Research on the Treatment of Soil Leachate by Using Microalgae

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Abstract. In order to remove and recover nitrogen and phosphorus of water from soil surface runoff, the combination method of cultivation and harvesting of microalgae was used in this research. Microalgae was cultured in the leachate of soil which was collected from vegetable greenhouse; culture solution was sampled and detected at different intervals. The results showed that leachate contained nutrients which could meet the needs of algal growth, the algal density increased in line with increase of culture time; pH of culture solution was alkaline and increased fast in the first 5 days; total and soluble phosphorus, total nitrogen and nitrate decreased fast in the first 5 days which showed assimilation of microalgae could remove phosphorus and nitrogen in the leachate.

1. Introduction

Nitrogen and phosphorus were macroelements for plant growth. In order to get high crop yields, excessive chemical fertilizer was added to the soil of greenhouse. Chemical fertilizer entered into surface water with surface runoff which caused water eutrophication [1]. According to bulletin of the first national census of pollution sources, total nitrogen and phosphorus from agricultural sources accounted for 57.2% and 67.4% of surface water pollution [2]. Compared to point source pollution, agricultural non-point source pollution was characterized by dispersion, concealment, randomness and hard detection which increased difficulty of treatment. In order to inhibit the leaching of nitrogen and phosphorus, the main controlling methods included source reduction and process control. Source reduction was to reduce dosage of chemical fertilizer, which might lead to reduction of crop yield. Based on the reduction of fertilizer, some in-situ process controls could improve fertilizer utilization such as reasonable irrigation, change of planting pattern, fertilizer inhibitor, soil amendment [3].

In order to control non-point pollution of soil and recover nitrogen and phosphorus from surface runoff, microalgae was cultured in the leachate of soil, microalgae growth could assimilate nitrogen and phosphorus, then microalgae was harvested by flocculation, so the phosphorus and nitrogen in the leachate was removed. This research combined removal and recover of nitrogen and phosphorus of leachate, which not only protected environment but also economized resources.
2. Materials and methods

2.1 Soil and leachate
Soil was collected from vegetable greenhouse in Wuqing district. The method of preparing leachate was as follows: the soil was put in the plastic beaker, then 2 fold of water was added into beaker, the mixture was agitated for 30min on a magnetic stirrer at room temperature and stand for 12h, the supernatant was collected after centrifugation, the supernatant was leachate.

2.2 Algae species
*M. aeruginosa* (FACHB-469) and *chlorella* (FACHB-8) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan, Hubei Province, China. The algae was cultured in BG11 medium.

2.3 Laboratory culture experiments
500mL leachate was added into 1L glass beaker, autoclaved at 121℃ for 20min, sterilized by UV radiation on a clean bench for 30min. Then 50 mL of M. aeruginosa and chlorella suspension liquid (50 mL of starter-culture was placed in a 50 mL centrifuge tube and centrifuged for 10 min at 10000 rpm, and then 25 mL sterile water was added and mixed) was inoculated into the leachate. The glass beaker was put into incubator, the growth conditions were as follows: 25℃, 2200 lx light intensity at 12 h/d. Then, 50mL of algal culture was sampled at different intervals and used to determine biochemical properties. At last, the algal cells were harvested by flocculation with biological flocculants.

2.4 Detection methods
*M. aeruginosa* cultures were examined using a Motic digital microscope and the cell density (cells/mL) enumerated using Motic Image Advance Software; The pH was determined using pH meter; the phosphorus was analyzed by the molybdenum-antimonyanti-spectrophotometric method; the total nitrogen and nitrate were detected by Ultraviolet Spectrophotometry.

3. Results and discussions

3.1 The change of algal density over time
The change of algal density was showed in Figure 1. The algal density increased in line with increase of culture time, growth rate of *M. aeruginosa* and *chlorella* was about 3.6×10^5 and 3.1×10^5 cells/(day·mL) respectively. Chlorella grew faster than *M. aeruginosa* in the first 10 days, but reverse after 10days. It is well-known that algae growth needed nutrient elements such as nitrogen, phosphorus and some trace elements. The algae grew quickly in the leachate, which illustrated leachate was rich in nutrients.

![Figure 1. The change of algal density over time](image-url)
3.2 The change of pH over time

The change of pH over time was showed in Figure 2. The soil was saline-alkali soil, so the leachate was alkaline. Algal growth could strengthen the alkaline of solution. pH increased fast in the first 5 days and increased slowly afterwards. Some research reported that photosynthesis of cyanobacteria increased pH value of solution [4], the reaction mechanism was showed as follows:

$$6\text{HCO}_3^- + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 6\text{OH}^-$$

(1)

Cyanobacteria grew quickly in the alkaline environment, so it was feasible that the microalgae was used to treatment water from saline-alkali soil.

3.3 The change of phosphorus of leachate over time

Phosphorus was limiting factor of algae growth, cyanobacteria grew quickly when phosphorus concentration was between 0.10 and 1.00mg/L, phosphorus was not limiting factor when phosphorus concentration was above 0.20mg/L [5]. Soluble phosphorus of leachate was 4.83 mg/L, so leachate supplied abundant phosphorus for algae. Soluble phosphorus was lower than total phosphorus for every sample, but difference was very small, this result indicated that inorganic phosphorus was main form of leachate and cell-free filtrate. The change of total phosphorus and soluble phosphorus over time was same between M.aeruginosa and Chlorella. Total phosphorus and soluble phosphorus decreased sharply in the 5 days, but algal density maintained steady growth. The reason was that algae not only assimilated phosphorus, but also adsorbed phosphorus for subsequent assimilation.
3.4 The change of total nitrogen and nitrate of leachate over time

The change of total nitrogen and nitrate of leachate over time was showed in figure 5 and 6. Nitrogen was another macronutrient for algal growth. The total nitrogen and nitrate concentration of leachate was 6.68 and 4.16mg/L, the total nitrogen was higher than nitrate and the same change to free-cell filtrate. This result showed that leachate contained different form nitrogen beside nitrate, and algae mainly assimilate inorganic nitrogen. Different form nitrogen decreased fast in the first 5days and decreased slowly afterward, which indicated that algae assimilated abundant nitrogen at first, and insufficient supply of nitrogen would inhibit algal growth. Besides, the ratio of nitrogen to phosphorus was low, so the concentration of phosphorus was high and nitrogen was low in the leachate in the end. In order to increase assimilation of phosphorus, exogenous nitrate was added into the medium containing leachate, the concentration of nitrogen and phosphorus was low in the end, so optimum ratio of nitrogen to phosphorus played a key role in removing nitrogen and phosphorus of leachate.
3.5 Harvesting of microalgae by flocculation

Previous studies showed that microbial flocculant and ferric trichloride facilitates floating aggregation of *Microcystis aeruginosa* [6]. Ferric trichloride might cause residual of iron, so we used chitosan as substitute of ferric trichloride. The results showed that microbial flocculant and chitosan could flocculate microalgae, all algal cells aggregated together which facilitated harvest of algae.

4. Conclusions

The study mainly researched the treatment effect of microalgae on leachate of soil from vegetable greenhouse. The results showed that microalgae could grow in the leachate of soil without adding exogenous nutrient, optimum ratio of nitrogen to phosphorus could increase removal of nitrogen and phosphorus; algal density increased with increase culture time; the culture solution was alkaline; nitrogen and phosphorus of leachate decreased fast in the first 5 days.

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