

Effects of Streptomycin Wastewater on the Activity of GSH-PX in Zebrafish before and after Aerobic Biological Treatment

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Abstract. Most wastewater treatment plants now use aerobic biological treatment to dispose wastewater, but the toxicity of influent and effluent is unclear. In this study, subacute toxicity test was used to set four exposure groups and one control group to measure GSH-PX activity when zebrafish was exposed for 3,6,9,12,15 days at the indicated volume ratio, which is 10%, 30%, 50%, 70% respectively for influent and 10%, 20%, 30%, 40% for effluent. For influent, because of the response to the oxidative stress, the GSH-PX activity was activated in all other exposure groups except for the 70% volume group. The GSH-PX activity in 70% volume was significantly lower than the control group indicates that because of the sustaining increase of volume ratio, antioxidant system in zebrafish was destroyed and antioxidant enzyme activity was reduced. But for effluent, GSH-PX activity in each group was less than that in influent but still slightly induced, which means that after aerobic biological treatment, the sewage can reduce oxidative damage to zebrafish, but still have induction effect. For both influent and effluent, maximum of GSH-PX activity was both at the 30% exposure volume (18.7523U/mgprot for influent, 10.8023 U/mgprot for influent) which can provide the data basis for future research. All in all, GSH-PX activity in zebrafish muscle tissue can response to the oxidative stress and the changes can be used as an indicator of oxidative damage. And it is necessary to use the anaerobic method for the secondary treatment.

1.Introduction

Antibiotic wastewater is a kind of high-concentration organic wastewater containing refractory materials and biological toxic substances [1]. In China, over 700 kinds of antibiotics are produced and used, with an annual output of approximately 210,000 tons of raw materials, as well as 30,000 tons for export in 2016 [2]. Shijiazhuang, the provincial capital of HeiBei, as the most productive place for antibiotics, its production accounts for one sixth of the country [3]. Streptomycin is one of the common antibiotics, it extracted from the culture medium of streptomyces lividans [4]. Nowadays, there are still many technical difficulties in the production of antibiotics, resulting in many problems such as low utilization rate of raw materials, low purification purity and high residual antibiotic content in wastewater [5]. Discharge of wastewater is large. Most of sewage treatment plants now use aerobic biological treatment to dispose wastewater with considerable economic, environmental and social benefits. However, the toxicity of the influent and effluent is unclear [6].



The discharge of antibiotic wastewater released into the water can consume the oxygen in water and then cause the oxygen deficiency and destroy the self-purification function of water. It may lead to the eutrophication and have certain impacts on the aquatic organisms. Zebrafish is one of the model organisms, which has the characteristics of easy feeding, rapid development, annual spawning, large fecundity and easy collection of eggs [7]. In 1996, the Organization for Economic Cooperation and Development (OECD) included zebrafish in its testing standards for health toxicology and environmental toxicity [8]. GSH-PX activity in muscle tissue of zebrafish is one of the most important antioxidant enzymes in the fish body. The change of its activity directly affects oxygen free radical in the intracellular and its final product-lipid peroxides (LPO) [9].

This study selected a sewage treatment plant's influent and effluent as the water samples which deals with streptomycin wastewater primarily and using zebrafish as the toxicity test organism to evaluate the effect of streptomycin wastewater on GSH-PX activity in muscle tissue of zebrafish.

2. Materials and methods

2.1. Experimental Materials

2.1.1. Instruments and reagents. UV-visible light spectrophotometer (UV-2550); high speed centrifuge (TG16-WS); hypothermia refrigerator (BBC-226STV); high purity water distiller (SYZ-A). Protein kits, GSH-PX were purchased from a biological engineering research institute.

2.1.2. Test animals. Zebrafish were obtained from aquafarm in Shijiazhuang. In this experiment, mean length of fish used in this experiment was 34.0 ± 0.3 mm and mean weight of fish used in this experiment was 0.24 ± 0.03 g. Before the experiment, disinfection treatment employ 5% salt water, and then domesticate a week in 48h of aeration tap water. Feeding was halted 7d prior to testing. Healthy individuals of fish with similar size and weight were employed for test.

2.2. Experimental Methods

2.2.1. Preliminary experiments. Health zebrafish were put into 100% streptomycin wastewater (water volume concentration percentage), and no death within 96 h. For the influent, subacute experiment set up four experimental groups, volume ratio is 10%, 30%, 50%, and 70% respectively. For the effluent, subacute experimental set up four experimental groups, volume ratio is 10%, 20%, 30%, 40%, respectively. The effects of streptomycin wastewater with different volume fraction on antioxidant capacity of zebrafish muscle tissue are determined.

2.2.2. Exposure experiment. The subacute toxicity experiments lasted 15d. There are one control group and four experimental groups in the subacute experiments. There are three parallel experiments in each group, and put in 20 fish randomly. The experiment uses the static displacement method and changes the water every day. Besides, experiment assay is carried on 3, 6, 9, 12, and 15d.

2.3. Assay Methods

Zebrafish was dissected. 0.20g muscle tissue was obtained and rinsed in physiological saline. Muscle tissue was blotted with water and weighed. Samples was added to the mass ratio of 1:9 saline and grinded in the ice-water bath for 6-8min. 10% of the tissue samples were removed for experimentation and centrifugated for 10 minutes at speed of $3500 \text{ r} \cdot \text{min}^{-1}$ to get supernatant for future determination. The supernatant was collected for the experiment.

2.4. Statistical Analysis

All statistical analyses were expressed as mean \pm standard deviation (SD). One-way Anova of all data were checked using SPSS 17.0. Significant difference analysis uses the method of least significant difference (LSD). It analyzes the data in the groups on the same day. $P < 0.05$ was considered statistically different, $0.01 < P < 0.05$ statistically significantly different, and $P < 0.01$ statistically very significantly different.

3. Results and Discussion

GSH-PX is found in almost all tissues of animals and has anti-oxidant functions to protect cell membranes from the irritation and damage of peroxides. It can reduce the incidence of cell mutations and clear out H_2O_2 that produced in tissues ability [10]. If the GSH-PX activity is suppressed, it may affect the body's antioxidant function and cause tissue damage [11]. This study selected a sewage treatment plant's influent and effluent as the water samples. Subacute toxicity method was used to set four exposure groups and one control group to measure GSH-PX activity when exposed for 3,6,9,12,15 days at the indicated volume ratio, which is 10%, 30%, 50%, 70% respectively for the influent and 10%, 20%, 30%, 40% for the effluent. Almost all exposure groups had higher GSH-PX activity than the controls either in the influent or in the effluent, except for the exposure to 70% influent water.

3.1. Effects of Streptomycin Influent and Effluent on GSH-PX Activity in Zebrafish

3.1.1. *Effects of Streptomycin Influent on GSH-PX Activity in Zebrafish.* The activity of GSH-PX in zebrafish muscle with different concentrations of streptomycin influent in different exposure times is shown in Figure.1(a). For influent, when treated with 10% exposure volume, the activity of GSH-PX was significantly induced ($P<0.01$) from the ninth day. When treated with 30% exposure volume, the activity of GSH-PX was activated at all days of exposure, especially when exposed to the 6th, 9th and 12th days. When the number of exposure days was identical, the maximum enzyme activity all appeared in the 30% exposure group. The remarkable increase of GSH-PX activity indicates that when exposed to a certain concentration of streptomycin wastewater, zebrafish body may increase the intracellular enzyme activity and improve the body's antioxidant protection mechanism, so that H_2O_2 and oxygen free radicals can be promptly cleared and non-accumulation in cells. Avoid cell lipid peroxidation caused by cell damage simultaneously. Due to the reduction of lipid peroxidation products and the continual improvement of cellular anti-oxidant functions, oxidative stress damage in tissues can be minimized [12]. Li et al studied the effects of diethylstilbestrol on GSH-PX of Cadmium-contaminated Grass Carp. They found the maximum GSH-PX activity appeared at the mid-value of exposure concentration (1mg/L) and proposed that grass carp had the strongest ability to scavenge oxygen free radicals when exposed to the concentration of 1mg/L [9], which is consistent with the results of this study.

When treated with 50% exposure volume, the GSH-PX activity was significantly induced ($P<0.01$). However, the maximum was reduced by 21.40% compared to the 30% exposure group (When exposed to the 12th day). With the continuous increase of exposure concentration, the activity of enzyme gradually decreased, which may lead to the inadequate antioxidant capacity and the damage of antioxidant system [13]. This phenomenon was even more evident when exposed to 70% exposure group. Except for this group which exposed for 6 days, the enzyme activity of other groups was significantly lower than that of the control group. Especially, on the 15th day of exposure, the enzyme activity was only 27.40% of the control group. An assumption that the increase of concentration and prolongation of exposure time, the scavenging capacity of zebrafish's own cells is not enough to eliminate the free radicals, even damage the cells irreversibly, which hinders the synthesis of GSH-PX. So that enzyme activity was significantly lower than the control group. We have tried to compare our predictions with available conclusions made from another study. Liu et al studied the effect of ammonia nitrogen stress on activities of SOD and GSH-PX in muscle tissue of loach. According to the concentration of NH_3-N , four experimental groups and one control group were set up. Concentration ratio were 50 mg/L, 150 mg/L, 250 mg/L, 450 mg/L. Muscle tissue was grinded for measure the GSH-PX activity at 2, 7, 14 and 21 days. They found when the concentration was 250mg/L, compared with the 150mg/L concentration group, GSH-PX activity was seriously decreased. GSH-PX activity was 57.46% of that in the control group when exposed to 450 mg/L for 21 days. They proposed that the activity of GSH-PX in muscle is affected by the exposure time and concentration of ammonia nitrogen. With the extension of exposure time, the antioxidant capacity of muscle tissue is inadequate, which will accumulate free radicals and decline the enzyme activity. Therefore, the enzyme activity of the

high concentration group showed a significant decrease at 21d [14]. That can provide the basis for this research's conjecture.

3.1.2. Effects of Streptomycin Effluent on GSH-PX Activity in Zebrafish. For effluent, streptomycin effluent within 15d of GSH-PX activity in zebrafish muscle was shown in Figure.1(b). GSH-PX activity in each group was less than that in influent and the change trend was stable. Indicating the oxidative damage to zebrafish was reduced after aerobic biological treatment. However, the zebrafish muscle tissue was still slightly induced ($0.01 < P < 0.05$) compared with the control group.

It can be seen in the Figure.1(b), under the same exposure conditions in different groups, the trend in the activity of GSH-PX changed variously with the increase of exposure time. The results of this experiment are keeping with those of Yao et al, who proposed that GSH-PX clears lipid peroxides induced by reactive oxygen species and hydroxyl groups in order to protect the integrity of cell membrane structure and function [15]. And changes in intracellular lipid peroxides are also affected by the cell's own metabolites [16].

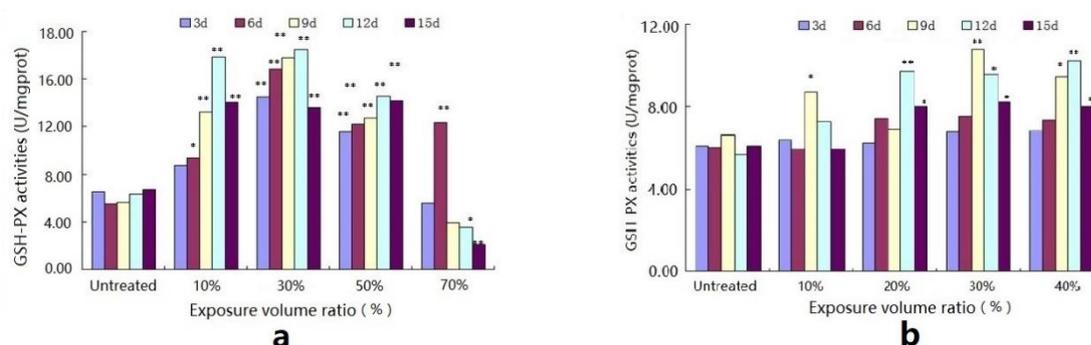


Figure 1. Effect of streptomycin wastewater (a for influent, b for effluent) on GSH-PX activities in zebrafish muscle tissue.

(The same day all the data are compared with control group, * $p < 0.05$, ** $p < 0.01$.)

3.2. Comparison of GSH-PX activity with influent and effluent

When treated with the same exposure volume (10%,30%) of influent and effluent, different results can be observed. When treated with 10% exposure volume of influent, the activity of GSH-PX increased at first and then decreased with the extension of exposure time, which accord with "bell-shaped" distribution. However, GSH-PX activity changes are not regular when treated with the effluent (10%), GSH-PX activity decreased on 6th day, then increased on 9th day, and decreased again on 12th day and 15th day. For both influent and effluent, the maximum of GSH-PX activity was both at the 30% exposure volume (18.7523 U/mgprot in influent, 10.8023 U/mgprot in effluent) indicating that when zebrafish exposed to 30% volume of wastewater, body's enzyme activity and antioxidant capacity are the largest, which can be used as a threshold for future research.

On the other hand, for the influent, under the same exposure time, the activity of GSH-PX also showed a "bell-shaped" distribution as the exposure concentration increased. Shen et al studied the effect of streptomycin in influent on SOD activity and MDA content in zebrafish muscle. They found the SOD activity in zebrafish muscle was significantly induced on the 3rd day, and the SOD activity in the 50% volume fraction reached 30.56U/mg. 70% of the volume fraction of SOD activity in the experimental group gradually decreased with the extension of exposure time which SOD and GSH-PX can indicate the degree of oxidative damage in zebrafish [17], which is similar to the results of this study. Heleen Van Acker proposed that different concentrations of antibiotic wastewater produced reactive oxy-gen species (ROS) at a rate greater than the clearance rate, unbalancing the oxidation and antioxidant systems of zebrafish muscle tissue, causing tissue damage eventually[18]. But it can be seen in Figure.1(b), no similarities were observed in the effluent. Zhao et al discussed the effect of biochemical method on streptomycin effluent on the activity of SOD in zebrafish muscle. They found that when exposed for 9~15 days, 10%, 20%, 40% of the experiment group had no significant difference from the control group [19]. These experimental results indicate that the zebrafish

antioxidant defense system eventually reached a new balance and the fish antioxidant defense system has a function of self-regulating [20].

4. Conclusion

In this study, streptomycin Wastewater was used for subacute toxicity test by static laboratory exposure method, and experiments determined the zebrafish muscle GSH-PX activity. For influent, except for the 70%, the GSH-PX activity was induced in all other exposure groups because of the response to the oxidative stress. Owing to the continuous increase of volume ratio, antioxidant system in zebrafish was destroyed and antioxidant enzyme activity was reduced. Therefore, the GSH-PX activity in 70% volume was significantly lower than the control group. For effluent, GSH-PX activity in each group was less than that in influent but still slightly induced indicates that after the aerobic biological treatment, the sewage can reduce oxidative damage to zebrafish, but still have induced effect. It is essential to use the anaerobic method for the secondary treatment. For both influent and effluent, maximum of GSH-PX activity was both at the 30% exposure volume, which can be used as a threshold for future research. GSH-PX activity in zebrafish muscle tissue can response to the oxidative stress and the changes can be used as an indicator of whether oxidative damage occurs.

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6. References

- [1] Shi Ruiming, Wang Feng, Yang Yuping, et al. Status and Research Progress of Antibiotic Wastewater Treatment [J]. *Journal of Shan Dong Chemical Industry*, 2007,36(9):10-14.
- [2] Cheng Xianwei, Liang Yinxiu, Zhu Hui, et al. Research Progress on Constructed Wetlands for Treatment of Antibiotics in Water [J]. *Journal of Wetland Science*, 2017,15(1):125-132.
- [3] Xu Yan, Zhang Yuan, Guo Changsheng, et al. Characteristics of Antibiotics, Resistant Bacteria and Resistance Genes in WangYangGou, Shijiazhuang [J]. *Journal of Agro-Environment Science*, 2014,33(6):1174-1182.
- [4] Su Jianjiang, Huang Fuyi, Zhu Yongguan, et al. Research Progress on Environmental Antibiotic Resistance Genes [J]. *Journal of Biodiversity Science*, 2013,21(4):481-487.
- [5] Zeng Linxuan, Zhang Qiuyun, Liu Peihong, et al. Progress of Antibiotic Pharmaceutical Wastewater Treatment Technology [J]. *Journal of Safety and Environmental Engineering*, 2005,12(4): 62-68.
- [6] Shao Yiru, Xi Beidou, Cao Jinling, et al. Distribution and Removal of Antibiotics in Urban Sewage Systems [J]. *Journal of Environmental Science and Technology*, 2013,36(7):85-94.
- [7] Alexander J, Stainier DY. A Molecular Pathway Leading to Endoderm Formation in Zebrafish [J]. *Journal of Current Biology*, 1999,9(20):1147-1157.
- [8] Wang Jiajia , Xu Chao, Tu Yunjie, et al. Experimental Research and Application of Zebrafish and Its Embryo in Toxicology [J]. *Asian Journal of Ecotoxicology*, 2007,2(2):123-136.
- [9] Li Yuanling, Lan Xin, SiWantong, et al. Effects of Diethylstilbestrol on GSH-PX, SOD and MDA of Cadmium-contaminated Grass Carp [J]. *Journal of Agro-Environment Science*, 2008,27(1):0350-0353.
- [10] Yao Xin, Qian Yuanshu. Effects of Fluoroquinolones on Activities of Extracellular GST, GR and GSH-PX in Extrahepatic Tissue of Rats [J]. *Journal of Chinese Antibiotics*, 2005,30(7):423-427.
- [11] Schauer R, Sommer U, K rüger D, et al. The Terminal Enzymes of Sialic Acid Metabolism [J]. *Journal of Biosci Rep*, 1999,19(5):373.

- [12] Ding Limin, Huang Xiaomin, Zhang Zhuoyi. Effects of Procyanidin on The Activity of SOD and GSH-PX Induced by Hydrogen Peroxide in Endothelial Cells [J]. *Journal of Chinese Medicine Emergency*, 2013,22(5):714-717.
- [13] Yin Jin, Hu Yixiu, Hu Yuming, et al. Effect of Grape Seed Proanthocyanidins Extract on MDA, SOD and GSH-PX in Mice [J]. *Journal of China Tropical medicine*,2007,8(7):1285-1287.
- [14] Liu Yang, Leng Yufei, Yu Lianyang. Effect of Ammonia Nitrogen Stress on Activities of SOD and GSH-PX in Different Tissues of Loach [J]. *Journal of Anhui Agriculture*, 2011,39(2):1069-1072.
- [15] Yao Xin. Effects of Fluoroquinolones on Extrahepatic Drug Metabolizing Enzymes and GR, GSH-PX Activities [D]. Chongqing Medical University, 2003.
- [16] P.A. Olsvik, T. Kristensen, R. Waagbø, et al. mRNA Expression of Antioxidant Enzymes and Lipid Peroxidative Stress in Liver of Atlantic Salmon Exposed to Hyperoxic Water During Smoltification [J]. *Journal of Comparative Biochemistry and Physiology*, 2005, PartC:314-323.
- [17] Shen Hongyan, Wang Linxin, Yang Jindi, et al. The Effect of Streptomycin in Influent on SOD Activity and MDA Content in Zebrafish Muscle [J]. *Journal of Hebei University of Science and Technology*, 2014,35(3):303-309.
- [18] Heleen Van Acker, Tom Coenye. The Role of Reactive Oxygen Species in Antibiotic-Mediated Killing of Bacteria [J]. *Journal of Trends in Microbiology*, 2017,25(6):456-466.
- [19] Zhao Yue, Cao Zhihui, Wang Bing, et al. Effect of Streptomycin Wastewater Effluent Treated with Biochemical Method on SOD Activity and MDA Content in Zebrafish [J]. *Journal of Jiangsu Agricultural Sciences*, 2015,43(8):348-351.
- [20] Li Min, Zhao Yue, Cao Zhihui, et al. Effect of Streptomycin Wastewater on POD Activity and GSH Content in Zebrafish [J]. *Journal of Safety and Environmental Engineering*, 2015,22(3):1-11.