

Nitrogen Transformation and Microbial Spatial Distribution in Drinking Water Biofilter

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Abstract. Well understanding the rule of nitrogen mutual transformation in biofilters is important for controlling the DBPs formation in the subsequent disinfection process. Ammonia nitrogen removal effect and nitrogen transformation approach in biofilter of drinking water was researched in the study. The biofilter removed ammonia of 48.5% and total phosphorus of 72.3%. And the removal rate of TN, NO₃⁻-N, DON were 37.1%, 33.1%, 46.9%, respectively. Biomass and bioactivity of different depth of the biofilter were determined, too. The overall distribution of biomass showed a decreasing trend from top to bottom. The bioactivity in lower layer gradually increased. Especially the bioactivity of heterotrophic microorganisms showed a gradual increase trend. The amount of the nitrogen loss was 3.06mg/L. Non-nitrification pathway of "nitrogen loss" phenomenon in biofilter might exist assimilation, nitrification and denitrification in autotrophic.

1. Introduction

Filtration is an important process in the drinking water treatment. Pollutants remaining after the coagulation and the sedimentation process could be removed by the filtration. Biofilm forms during the filter running, so the filter becomes a kind of biofilter. Reducing the dissolved organic nitrogen (DON) concentration in water before the disinfection process facilitates the control of DBP formation. However, the traditional biofilter could produce DON. Soluble Microbial Products (SMPs) are produced by microbes via degradation of the matrix or are the intermediate or final products of endogenous cell decomposition^[1, 2]. SMPs and extracellular polymeric substances (EPS) constitute new DON^[3]. The media type and character determine the micro-environment in biofilter, and thus affect the character of the microorganisms attached to the media. Our previous work illustrated that DON concentration significantly changed with depth in quartz sand filters and activated carbon filters^[4]. So an improved biofilter was set up with two-layer columns with activated carbon and quartz sand. Compared with the single-layer, the two-layer biofilter controlled the DON concentration more efficiently^[5].

The major forms of nitrogen in water are NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, and organic nitrogen^[6]. NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N are commonly referred to as "3N" or dissolved inorganic nitrogen (DIN). Organic nitrogen is the generic term for nitrogen-containing organic compounds, including DON and non-dissolved organic nitrogen (NDON)^[7, 8]. There is mutual transformation among DIN, DON, and NDON by biofilter microbes. Zhang found the biomass at different depths in the biofilter showed a linear positive correlation with the decrease in TN at the corresponding depth, and the correlation coefficient was



highest for the top layer of the filter^[9]. It is very important that understanding the rule of nitrogen mutual transformation in biofilters to control over the formation of DBPs in the subsequent disinfection process.

In this study, an improved biofilter with two-layer columns of activated carbon and quartz sand was set up. Tap water added nitrogen, phosphorus and other elements was simulated the water of sedimentation tank. The removal efficiency of ammonia and other pollutants and the mechanism of "nitrogen loss" in the biofilter were discussed. The microbial properties of the biofilter were analyzed, too. This would provide a reliable basis for removing the related pollution, and controlling the safety and stability of drinking water quality.

2. Materials and methods

2.1 Experimental site and installations

Some text. The experiment was conducted in the pilot hall in the Ningbo Institute of Technology, Zhejiang University (see Fig. 1). Biofilter columns with 10 cm diameter and 100 cm height. The filter layer were filled with different media and equipped with a flow meter. The two-layer media biofilter with activated carbon in the upper layer and quartz sand in the lower layer, with 20 cm layer height of active carbon and 80 cm layer height of quartz sand. Sampling ports of media and water were arranged along the biofilter columns in order to determine the features of water and media at different heights. As same as the most drinking water plant filter, the biofilters were designed to down flow, i.e., the inlet was at the top and the outlet was at the bottom. The raw water was the tap water of mother liquid added ammonium chloride and potassium dihydrogen phosphate to simulate the sedimentation tank effluent in drinking water plant. The equipment run and backwash simulated the traditional drinking water plant and the literature^[5]

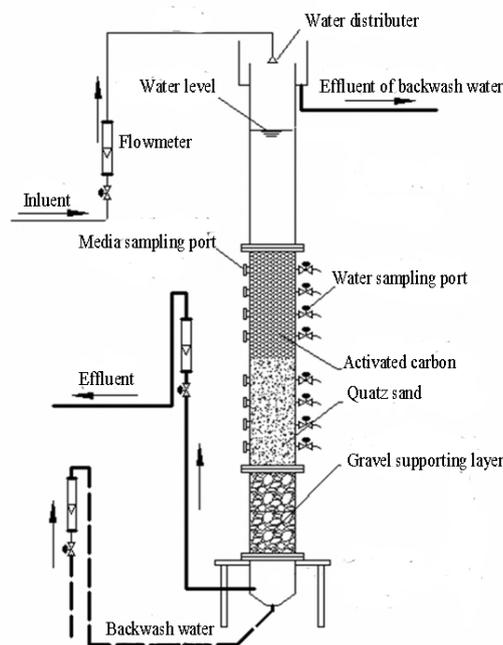


Fig.1 Experimental installations and schematic^[5]

2.2. Analytical methods

Ammonia, nitrite, nitrate and total dissolved nitrogen (TDN) were measured using the salicylate-hypochlorite method, N-(1-naphthyl)-ethylenediamine photometric method, UV spectrophotometry method, alkaline potassium persulfate digestion-UV spectrophotometric method, respectively. These determinations were carried out according to the Chinese National Standard Methods^[10]. DON was

quantified as TDN minus ammonia, nitrite and nitrate. A turbidity meter (AQ4500, Thermo Orion) and a pH meter (HQ40d, HACH) for the measurement of turbidity and pH were calibrated prior to use. Lipid phosphorus method was used in determining media biomass^[11]. The total phosphorus was determined by an ammonium molybdate spectrophotometric^[12]. The media were dried for 2 h at 105 °C in oven, and weighted after cooling to calculate biomass per unit volume of the media. Biological activity was determined by the method of microbial specific oxygen uptake rate (SOUR). SOUR was defined as the unit of oxygen consumed by the unit of biofilter media in the unit time, i.e. $\text{mg}(\text{O}_2)/\text{cm}^3(\text{filter})\cdot\text{h}^{[13]}$.

3. Results and discussion

3.1 The effect of the biofilter

Tab. 1 The quality of raw water and running conditions of the biofilter

$\text{NH}_4^+\text{-N}$ mg/L	$\text{NO}_2^-\text{-N}$ mg/L	$\text{NO}_3^-\text{-N}$ mg/L	TN mg/L	DON mg/L	TP mg/L	DO mg/L	T °C	pH	Turbidity NTU
5.38	0.003	3.66	11.47	1.05	0.44	8.7	11.2	10.4	3.71

The quality of raw water and running conditions of the biofilter was shown in the Tab.1. After 40 days running of the biofilter, the removal rate of $\text{NO}_2^-\text{-N}$ was 57.7% steadily. Then the membrane of biofilter was mature.

Stable enrichment of microorganisms in the biofilters can be divided into heterotrophic bacteria and nitrifying bacteria. The heterotrophic bacteria can remove organic matter and grow faster. The nitrifying bacteria can remove $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ through the nitrification. Among them, nitrifying bacteria belong to autotrophic bacteria, which are composed of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Generally speaking, the growth rate of nitrifying bacteria is much smaller than that of heterotrophic bacteria. The biased relationship between NOB and AOB determines the growth rate of NOB and is smaller than that of AOB^[14]. Therefore the nitrifying bacteria in the biofilter, especially the growth and enrichment of NOB, can serve as the main mark for the mature of the biofilter in the biofilm of biofilter phase.

After the biofilm of biofilter was mature, the concentration and removal rate of TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DON, $\text{NO}_2^-\text{-N}$, and TP in the influent and effluent of the biofilters were measured. The removal rate of the indexes were measured, too. The results are shown in Fig. 2. As a bioactive filter, the biofilter has a good removal of ammonia nitrogen. At the early running period, the biofilter could remove part of the ammonia nitrogen relying on the bacteria retained to the biofilter. The biofilter has a good effect of removing ammonia and total phosphorus, with the removal of 48.5% and 72.3%, respectively. And the value did not appear fluctuations during monitoring. The removal rate of TN, $\text{NO}_3^-\text{-N}$, DON were 37.1%, 33.1%, 46.9%, respectively.

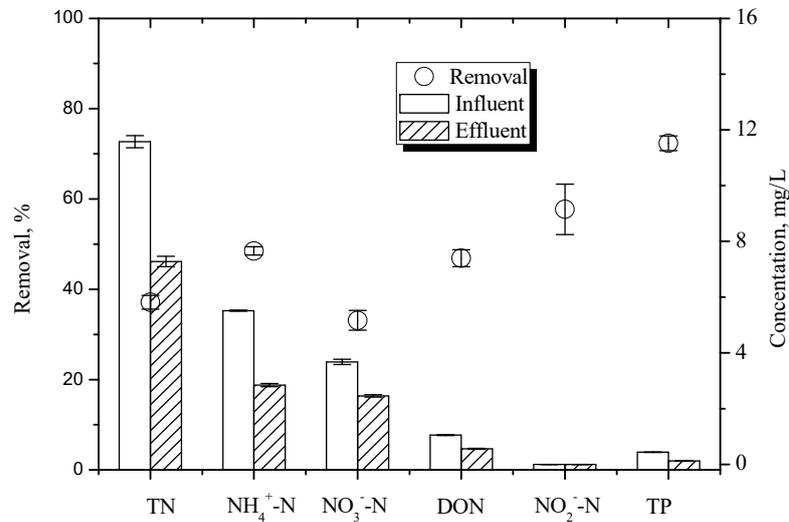


Fig. 2 The removal rate and the indexes of the influent, effluent of the biofilters (n=3)

3.2 Biomass and bioactivity distribution in the biofilter

The biomass attached to the media was detected using the lipid phosphorus method. Bioactivity of total bacteria and heterotrophic bacteria was detected using the method of microbial specific oxygen uptake rate (SOUR). The results were shown in Fig.3.

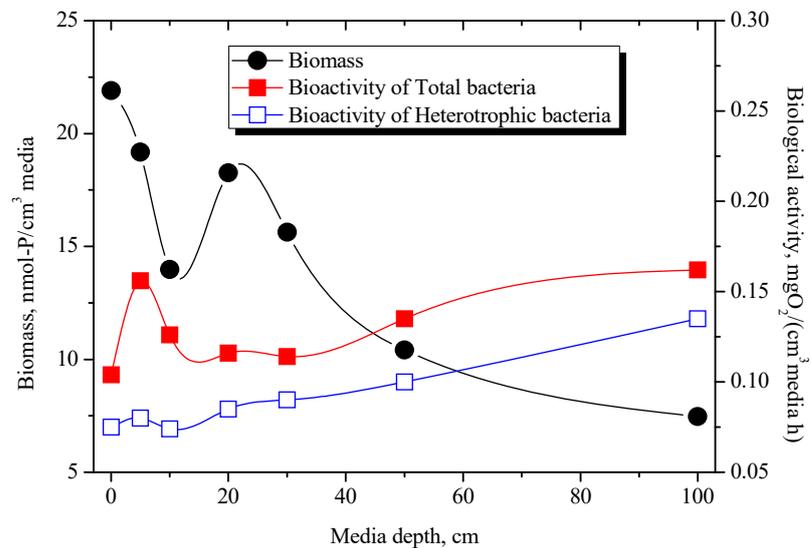


Fig. 3 Biomass and bioactivity distribution in the biofilter

The overall distribution of biomass showed a decreasing trend from top to bottom in the biofilter. The biomass at 0 cm depth was 21.90 nmol-P/cm³ (media), while the lowest was 7.47 nmol-P/cm³ (media), with a difference of 2.93 times between the upper and lower layers. The trend of biomass was

similar to Zhang et al result^[5]. The organic matter of influent and the oxygen were decreasing by biofilm oxidation degradation when the water flowed from the top down to the bottom in the biofilter. There was a significant rebound of the biomass at 20 cm depth of the junction of activated carbon and quartz sand in the biofilter. The particle size of quartz sand in the lower layer was smaller than that of activated carbon in the upper layer. So the porosity become smaller, and the lower media could retained more bacteria and suspended solids. The phenomenon showed that the film performance of quartz sand was better than granular activated carbon.

Although the biomass reduction, the bioactivity in the second half gradually increased. Especially the bioactivity of heterotrophic microorganisms showed a gradual increase trend. The rate of bioactivity of heterotrophic microorganisms increased along the depth of biofilter. Bioactivity and biomass had a certain degree of correlation, but the high biomass did not necessarily indicate the high biological activity. Different types of microorganisms had different metabolic intensities, even if the same microorganisms in their different growth stages would also show different bioactivities. In addition to biomass, the bioactivity related to water temperature, DO, pH and other environmental factors. The biofilter forming a stable ecosystem within the equipment. The degradation of organic matter and ammonia nitrogen has a significant removal in the biofilter.

3.3 "Nitrogen Loss" phenomenon and possible pathway analysis

Liu and Yu's study showed that "three inorganic nitrogen concentration" of the influent was higher than the average concentration of "three inorganic nitrogen concentration" in the effluent by 1 mg/L in the pilot experiment biofilter. It resulted in a "nitrogen loss" with part of the nitrogen was removed by non-nitrification^[15, 16].

Bio-denitrification is an important research direction in the micro-polluted water biological treatment technology. The denitrification mechanism has important reference value for the removal of ammonia nitrogen in water treatment. For drinking water biofilters, the removal of nitrogen may be more complex than usual. The principle of denitrification might be due to the oxidative degradation of the biofilm, the physical closure of the quartz sand, biological assimilation, biological denitrification, etc., so as to achieve the desired effect of nitrogen removal.

The average concentration of inorganic nitrogen in the influent was 9.04mg/L, and that in the effluent was 5.98 mg/L. So the total concentration of "three inorganic nitrogen" in the influent was higher than that in the effluent 3.06 mg/L. The amount of nitrogen loss was 3.06 mg/L, indicating that part of the nitrogen was removed by non-nitrification.

After the water passed through the biofilter, the conversion of N in the water had physical adsorption, volatilization, biological nitrification, biological denitrification and assimilation, leading to the change of its molecular structure and the decrease of the total amount of effluent N. In the course of the experiment without aeration link, so the volatile loss can be directly ignored. The physical adsorption of ammonia of the biofilter is limited, the resulting significant reduction in the possibility of ammonia is also less likely.

(1)Assimilation. In addition to nitrifying bacteria oxidizing of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$, there were a large number of heterotrophic bacteria oxidizing organic matter in the biofilter after the mature of the biofilm. The heterotrophic bacteria got the energy through the oxidation of electronic donors, while they absorbed the nutrients from the external environment to maintain their growth. The bacteria intaked C, N, P and other trace elements for metabolism by the assimilation. Since the carbon source removed by alienation and assimilation could not be distinguished, it was necessary to discuss the relationship between the loss of N in the system and the P required for assimilation. The bacterial cell formula $\text{C}_{60}\text{H}_{87}\text{O}_{23}\text{N}_{12}\text{P}$ of the reference bacteria showed that the cells were collected every $14 \times 12 = 168$ mass units of nitrogen by assimilation, $31 \times 1 = 31$ mass units of phosphorus should be taken at the same time. The nitrogen loss in the biofilter was 3.06 mg/L. According to the ratio, the corresponding P should be 0.56 mg/L. However only 0.33 mg/L of total phosphorus removal was detected in the biofilter. Therefore, it could be explained that the assimilation of microorganisms was not the only reason for the "nitrogen loss" phenomenon.

(2) Denitrification. In the case of hypoxia, the denitrifying bacteria produces nitrogen and nitric oxide using organic matter as an electron donor, and using nitrate nitrogen or nitrite nitrogen as the electron acceptor. The processes was called denitrification. In the study, the average removal rate of nitrate nitrogen was only 33.1%. So "Nitrogen loss" phenomenon was not entirely due to denitrification caused in the biofilter.

(3) Autotrophic denitrification. Researchers found coupling effect of short-range nitrification and anaerobic ammonium oxide bacteria. The decrease of nitrogen in the biofilters was due to AOB, and a reaction similar to oxygen-limited autotrophic nitrification denitrification might occur in the biofilter^[17]. AOB could utilize H₂ and NH₄⁺-N as electron donors to initiate the NO₂⁻-N reduction reaction, when DO was limited^[18]. It was reported that AOB utilized O₂ as an electron acceptor and oxidize some NH₄⁺-N into NO₂⁻-N under the condition of limited DO^[19]. NH₄⁺-N was oxidized into nitrite nitrogen by aerobic nitro bacteria located in the outer layer of media. When NH₄⁺-N as an electron donor and NO₂⁻-N as an electron acceptor were through the anoxic biofilm, the two substances were converted into gaseous N₂ by anaerobic ammonium-oxidizing bacteria such as *Brocadia anammoxidans* and *Kuenen stuttgartiensis*. Then nitrogen loss occurred^[20]. This mechanism was a complete autotrophic condition under biological denitrification. The autotrophic denitrification conditions were low C/N, low-carbon and high nitrogen water. The influent of the biofilter in the experiment was a typical high ammonia nitrogen, with the average concentration of ammonia of 5.38 mg/L. The relative concentration had exceeded the general concentration of polluted water. In the absence of oxygen in the biofilm (anaerobic) environment, part of the nitrogen transformed into gaseous nitrogen from the biofilter, resulting in the nitrogen loss phenomenon.

4. Conclusions

The biofilter had a good removal of ammonia nitrogen with the removal of 48.5%, and had a good removal of the total phosphorus, with the stability of about 72.3%. The removal rate of TN, NO₃⁻-N, DON were 37.1%, 33.1%, 46.9%, respectively.

The overall distribution of biomass showed a decreasing trend from the top to the bottom. The biomass at 0 cm was 21.90 nmol-P/cm³ (media), and the lowest was 7.47 nmol-P/cm³ (media), with a difference of 2.93 times between the upper and the lower layers. The bioactivity in lower layer gradually increased. Especially the bioactivity of heterotrophic microorganisms showed a gradual increase trend. The rate of bioactivity of heterotrophic microorganisms increased along the depth of biofilter.

The amount of the Nitrogen loss was 3.06mg/L. The analysis of non-nitrification pathway "nitrogen loss" phenomenon in biofilter might exist assimilation, nitrification and denitrification in autotrophic.

Acknowledgements

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