

Article

Design, Synthesis, and Soil Application of Copper-Based Coordination Polymers as Efficient Urease Inhibitors

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Abstract: Copper-based coordination polymers (Cu-CPs) have emerged as promising urease inhibitors with potential applications in agriculture to enhance nitrogen use efficiency and reduce environmental pollution. This review comprehensively summarizes the chemistry and design of Cu-CPs, focusing on their metal centers, auxiliary ligands, molecular architectures, and structure-function relationships. The mechanisms by which Cu-CPs inhibit urease activity are discussed alongside traditional inhibitors, highlighting their superior stability and tunable bioactivity. Furthermore, the application of Cu-CPs in soil environments is evaluated, including their stability, transport, and effects on plant growth and soil microbial communities. Challenges such as cost, controlled release, environmental impact, and scalable synthesis are addressed, with future perspectives emphasizing green chemistry approaches and smart responsive materials. Multidisciplinary collaboration is essential to translate these advances into practical solutions for sustainable agriculture.

Keywords: copper-based coordination polymers; urease inhibition; auxiliary ligands; nitrogen use efficiency; soil application

1. Introduction

Urea is one of the most widely used nitrogen fertilizers in global agriculture due to its high nitrogen content and cost-effectiveness. However, its efficiency is often compromised by rapid enzymatic hydrolysis catalyzed by urease, an enzyme ubiquitously present in soil [1]. This hydrolysis leads to the formation of ammonia and carbon dioxide, resulting in significant nitrogen loss through volatilization and leaching, and thereby reducing fertilizer use efficiency and causing environmental pollution such as eutrophication and soil acidification.

To mitigate these issues, urease inhibitors have been developed and integrated into fertilization strategies. These inhibitors aim to delay the hydrolysis of urea, thereby improving nitrogen retention in the soil and enhancing crop uptake [2]. Traditional urease inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), have shown effectiveness, but concerns regarding their environmental persistence, bioavailability, and long-term ecological safety have prompted the search for novel alternatives.

In recent years, copper-based coordination polymers (Cu-CPs) have emerged as a promising new class of urease inhibitors. These materials combine the inherent biological activity of Cu²⁺ ions with the structural tunability of coordination frameworks, offering multiple modes of interaction with urease enzymes. Their extended architectures allow for multivalent interactions and potential controlled release behavior, making them suitable candidates for sustainable agriculture applications.

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A representative example of such a compound is illustrated in Figure 1, which shows a copper(II)-based coordination structure retrieved from the PubChem database (CID: 899149). The central Cu^{2+} ion is coordinated by oxygen and nitrogen donor ligands, forming a stable complex. This type of structure represents the foundational motif in many Cu-CPs currently being investigated for their biochemical and environmental performance.

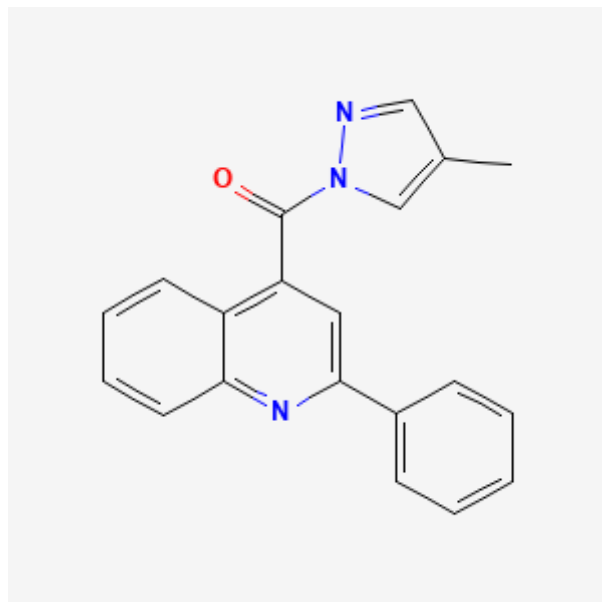


Figure 1. Representative Cu(II) coordination compound from PubChem (CID: 899149), exemplifying the structure of Cu-based coordination polymers.

This review aims to provide a comprehensive overview of the design, synthesis, and soil application of Cu-CPs as effective urease inhibitors. We highlight recent advances in their structural development, mechanisms of enzyme interaction, and field-relevant performance, as well as discuss challenges and opportunities in optimizing these materials for large-scale agricultural use.

2. Mechanism of Urease and Inhibition Strategies

2.1. Structure and Catalytic Mechanism of Urease (Ni^{2+} Active Center)

Urease is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide, playing a critical role in nitrogen cycling in agricultural systems. The crystal structure of pigeon pea urease (PDB ID: 4G7E) resolved at 2.2 Å reveals a highly conserved active site containing two nickel ions coordinated by histidine and other amino acid residues (Figure 2). These Ni^{2+} ions are essential cofactors that activate the urea molecule by polarizing its carbonyl group, facilitating nucleophilic attack and subsequent hydrolysis [3]. The enzyme adopts a trimeric quaternary structure, where the nickel ions reside within a well-defined catalytic pocket, highlighting the importance of precise metal coordination for enzymatic function.

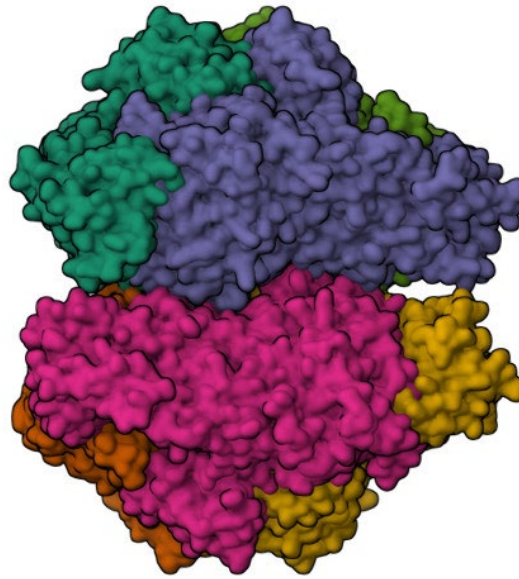


Figure 2. Crystal structure of pigeon pea urease (PDB ID: 4G7E) showing the Ni^{2+} -centered active site responsible for catalyzing urea hydrolysis.

Similar structural features have been observed in bacterial ureases, such as from *Yersinia enterocolitica* (PDB ID: 4Z42), which show conserved nickel-binding sites and multimeric enzyme organization [4]. Moreover, the nickel chaperone protein UreE (PDB ID: 1GMU) plays a pivotal role in the delivery and incorporation of Ni^{2+} into the urease apo-enzyme during maturation, ensuring full enzymatic activity [5]. Understanding these structures provides critical insights into the metal-dependent catalytic mechanism of urease and offers potential targets for inhibition.

2.2. Conventional Urease Inhibitors: Types and Limitations

Conventional urease inhibitors, including N-(n-butyl) thiophosphoric triamide (NBPT) and hydroquinone (HQ), have been employed to reduce nitrogen loss from urea fertilizers by delaying urea hydrolysis. NBPT acts as a competitive inhibitor by binding to the urease active site, whereas HQ exhibits a different mode of action by interfering with the enzyme's structure. However, these inhibitors often suffer from limited stability in soil environments and rapid degradation, reducing their long-term effectiveness. Additionally, concerns about environmental toxicity and non-specific interactions have motivated the search for novel inhibitors with improved performance.

2.3. Emerging Metal-Based Inhibitors: Advantages and Mechanisms

Recent research highlights the potential of metal-based inhibitors, particularly copper-based coordination polymers (Cu-CPs), as effective urease inhibitors. Copper ions can competitively bind to the active site or induce structural changes that disrupt the nickel coordination environment, leading to enzyme inactivation [4]. Compared to traditional inhibitors, Cu-CPs exhibit enhanced stability and sustained inhibitory activity in soil-plant systems, making them promising candidates for agricultural applications [2]. The tunable chemical structure of Cu-CPs also allows for optimization of their binding affinity and selectivity towards urease, providing a versatile platform for designing next-generation urease inhibitors.

3. Copper-Based Coordination Polymers (Cu-CPs): Chemistry and Design

3.1. Basic Building Blocks: Metal Centers and Organic Ligands

Copper-based coordination polymers (Cu-CPs) are an emerging class of materials characterized by the coordination of copper ions with organic ligands to form extended

network structures. The copper ion typically exists in the +2 oxidation state, enabling versatile coordination geometries such as square planar, tetrahedral, and octahedral, depending on ligand type and coordination environment. The choice of organic ligands—often containing nitrogen, oxygen, or sulfur donor atoms—plays a critical role in defining the polymeric framework's dimensionality (1D, 2D, or 3D), porosity, and chemical reactivity.

The metal-ligand coordination imparts stability and functionality to the Cu-CPs, making them promising candidates for biological applications such as urease inhibition. Notably, the ability of copper to interact with enzyme active sites, either by competing with native metal cofactors or by direct coordination with amino acid residues, underpins their inhibitory potential. Understanding the structural features of both the urease enzyme and Cu-CPs is essential for rational design (As shown in Figure 2).

3.2. Role of Auxiliary Ligands in Structural Modulation

Auxiliary ligands serve as key modulators in the assembly and final topology of Cu-CPs. Ligands with specific shapes and functional groups, such as the “V”-shaped second auxiliary ligands reported by Ding et al., enable precise control over intermetallic distances, polymer dimensionality, and flexibility [1]. These ligands influence the self-assembly process by directing the spatial orientation of copper centers, thereby tuning the physical and chemical properties of the resulting coordination polymers.

In addition, auxiliary ligands can enhance solubility and facilitate interactions with urease enzymes by providing additional binding sites or hydrophobic/hydrophilic balance. The careful design of auxiliary ligands is therefore crucial to optimizing Cu-CPs' bioactivity and stability in aqueous environments, where enzyme inhibition occurs. The chemical structure of a representative ligand utilized in Cu-CP fabrication is depicted in Figure 3.

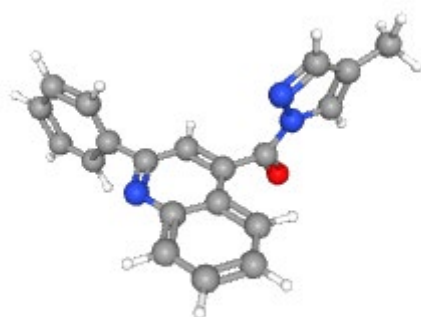


Figure 3. Chemical structure of (4-methyl-1H-pyrazol-1-yl)(2-phenylquinolin-4-yl)methanone, a representative auxiliary ligand used in Cu-CP synthesis.

3.3. Structure-Function Relationship: Molecular Architecture and Activity

The inhibitory performance of Cu-CPs against urease is intricately linked to their molecular structure and topology. Urease enzymes depend on a dinuclear nickel center for catalyzing the hydrolysis of urea into ammonia and carbon dioxide, a process critical in agriculture but responsible for nitrogen loss and environmental pollution [3]. Cu-CPs can mimic or disrupt this active site by coordinating to nickel ions or by occupying the active pocket, thereby hindering enzyme activity.

Studies indicate that Cu-CPs with planar aromatic ligands and accessible metal centers show enhanced binding affinity towards urease, effectively blocking substrate access or inducing conformational changes that reduce catalysis. Structural rigidity and extended frameworks facilitate multivalent interactions with the enzyme surface, increasing inhibition potency. Figure 4 illustrates a detailed bacterial urease structure providing insight into possible interaction sites for Cu-CPs [1].

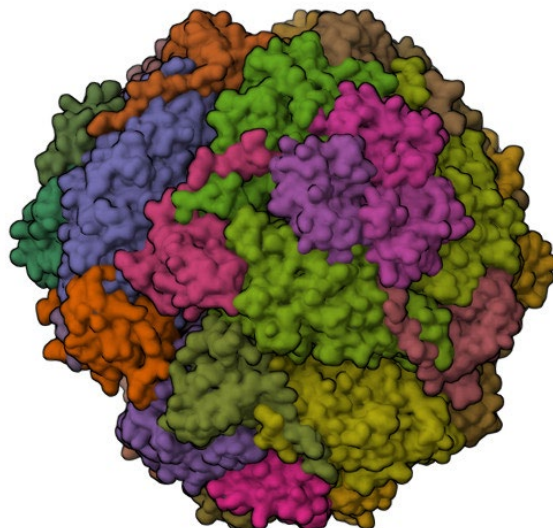


Figure 4. Crystal structure of bacterial urease from *Yersinia enterocolitica* revealing subunit arrangement and nickel ions at the active site (PDB ID: 4Z42).

3.4. Case Studies

It was synthesized novel two-dimensional Cu-CPs regulated by specifically designed auxiliary ligands that demonstrate high urease inhibition efficiency *in vitro* [6,7]. Their work exemplifies how ligand engineering can be exploited to modulate coordination polymer properties, leading to improved biological performance.

The combination of copper centers, tailored ligands, and controlled polymeric architecture offers a promising route for developing next-generation urease inhibitors with potential applications in agriculture and environmental management. These advances underscore the importance of understanding both coordination chemistry and enzyme structure for effective inhibitor design.

4. Urease Inhibition Activity of Cu-CPs

4.1. Evaluation Methods of Inhibitory Activity

Urease inhibition by Cu-based coordination polymers (Cu-CPs) is commonly evaluated using *in vitro* enzymatic assays, which quantify the enzyme's catalytic conversion of urea into ammonia and carbon dioxide. Standard protocols often involve measuring the released ammonia using colorimetric assays such as the indophenol method, where ammonia reacts with phenol and hypochlorite under alkaline conditions to produce a blue-colored complex measurable by spectrophotometry at ~630 nm.

The inhibitory potential is usually expressed in terms of percentage inhibition compared to a control without inhibitors. Critical parameters like IC_{50} values (concentration of inhibitor causing 50% enzyme activity reduction) and K_i (inhibition constant) are calculated through dose-response curves, helping to distinguish between competitive, non-competitive, and mixed modes of inhibition [8]. Advanced techniques such as isothermal titration calorimetry (ITC) or surface plasmon resonance (SPR) are occasionally used to directly measure binding affinities and thermodynamic properties of Cu-CPs interacting with urease. Furthermore, spectroscopic methods (e.g., UV-Vis, FT-IR) and molecular docking simulations complement these assays by elucidating binding modes and interaction sites at the molecular level.

4.2. Relationship Between Inhibitory Efficiency and Structure

The urease inhibition efficiency of Cu-CPs is highly dependent on their structural features and chemical composition. The Cu(II) metal centers coordinate with nitrogen and oxygen atoms from organic ligands, forming extended networks whose dimensionality

(1D, 2D, 3D) and topology modulate their accessibility and interaction with the urease active site.

In particular, ligands with planar aromatic systems and heteroatoms (e.g., nitrogen in pyrazole or quinoline moieties) enable π - π stacking interactions and hydrogen bonding with amino acid residues in the urease pocket, enhancing binding affinity. The "V"-shaped auxiliary ligands introduced by Ding et al. provide spatial orientation control that optimizes the proximity of copper centers to the enzyme, promoting stronger inhibitory effects [9].

The inhibitory mechanism often involves Cu-CPs competing with the native dinuclear nickel center in urease, either by chelating nickel ions or by directly interacting with key histidine and cysteine residues in the active site, disrupting substrate binding or catalysis. Structural rigidity of the polymers supports multivalent interactions, increasing binding strength and leading to potent and selective inhibition. However, excessive polymer cross-linking may reduce flexibility and limit enzyme accessibility, highlighting the need for fine-tuned polymer design.

4.3. Longevity, Release Behavior, and Improvements

For effective agricultural application, the stability and longevity of urease inhibition are crucial, as rapid degradation or leaching of inhibitors limits their practical use. Cu-CPs exhibit inherent advantages by virtue of their polymeric matrix, which can act as a reservoir, gradually releasing active copper ions or ligand fragments into the soil environment over time.

However, environmental variables such as soil pH, moisture content, microbial activity, and temperature fluctuations significantly affect release kinetics. The interaction of Cu-CPs with soil components may also alter their bioavailability and inhibitory function. To address these challenges, Ding et al. proposed the incorporation of chemical stabilizers, which protect Cu-CPs from rapid breakdown or immobilization in soil, thereby prolonging urease inhibition [10].

Additionally, modification of the ligand environment to increase hydrophobicity or introduce responsive functional groups enables controlled release triggered by specific environmental stimuli [11]. These advances aim to optimize dose efficiency, minimize environmental toxicity, and reduce the frequency of inhibitor application.

4.4. Case Study

In their 2022 study, Ding et al. synthesized novel two-dimensional Cu-CPs using "V"-shaped auxiliary ligands and systematically evaluated their urease inhibition activity through in vitro enzyme assays and soil-plant simulation experiments [12]. The results demonstrated that these Cu-CPs exhibit high inhibition efficiency, with IC_{50} values significantly lower than those of traditional inhibitors like NBPT.

Moreover, the polymeric inhibitors showed enhanced stability in soil, maintaining inhibitory effects over extended periods compared to conventional small-molecule inhibitors. Soil incubation tests confirmed reduced ammonia volatilization and improved nitrogen use efficiency in treated samples, indicating practical benefits for agriculture. These findings highlight the potential of Cu-CPs as next-generation urease inhibitors offering effective, sustainable, and environmentally friendly alternatives.

The study also underscored the importance of integrating coordination chemistry, materials science, and enzymology to design effective inhibitors that meet agricultural needs while mitigating environmental impact.

5. Soil Application and Environmental Impacts

5.1. Stability and Degradation Behavior of Cu-CPs in Soil

Copper-based coordination polymers (Cu-CPs) introduced into soil environments must maintain sufficient chemical stability to ensure prolonged urease inhibition while eventually undergoing controlled degradation to prevent accumulation and toxicity. The stability of Cu-CPs is influenced by soil pH, moisture content, temperature, and microbial

activity. For instance, acidic soils may promote ligand protonation and polymer breakdown, whereas alkaline conditions tend to stabilize coordination bonds [13].

Studies indicate that Cu-CPs exhibit moderate resistance to hydrolytic and microbial degradation, attributed to their extended polymeric networks and strong metal-ligand coordination bonds. However, over time, environmental factors facilitate gradual breakdown, releasing copper ions and organic ligands into the soil matrix. This degradation not only controls the release of active inhibitory species but also reduces potential long-term environmental burden. The design of Cu-CPs with tailored ligand composition and polymer cross-linking is thus essential to balance stability and biodegradability.

5.2. Transport and Bioavailability in Soil-Plant Systems

The mobility and bioavailability of Cu-CPs or their degradation products in the soil-plant system critically affect their efficacy as urease inhibitors and potential ecological impacts. Cu-CPs typically interact with soil components such as clay minerals, organic matter, and metal oxides, which can immobilize or adsorb these polymers, limiting their transport but also reducing leaching risks.

In rhizosphere environments, root exudates and microbial secretions may alter Cu-CP speciation and solubility, influencing uptake by plants or microorganisms. Bioavailability studies demonstrate that copper ions released from Cu-CPs can be absorbed by plant roots, entering metabolic pathways or accumulating in tissues. Therefore, assessing Cu bioaccumulation and translocation dynamics is vital to ensure that Cu-CP use does not adversely affect crop safety or soil health.

5.3. Impact on Plant Growth and Safety Assessment

The application of Cu-CPs has shown promising results in improving nitrogen use efficiency by reducing urease-driven ammonia volatilization. However, excessive copper accumulation may exert phytotoxic effects, such as oxidative stress, growth inhibition, and altered nutrient uptake. Controlled application rates and formulations of Cu-CPs are thus critical to maximize beneficial effects while minimizing toxicity risks.

Greenhouse and field trials indicate that properly formulated Cu-CPs can enhance plant growth parameters, including biomass accumulation and yield, by optimizing nitrogen availability. Safety assessments must include dose-response analyses, phytotoxicity screening, and evaluations of residual copper in edible plant parts to comply with food safety standards.

5.4. Potential Effects on Soil Microbial Communities and Nitrogen Cycling

Soil microorganisms play pivotal roles in nutrient cycling, organic matter decomposition, and plant health. The introduction of Cu-CPs may affect microbial community composition and function due to copper's known antimicrobial properties. While moderate copper levels can inhibit pathogenic microbes, they may also negatively impact beneficial bacteria and fungi involved in nitrogen fixation, nitrification, and denitrification.

Metagenomic and enzymatic activity assays reveal that Cu-CP application can lead to shifts in microbial diversity, favoring copper-tolerant species. These changes may alter nitrogen transformation pathways, potentially influencing overall soil fertility. Therefore, evaluating long-term impacts on microbial ecology and nitrogen cycling is crucial for sustainable Cu-CP deployment.

Integrated studies combining soil chemistry, microbiology, and plant physiology are necessary to comprehensively understand environmental consequences and guide the design of eco-friendly Cu-CP formulations.

6. Challenges and Future Perspectives

6.1. Challenges in Field Application: Cost, Controlled Release, and Environmental Residues

While copper-based coordination polymers (Cu-CPs) exhibit promising urease inhibition activity in controlled laboratory settings, their large-scale field application faces several significant challenges.

Cost-effectiveness is a primary barrier; the synthesis of Cu-CPs often involves expensive ligands and complex reaction conditions, which may hinder widespread agricultural adoption. To compete with conventional urease inhibitors, production costs must be reduced through optimized synthetic routes and the use of readily available, low-cost raw materials.

Another critical challenge is the controlled release of active components. Cu-CPs must balance maintaining sufficient stability to inhibit urease effectively while allowing gradual degradation to minimize toxicity and environmental accumulation. Current formulations may suffer from premature degradation or overly slow release, reducing efficacy or causing residue buildup. Developing formulations with tunable release kinetics responsive to environmental stimuli (e.g., moisture, pH) is necessary for precise field performance.

Environmental concerns about metal accumulation and residual toxicity remain. Copper ions released into the soil must not exceed safe thresholds to avoid phytotoxicity and disruption of soil microbiomes. Monitoring and mitigating such environmental residues are essential for sustainable use.

6.2. Green and Scalable Synthetic Strategies

Advancing Cu-CPs for practical applications demands the development of green and scalable synthetic methodologies. Traditional coordination polymer synthesis often relies on organic solvents, elevated temperatures, and long reaction times, raising environmental and economic issues.

Emerging strategies such as solvent-free synthesis, mechanochemistry, and room-temperature aqueous-phase reactions hold promise to reduce environmental impact and energy consumption. Moreover, continuous flow reactors and automated synthesis platforms can facilitate large-scale production with reproducibility and efficiency.

Adopting these green chemistry principles will not only improve sustainability but also enable industrial-scale manufacturing, critical for translating laboratory successes into commercial products.

6.3. Development of Smart, Stimuli-Responsive Cu-CPs

Future research should focus on intelligent Cu-CP systems capable of stimuli-responsive behavior for enhanced agricultural performance. These smart materials can adapt to environmental cues—such as soil moisture, temperature, or enzymatic activity—to modulate the release of copper ions and ligands precisely when urease inhibition is needed most.

For instance, Cu-CPs integrated with pH-sensitive linkers could release active species preferentially under alkaline conditions, coinciding with peak urease activity. Similarly, enzyme-triggered degradation mechanisms may allow on-demand inhibitor release, reducing off-target effects and environmental accumulation.

The design of such adaptive materials requires multidisciplinary approaches, combining coordination chemistry, materials science, and soil biology, offering exciting avenues for next-generation agrochemicals.

6.4. Potential for Synergistic Design with Other Fertilizer Additives

Combining Cu-CPs with other fertilizer additives or soil amendments may enhance overall nutrient management efficiency and crop productivity. For example, integrating Cu-CPs with nitrification inhibitors, phosphorus solubilizers, or biofertilizers could provide complementary benefits, reducing nitrogen losses while improving nutrient availability and soil health.

Synergistic formulations can also address multiple agronomic challenges simultaneously, such as nutrient leaching, soil acidification, and pathogen suppression. However, designing such complex systems requires careful consideration of chemical compatibility, release profiles, and ecological impacts to avoid antagonistic interactions or toxicity.

Future studies should explore multifunctional coordination polymers or composite materials that incorporate multiple active components, offering tailored and holistic solutions for sustainable agriculture.

7. Conclusion

Copper-based coordination polymers (Cu-CPs) represent a highly promising class of urease inhibitors with significant potential to improve nitrogen use efficiency in agriculture. Their unique structural features, including versatile metal-ligand coordination and tunable molecular architectures, enable effective interaction with urease enzymes, resulting in potent inhibition of urea hydrolysis. Compared to traditional inhibitors, Cu-CPs offer advantages such as enhanced stability, adjustable release profiles, and the possibility of molecular-level design through auxiliary ligands, which can optimize both efficacy and environmental safety.

Despite these promising attributes, several challenges remain before Cu-CPs can be widely adopted in field applications. Issues related to synthesis cost, controlled release behavior, environmental impact, and scalability need to be addressed through innovative research efforts. The development of green synthetic routes, stimuli-responsive materials, and synergistic formulations with other fertilizer additives are important future directions.

To fully realize the agricultural and environmental benefits of Cu-CPs, multidisciplinary collaboration among chemists, soil scientists, agronomists, and environmental researchers is essential. Such cooperation will accelerate the translation of fundamental coordination chemistry advances into practical, sustainable urease inhibition technologies. By bridging laboratory innovation with real-world application, Cu-CPs can contribute significantly to reducing nitrogen losses, mitigating environmental pollution, and promoting sustainable agriculture globally.

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