Zebrafish as a Model Animal to Strengthen the Discipline Understanding and Interest Guidance of Students Majoring in Green Chemical Engineering and Biomedicine

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Abstract. Due to various reasons, there has always been a misleading understanding of the "non green" of chemical engineering and processes in the whole society, which has directly led to the low interest, confusion and even exclusion of many freshmen in the discipline and major after they entered into campus. Zebrafish has a high degree of similarity with human genes, and shows a prominent advantage as a model organism. This study takes this model as the ideal object; through the designated practice and demonstration experiment activities, it led all professional students to explore and understand the problems with high concern and more reflection, such as "whether chemical industry affects the environment and human health", "what is a green process" and "the magical effect of drugs". As the results, its implementation is proved conducive to creating a campus atmosphere of green education concept, building a green curriculum platform, and promoting green teaching and scientific research and green talent training.

Keywords: Zebrafish · Discipline Understanding · Interest Guidance · Chemical Engineering · Biomedicine · Sichuan University (SCU)

1 Introduction

In June 2016, China officially became the 18th full member of the Washington Agreement, marking that China's higher engineering education has been widely recognized by the international community [1, 2]. In order to actively support innovation driven development and aim at the "made in China 2015", "One Belt and One Road" and other major national strategic layout, China Ministry of Education put forward the concept of "New Engineering and Technical Disciplines" in 2017 to further promote the reform of engineering education in domestic colleges and universities [3], form a world-class

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engineering education China program and experience, and cultivate a large number of emerging engineering science and technology talents. Currently, green manufacturing technology is growing rapidly under the background of sustainable development [4]; and the major of green chemical engineering and biomedicine is attracting more and more attention. It has been five years since the School of Chemical Engineering of Sichuan University began to enroll students majoring in green chemical engineering and pharmaceutics (later changed to biomedicine) in 2017. Relying on the “Double First-Class” disciplines of SCU [5], the school reinterprets the connotation of green engineering talents and continuously guides the direction of talent training mode. At present, more than 1000 students have been enrolled in this major. However, there has always been a misleading understanding of the "non green" of chemical engineering and process in the society due to various reasons [6], which has directly led to the low interest, confusion and even exclusion of many freshmen after they entered into the campus, and thus it unavoidably resulted in the loss of a considerable number of excellent students in the process of specialty changing application at the end of freshman year, which has had a noticeable impact on the cultivation of talents in the specialty and even the whole discipline. Up to now, it also becomes a long-term problem that puzzles the development of the whole school. In this context, domestic fraternal colleges and departments generally adopt the way of strengthening publicity to guide, but the overall effect is not as good as expected. The reason is that students lack practical experience and direct feeling, so it is difficult to have a strong resonance and identity, meanwhile it is more difficult to stimulate their interest in learning from the heart; the contents of many introductory courses offered in the freshman year are too broad and professional, so it is inevitable that they will feel "too high and too few". At the same time, the contents also fail to respond to the society focus comprehensively and highlight the key points with focused goals. Under these backgrounds, the corresponding effective teaching means and educational researches are highly expected.

Zebrafish has a high similarity of 87% with human genes, which has a prominent advantage as a model organism [7]. It is highly similar to mammalian biological structure, physiological function and signal transduction pathway, which is often used as a popular model of biology and genetics. Since the 21st century, zebrafish has also been used as a screening model of drug activity [8]. Nowadays, this animal has become a "wind vane" for detecting the toxicity of various substances and changes in the aquatic environment due to its sensitivity to changes in the water environment on the basis of a series of relatively complete test methods. It has been widely used in the fields of industrial safety, toxicology, pharmaceutics and environmental protection. If teachers can take it as a carrier to replace publicity and eloquence with practice and data, and then stimulate students’ enthusiasm for knowledge and interest in learning with real and vivid animal responses, and finally make it serve as a "stepping stone" for subsequent professional courses in senior grade (such as the "E-H-S introduction" for the relationship among enviromnet, health and safety), it will be a meaningful way worth exploring. Through literature collection, investigation and interview, it is found that the relevant teaching reform projects have not been carried out in domestic colleges and universities, which have novelty, operability, practicality and wide participation. It can play a beneficial role in improving classroom teaching, promoting professional talent training

and even discipline development, enriching talent training modes and assisting "mass entrepreneurship and innovation" activities in campus.

2 Methods

In the design of this teaching research project, we have fully implemented the OBE (Outcome Based Education) concept [9]. This idea is oriented by social needs and focuses on the improvement of students' overall quality. It is also an important direction of higher education teaching reform in China. The OBE concept is oriented by output, and the corresponding teaching idea is reverse design. It selects familiar experimental materials, interesting practical processes and expected experimental results in daily life to stimulate students' enthusiasm for learning their major.

It was found most of the students are interested in experimental animals after a thorough investigation in the 'freshmen seminar' and class meeting in the early stage. Because the model animal zebrafish is meek, safe, intuitive, easily governable and strong practicality, meanwhile the freshmen need little basic knowledge and low experimental requirements for it, the model has been carefully selected as the suitable object of the project after comparison with other common experimental animals. Ideal model is very crucial to guide them to have a correct understanding of the subject and major, which can stimulate their interest in learning and lay a foundation for professional study effectively. The specific arrangement of related practical activities were determined by repeated discussion with the Engineering Experiment Center of the school and the teachers of relevant professional courses; taking the concentrated propaganda after their admission as the first step, it aimed to lead all professional students to explore and understand the problems with high concern and more reflection around "whether chemical industry affects the environment and human health", "what is a green process" and "the magical effect of drugs" during the implementation of designated practice and demonstration experiments. It was hereby designed as follows:

First, necessary pre-experiments and basic training were carried out to make students understand the living and reproductive habits of zebrafish, the process of their fertilization and embryonic development, and their similarities and differences with humans; after all the conditions were ripe, it would be promoted for the implementation of zebrafish acute toxicity experiment, safety evaluation experiment and drug activity evaluation experiment by stages, which could guide students to conduct visual observation, microscopic observation, anatomical observation, behavioral analysis and index evaluation on model animals. In this way, a correct understanding and macro understanding of "Chemical Industry-Environment-Health-Safety-Medicine-Green Manufacturing" is expected to establish as soon as possible. Among them, the simple and safe experimental process was all participated in and completed in groups, while a little complex or professional operation was demonstrated by teachers and mainly observed by all the freshmen. After all the practice activities, the students were required to sort out, analyze and summarize the recorded phenomena and data according to the regulations and standards, then submit the final report and write a detailed experience/summary of discipline understanding (teachers would guide them to collect and consult literatures and discuss in groups in the process). At the same time, the principals and well-known professors of

the schools and related institutes were invited from time to time to provide science popularization propaganda, face-to-face consultation and other activities for the students; and the teachers of the project team were organized to summarize project progress, analyze goal achievement and deal with the existing problems. They kept collecting students' feedback, discussing and evaluating the effects of the implementation of practical activities. All the involved parties would be coordinated, and a scientific and perfect activity development together with assessment system was established finally by the common effort.

3 Results and Discussion

IN the acute toxicity experiment, the zebrafishes contacted with the aqueous solution containing the tested substances (heavy metal ions and green solvents) of different concentrations under the specified conditions. In an experimental cycle, the mortality of experimental fishes was recorded every 6 h, the LC_{50} concentration of each tested substance was determined when the mortality of fishes reached 50% [10], and the acute toxicity grade was also judged on the basis of IUPAC classification standards for toxicity of chemical reagents. The test method was carried out according to the Organization for Economic Cooperation & Development (OECD) test method for acute toxicity of fishes and China's National Standard (GB/T 13267-91) method for determination of acute toxicity of water quality substances to freshwater fishes including zebrafish.

In another example of the experiment of *Salvia miltiorrhiza* (Danshen, a very famous traditional Chinese medicine) extract (abbreviated as SME) against zebrafish hypoxia, students first took an appropriate amount of *Salvia miltiorrhiza* powders and extracted them under four different conditions [11], the filtrate was collected after extraction and filtration for comparison in the following experiments. Subsequently, the students took the fishes out of the tank. And then observe their anoxic state and record it. After that, the anoxic fishes were put into a beaker filled with 50 mL pure water, which was added with the SME obtained from different ways dropwisely. During this process, the teachers guided students to observe the recovery of the fishes, record the relevant experimental data (e.g. time for dying fishes to resume normal swimming) and images, and compare them with the results of anatomy and microscopic analysis. Representative scenes can be found in Fig. 1.

It is worth mentioning that this experiment utilized the basis of a common biology experiment in high school - the anatomy of crucian carp. This is both familiar and strange to freshmen, and the fishes here are much smaller. The teachers trained students to follow the five steps: 1) take a normal fish and a zebrafish that died due to hypoxia for dissection; 2) put the fish on the dissecting pad and pat it dry; 3) start dissection from the abdominal fin of the zebrafish with a blade, and cut its abdomen to the head with a scalpel, and then the internal liver of the zebrafish can be clearly seen; 4) remove the gill cap of zebrafish and the side muscle of successively, so that the internal structure of zebrafish can be directly and clearly observed; 5) check the internal organs of fish with microscope or magnifying glass, dissect and compare the recovered fish and the suffocated fish, and record the differences through micrographs. Even, some students were surprised to find that a few fish were deformed under the continuous influence of

Fig. 1. Representative photos in the project activities (including observation and personal experience).

Fig. 2. Deformed zebrafish under hazardous simulation.

harmful substances before the dissection (see Fig. 2), which shocked them and deepened their internal awareness of environmental protection. Intuitive understanding is far better than the text of textbooks.

As a result, the students clearly found that the toxicity of green solvents was indeed not obvious, while the harmful substances in the traditional chemical industry caused obvious (fatal) damage. The latter system exhibited a certain dose-toxicity and toxicity-time relationship. The new reagents represented by deep eutectic solvent have better biocompatibility and safety, and is an important implementation medium of current green technology. In addition, zebrafishes were close to death when they were seriously anoxic; however, after the extract of *Salvia miltiorrhiza* Bunge was added into their living environment, the anti-hypoxia reaction appeared in different degrees. They could not only quickly return to normal state, but also showed continuously-enhanced ability to resist hypoxia, which make students have a deeper understanding of the great treasure house of traditional Chinese medicine and enhance their national pride and self-confidence.

Table 1. Content design of questionnaire for students and results (n = 90).

No.	Questions	Answers	Results
1	How much did you know about green chemicals before you entered the University	A. much B. general C. little	A (71%), B (26%), C (3%)
2	How much did you know about biomedicine before you entered the university?	A. much B. general C. little	A (45%), B (52%), C (3%)
3	Was green chemical engineering and biomedicine your first choice for the college entrance examination?	A. yes B. no	A (68%), B (32%)
4	What aspects of traditional chemical industry do you care about most?	A. pollution B. explosion C. inhalation hazard D. exposure hazard E. future development	A (55%), B (10%), C (35%), D (42%), E (58%)
5	Did you think that traditional Chinese medicine was effective through observation?	A. yes B. no C. uncertain	A (97%), B (0%), C (3%)
6	Do you like animal experiments?	A. yes B. no C. just so so	A (55%), B (10%), C (35%)
7	Are you worried about the current water quality in your environment?	A. yes B. no C. uncertain	A (68%), B (13%), C (19%)
8	Have you ever used water purification equipment in your home?	A. yes B. no	A (55%), B (45%)
9	Do you have any family members or relatives working in the chemical or pharmaceutical industry	A. yes B. no	A (3%), B (97%)

(*continued*)

Table 1. (*continued*)

No.	Questions	Answers	Results
10	Through this activity, will you continue to study in green chemical engineering and biomedicine in the future?	A. yes B. no C. uncertain	A (75%), B(0%), C(25%)
11	What other industries do you think are likely to cause pollution to the water environment (multiple choices)	Free answer	Papermaking, tanning, energy, textile dyeing and finishing, brewing and fermentation, etc.
12	Do you have any more specific comments or suggestions?	Free answer	Hope it can be normalized, content diversification, etc.
13	Please give your final score for this project:	very satisfied:☆☆☆☆☆, very satisfied: ☆☆☆☆, just ok: ☆☆☆, unsatisfactory: ☆☆, very unsatisfactory: ☆.	☆☆☆☆☆ (33%),☆☆☆☆ (57%), ☆☆☆ (10%)

During the whole activities, all the students were encouraged to conduct the experiments with as few reagents as possible to reduce the pollution and unnecessary waste. On the other hand, the students were required to pour the waste liquid into the waste liquid tank after the experiment. Besides that, the teachers guided them to complete harmless treatment of dead fishes. After the experiment, the participants were inspired to discuss the "three wastes" treatment scheme corresponding to the objects and contents.

At the end of the experiment, the questionnaire (see Table 1) was sent to each student immediately, and then it was completed on the spot. According to the result analysis, it can be found although this major was not the first choice of many students, they were basically determined to stay in this major at present. Their understanding and interest in green chemical industry and biomedicine are being effectively strengthened, and they have a deeper understanding of traditional Chinese medicine and environmental issues. Most of the participants gave very positive comments on this activity and project. Besides that, all students also need to fill in Likert scale to quantitatively score whether the teaching content and teaching methods can effectively inspire discipline interest, cultivate practical ability, improve learning initiative and participation. The reliability is evaluated according to Cronbach's alpha [12]. Then the paired sample t-test is used to analyze the impact of this kind of guided teaching mode on the learning effect compared with the traditional propaganda.

At last, this is also the first learning and practice experience of these students after they entered the campus. They begin to collect and learn the knowledge related to the experimental project before experiments, and start to find problems and put forward valuable solutions. They are also beginning to contact the teamwork, and the group

activity can enable effective communication and collision of ideas; and their ability has also been improved to think independently, solve problems and deal with emergencies. Each experimental link with practical application value has greatly stimulated the students' interest and exploratory thinking, and promoted them to maintain the passion of young men and active learning motivation throughout the practical process. Moreover, they also had a preliminary understanding of the regulations, rules and requirements in the laboratories of Sichuan University. Such "enlightenment" project made a good start to carry out relevant professional experiments of chemical engineering or biomedicine in their higher grade.

4 Conclusions

Accordign to above results, it infers the project can integrate many functions, such as entrance education, discipline publicity, initiation (inspiration) learning and interest stimulation. Based on the guidance team composed of chemical, pharmaceutical and bioengineering teachers of the school and the Sichuan Key Laboratory of pharmaceutical engineering technology, the project has been constructed with relevant foundation and resources.

The development of such a project is conducive to creating a campus atmosphere of green education concept, building a green curriculum platform, and promoting green teaching & scientific research together with talent training. Its final direction is expected to be a science popularity-foucsed open experiment available for promotion and demonstration, which can be oriented to more students of different grades in chemical engineering, pharmaceutics, pharmacy, environment, medicine, life science and other related majors.

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A. Yohannes–Visualization, Writing-Original Draft.
Y. Li—Writing - Review & Editing.
Q. Sun—Quantitation, Validation.
J. Liu—Data curation and analysis.
T. Yao—Survey.
S. Yao—Conceptualization, Methodology, Project administration.

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