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Experimental Design for the Acute Toxicology Assessment of Nanoscale Ultrafine Materials Based on Virtual Simulation Experiments and A Diverse Interactive Blended Teaching Approach

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***Abstract:* -** The escalating production of nanoscale ultrafine materials has led to a greater release of nanoparticles into aquatic environments, raising concerns about their environmental impact and potential health risks. As a cross-disciplinary field, environmental functional material toxicology seeks to blend professional courses and foster innovative talents in environmental health. This study introduces a novel experimental teaching program in nanoscale ultrafine material toxicology, centered on microalgae cultivation. A comprehensive experiment assessing the biological toxicity of ultrafine materials was developed, focusing on their impact on microalgae growth inhibition. By incorporating varied teaching methods like "online virtual simulation experiments with traditional offline teaching, offline experiments, and post-learning reinforcement", this study advocates for a diverse interactive teaching approach for environmental toxicology experiments. This student-centric teaching model prioritizes interaction through virtual simulations, sustains course relevance, enhances classroom instruction, nurtures student individuality, underscores disciplinary advancements, and cultivates students' abilities to pose, analyze, and resolve issues within a broad academic framework, thereby fostering innovative thinking capabilities.

Keywords: Nanoscale ultrafine materials; environmental toxicology; virtual simulation experiments; experimental teaching design

I. INTRODUCTION

The emergence of the "New Engineering" field has imposed fresh demands on nurturing talents in environmental studies, grounded in the novel requisites of national strategic development, evolving international competition, and the heightened emphasis on moral character building. This paradigm shift calls for educational reforms in higher education institutions, emphasizing the cultivation of highly skilled, innovative, and globally competitive multifaceted individuals. Experimental teaching stands as a pivotal component of theoretical instruction in urban environmental disciplines, offering a distinctive function that conventional foundational courses cannot replace. It serves as a crucial bridge between theory and practice, playing an indispensable role in fostering students' comprehensive application abilities, practical skills, and entrepreneurial spirit[1]. Not only does it aid students in enhancing their grasp of fundamental knowledge, but it also becomes a vital conduit for nurturing scientific thinking, investigative spirit, creativity, and modern societal acumen[2]. Environmental toxicology, as an intensely practical applied discipline, delves into the detrimental impacts and mechanisms of harmful pollutants on biological systems in the environment, conducting safety assessments and risk evaluations. It embodies characteristics of both foundational and applied sciences. The integration of environmental toxicology experimental teaching with theoretical instruction is essential, as they complement each other harmoniously. Practical engagement in specific experimental procedures, data analysis, and results discussion are imperative for a deeper understanding of theoretical concepts pertaining to environmental pollutant management. Presently, environmental toxicology experimental courses predominantly feature confirmatory and demonstrative experimental projects, characterized by outdated experimental concepts that lack operability, comprehensiveness, and innovation[3]. Given the evolving educational perspective that prioritizes students and fosters innovative applied talents, interdisciplinary integration has become a prevailing trend in the development of professional

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fields in Chinese universities[4]. Within environmental toxicology, there is a pressing need for the incorporation of more pertinent interdisciplinary requirements, fortified knowledge reserves, practical utility, and swiftly evolving teaching contents[5] that cater to the demands of both interdisciplinary advancement and student needs. Consequently, reform in environmental health experimental teaching programs is urgently warranted.

Within the current educational context, the “Nanoscale Ultrafine Material Acute Toxicology Assessment Experiment” in experimental teaching represents a fusion of “foundational experiments - innovative experiments”. This integration is tailored to the cognitive levels of students and the research interests of teachers. The experiment addresses the health implications of nanoscale materials within the environment, specifically focusing on ultrafine zero-valent iron—a prominent nanomaterial in environmental studies. By employing standard characterization techniques like XRD and XPS, students are tasked with delving into critical properties of nanoscale ultrafine zero-valent iron, including phase information and elemental composition. A comprehensive experiment is crafted around the biological toxicity of ultrafine materials, specifically designed to inhibit microalgae growth. This endeavor not only encompasses bio-nanomaterial characterization but also incorporates toxicity risk assessment. The experiment is structured to provide practical and feasible procedures, ensuring ease of implementation. Moreover, it adopts a multifaceted teaching approach that combines “online virtual simulation experiments with traditional offline teaching, offline experiments, and post-learning reinforcement”. This student-centric approach emphasizes interaction during virtual simulations, ignites students’ passion for research, fosters proactive and self-directed learning, and enables students to acquire a diverse range of professional knowledge within a constrained timeframe.

II. Design of Offline Experiments

Ultrafine materials represent a crucial category of emerging pollutants, given their numerous novel physicochemical characteristics and complex environmental behaviors. The biological safety and environmental toxicological effects of nanoscale ultrafine materials have become a focal point of scientific inquiry[6]. Throughout their production, transportation, usage, and disposal processes, these materials inevitably enter water environments where they interact with soluble organic substances and aquatic organisms, inducing alterations in their physicochemical properties[7]. Due to their small size and lipophilic nature, ultrafine materials can disseminate through sedimentation, transport, and even biomagnify within food chains, ultimately posing risks to ecosystems and human health[8]. Nanoscale zero-valent iron (nZVI) refers to zero-valent iron ultrafine particles with diameters around 1-100 nanometers. Possessing remarkable reduction capabilities, a large surface area, strong adsorption capacities, as well as excellent heat absorption properties and redox reactivity, nZVI has been widely applied in environmental pollution remediation[9, 10]. However, existing scientific studies have demonstrated that nZVI exerts toxic effects on algae, animals, plants, and microbial populations[11]. Algae serve as primary producers in aquatic ecosystems, playing a pivotal role in ecological balance and displaying sensitivity to environmental changes. Single-celled green algae, due to their short reproductive cycles, ease of isolation, cultivation, and the observable toxic effects at the cellular level, are often considered ideal experimental subjects in environmental toxicology research. They are commonly employed in acute cytotoxicity tests[12, 13]. Hence, toxicological experiments based on microalgae cultivation can serve as valuable tools in foundational and comprehensive undergraduate laboratory teaching, offering insights and guidance for the instruction and cultivation of talent in environmental toxicology.

2.1 Experimental Content

The experimental focus involves utilizing X-ray diffraction analysis (XRD) technology for phase identification, structural analysis, and characterization of the amorphous state of ultrafine materials. Techniques such as X-ray photoelectron spectroscopy (XPS) are employed to investigate the physicochemical structure and elemental chemical states of the surface of ultrafine materials. By using single-celled algae as the test organism, growth inhibition experiments are conducted to ascertain the effective concentration of ultrafine materials impacting the growth of the algae. Furthermore, the half-maximal effective concentration value (EC50) of the ultrafine materials at 24 hours is calculated, allowing for the quantitative assessment of the toxic effects and ecological risks associated with the ultrafine materials.

2.2 Reagents and Equipment for Experiments

Chemical reagents and substances such as sodium nitrate, trisodium phosphate, citric acid, ammonium iron citrate, magnesium sulfate heptahydrate, calcium chloride dihydrate, ethylene diamine tetraacetic acid, zinc sulfate heptahydrate, iron sulfate heptahydrate, sodium carbonate, boric acid, manganese sulfate monohydrate, copper sulfate pentahydrate, ammonium molybdate tetrahydrate, ultrapure water, ammonium molybdate, nitric acid, anhydrous ethanol, sodium hydroxide, hydrochloric acid, and others are all of analytical grade and were procured from Shanghai Sinopharm Chemical Reagent Co., Ltd. *Chlorella pyrenoidosa* were sourced from the Freshwater Algal Resource Repository of the Institute of Hydrobiology, Chinese Academy of Sciences. Nanoscale zero-valent iron (50nm) was obtained from Jiangsu Annatai Environmental Technology Co., Ltd.

The experimental setup includes electronic balances, magnetic stirrers, beakers, glass rods, pipettes, micropipettes, graduated cylinders, centrifuges, autoclaves, culture dishes, light incubators, filtration setups, hydrothermal reactors, ovens, muffle furnaces, ultrasonic oscillators, vacuum drying ovens, pH meters, UV-visible spectrophotometers, X-ray diffractometers, X-ray photoelectron spectrometers, transmission electron microscopes, scanning electron microscopes, and other essential laboratory tools and instruments.

2.3 Experimental Methods

1) Material Characterization

(1) X-Ray Diffraction Analysis (XRD)

The crystalline structure and crystallinity of ultrafine materials were assessed using the X-ray powder diffractometer from Bruker, Germany. The measurement conditions involved utilizing Kα radiation with Cu target, λ=1.5406 Å, operating at a current of 50 mA and an accelerating voltage of 40 KV. The scanning parameters included a step size of 0.02°, a scanning range of 10-80°, and a scanning speed of 5°min^{-1}.

(2) X-Ray Photoelectron Spectroscopy (XPS)

For surface element composition and chemical valence states of the ultrafine material samples, analysis was conducted using the X-ray photoelectron spectrometer (PHI-5000C) from Perkin Elmer, USA. The instrument employed Al Kα X-ray (1486.6 eV) as the light source, complemented with a hemispherical analyzer operating in a fixed pass energy mode (Retarding). Measurements were performed at a depth of 5-10 nm with a scanning step of 0.05 eV, referencing the C1s peak at 284.6 eV for energy calibration. Peak deconvolution was achieved using AugerScan or XPS peak software.

2) Cultivation of Chlorella

Chlorella cells were inoculated in BG11 medium (refer to Table 1) and cultivated at 25 °C under a light intensity of 2000 Lux with a 12:12 light-dark cycle. The cultivation proceeded until reaching the exponential growth phase before commencing the experimental procedures.

Table 1 Components of BG-11 culture media

Component	Concentration (mg/L)
$NaNO_3$	150
$K_2HPO_4\cdot 3H_2O$	40
$MgSO_4\cdot 7H_2O$	75
$CaCl_2\cdot 2H_2O$	36
Citric acid	6
Ferric citrate	6
Na_2EDTA	1
Na_2CO_3	20
A5 solution (consisting of the following)	
H_3BO_3	2.86
$MnCl_2\cdot 4H_2O$	1.86
$ZnSO_4\cdot 7H_2O$	0.22
$Na_2MoO_4\cdot 2H_2O$	0.39
$CuSO_4\cdot 5H_2O$	0.08
$Co(NO_3)_2\cdot 6H_2O$	0.05

3) Determination of Chlorella Pyrenoidosa Growth

Different concentrations of algal suspensions were chosen for analysis. Under an optical microscope, cell numbers were determined using a hemocytometer, and the absorbance value (OD680) of the algal suspension was measured at 680 nm using a spectrophotometer. The absorbance value was plotted on the y-axis, while the cell quantity per unit volume was plotted on the x-axis. A linear equation was established correlating these two parameters, enabling the calculation of algal cell density in each system. However, the presence of ultrafine materials will affect the absorbance value of the algal cells, necessitating the deduction of the background value solely comprising a mixture of ultrafine materials and BG-11 culture medium.

4) Toxicity effects of ultrafine materials on chlorella pyrenoidosa

Chlorella was exposed to ultrafine material systems at concentrations of approximately 100 mg/L. Additionally, experimental groups were set up where Chlorella was solely cultured in BG11 medium as a blank control. The cultures were maintained for approximately 24 hours, following which the growth of the algae in each group was assessed.

5) Growth inhibition experiment: ultrafine material on chlorella pyrenoidosa

Following the acute cytotoxicity testing method for algae, a growth inhibition experiment was conducted on chlorella pyrenoidosa to assess the effective concentration of ultrafine materials that influence the growth and development of chlorella pyrenoidosa. Various weights of ultrafine materials were measured into sterilized and dried conical flasks. These were then combined with algal suspensions reconstituted in BG11 medium, resulting in consecutive concentrations of ultrafine materials in the algal-cell and material mixture system: 0, 100, 250, 500, 1000, and 2500 mg/L. Three replicates were prepared for each concentration, and chlorella pyrenoidosa in the logarithmic growth phase was inoculated. The initial cell density of the algae was set at 1.0×10^7 cells/L, and the cultures were nurtured for 24 hours in a light-regulated incubation chamber. Subsequently, the inhibition rate of the ultrafine materials on chlorella cells was calculated using a formula based on relative growth rate. This formula was utilized to generate a dose-response curve, from which the Half Maximum Effective Concentration (EC50) of the ultrafine materials at 24 hours was determined. The formulas for calculating the relative growth rate (1) and growth inhibition rate (2) of the algae are provided below:

$$\mu_{i-j} = \frac{lnX_j - lnX_i}{t_j - t_i} \tag{1}$$

$$I_{r=} \frac{\mu_c - \mu_T}{\mu_c} \times 100\% \tag{2}$$

Where,

μ_{i-j}— the relative growth rate from time i to time j;

X_i— the cell density at time i;

X_j— the cell density at time j;

I_r—the inhibition rate based on relative growth rate;

μ_c— the average of the relative growth rates of parallel samples in the control group;

μ_T—the relative growth rates of parallel samples in the experimental group.

2.4 Experimental Results and Discussion

1) Characterization of ultrafine materials

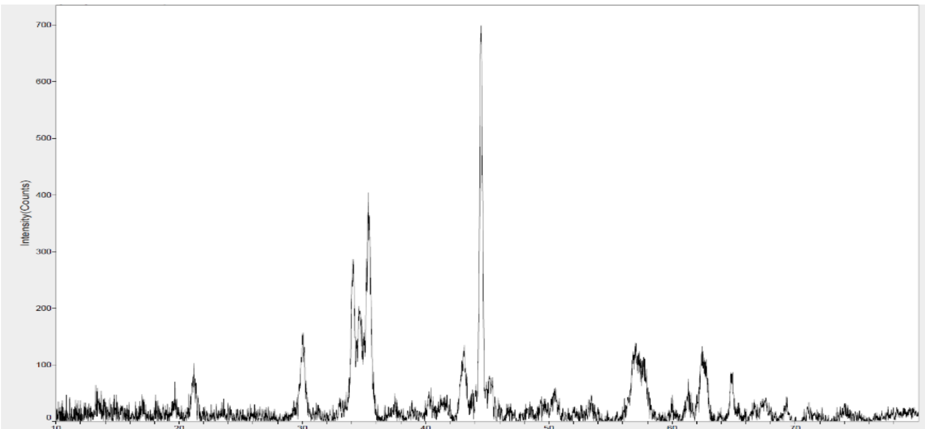

Figure 1 XRD patterns of nZVI

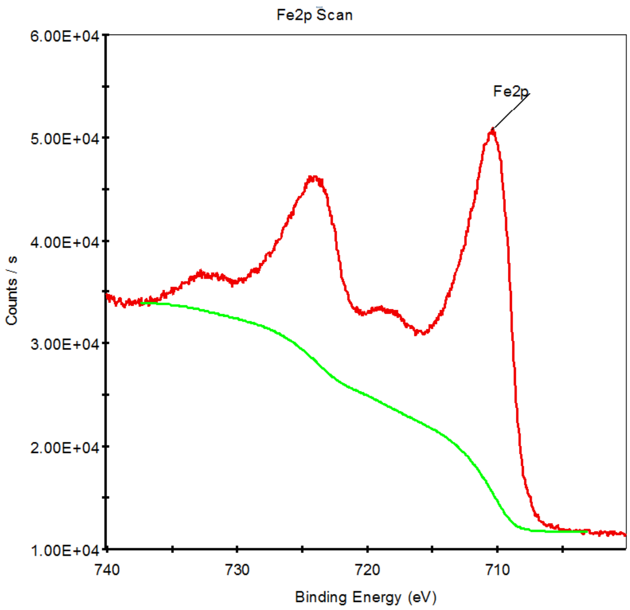

Figure 2 XPS map of nZVI

Characterization of nZVI materials was conducted using XRD and XPS techniques. XRD analysis provides phase information about nZVI composition. Figure 1 displays the XRD spectrum of nZVI, showing a diffraction peak at 2θ angle of 44.5°, corresponding to α-Fe^0 in the standard database (JCPDS No.87-722), indicating that nZVI primarily consists of elemental iron. XPS analysis detects elemental data information of the nZVI shell layer, complementing the limitations of XRD to some extent. Figure 2 presents the Fe2p spectrum of nZVI, where the peak at a binding energy of 725.4 eV corresponds to Fe2p1/2, indicating the presence of iron oxides on the material surface. The peak at 720.5 eV corresponds to Fe0. Additionally, the peak at 711.3 eV represents Fe2p3/2, suggesting the presence of Fe2O3. Therefore, it is evident that nZVI materials predominantly consist of iron oxides, with zero-valent iron enveloped by organic compounds and iron oxides on the surface.

2) Relationship between the number of chlorella pyrenoidosa cells and absorbance

A certain amount of Chlorella pyrenoidosa solution was selected and sequentially diluted at different dilution factors for cell counting, resulting in the corresponding number of algal cells. The obtained series of algal solutions were measured for absorbance at 680 nm using a spectrophotometer. A standard curve was plotted of cell numbers against absorbance values (OD) (see Figure 3), indicating a strong linear relationship between the microalgal quantity and cell absorbance.

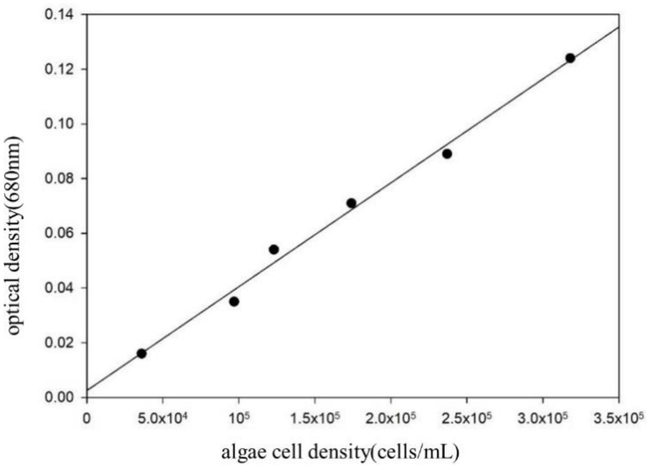

Figure 3 Standard curve of algal cell number

3) Influence of ultrafine materials on the growth of chlorella pyrenoidosa

Figure 4 illustrates the impact of ultrafine materials on the growth of Chlorella pyrenoidosa. In the nZVI experimental group, the specific growth rate of chlorella pyrenoidosa was determined to be0.213 d^{-1}, significantly lower compared to the control group ($P < 0.05$), with an inhibition rate of 14.59%.

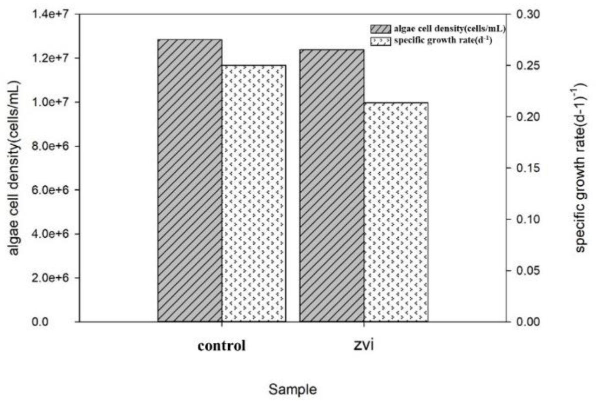

Figure 4 Effect of nano-zero iron on Chlorella pyrenoidosa growth

4) Toxicity assessment of ultrafine materials

The dose-response curve of nZVI on the growth inhibition effect of Chlorella pyrenoidosa over 24 hours is depicted in Figure 5. The results were linearly fitted, yielding the corresponding equation: I_r=0.0759X+5.6548, with an R-squared value of 0.96. Through the linear fitting equation, the concentration of nZVI corresponding to a 50% inhibition effect (EC_{50}) was calculated to be 584.26 mg/L. According to literature, the EC_{50} value for graphene oxide on Chlorella pyrenoidosa is 37.3 mg/L[14], while for single-walled carbon nanotubes, it is 261.5 mg/L[15]. This suggests that nZVI exhibits relatively good short-term biocompatibility.

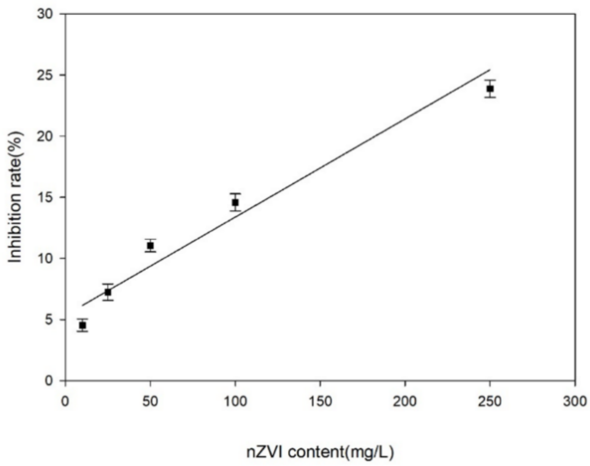

Figure 5 The curve of nZVI content to inhibitory effect on Chlorella pyrenoidosa in 24 h

III. A Diverse Interactive Blended Teaching Approach

In the face of increasingly complex issues in environmental toxicology and environmental health science, the connotations and extensions of these disciplines have undergone profound changes: environmental toxicology and environmental health science are displaying characteristics that are progressively more open, diverse, and international. With the continuous occurrence of new environmental hazards, traditional educational concepts and methods have struggled to meet the new demands of the times. Open, diverse, and heuristic teaching methods have become crucial for disciplinary development and professional education reform. Currently, in pursuit of national innovation and development strategies and the establishment of “Double First-Class” initiatives, it is imperative to explore the establishment of effective mechanisms for nurturing top-notch innovative talents. This endeavor aims to promote the emergence of outstanding innovative talents, which is not only a historical requirement for the great rejuvenation of the Chinese nation but also an urgent demand for the ongoing reform in higher education teaching. Courses in environmental toxicology form a pivotal cluster within the field of environmental science and have long been a focal point in environmental science and engineering education. Confronted with new challenges in disciplinary development, traditional environmental toxicology course teaching systems and curriculum designs are undergoing substantial transformations.

Virtual simulation teaching represents an innovative instructional method that leverages computer modeling and three-dimensional visualization technologies to furnish students with a learning environment that is close to reality yet safe and controlled. This approach surpasses the confines of traditional teaching, especially in fields like environmental toxicology and materials science that demand intricate experimental conditions and high-cost equipment[16]. Through virtual simulation technology, students can engage in high-risk or difficult-to-implement experimental operations without actual peril, thereby deepening their comprehension and application of theoretical knowledge. Furthermore, virtual simulation experiments permit students to practice repeatedly, aiding in the consolidation of learning outcomes and enhancement of experimental skills. Throughout the teaching process, virtual simulation technology also ignites students' curiosity and desire for exploration. Through interactive learning, students can actively engage in teaching activities, augmenting their learning experiences. Additionally, it equips teachers with abundant educational resources and flexible teaching methodologies, rendering teaching content more vivid and illustrative, thus enhancing students' learning interests and motivations. Moreover, virtual simulation teaching supports personalized learning, enabling students to select various experimental operations based on their learning pace and interests. This fosters the development of students' autonomous learning capabilities and innovative thinking. In today's globalized and digitized era, virtual simulation teaching can advance the progress of distance education and online learning, enabling a broader dissemination of high-quality educational resources. Consequently, with ongoing technological advancements, virtual simulation teaching is poised to play an increasingly crucial role in the educational realm.

This course addresses the disparity between computational dose-effect calculations for nanomaterials and students' real-world experiences, as well as the complexity of pollutant molecular structures. By incorporating virtual simulation experiments into the curriculum, various detection methods such as growth inhibition tests, high-throughput sequencing, Ames tests, comet assays, and frozen biological tissue section immunofluorescence have been developed to obtain corresponding physicochemical, ecological, and human health indices for nanomaterials. This integration constructs a framework that combines physicochemical, ecological, and health indicators to digitally replicate the simulation of environmental pollution and its health impacts, partially alleviating the high costs and resource consumption associated with human or animal health experiment teaching in the field of environmental toxicology. In applying digital scenario calculations in environmental toxicology teaching, the inherent laws of knowledge are elucidated through digital technology. This approach creates a diversified learning environment that accommodates various learning styles, interests, and abilities, thereby shifting from the traditional didactic teaching approach to guiding students to actively engage in virtual simulation teaching. By fostering students' subjective initiative and enthusiasm, this method subtly internalizes abstract theories during the virtual simulation teaching process. Through students' active participation, independent research, knowledge expansion, and the gradual cultivation of systematic and interdisciplinary thinking, this approach facilitates the development of critical thinking skills.

The efficacy and rewards of multifaceted interactive blended teaching encompass several key facets:

1. Experiment Design: In the process of experiment design, encompassing literature review, research focus delineation, and method determination, students grasp the fundamental principles of experimental design. This practice hones their logical thinking skills in formulating research questions. By delving into cutting-edge research findings within the experiment's domain, students further refine their logical thinking abilities, fostering an active interest in exploration. This not only solidifies their foundational knowledge but also deepens their understanding of disciplinary advancements, nurturing their sense of autonomous innovation and creative thinking.

2. Hands-On Experimentation: Throughout the experimental process, students personally engage in all operational procedures. Depending on the experiment's requirements, they collaborate effectively, independently handling tasks such as procuring and preparing materials, cleaning glassware, sterilizing, aseptic operations, counting, and recording results. Confronted with various challenges during experiments, students learn to adjust and respond autonomously under the guidance of teachers, thereby enhancing their independent experimental capabilities.

3.Utilization of Advanced Equipment and Virtual Simulation Software: Leveraging the existing resources of large-scale instrument platforms and virtual simulation experiment software aids in comprehending cutting-edge technologies, igniting students' professional cognition, unearthing their innovative potential, and improving their ability to link theory with practice and solve problems. Throughout project execution, students foster teamwork, communication, practical skills, and organizational coordination capabilities.

4. Cultivation of Experimental Summary Proficiency: Students' ability to summarize experiments is honed. Following both offline and virtual simulation experiments, students, working in groups, are tasked with drafting experiment reports. They employ appropriate methods to analyze and process data, reflecting on encountered

challenges during experiments, drawing conclusions, and creating succinct PPT presentations for exposition. This process teaches students how to independently analyze results, distill conclusions, propose discussion viewpoints, suggest avenues for further research, and enhances their logical thinking and verbal expression skills simultaneously.

IV. CONCLUSION

In today's academic landscape, disciplines are increasingly converging towards comprehensive integration, with interdisciplinary intersections and fusion emerging as the predominant trend. As a result, revolutionary changes in research environments and educational approaches are inevitable. The realm of environmental pollution and health risks represents a multidisciplinary crossroads, seeking to delve deeply into the interconnectedness between emerging environmental contaminants and human health. This field of study spans disciplines such as environmental toxicology, materials science, and life sciences, requiring specialized backgrounds. Within this context, the experiment combines students' cognitive levels with teachers' research topics. It focuses on the health effects of ultrafine materials in the environment, specifically investigating the nano-scale zero-valent iron, a typical ultrafine material in environmental domains. By utilizing conventional characterization methods like XRD and XPS, students are tasked with analyzing key properties such as the phase and elemental information of nano-scale zero-valent iron. The experiment is designed to comprehensively examine the biotoxicity of ultrafine materials based on the growth inhibition of small algae. This experiment seamlessly integrates biological ultrafine material characterization with toxicity risk assessment. Its straightforward procedures engage students, stimulating their research interests and fostering proactive autonomous learning. Within a limited timeframe, students acquire a broad spectrum of professional knowledge. Through the exploration of diverse teaching models incorporating "online virtual simulation experiments, offline traditional teaching, offline experiments, and post-class consolidation", the experiment contributes to the reform and practical application of a multifaceted interactive teaching approach in the series of environmental toxicology laboratory courses. This student-centered teaching model promotes interaction during virtual simulations, maintains the metabolic capacity of the curriculum, optimizes classroom instruction, nurtures individual student development, highlights disciplinary frontiers, and cultivates students' abilities to raise, analyze, and resolve issues within a comprehensive and expansive academic scope. Ultimately, this approach fosters innovative thinking among students.

Harnessing the power of interdisciplinary cross-disciplinary experimental teaching can uplift students' overall refinement and enhance the quality of talent cultivation. The ultrafine materials toxicology experiment not only reinforces students' understanding of environmental chemistry, physical chemistry, instrumental analysis, environmental microbiology, materials toxicology, and associated knowledge domains, but also nurtures their learning aptitudes and research interests. It sparks students' interest in scientific inquiry, facilitating a deeper exploration of various facets within the environmental science domain. By mastering specialized knowledge and experimental skills, students are empowered to unleash their initiative and creativity fully. Moreover, this experiential approach cultivates students' ability to apply their expertise and skills to address real-world environmental challenges, fostering a sense of professional identity and social responsibility, and meeting the nation's demand for versatile talents. Moving forward in our experimental teaching endeavors, we shall continue to innovate and explore, refining our strategies based on various challenges and circumstances encountered during the teaching process. Through continuous reflection and enhancement, we endeavor to elevate the instructional quality of experimental courses, striving to nurture a greater number of high-caliber research-oriented professionals.

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REFERENCES

[1] Wang Xiaolin, Li Mei. On the Exploration of Reform in Environment Toxicology Experiment Teaching. Journal of Linyi University. 2018, 40(06): 135-141.

[2] Zuo Jialiang, Zhu Jinwei, Xiao Runmin, et al. Self-design experiment of environment toxicology and cultivation of innovative ability. Laboratory Science. 2020, 23(05): 52-55.

[3] Lv Nianling, Yuan Yancheng, Huang Xiaomei, et al. Focusing on process for experimental teaching. Experimental Technology and Management. 2017, 34(06): 154-156.

[4] Bao Shuimei. The Paths of Higher Education Development Based on Interdisciplinary Integration. Journal of National Academy of Education Administration. 2021(09): 39-46.

[5] Hong Haizheng. Discussion on Teaching in Environmental Toxicology—Foundation and Intersections. Journal of Higher Education. 2017(11): 96-97.

[6] Bo Yang, Jin Chengyu. Influence of Nanoparticles on Cell Substructures. Laboratory Research and Exploration. 2013, 32(10): 308-311.

[7] Fan Gongduan, Chen Wei, Zheng Xiaomei, et al. The Cytotoxic Effects of Nanomaterials on Algae and Its Mechanisms. Asian Journal of Ecotoxicology. 2018, 13(02): 23-33.

[8] Miao Lingzhan, Wang Peifang, Hou Jun, et al. Research progress on toxicity of metallic nanomaterials to different microbial aggregates. Water Resource Protection. 2019, 35(01): 73-78.

[9] Sheng Jie, Fu Haoyang, Wang Wei, et al. Research progress on the characterization and modification of nanoscale zero-valent iron for applications. Environmental Chemistry. 2020, 39(11): 2959-2978.

[10] Liang Wen, Zhou Nianqing, Dai Chaomeng, et al. Experimental Study on Degradation of Diclofenac by Combined Processes Based on Nanoscale Zero-Valent Iron. Safety and Environmental Engineering. 2021, 28(03): 123-129.

[11] Zeng Ying, Huang Shuangqiu, Liao Jinxin, et al. Toxicity of Supported Nanoscale Zero-valent Iron on Green Alga Chlorella pyrenoidosa. Asian Journal of Ecotoxicology. 2021: 1-15.

[12] Zhou Xiaojun, Xu Yanghui, He Qiang, et al. Effects of Nanoparticles on Growth of Freshwater Algae: Toxicity Mecha-nism and Combined Toxicity. Asian Journal of Ecotoxicology. 2021, 16(03): 128-143.

[13] Wang Zhuang, Jin Shiguang, Zhang Fan, et al. Single and joint toxicity of zinc oxide and titanium dioxide nanoparticles for freshwater algae. Journal of Agro-Environment Science. 2021, 40(10): 2095-2105.

[14] Zhao J, Cao X, Wang Z, et al. Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae. Water Res. 2017, 111: 18-27.

[15] Yang Xiaojing, Chen Hao, Yan Hai, et al. Effects of Nano-TiO2 and Single-Walled Carbon Nanotubes on the Growth of Chlorella vulgaris. Asian Journal of Ecotoxicology. 2010, 5(01): 38-43.