

Effects of Degradation of Nonylphenol in Waste Water by Strong Ionization and Its Degradation Products on Sex Differentiation in Zebrafish

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Abstract. Nonylphenol (NP), as a typical environmental endocrine disruptor, exists widely in the natural environment. It has a high toxicity with a low concentration. NP at the level of $\mu\text{g/L}$ is enough to interfere with the sex differentiation of many aquatic organisms. The effects of degradation of NP in waste water by strong ionization (SID) and its degradation products on sex differentiation in zebrafish was studied in this paper. The NP solution of 5mg/L was degraded by SID device, and the 20d zebrafish were exposed to NP wastewater of different concentrations before and after degradation until their sexual maturity. The body length, body weight and sex differentiation ratio of zebrafish were recorded, and the sex hormone levels of zebrafish were extracted and detected. The gonadal glands of zebrafish were slices and analysed. This study found that the effects of nonylphenol on male zebrafish were much greater than that of female, and there was no significant positive correlation between toxicity and dose. In addition, the effects of 5mg/L NP wastewater degraded by SID for 60min were not significantly different from those of the control group, indicating that SID could effectively degrade NP and alleviate its biological toxicity.

Keywords. Strong ionization, waste water, degradation

1. Introduction

Nonylphenol is a typical environmental endocrine disruptor, which has been detected in all watersheds in China. In some watersheds, such as the Pearl River (33200ng/L) [1], the concentration is much higher than the $6.6\mu\text{g/L}$ standard set by the U.S. Environmental Protection Agency (EPA). NP structure is similar to estradiol, so it can mimic natural estrogen, affect the sex differentiation and reproduction of the organism through interaction with estrogen receptor, thus affecting the dynamic balance of the whole population [2], and damage the liver, kidney and gonad tissues of the organism [3]. Other studies have found that NP can induce cell apoptosis, autophagy and necrosis, and induce the generation of cancer cells to a certain extent [4]. At present, researchers have detected the presence of NP in the decidua and early embryos of

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pregnant women, and found that NP has obvious characteristics of maternal transfer [5], indicating that NP contamination cannot be ignored.

Due to the high toxicity and stable structure of NP itself, the degradation effect of NP by traditional sewage treatment methods is not ideal. Studies have shown that NP with complex side chain structure has very low biodegradability and can hardly be degraded in SBR [6]. In recent years, some advanced oxidation degradation of refractory organic pollutants in wastewater has become a hot research technology. Traditional advanced oxidation technologies, such as Fenton method, do not have a high degradation efficiency for NP, while some methods combining nanoscale catalysts have improved the degradation efficiency of NP, but the service life [7] and recovery of catalysts [8] have become new problems. Some studies have shown that strong ionization dielectric barrier discharge (SID) has a high removal effect on the refractory organic pollutants in sewage without the addition of reagents or catalysts. Such as pyridine (20mg/L, 96%) [9] and bisphenol A (20mg/L, 99.59%) [10], the degradation efficiency was higher than 95%. Therefore, in this experiment, the SID method was chosen to degrade NP in sewage, to determine the degradation efficiency of NP by SID method, and to explore the rule of toxicity change before and after NP degradation. In this study, zebrafish were used as the model organism, and zebrafish were exposed to NP wastewater with different concentrations and degradation time. By measuring the body length and weight of zebrafish, the influence on its growth and development was analysed; By recording the sex ratio and detecting the content of sex hormones to explore the influence of sex differentiation; The influence of gonad development was studied by section observation and pathological analysis of gonad. On the one hand, this experiment is used to verify the previous studies on the pure toxicology of NP, on the other hand, it also improves the shortcomings of the study on pure degradation, and provides a basis for the toxicology study on the degradation of NP by strong ionization discharge.

2. Experiments

2.1. Experimental Device

In this experiment, SID technology is used to convert the power frequency voltage of 220V/50Hz into the high frequency voltage of 2.5-4kV /15~23kHz by high frequency and high voltage power supply and input it to the dielectric barrier discharge (DBD) generator. The raw material O₂ is ionized and injected into the water phase through the Venturi nozzle to react with water molecules, forming O₃ and ·OH as the main active substances, oxidizing and degrading the pollutants in water, so as to achieve the purpose of purifying organic pollutants.

2.2. Equipment and Drugs

UV-visible spectrophotometer, TOC detector, tissue leveller; Ultra low temperature refrigerator; Electronic Balance; Ultra-micro nucleic acid protein analyzer.

NP was purchased from Shanghai AlADDIN Biochemical Technology Co., Ltd, BCA protein concentration determination kit, Fish estradiol (E2) ELISA kit, Fish testosterone (T) ELISA kit from Shanghai New Platinum Chemical Technology Co.,

Ltd. The rest reagents were purchased from Sinopharm Group and were all analytical pure. All solutions were prepared with pure water from the ultra-pure water system (UPPLUS-100L, LICHEN, CHINA).

2.3. Methods

2.3.1. NP Degradation Analysis

Nonylphenol solution was scanned by UV-Vis spectrophotometer to determine its maximum absorption wavelength, and its concentration was determined by the absorbance at the maximum absorption wavelength. TOC value was measured with TOC detector to determine the degree of mineralization.

2.3.2. Toxicity Test of Zebrafish

180 20d AB zebrafish were selected. They were divided into six groups: CK (blank), CK+(solvent control), 20 μ g/L (NP concentration was 20 μ g/L), 200 μ g/L (NP concentration was 200 μ g/L), 30min (5mg/LNP degradation for 30min), and 60min (5mg/LNP degradation for 60min), with three parallel groups in each group. They were exposed to the corresponding concentration for 60 days. Deformities and death of zebrafish were recorded during exposure period, and body length, weight and sex ratio of each group were measured after exposure. Six zebrafish (three males and three females) were taken from each group, and the contents of estradiol and testosterone were extracted and detected respectively according to the corresponding kit instructions after exposure. The gonadal sections of female and male zebrafish were stained, respectively, and the gonadal sections of female and male zebrafish were photographed under a 100x and 200x microscope for pathological analysis.

3. Results and Discussion

3.1. NP Degradation Analysis

The SID method was used to degrade the NP wastewater with an initial concentration of 5mg/L. It was found that the degradation rate of NP reached about 95% at 30min, and the characteristic peak of NP was almost not detected at 60min, so it was completely degraded by default. The results of mineralization analysis by TOC showed that the mineralization rate of NP was about 44.84% after degradation for 60min, indicating that many small molecule intermediate products had not been completely degraded into H₂O and CO₂.

3.2. Toxicity Test of Zebrafish

3.2.1. Body Length, Body Weight and Sex Differentiation Ratio of Zebrafish

There was no obvious deformity and death in each group during exposure. In terms of sex differentiation, we found that 20 μ g/L nonylphenol significantly increased the proportion of females in zebrafish (73%), while 200 μ g/L nonylphenol had no significant effect. It seemed that there was no positive correlation between NP toxicity

and dose in this respect. Previous studies have also shown that lower concentrations of NP tend to be more toxic in terms of influencing sex ratio.

The growth and development of zebrafish is one of the important indicators of their reproductive health. Nonylphenol can cause a series of effects on the body length and weight of zebrafish through endocrine disruption. After exposure, the body length and weight of zebrafish in each group were shown in figure 1. Compared with the CK group, the body length and weight of zebrafish in the 20 $\mu\text{g/L}$ group were increased, and the body length and weight of the 200 $\mu\text{g/L}$ and 30min groups were significantly decreased. From the Angle of body length and body weight, it was found that 20 $\mu\text{g/L}$ nonylphenol had a little promotion effect on the growth and development of zebrafish, while 200 $\mu\text{g/L}$ nonylphenol significantly inhibited the growth and development of zebrafish. To some extent, NP promoted the growth and development of zebrafish at low concentration but inhibited it at high concentration.

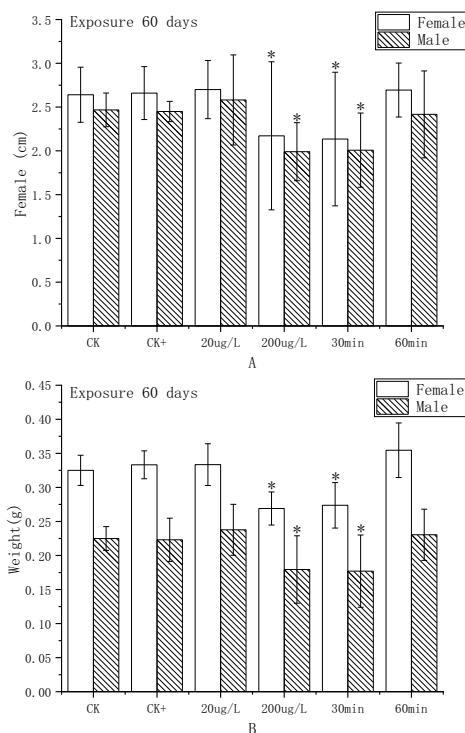


Figure 1. Effects of NP on body length (A) and body weight (B) of zebrafish. Values represent the mean \pm SD of three replicate samples from each group. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences between exposure groups and CK+ group.

3.2.2. Sex Hormone Level of Zebrafish

Estradiol (E2) and testosterone (T) were extracted from zebrafish according to the corresponding kit instructions. The results are shown in figure 2 below. As can be seen from the chart, after exposure, T in each concentration group in males was significantly lower than that in the control group, especially in the 200 $\mu\text{g/L}$ and degradation group for 30min, while T in each concentration group in females was slightly higher than that

in the control group. Studies have shown that the imbalance of T and E2 ratio will affect gonadal development, sex differentiation and reproduction of fish [11]. In this study, with the increase of NP exposure concentration, E2 increased and T decreased significantly in male zebrafish, and the ratio of T to E2 was unbalanced. Therefore, gonadal development, sex differentiation and reproductive ability would be greatly affected.

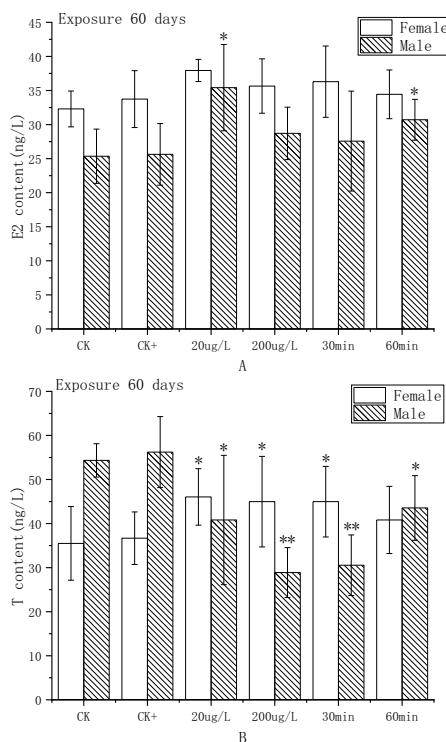


Figure 2. The effect of NP on E2(A) and T(B) of zebrafish. Values represent the mean \pm SD of three replicate samples from each group. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences between exposure groups and CK+ group.

3.2.3. Gonadal Development of Zebrafish

The ovary is the reproductive organ of female animals, where follicle formation, fertilized egg maturation, steroid production, and steroid hormone synthesis occur [12]. In this paper, the ovarian sections of female zebrafish in each group were shown in figure 3 below. In each concentration group, oocyte development at each stage was found, and oocyte development was normal without obvious pathological changes. It can be seen that NP has no significant effect on ovarian development.

In addition, the same analysis was made on the gonadal gland of male zebrafish. As shown in figure 4 of the gonadal section, the testis of zebrafish of CK and CK+ was covered with a large number of spermatozoa, and each spermatogenic capsule contained germ cells at different stages. Although there were germ cells in different periods in each concentration group, the number of sperm was significantly reduced and intercellular space appeared, especially in the testicular sections of zebrafish in the

200 μ g/L and 30min groups. The number of sperm in spermatogenic vesicles was significantly reduced and fragmented, and a large number of intercellular spaces appeared. Studies have shown that sex hormones can directly regulate sperm production in fish [13], while the imbalance of T and E2 ratio may interfere with gonadal development, sex differentiation and reproductive function in fish [14]. In this study, the ratio of T to E2 was significantly decreased in both groups of 200 μ g/L and 30min, which may be one of the reasons for the testis lesions of zebrafish.

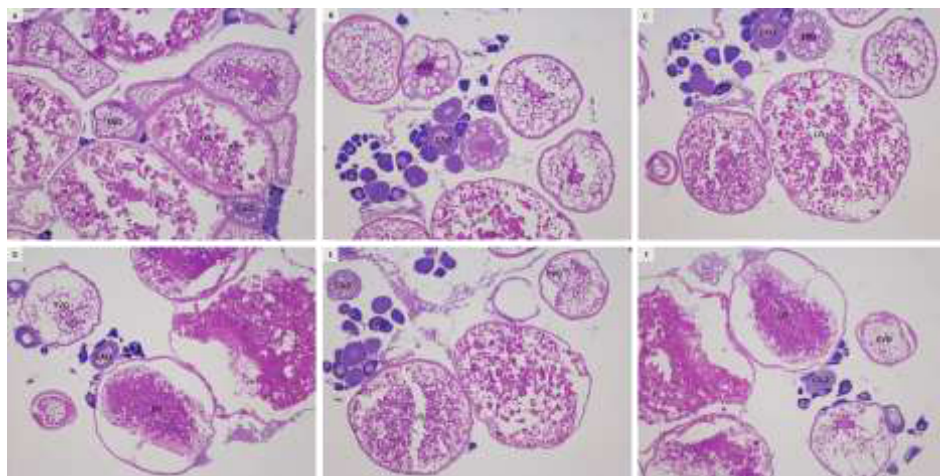


Figure 3. Histological section of zebrafish ovary. (A) Control (B) Control+ (C) 20 μ g/L (D) 200 μ g/L (E) NP Wastewater degraded by SID for 30 min (F) NP Wastewater degraded by SID for 60 min. The ovaries were categorized into the stages: perinucleolar oocytes (PO), cortical alveolar oocytes (CAO), early vitellogenic oocytes (EVO), and latemature oocytes (LMO) (100 \times magnification).

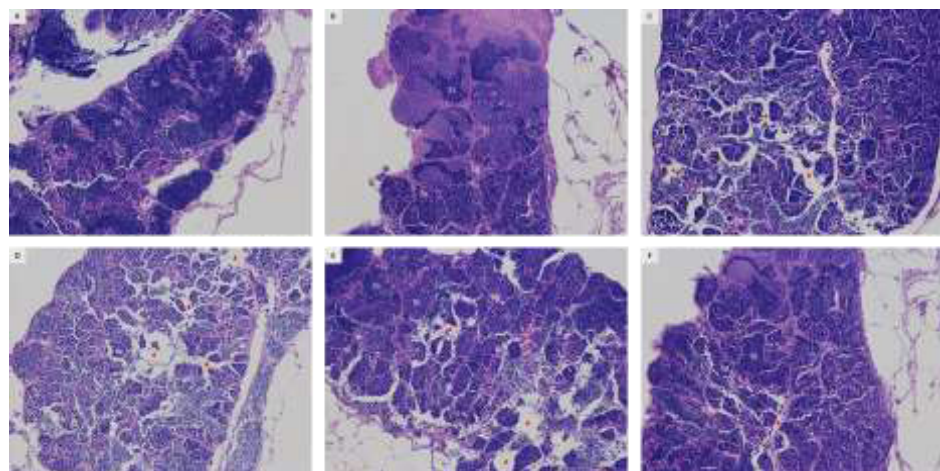


Figure 4. Histological section of zebrafish testis: (A) Control (B) Control+ (C) 20 μ g·L⁻¹ (D) 200 μ g·L⁻¹ (E) NP Wastewater degraded by SID for 30 min (F) NP Wastewater degraded by SID for 60 min. The triangle shows that the cell gap is widened. The testis was categorized into the stages: spermatogonia (Sg), spermatocytes (Sc) and spermatozoa (Sz). (200 \times magnification)

4. Conclusion

In this paper, the SID method was used to degrade the 5mg/L NP wastewater. After 60 minutes of degradation, the UV-Vis spectrophotometer could not scan the characteristic peak of NP, the mineralization analysis results showed that the mineralization rate was 48.84%, indicating that many small molecular intermediates had not been completely degraded. By exposing zebrafish to NP wastewater with different concentration and degradation time, the toxicological experiments on zebrafish showed that nonylphenol had a great influence on the growth and development of zebrafish, especially male zebrafish: high concentration of nonylphenol significantly inhibited the growth and development of zebrafish. However, the lower concentration of nonylphenol significantly increased the proportion of females in the process of sex differentiation in zebrafish. Male zebrafish had delayed gonad development, reduced sperm count and enlarged testis cavities under the effect of nonylphenol, and these problems became more serious with the increase of NP concentration. The control of each concentration group showed that the effects of 30min group on various indexes of zebrafish were close to those of 200 µg/L group, which was also consistent with the degradation rate of NP. The parameters of the 60min degradation group were almost the same as those of the control group, which proved that the small molecular intermediates which had not been completely mineralized in the mineralization analysis had been completely degraded after 24h aeration, or had no toxic effects on the growth and development of zebrafish and sex differentiation. It can be inferred that no more toxic intermediates appear in the degradation process of NP by SID technology, and NP in wastewater can be almost completely degraded, which plays a great role in alleviating its environmental toxicity.

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