminescence. Structural analysis revealed amorphous, near-spherical particles with an average diameter of approximately 7 nm. Surface inspection revealed the presence of carbonyl and other functional groups. These groups enabled straightforward modification with ethylenediaminetetraacetic acid, after which chromium was detected by a chemo-sensor probe.

As with other bottom-up routes, pyrolysis has been favored because procedures are simple, a solvent-free operation is feasible, and costs can be reduced. A drawback is the requirement for high temperatures during the synthesis process (Chen et al., 2022). For this reason, hydrothermal processing has often been selected when lower thermal budgets are needed (Cheng et al., 2017).

Hydrothermal and solvothermal routes have been widely adopted for producing biomass-derived carbon dots. Precursors are heated in sealed vessels at elevated temperature and pressure, usually in solution, to form the dots. The procedures are viewed as cost-

efficient and low-hazard, and high quantum yields are often achieved, which supports their use at scale (Flores-Oña & Fullana, 2020).

As an example, Atchudan prepared nitrogen-doped carbon dots from dwarf banana peels by a hydrothermal process (Atchudan et al., 2020). The products exhibited a predominantly graphitic framework with a minor amorphous fraction and a uniform average size of approximately 4 nm. Good water dispersibility was attributed to surface groups such as -COOH and -OH. The dots displayed excitation-dependent emission with a quantum yield of approximately 23%, and the signal remained stable against photobleaching during the testing period. These features enabled use as fluorescent ink for writing and as probes for metal ions through fluorescence quenching.

CDs/hydroxyapatite (CD-HAP) composites were prepared by Chung from sugarcane bagasse char under hydrothermal conditions (Chung et al., 2020). A mixture of charred bagasse and NaOH was first heated in an autoclave. After filtration, varying volumes of the obtained CDs were added to preformed phosphate mixtures and then introduced slowly into a calcium nitrate tetrahydrate solution in HCl. The optimal CD synthesis temperature was identified as 190 °C, giving particles with an average diameter of  $4.72 \pm 1.1$  nm. The sugarcane-derived CDs were monodispersed, whereas CD-HAP displayed a rod-like morphology. Similar surface functional groups were observed in both materials. The CDs showed strong fluorescence and increased the optical response of the CD-HAP composite. An improvement in drug-loading capacity was also reported.

Water-soluble, nitrogen-doped CDs were produced hydrothermally by Paul and Kurian from two peels: jackfruit and tamarind (Paul & Kurian, 2021). The jackfruit-derived N-CDs had an average size of 6.4 nm and a quantum yield of 13.04%. The tamarind-derived N-CDs measured 5.3 nm with a quantum yield of 6.13%. Both samples emitted blue fluorescence under 350 nm excitation. FTIR analysis revealed the presence of hydroxyl and nitrogen functionalities, which contributed to the photoluminescence behavior.

Zhang used N-doped CDs to detect chlortetracycline (CTC) in pork (Zhang et al., 2021). A one-step hydrothermal process was performed with hawthorn fruit and diethylenetriamine as the dopant. The resulting dots were quasi-spherical, with an average diameter of 3.8 nm, and exhibited excitation-independent emission with a quantum yield of 22.96%. Hydrophilic surface groups confirmed successful doping. Fluorescence quenching enabled rapid CTC detection with recoveries of 93.62–103.18%.

Taken together, these reports demonstrate that hydrothermal and solvothermal processes are widely employed for biomass-derived CDs, as they enable the use of aqueous media, require moderate equipment, and achieve competitive quantum yields.

**Table 2. Representative Examples of Biomass-Derived Carbon Dots via Bottom-Up. Routes.**

Biomass Source	Method	Conditions	Application	Ref
Sesame seeds	Microwave	1-step, base medium	Fe <sup>3+</sup> sensing	(Roshni & Divya, 2017)
Palm kernel shell	Microwave	DEG, 1-5 min	Cellular imaging	(Ang et al., 2020)
Eggshell membrane	Microwave	NaOH, charred	Photocatalysis	(Jusuf et al., 2018)
Bamboo leaves	Pyrolysis	High temp, modified	Tumor imaging	(Fahmi et al., 2018)
Mangosteen peel	Pyrolysis	With urea	N-doping study	(Aji et al., 2017)
Turtle shells	Pyrolysis	Controlled	Anti-counterfeiting	(Guo et al., 2019)
Dwarf banana peels	Hydrothermal	N-doped	Fluorescent ink	(Atchudan et al., 2020)
Sugarcane bagasse	Hydrothermal	190°C, NaOH	Drug delivery	(Chung et al., 2020)
Hawthorn fruit	Hydrothermal	With DETA	Antibiotic detection	(H. Liu et al., 2021)

*Top-down pathway*

**Table 3. Top-Down Routes for Carbon Dot Synthesis from Carbonized Biomass.**

Method	Principle	Advantages	Limitations	Biomass Example	Ref
Arc discharge	Plasma vaporization between electrodes	Fast, continuous process	High energy, low QY, poor size control, requires bulk carbon	Carbon nanotube precursor (2004 discovery)	(Chao-Mujica et al., 2021)

Method	Principle	Advantages	Limitations	Biomass Example	Ref
Laser ablation	Pulsed laser vaporization	Tunable, fast, clean	Low QY, expensive equipment, requires pre-carbonization	Soybean (with carbonization + annealing)	(Yu et al., 2020)
Chemical oxidation	Acid treatment of carbonized biomass	Accessible, scalable, and multicolor possible	Harsh reagents, contamination risk, waste generation	Tomato ( $\text{H}_2\text{SO}_4$ or $\text{H}_3\text{PO}_4$ )	(Kailasa et al., 2019)

Arc discharge has been used to make nanoscale carbons, including carbon nanotubes. In this method, a plasma is formed between an anode and a cathode. The plasma vaporizes a carbon source, and carbonaceous products are deposited at the cathode. Carbon dots were first observed by this route in 2004 during the preparation of single-walled carbon nanotubes. During preparative electrophoresis, a fluorescent band was noted. Under UV light, multiple colors were observed, revealing a new class of photoluminescent nanoparticles (Su & Zhang, 2015).

A modified process called submerged arc discharge in water has also been applied. Using this variant, Chao-Mujica obtained carbon dots with an average diameter of about 2.3 nm. A quantum yield of nearly 16% was reported, which is higher than the values often described for arc-discharge products (Chao-Mujica et al., 2021).

High temperature and pressure are applied during laser-based synthesis. A pulsed laser vaporizes the carbon precursor. At high flux, a plasma is formed. On cooling, the vapor condenses to nanoparticles. Laser ablation has been used to prepare carbon dots due to its fast and tunable nature. Limits have been noted, including low quantum yields and poor control of particle size (Kumar et al., 2022; Y. Liu et al., 2021).

In 2011, size control was improved by Hu and co-workers through the adjustment of laser parameters during the ablation of graphite flakes (Hu et al., 2011). In 2020, Wang reported a multi-step route for soybean-derived carbon dots that combined laser ablation in liquid with prior carbonization steps (S. Wang et al., 2020). The precursor was first hydrothermally carbonized and then annealed at high temperature. An Nd: YAG laser was applied in  $\text{NH}_4\text{OH}$  (pulse energy 100 mJ, 1 h). Nanoparticles with an average diameter of 9.57 nm were obtained. Nitrogen-rich surface groups were identified, and photoluminescence was enhanced. Bright blue emission with a quantum yield of 1.81% was measured. A

control sample annealed without ablation showed no emission. These results indicate that process tuning can increase the quantum yield and yield more consistent sizes.

Chemical oxidation has been applied to convert carbonized precursors into carbon dots. Oxidizing acids such as nitric acid and sulfuric acid have been used in this route. The method has been favored due to its accessibility and scalability. It has also been criticized. Harsh conditions, multistep neutralization and purification, limited control of particle size, and risk of residue-derived contamination have been noted (Lin et al., 2021).

A greener variant was described by Kailasa and co-workers using tomato (*Solanum lycopersicum*) as the source (Kailasa et al., 2019). A dispersed mixture of frozen tomato slices and  $\text{H}_2\text{SO}_4$  was heated at 100 °C for 1 h to yield blue-emissive dots. Green- and yellow-emissive dots were produced by heating sonicated mixtures of frozen tomato with  $\text{H}_3\text{PO}_4$  at 80 °C for 25 min and 20 min, respectively. The three products were neutralized with NaOH and purified by dialysis. Carboxyl, hydroxyl, and amine groups were identified on all samples. The sizes were approximately 5–10 nm. The emission depended on the excitation wavelength. Quantum yields were ~12.70% (blue), 4.21% (green), and 2.76% (yellow).  $\text{Fe}^{3+}$  was detected in biofluids and pharmaceutical samples by fluorescence quenching. Low cytotoxicity and acceptable compatibility were shown in HeLa cell imaging (Cheng et al., 2017).

Although biomass-sourced dots have been obtained by chemical oxidation, concerns tied to corrosive reagents, residues, and reproducibility have remained and should be addressed in future work.

### Critical Evaluation of Reported Findings

The rapidly expanding literature on biomass-derived carbon dots has revealed several persistent challenges that warrant critical discussion. First, the reported quantum yields exhibit substantial variability, ranging from below 2% to above 45% for BDCDs synthesized via similar routes and from comparable biomass sources. This wide distribution suggests that many factors beyond the nominal synthetic route—including unreported variables such as reactor materials, trace minerals from biomass ash, water purity, and precise thermal profiles—exert considerable influence on optical properties. Without standardized measurement protocols and reference materials, direct comparison across studies remains problematic.

Second, there is an ongoing debate regarding the structural nature of many reported BDCDs. Transmission electron microscopy and photoluminescence data alone cannot distinguish true carbon dots, which possess graphitic or amorphous carbon cores with

quantum confinement effects, from carbonized polymer dots (CPDs), which may derive their emission primarily from molecular fluorophores embedded within a carbonaceous matrix. The latter are often produced under milder conditions and exhibit strong excitation-dependent emission, features commonly attributed to surface states. While both material classes have practical utility, their different photophysical origins have important implications for property optimization and application performance. More rigorous structural characterization, including high-resolution TEM, X-ray photoelectron spectroscopy, X-ray diffraction, and time-resolved spectroscopy, is required to clarify this distinction in future reports.

Third, reproducibility remains a significant concern. Biomass feedstocks are inherently heterogeneous in composition, moisture content, and ash fraction. Even when the same botanical source is used, seasonal variations, geographic origin, and post-harvest processing can alter the chemical composition of the starting material. This variability can lead to markedly different BDCD properties under nominally identical synthetic conditions. Several studies have reported batch-to-batch differences in quantum yield exceeding 10 percentage points. To address this issue, future work should emphasize feedstock standardization, including pre-treatment steps to remove ash, controlled drying protocols, and detailed compositional analysis of the precursor material.

Fourth, methodological inconsistencies hinder progress toward design rules. Many reports do not specify critical parameters such as heating rates, cooling protocols, vessel materials, or the exact geometry of reactors. For instance, in microwave synthesis, the power density, cavity mode (single vs. multimode), and vessel volume can all impact the temperature distribution and reaction kinetics; yet, these details are frequently omitted. Similarly, hydrothermal studies often fail to report the fill fraction of the autoclave, which influences the autogenous pressure and can significantly alter carbonization pathways. Without this information, independent replication and mechanistic interpretation are severely constrained.

Finally, the environmental credentials of BDCDs are sometimes overstated. While biomass feedstocks are renewable and can valorize waste, the overall “greenness” of a synthetic route must also account for energy consumption, solvent use, reagent toxicity, and waste generation. Pyrolysis at 600°C, hydrothermal processing at high pressure, or chemical oxidation with concentrated acids all have substantial environmental footprints. Life-cycle assessment (LCA) studies comparing BDCDs to conventional alternatives (organic dyes, metal-based quantum dots) are largely absent from the literature. Future work should incorporate quantitative environmental metrics, such as energy consumption per gram of product and carbon footprint, to substantiate claims of sustainability.

### *Design Rules Extracted from Comparative Analysis*

Based on the systematic comparison of synthetic routes and reported properties, several empirical design rules can be extracted to guide future BDCD synthesis (Šafranko et al., 2021):

Rule 1: For blue emission with quantum yield exceeding 20%, hydrothermal or solvothermal processing at 180-200°C for 3-6 hours is recommended. This temperature range promotes sufficient carbonization to form graphitic domains while preserving surface functional groups that enhance quantum yield. Temperatures above 220 °C tend to increase graphitization but may reduce surface-state contributions, leading to excitation-independent emission with lower overall QY in aqueous dispersion.

Rule 2: For nitrogen doping and red-shifted emission, combine nitrogen-rich biomass (e.g., protein-containing sources such as eggshells, collagen, or legume residues) with nitrogen-containing additives such as urea, ethylenediamine, or ammonia. Nitrogen incorporation is most effective when carried out during the primary carbonization step rather than via post-synthetic modification. Pyrolysis above 350 °C in the presence of urea has been shown to introduce graphitic nitrogen, which shifts emission toward green and yellow wavelengths.

Rule 3: For enhanced water dispersibility and colloidal stability, ensure the presence of carboxyl (–COOH) and hydroxyl (–OH) groups on the BDCD surface. This can be achieved via mild post-treatment with dilute nitric acid or hydrogen peroxide, or by conducting the synthesis in alkaline media (NaOH or KOH), which promotes surface oxidation. Over-oxidation should be avoided, as excessive carboxyl content can lead to aggregation via hydrogen bonding and may quench photoluminescence.

Rule 4: For reproducibility and minimization of batch-to-batch variability, pre-process biomass feedstocks to remove inorganic ash, standardize moisture content, and ensure consistent particle size of the starting material. Washing with deionized water followed by freeze-drying or controlled oven-drying to a defined moisture content (e.g., 5-10%) is recommended. Chemical composition analysis (elemental analysis for C, H, N, O content) should be performed on each feedstock batch and reported alongside synthetic conditions.

Rule 5: For applications requiring excitation-independent emission (e.g., biomedical imaging), favor pyrolysis at temperatures above 400 °C or hydrothermal treatment above 220 °C, both of which promote higher crystallinity and reduce the contribution of surface states. Conversely, for applications requiring tunable multicolor emission from a single material (e.g., multiplexed sensing or anti-counterfeiting), lower-temperature hydrothermal (150-180 °C) or microwave routes are preferable, as these yield predominantly surface-state emission.

These design rules, while empirical, provide a starting framework for rational BDCD synthesis. Further refinement will require systematic studies that isolate individual variables and employ multivariate analysis to quantify the relative contribution of each parameter to final properties.

#### *Outlook of Carbon Dots*

Biomass-derived carbon dots have been established as a versatile class of photoluminescent nanomaterials that sit at the intersection of biomass valorization and functional carbon nanotechnology. The body of work reviewed here demonstrates that BDCDs are not a single material but rather a tunable materials space defined by synthetic routes and processing decisions. The most significant factor governing BDCD performance is not the biomass label itself, but the synthetic history encoded in each sample - including precursor selection, reaction medium, temperature, time, and post-treatments.

#### *Current Limitations and Challenges*

Despite the substantial progress documented in this review, several critical challenges must be addressed before BDCDs can transition from laboratory curiosities to reliable components in commercial sensing, catalytic, and biomedical technologies.

#### *Batch-to-Batch Reproducibility*

One of the most frequently cited concerns is poor reproducibility across synthetic batches. Biomass feedstocks are inherently heterogeneous, with variations in composition, moisture content, and inorganic ash fraction arising from differences in botanical species, geographic origin, harvest season, and post-harvest processing. Even when the same biomass type is used, these factors can lead to substantial differences in BDCD properties under nominally identical synthetic conditions. Reported variations in quantum yield of 10 percentage points or more between batches are not uncommon. This variability limits the practical applicability of BDCDs, particularly for quantitative sensing applications where a consistent signal response is essential.

Contributing factors include: (1) uncontrolled variations in precursor composition, particularly the C/N/O ratio and trace metal content; (2) reactor-to-reactor differences in heat transfer, pressure profiles, and mixing; (3) subtle differences in water quality (deionized vs. distilled vs. tap) that affect pH and ionic strength; and (4) lack of standardized purification protocols, leading to variable removal of unreacted precursors and low-molecular-weight fluorophores. Addressing this challenge will require systematic feedstock characterization, controlled pre-treatment to remove ash and standardize moisture, and adoption of continuous-flow or microfluidic reactors that provide more uniform reaction conditions than batch autoclaves.

### *Lack of Standardized Reporting*

A second major issue is the absence of standardized reporting practices in the BDCD literature. Many studies do not provide sufficient detail to enable independent replication. Critical parameters - such as heating rates, cooling protocols, vessel materials, reactor geometry, exact solvent compositions, and purification steps - are frequently omitted. For quantum yield measurements, reference dyes, correction for reabsorption, and solvent refractive indices are not always specified, making cross-study comparisons unreliable. This lack of standardization hinders meta-analysis and the extraction of general design principles.

To remedy this, the community should adopt minimum reporting standards similar to those used in nanomaterial synthesis (e.g., MIRIBEL guidelines for biological nanomaterials). Proposed standards should include: complete characterization of starting biomass (elemental analysis, moisture content, ash analysis); detailed description of reactor setup (volume, geometry, material of construction); full temperature and pressure profiles; solvent specifications (purity, pH, ionic strength); purification procedures (dialysis molecular weight cutoff, chromatography conditions); and standardized quantum yield measurement protocols with specified reference materials and correction factors. Journals and funding agencies can play a role by requiring adherence to these standards.

### *Influence of Biomass Ash and Trace Elements* (Abraham et al., 2016)

A largely underexplored factor is the role of inorganic ash and trace elements present in biomass. Agricultural residues and plant materials typically contain 1-10% ash (on a dry weight basis), comprising silica, calcium, potassium, magnesium, iron, and other elements. During carbonization, these elements can act as unintended catalysts or dopants, influencing particle nucleation, growth, and final optical properties. For instance, trace iron can catalyze graphitization, while calcium and magnesium can form carbonate species that alter surface chemistry. Phosphorus from biomass ash may introduce unexpected heteroatom doping. Few studies have systematically investigated the effect of ash content, and even fewer have attempted to correlate ash composition with BDCD properties.

Future work should include ash analysis (via inductively coupled plasma mass spectrometry or X-ray fluorescence) as a routine characterization step, and should compare BDCDs derived from ash-rich versus demineralized biomass to isolate the effects of ash. Controlled doping studies using purified biomass with deliberate addition of specific trace elements could also help disentangle these variables.

### *Long-Term Stability* (Basu & Hazra, 2017)

While many reports demonstrate short-term photostability (hours to days under continuous UV irradiation), data on long-term colloidal stability, shelf-life, and performance

degradation over weeks to months are scarce. In practical applications - particularly environmental monitoring and point-of-care diagnostics - sensors must remain functional over extended periods. Aggregation, photobleaching, and oxidative degradation of surface groups are potential failure modes that have not been systematically characterized. Accelerated aging studies, stability testing under varying pH and ionic strength conditions, and performance evaluation in complex matrices (e.g., serum, river water, soil extracts) are necessary to assess real-world viability.

#### *Scale-Up and Process Intensification*

Most reported BDCD syntheses are conducted at laboratory scale (milliliter to tens of milliliters per batch), with product yields ranging from milligrams to a few grams. Scaling to kilogram or ton quantities required for industrial applications poses significant challenges. Batch-to-batch variability is likely to worsen at larger scales due to non-uniform heat and mass transfer in conventional reactors. Energy consumption per unit mass may increase, eroding the environmental benefits of biomass valorization. Capital costs for autoclaves, microwave reactors, or pyrolysis furnaces capable of processing large volumes are substantial.

Process intensification strategies—such as continuous-flow reactors, microfluidic synthesis, and plug-flow systems—offer potential solutions by providing better control over reaction conditions and enabling modular scale-up. Preliminary studies using continuous hydrothermal flow reactors have shown promise for producing carbon dots with improved batch-to-batch consistency. However, adaptation of these technologies to biomass feedstocks, which are often heterogeneous solids or viscous slurries, remains a technical hurdle. Further engineering research is required to develop scalable, energy-efficient processes.

#### *Life-Cycle Environmental Impact*

While BDCDs are often marketed as “green” nanomaterials, a comprehensive environmental assessment requires consideration of the entire life cycle, including feedstock cultivation or collection, transportation, preprocessing, synthesis, purification, and end-of-life disposal. Energy consumption is a critical factor: hydrothermal processing at 200 °C under pressure for several hours, or pyrolysis at 600 °C, consumes substantial energy (2-15 kWh per gram of product, depending on the route and scale). If this energy is derived from fossil fuels, the carbon footprint may be comparable to or greater than that of conventional organic dyes or metal-based quantum dots.

Life-cycle assessment (LCA) studies comparing BDCDs to alternative fluorescent materials are scarce in the literature. Such studies should account for energy inputs,

greenhouse gas emissions, water use, reagent toxicity, and waste generation. They should also evaluate the environmental benefits of valorizing waste biomass versus using it for other purposes (e.g., composting, anaerobic digestion for biogas, or direct combustion for energy). Only with this holistic perspective can the true “greenness” of BDCDs be evaluated. Future work should include LCA as a standard component of process development, and should prioritize routes with low energy intensity and minimal hazardous reagent use.

#### *Regulatory and Toxicological Considerations* (Abraham et al., 2016)

For biomedical applications, BDCDs must meet stringent regulatory requirements. In vitro cytotoxicity assays (e.g., MTT, LDH release) are commonly reported and generally show favorable results (>80% cell viability at concentrations up to 100-200 g/mL). However, in vitro assays do not capture the full spectrum of potential toxicity. In vivo biodistribution, long-term accumulation, immune response, and genotoxicity data are extremely limited for BDCDs. Concerns include: (1) potential contamination with trace metals from biomass ash; (2) presence of unreacted or partially carbonized organic precursors; (3) unknown metabolic fate of ingested or systemically administered BDCDs; and (4) long-term effects of chronic exposure.

Before BDCDs can be approved for clinical use, comprehensive toxicological profiling in animal models is required, including studies on acute and chronic toxicity, reproductive toxicity, carcinogenicity, and immunogenicity. Standardization of BDCD composition and surface chemistry is also necessary to ensure that toxicology results are reproducible and generalizable. Regulatory pathways for carbon-based nanomaterials are still evolving, and close collaboration with regulatory agencies (e.g., the FDA, EMA) will be essential to navigate the approval process.

#### *Future Research Directions*

Building on the limitations identified above, several future research directions are likely to have a high impact: Process Innovation. Continuous-flow synthesis systems, including microreactors and tubular flow reactors, should be developed and optimized for biomass feedstocks. These systems offer improved temperature and residence time control, enabling more reproducible BDCD synthesis and facilitating scale-up. Microfluidic platforms can be used for high-throughput screening of synthetic conditions, accelerating the identification of optimal parameters for specific applications.

Process intensification strategies such as ultrasound-assisted carbonization, plasma-enhanced synthesis, and electrochemical methods should be explored as alternatives to conventional heating. These techniques can reduce reaction times and energy consumption while enabling in-situ surface functionalization.

### *Characterization and Mechanistic Understanding*

In-situ and operando characterization techniques should be applied to elucidate carbonization mechanisms. Time-resolved spectroscopy (fluorescence lifetime, transient absorption) can distinguish between quantum confinement, surface-state, and molecular fluorophore contributions to emission. Isotope labeling ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) combined with NMR or mass spectrometry can track carbon and nitrogen incorporation pathways during synthesis. Advanced electron microscopy (aberration-corrected HRTEM, electron energy loss spectroscopy) can provide atomic-level structural information.

The establishment of structure-property correlation databases compiled from systematically varied synthetic conditions would enable predictive modeling and machine learning approaches to BDCD design.

### *Standardization and Reproducibility*

The community should work toward consensus protocols for quantum yield measurement, including specification of reference dyes, correction procedures, and uncertainty estimation. Interlaboratory comparison studies (round-robin tests) using a common set of BDCD samples would help identify sources of measurement variability. Minimum reporting standards for BDCD synthesis and characterization should be formally proposed, discussed, and adopted by major journals in the field. These standards should cover feedstock characterization, detailed synthetic protocols, purification procedures, and photophysical measurements.

### *Greener Synthetic Routes*

Life-cycle assessment should be integrated into synthetic route development from the outset. Energy metrics (kWh per gram of product), carbon footprint, and water use should be quantified and reported alongside optical properties. Routes with the lowest environmental impact should be prioritized for scale-up.

Replacement of harsh reagents (concentrated acids, high-boiling solvents) with milder alternatives is a key goal. Enzymatic carbonization, electrochemical activation, and supercritical water processing are promising directions. Solvent-free or aqueous-only routes should be favored whenever possible.

Integration of renewable energy sources (solar thermal, photochemical initiation) into BDCD synthesis represents a frontier area that could further reduce carbon footprints.

### *Application-Driven Design*

Future BDCD development should adopt a “reverse engineering” approach, starting from the desired application requirements (e.g., detection limit, emission

wavelength, biocompatibility, photostability). Synthetic routes and feedstocks can then be selected rationally using the design rules established in this review. For instance, a sensing application requiring low detection limits for heavy metal ions would prioritize hydrothermal synthesis at 180-200°C to maximize quantum yield and surface carboxyl groups for metal ion coordination.

Performance testing in complex, application-relevant matrices (such as seawater, blood serum, soil leachate, and food extracts) should become standard practice. These tests often reveal interference effects, matrix quenching, and stability issues not apparent in buffer solutions.

Long-term operational stability studies (months to years) are needed to assess whether BDCDs can meet the performance requirements of commercial sensors and imaging agents.

#### *Integration with Other Nanomaterials*

Hybrid materials combining BDCDs with metal nanoparticles, quantum dots, metal-organic frameworks, or polymers may offer synergistic properties. For example, BDCD-metal oxide composites have demonstrated enhanced photocatalytic activity, while BDCD-polymer conjugates offer improved stability and targeting for drug delivery. Systematic exploration of such hybrid architectures, guided by an understanding of individual component properties, could unlock new applications.

## Conclusions

Biomass-derived carbon dots should be viewed less as a single material and more as a tunable materials space defined by synthetic routes. When this perspective is adopted, biomass feedstocks are transformed from simple “green precursors” into a diverse library of building blocks whose composition and reactivity can be exploited through carefully selected processing strategies. The evidence reviewed here demonstrates that the properties of BDCDs-particle size, crystallinity, surface chemistry, quantum yield, emission wavelength, and photostability-are direct consequences of specific processing decisions rather than intrinsic attributes of the biomass source. Bottom-up routes from raw or minimally processed biomass have been favored because they can be implemented using simple equipment, in both aqueous and solvent-free systems, and at relatively low cost. Within this family, microwave, hydrothermal/solvothermal, and pyrolytic strategies have been shown to deliver dots with competitive quantum yields and tunable emission, often with heteroatom doping inherited from the parent biomass. Top-down approaches applied to carbonized biomass have provided complementary access to nanoscale carbons with higher crystallinity, demonstrating that process tuning in arc discharge, laser ablation,

or chemical oxidation can also tailor the optical response, albeit at the cost of harsher conditions and more complex purification.

A critical reading of the literature reveals characteristic trade-offs rather than clear winners. Bottom-up methods, often described as “green,” still rely on significant thermal or pressure inputs and, in some cases, concentrated bases or additives. Top-down oxidation routes are operationally accessible and scalable yet raise concerns related to corrosive reagents, batch-to-batch reproducibility, and residual impurities. An additional layer of complexity arises from the intrinsic variability of biomass feedstocks, which can obscure mechanistic interpretation and make cross-study comparisons difficult. As a result, the same biomass can yield markedly different BDCDs under nominally similar conditions, while different biomasses can converge to similar products if processed in a comparable way.

These observations suggest several directions in which future work is likely to have the most impact. First, more systematic “process-structure-property” studies are needed in which a single biomass is subjected to multiple synthetic routes under controlled and well-documented conditions. Such studies would help disentangle precursor effects from route effects and support the development of practical design rules for targeted properties. Second, greater standardization in reporting - covering not only quantum yield and basic spectroscopy but also feedstock pretreatment, reactor configuration, energy input, and purification protocols - would significantly enhance the comparability of BDCD studies and expedite the identification of genuinely scalable conditions.

Third, there is considerable room for innovation in the “greening” of both bottom-up and top-down routes. Opportunities include replacing strong mineral acids and bases with milder or recyclable alternatives, utilizing benign oxidants or electrochemical activation in place of conventional oxidation, and integrating continuous-flow or process-intensified reactors to reduce energy footprints. In parallel, closer coupling of synthesis with in-situ or operando characterization, along with simple process metrics such as energy consumption per gram of product, would enable environmental performance to be assessed alongside optical and functional metrics.

Ultimately, the connection between synthetic control and application performance needs to be strengthened under realistic conditions. Many reports have demonstrated impressive sensing, photocatalytic, bioimaging, or drug delivery behavior in model systems; however, performance in complex matrices, long-term stability, and compatibility with regulatory and safety requirements remain less explored. BDCDs that are designed from the outset with a clear end-use scenario-such as environmental monitoring, food safety, or biomedical imaging-are likely to benefit most from a synthesis-focused approach, in which feedstock choice and route optimization are guided by the desired operational environment and downstream processing constraints.

Continued progress in mapping the BDCD materials space – through systematic experimentation, greener process design, standardized reporting, and closer coupling between synthesis and application - can be expected to move these materials from laboratory demonstrations toward reliable components in future sensing, catalytic, and bioimaging technologies.

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