Toxicity assessment of chlorella vulgaris to landfill leachate before and after treatment

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Abstract

The basic physical and chemical indicators of landfill leachate treated by SND/UF/RO process, can reach the discharge standard. And the concentration of BPA and DMP can still be detected in the effluent. It is unclear whether the organism is safe owing to the effluent enters the environment directly. In this study, chlorella vulgaris was used as a biological evaluation material to evaluate the toxicity of landfill leachate before and after treatment according to the photosynthetic system of chlorella. The results of the study evaluated the effluent is very important for the environmental biosafety.

Keywords

Chlorella vulgaris, landfill leachate, photosynthetic system.

1. Introduction

Toxicological risk assessment of landfill leachate has become a hot topic in recent years. The toxic substances in leachate have been studied from the perspectives of chemical analysis[1], genetics[2], and toxicology[3]. Christian Khalil et al.[4] used chemical methods to assess the risk of municipal leachate. The genotoxicity of leachate which was analyzed by micronucleus test and *Ames* test on the organic pollutants of leachate in a comprehensive landfill[5]. Although the chemical method can detect the presence of environmental hormones, the toxicity of substances in leachate would be underestimated if the low concentration or trace toxic substances are below the detection limit and cannot be detected by chemical determination. In addition, chemical analysis does not reveal complex interactions between contaminants, while bioassays take into account factors such as bioavailability, synergy or antagonism[6], integrating all biologically relevant compounds. Detection to characterize various contaminants in leachate has become a powerful method in the field of environmental toxicology[7; 8].

The toxicity of landfill leachate after treatment with the treatment requires different bioreceptors to evaluate the toxicity of leachate[3]. At present, domestic and foreign research reports mainly use algae as receptor targets to evaluate the toxicity of leachate, and study the effects of leachate, especially the effluent, on the environment, ecology and humans. The effectiveness and feasibility of the process provide an important basis.

Algae can not only grow in rivers, streams, lakes and oceans, but also grow in short-term water or humid places. The growth range is very wide and the environmental conditions are not strict. The adaptability of algal is strong which need only very low nutrient concentration, low light intensity and relatively low temperatures. For the evaluation of endocrine disruptors, algae (especially microalgae) are currently used as evaluation objects in many literatures. As a primary producer, microalgae have the advantages of small individual, short life cycle, easy isolation and culture, relatively sensitive to environmental effects, and simple and easy to test experimental methods. Therefore, they are often used in domestic and foreign research. Microalgae, especially freshwater algae such as *Chlorella vulgaris*, is widely used as standard test organisms in aquatic ecosystems because of their short life cycle, widespread presence, good physiological tolerance and sensitivity. *Chlorella vulgaris* plays a vital role in the toxicity study of pollutants[9], and studies have reported the photosynthetic system in algae cells such as the photosynthetic pigment content. The chlorophyll

level decreased, and the psbB, psbC and rbcL transcript abundances of light and pigment decreased, which seriously affected the oxidation system and photosynthetic system of *Chlorella*[10]. Zhao et al[11] studied the toxicity of herbicide to pramezone to *Chlorella vulgaris*, and the results showed that herbicides have a major impact on algae growth, oxidation systems and photosynthetic systems.
At present, there are few studies related to the toxicity evaluation of *Chlorella vulgaris* for leachate, especially the toxicity study on the effluent. *Chlorella vulgaris* is used as the test object to evaluate the effectiveness of the treatment process.

2. Materials and Methods

2.1 Cultivation of Chlorella

Chlorella vulgaris is provided by the College of Life Science and Technology of Jinan University. *Chlorella* was cultured in BG-11 medium. The pH of the prepared medium was about 7.1. The medium was steam sterilized at 121 °C for 20 min before use. *Chlorella vulgaris* is placed in a light incubator for cultivation, and shaken three times a day at a fixed time. The setting conditions of the light incubator are follows: the temperature is set to 25℃, the light intensity is 40%, and the dark ratio of light is 12 h. : 12 h.

2.2 Growth inhibition assay of Chlorella vulgaris

In order to accurately and rapidly measure the biomass of algal cells[11], the absorbance is selected to indicate the growth of algae. The algae solution of *Chlorella vulgaris* was diluted to different concentrations of algae, and the number of algae cells was counted under a microscope using a hemocytometer[12], while using a dual beam UV-Vis near-infrared luminometer (Cary 500) at 680 nm. The absorbance of the corresponding algae solution was determined and the linear regression equation between the corresponding number of algae cells ($y \times 10^6$ cells/mL) and absorbance (x) was follows as $y = 21.476x + 0.453$ ($p < 0.01$, $R^2 = 0.988$).

The algae grown in the logarithmic phase was inoculated into a triangular flask containing the sterilized fresh medium. The corresponding absorbance was measured every day for 5 days when algal cells exposed to different concentrations of three leachates (5, 10, 15, 20, 25, 30%, v/v). Each set of experiments was run in parallel three times.

2.3 Determination of photosynthetic pigment content in algae cells

At the exposure concentration and exposure time set in 2.1, take 50 mL of algae solution treated with different concentrations of three leachates in a centrifuge tube, centrifuge at 6000 rpm for 10 min, discard the supernatant, and add 90% acetone solution (v/v) to resuspend, and then centrifuge at 6000 rpm for 5 min. After taking the supernatant, determine the corresponding absorbance at 664 nm and 647 nm on an ultraviolet spectrophotometer. The formula for calculating the chlorophyll content is as follows:

$$\text{Chlorophyll a (mg/L)} = 11.93A_{664} - 1.93A_{647} \quad (1)$$

$$\text{Chlorophyll b (mg/L)} = 20.36A_{647} - 5.50A_{664} \quad (2)$$

In the formula, A_{664} and A_{647} represent the absorbance at 664 nm and 647 nm, respectively.

3. Results and discussion

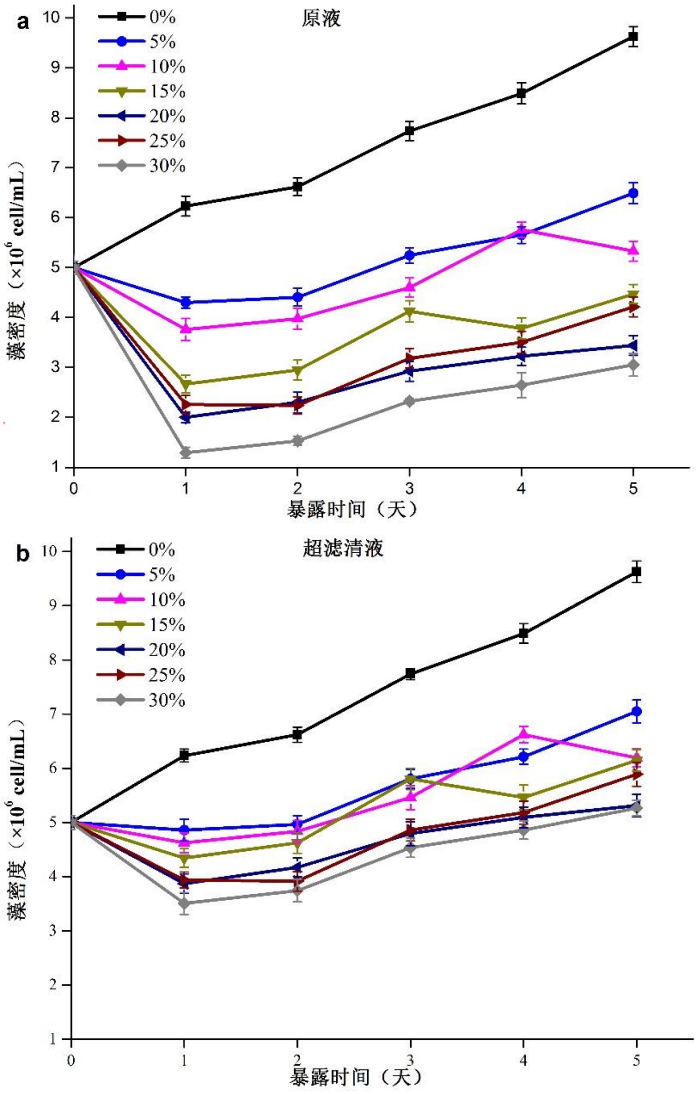
3.1 The inhibition growth of Chlorella vulgaris

The growth inhibition curves of *Chlorella vulgaris* exposed to three leachates were shown in Figure 1. It could be seen that the growth trends of *Chlorella vulgaris* exposed to three leachates were similar, and the inhibition rate of *Chlorella vulgaris* was increased with the increase of exposure concentration[13]. Figure 1a shows that the algae density was 1.29×10^6 cells/mL when algal exposed to raw leachate (30%, v/v) after 1 day, which was significantly different from the control group (6.23×10^6 cells/mL). After 5 days of exposure, the algae density increase was only 3.05×10^6 cells/mL when algal exposed to raw leachate (30%, v/v), while the control group increased to 9.63×10^6

cells/mL. And the algae cell growth inhibition rate was 68.3%, indicating that raw leachate of high concentrations severely inhibited the growth of algae cells and were very toxic to *Chlorella vulgaris*. It could be seen from Fig. 1b that the algae density is 3.51×10^6 cells/mL when algal exposure to ultrafiltration (30%, v/v) leachate which treated by biochemical treatment and ultrafiltration technology after 1 day, and the inhibition rate was lower than 30% raw leachate. The algae density was 5.27×10^6 cells/mL and the algal cell inhibition rate was 45.3% after 5 days of exposure, indicating that the leachate was reduced in biotoxicity after SND/UF treatment, and effectively removed partially toxic pollutants.

Fig. 1c showed that the algae density was 4.75×10^6 cells/mL after 1 day of exposure to the effluent (30%, v/v), and the algal cell density increases to 7.24×10^6 cells/mL after 5 days of exposure, which still exists weak growth inhibition. The assay indicates that the biotoxicity reduction in the effluent was very significant after treatment with the SND/UF/RO process.

Norazela Nordin[12] reported that the growth of *Chlorella* and *Scenedesmus* exposed to leachate was increased with the increase of leachate concentrations and chlorella was more sensitive to leachate. Algae cells showed good self-repairing ability at low concentration[12]. In this experiment, *Chlorella* also showed obvious self-repairing ability in the effluent and was severely inhibited in raw leachate, which was basically consistent with the reported literature. After treatment with SND/UF/RO, the growth inhibition of *Chlorella* exposed to the effluent significantly reduced, but still showed a weak inhibition at high concentrations (30%, v/v).



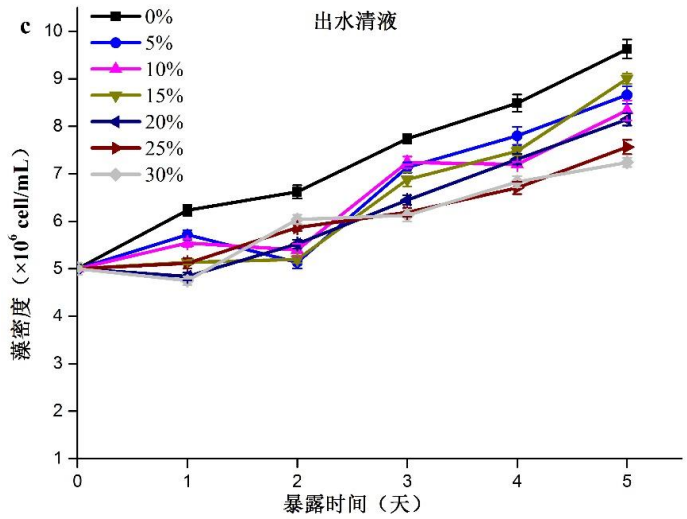


Figure 1 Growth inhibition curve of Chlorella vulgaris after exposure to
Three samples, (a) untreated leachate, (b) SND/UF treated leachate, (c) the effluent

3.2 Changes of chlorophyll content

Chlorophyll is the main pigment for photosynthesis in plants. It plays a central role in photosynthesis and is a family of lipid-containing pigments. At present, there are many literatures to determine the correlation between chlorophyll a and chlorophyll b in plants to reflect the impacts of the outside on the photosynthetic system of plants[14].

Fig. 2A and Fig. 2B respectively show the results of the effects of three leachates on the contents of chlorophyll a and chlorophyll b of *Chlorella vulgaris*. It could be seen from Fig. 2Aa that the chlorophyll a content of *Chlorella vulgaris* exposed to raw leachate displayed a significant concentration-dose-time effect with the increase of exposure time and exposure concentration. When algal exposed to raw leachate (5%, v/v) after one day, the chlorophyll a content decreased from 4.05 mg/L to 2.24 mg/L, and decreased from 5.13 mg/L to 1.09 mg/L after 5 days of exposure. After exposure to to raw leachate (30%, v/v) for 1 day, chlorophyll a decreased to 3.01 mg/L. With the extension of exposure time, the chlorophyll a content decreased to 0.40 mg / L after 5 days of exposure, all algae cells almost died, indicating that raw leachate could seriously damage the photosynthetic system of *Chlorella vulgaris*.

After the leachate treated by biochemical treatment and ultrafiltration technology, the photosynthetic pigment content of *Chlorella* exposed to SND/UF treated leachate was shown in Figure 2Ab and Figure 2Bb. It could be seen that the exposure concentration has little effect on the chlorophyll a content in the short-term exposure, but it gradually shows a significant concentration-dose-time effect after three days of exposure, the content decreased significantly with the increase of the concentration of chlorophyll a. After exposure to SND/UF treated leachate (5%, v/v) one day, the chlorophyll content in *Chlorella* decreased significantly to 2.19 mg/L. But as the exposure time prolonged, *Chlorella* gradually proceeded self-repairing ability at low concentrations, chlorophyll a content increased to 4.49 mg/L after 5 days of exposure, which was less than that of the control group. However, chlorophyll a content was 1.90 mg/L after exposure to high concentration (30%, v/v) SND/UF treated leachate for 1 day. And the chlorophyll a content only increased 2.63 mg/L with the prolongation of exposure time. The photosynthetic pigment content increased comparing to raw leachate, but there was a significant difference with the control group. The results showed that the leachate after being treated by SND/UF process could reduce the toxicity of leachate to some extent, but it still showed significant damage to the photosynthetic system of *Chlorella*.

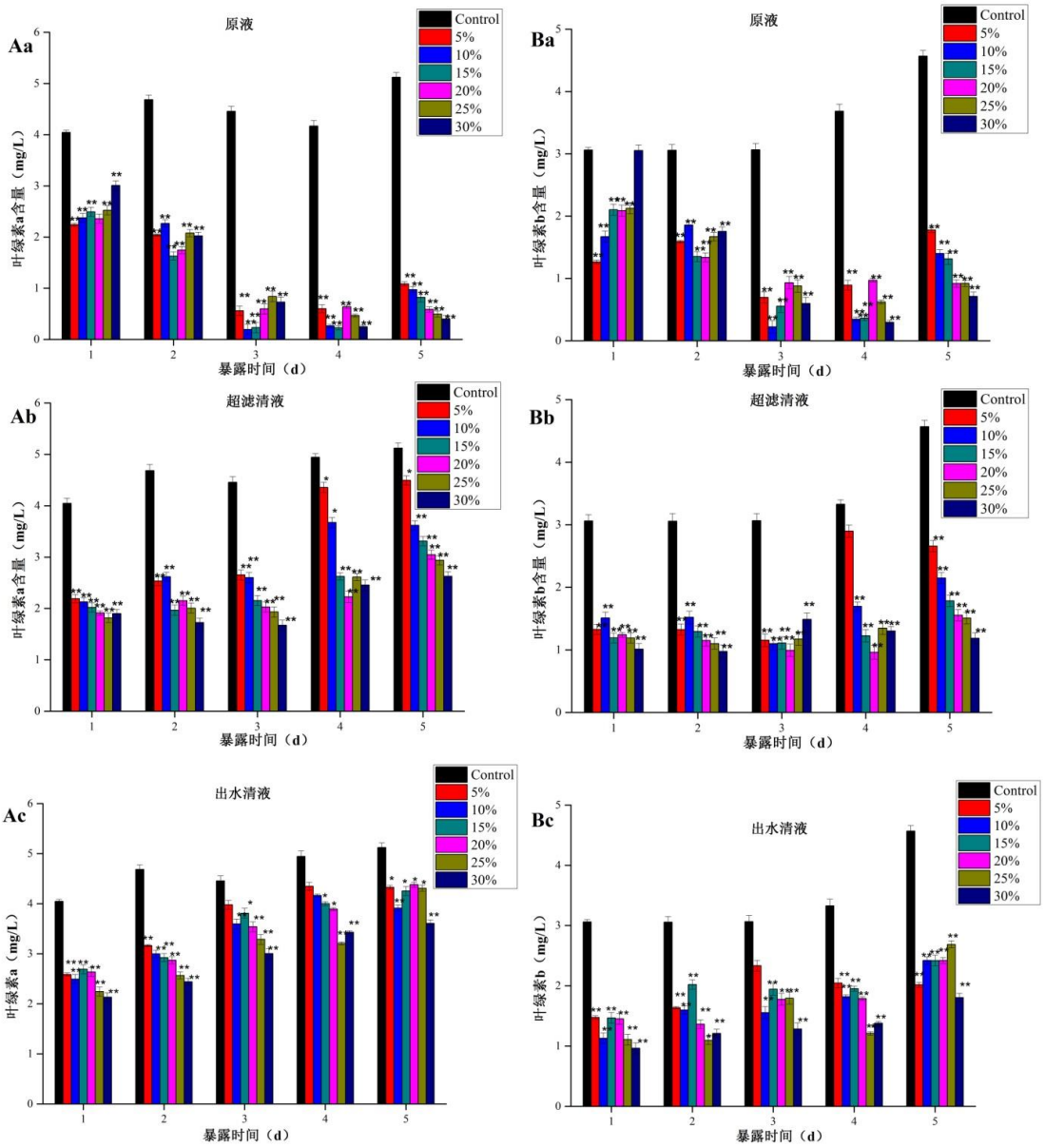


Figure 2 The contents of Chl a and Chl b in Chlorella after exposure to three samples, (A) Chl a, (B) Chl b, (a) untreated leachate, (b) SND/UF treated leachate, (c) the effluent

After SND/UF treated leachate was further treated by the reverse osmosis process, the effect of the effluent on the photosynthetic pigment of *Chlorella* was shown in Figure 2Ac and Figure .2Bc. It could be seen from Fig. 2Ac that the effluent has little effect on the chlorophyll a content in *Chlorella*, but in the short term (2 days), the effluent has a greater influence on the content of chlorophyll a with the exposure time. When prolonged, the level of chlorophyll a gradually increased, and the content at low and medium concentrations gradually approached the control group, and there was still a significant difference at high concentration. After one day of exposure to effluent (5%, v/v), the chlorophyll a content was 2.59 mg/L. Due to the trace amount of toxic pollutants in the effluent, the growth environment of *Chlorella* was affected. The self-repairing ability of *Chlorella* was obvious after prolonged exposure time. After 5 days, the chlorophyll a content increased significantly to 4.33 mg/L. When *Chlorella* exposure to the effluent (30%, v/v) for one day, the chlorophyll a content was 2.13 mg/L. After 5 days, the chlorophyll a content increased to 3.61 mg/L and till had significant difference with the control group (5.13 mg/L). The change trend of chlorophyll b content was similar to that of chlorophyll a, but the performance is more sensitive. The experimental results show that the biotoxicity of leachate treated with SND/UF/RO process to *Chlorella*, was greatly reduced, but it still

showed significant difference at high concentration, indicating that the effluent will slightly affect the synthesis of photosynthetic pigments in *Chlorella*.

Based on the changes of chlorophyll content of *Chlorella* in three leachates, it was found that the growth of *Chlorella* exposed to raw leachate was very serious damaged, even the algae cells died in a large amount and showed an irreparable state. The growth of *Chlorella* exposed to SND./UF treated leachate also inhibited, but it was much weaker than raw leachate and showed a partial repair trend in the late stage of exposure, indicating that the treatment process at this stage effectively reduced the toxicity of leachate to some extent. After SND/UF/RO treatment, *Chlorella* exposed to the effluent showed a weak inhibition. The algae cells showed good self-repairing ability with the time goes by[15], indicating that SND/UF/RO process could efficiently remove toxic contaminants in leachate, but the effluent still showed weak damage to the photosynthetic system.

4. Conclusion

This study mainly uses *Chlorella vulgaris* to evaluate the toxicity of three leachates and assesses the effect of the growth inhibition and the photosynthetic system of *Chlorella* exposed to SND/UF/RO leachate. The research, especially the toxicity assessment of the effluent, the main findings are as follows:

(1) Growth inhibition experiments of *Chlorella* showed that the algae density increased from 3.05×10^6 cells/mL to 7.24×10^6 after exposure to the effluent (30%, v/v). There was still a significant difference with the control group (9.63×10^6 cells/mL), indicating that the effluent had a weak inhibitory effect on the growth of *Chlorella*.

(2) Photosynthetic pigment experiments of *Chlorella* showed that the chlorophyll a content increased from 0.4 mg/L to 3.61 mg/L after exposure to the effluent (30%, v/v) for 5 days. There showed a significant difference with the control group (5.13 mg/L), indicating that a small amount of substances still exists in the effluent and had a weak effect on the photosynthetic system in *Chlorella vulgaris.*

Acknowledgements

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