

Study on the Way of Urea Removal by BAF

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Summary: Biofilm process is a kind of efficient way for sewage with high concentration of urea. A lot of researches on removal efficiency were carried out, but there existed less research for the process of urea removal. To understand the way of urea removal by biofilm process, biological aerated filter (BAF) was used to deal with sewage with high concentration of urea from a chemical plant, by studying adsorption property of the carrier, biomass and biological activity on the carrier, and contrastive analysis between hydrolysis product of urea and theoretical value was carried out and the microbial flora of urea removal was preliminarily determined using the method of inhibiting biological activity. The results showed that, removal of urea by BAF was mainly the result of biological action, the adsorption capacity of activated carbon for urea was limited, the dominant bacterial communities for the hydrolysis of urea were heterotrophic bacteria, and parts of nitrosobacteria and nitrobacteria also had a certain capacity of hydrolyzing urea.

Introduction

Urea is not only an important nitrogen fertilizer, but also a kind of important chemical raw materials [1]. With the rapid development of chemical industry the urea demand increases significantly. People pay more and more attention to the status and function in national agriculture and industry of urea. However, there are large amounts of wastewater containing urea in the urea production process [2]. It will do great harm to the environment if discharged directly without treatment. At present, the urea wastewater treatments mainly include thermodynamic hydrolysis method [3], chemical method [4], urease hydrolysis method [5, 6] and biological method [7-9]. Compared with other methods, biological method has some characteristics such as simplicity of operation, low cost, mild conditions, which attracted a lot of attention.

BAF is a biofilm process for sewage treatment crafts arisen in Europe and America at the end of the 1980s and early 1990s. It has advantages such as good and stable outlet wastewater quality, high treatment efficiency, small occupation area, low operation cost, and strong capacity of resistance to impact load. It has a variety of application and the present studies on BAF mainly focus on removal efficiency of pollutant [10, 11], operating parameter optimization [12], back wash characteristic [13, 14], choice of carrier [15-17]. Biological aerated filter not only can be used for water eutrophication treatment, but also can be widely used for the treatment of municipal wastewater and industrial wastewater with high concentration of Suspended Solids (SS), Chemical Oxygen Demand (COD), Bio-chemical

Oxygen Demand (BOD₅) and realized nitrification, denitrification, phosphorous removal [18-20]. Yan tried to use BAF for sewage with high concentration of urea in the previous studies, and achieved a good treatment effect [9]. However, the way of BAF removing urea was not clear, whether it was absorbed or transformed remained uncertain. In addition, the conclusion of metabolic type on the hydrolysis bacteria of urea had not been unified. It may be confirmed that most of the hydrolysis bacteria of urea were heterotrophic bacteria, as to whether autotrophic bacteria could remove urea in wastewater had different opinions. The research results of J. L. Campos [21] showed that the hydrolysis of urea was not observed in the separate nitrification reactor. P. Prosser [22] also reported that *Nitrosomonas* or *Nitrospira* could not decompose urea. In contrast, the research results of H. P. Koops [23] showed that five kinds of ammonia oxidizing bacteria could use urea as nitrogen source.

Based on the analyses above, the article on the basis of using BAF to study urine in the early days, analyzed the adsorption of urea on carrier, biomass and biological activity of the carrier and the concentration change of hydrolysis product of urea, and initially confirmed microbial flora of urea removal, with a view to provide a theoretical basis and technical support for the engineering application of high concentration urea wastewater.

Results and Discussion

According to the previous test results, BAF

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process can effectively remove more than 95% of urea in wastewater [9]. Removal of pollutants in the wastewater by BAF was mainly by the adsorption of carrier or adhering inside the biofilm of carrier surface, and then by degradation of the microbial metabolism. In order to investigate the carrier on the adsorption behavior of urea and the biological action of urea removal, tests of static adsorption of activated carbon for urea were carried out and content of ammonia nitrogen (hydrolysis product of urea) in the effluent of BAF was analyzed compared with the theoretical concentration of ammonia nitrogen, and then biomass and biological activity were analyzed inside the BAF respectively, in order to understand the process and approach of urea removal in a BAF.

Adsorption of Activated Carbon for Urea

When urea concentration was 187.34mg/L, pH value was 7.8, and the temperature was 23 °C, the adsorption of activated carbon for urea was investigated with the result showed in Fig. 2.

It could be seen from Fig. 1, activated carbon had certain adsorption for urea. But the adsorption capacity was very limited with maximum adsorption of only 1.69 mgCO(NH₂)₂/g carbon. Studies on the adsorption of the activated carbon for urea had been reported in 1990s [24]. The adsorption capacity was between 0.13mmol/g and 0.15mmol/g. The differences of adsorption capacity of the activated carbon for urea may be caused by using the activated carbon of different sources.

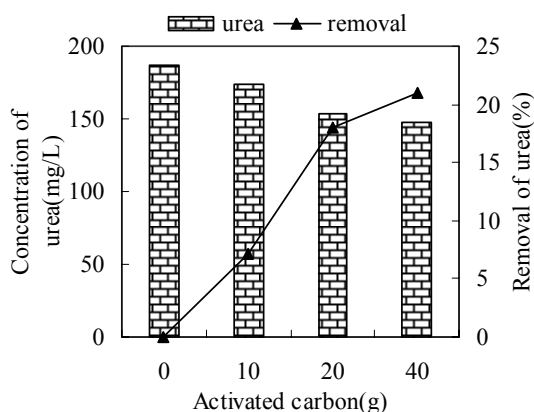


Fig. 1: Adsorption effect on urea with activated carbon.

In order to explain the adsorption of the

activated carbon for urea, the adsorption isotherm of activated carbon for urea were studied. The results of the activated carbon adsorbing urea were plotted, and Fig. 2 was obtained by using Freundlich empirical formula to fit for the results. It was generally believed that the greater n could cause better adsorption. When n was between 2 and 10, the adsorbent process was easy, while n was less than 0.5, the process was hard. While the greater k was, the greater the adsorption capacity is. As you could see from Fig. 2, when k was 5.73×10^{-5} , and n was 0.6574, the adsorption capacity of the activated carbon for urea was limited, and the adsorption process of the activated carbon for urea was not easy to carry out.

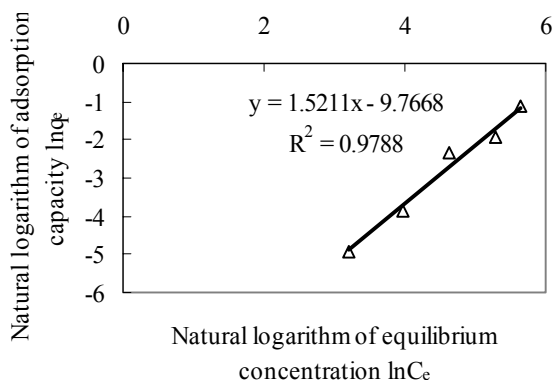


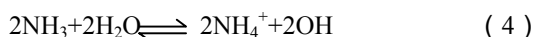
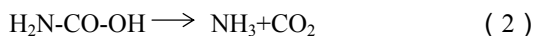
Fig. 2: Adsorption isotherm of the activated carbon for urea.

There are many factors affecting adsorption, mainly including nature of adsorbent, nature of adsorption, operating conditions. Generally, the greater the specific surface area of the adsorbent would result in better capacity; and non-polar adsorbent was easy to adsorb non-polar adsorbates, The more solubility of adsorbates would enhance the capacity for adsorbates. The great solubility of urea (1080 g/L under the temperature of 20 °C) made adsorption process easy. However, experiments with non-polar activated carbon as adsorbent, the adsorption capacity of urea with great solubility and powerful polar was limited due to its general rules of adsorption for organic matter.

Analysis of biological role for urea removal by BAF

Urea was hydrolyzed to NH₃ and CO₂ [25] under the action of urea aminohydrolase (Urease is for short) of microbial secretion. It had high activity and specificity, and rapidly catalyzed hydrolysis of urea at normal temperature and pressure, its catalyzed

hydrolysis rate was 10^{14} times of non-catalyzed hydrolysis. Related reactions (1) ~ (4) as follows:



Active site of urease is a dual-core nickel. One of the Ni^{2+} (1) ions is coordinated by two histidine (His) imidazole nitrogens, one aspartic acid (Asp) carbonyl oxygen and a H_2O molecule oxygen (or Hydroxyl of H_2O dissociation production), forming square pyramidal or trigonal bipyramidal geometry, which may be activated water molecules, producing nucleophilic group - hydroxyl ion (OH^-); another Ni^{2+} (2) is coordinated by two histidine imidazole nitrogen and a weak ligand, forming tetrahedral geometry, which may be the activated center of catalytic substrate activation. Urea first bonded with one of the Ni^{2+} [26], then was activated with water connected to another Ni^{2+} through deprotonation of the alkali, and hydroxy ligand as nucleophile attacking the carbonyl carbon was followed by, forming a transition state of tetrahedron. Transferring a proton from the protonated alkali or protonating an acid radical can generate ammonia and carbamic acid, and then urea is hydrolyzed. The comparative results of the ammonia nitrogen concentration of BAF effluent and the theoretical urea transformation (named as theoretical ammonia nitrogen concentration as follow) can be seen in Fig. 3.

It could be seen from Fig. 3, actual and theoretical ammonia nitrogen concentration of BAF effluent using the activated carbon as the carrier were very similar. The difference of the two was between 15 and 465 mg/L, and the higher urea concentration of inflow would enlarge the difference. These indicated that urea removal in the wastewater was mainly the result of biological function; at the same time it also could be seen from Fig. 3, actual ammonia nitrogen concentration and theoretical ammonia nitrogen concentration of BAF effluent had some differences. These may be caused by microbial assimilation, ammonia volatilization, ammonia nitrogen conversion, biofilm adsorption or existing simultaneously nitrification - denitrification in a biofilter.

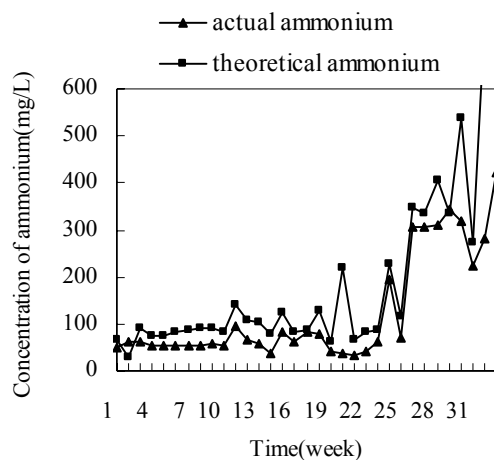


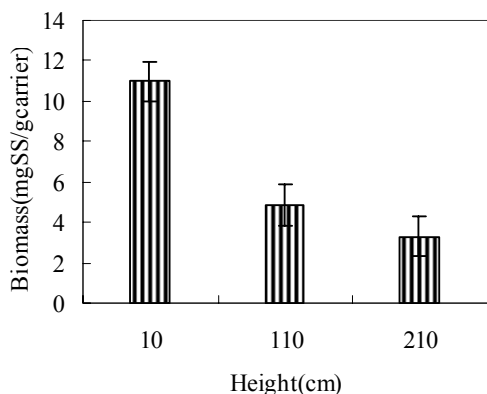
Fig. 3: Comparison between theoretical and actual effluent ammonium nitrogen concentration of biological aerated filter.

Analysis of Biomass and Biological Activity Inside the BAF

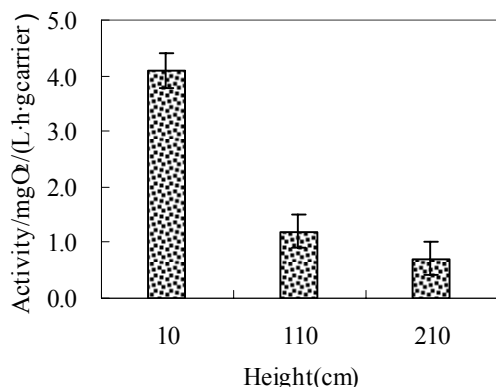
Different microbial groups have differences living space, dissolved oxygen, and the competitiveness of the substrates. They possess respectively specific space inside the BAF. There exists competition for space between heterotrophic bacteria and autotrophic bacteria. Heterotrophic bacteria with faster growers than autotrophic bacteria dominate the main space of BAF quickly. Many mathematical models had also reported the layered structure of biofilms: The most inner layer closing to packing carrier was nitrifying bacteria and the most outer layer was heterotrophic bacteria [27, 28]. It is thus clear that types and quantities of microbial flora in different space of the BAF are different because of the construction characteristics of plug-flow. Biomass and biological activity at different filter height can be seen in Fig. 4.

All of the BAFs have obviously biological effect with activated carbon as carrier. The biomass and total biological activity gradually decreased as the height of filter increased (As shown in Fig. 4). Biomass and biological activity were respectively 10.99, 4.86, 3.31 mg SS / g activated carbon and 4.09, 1.20, 0.71 mg O_2 / (L·h·g activated carbon) from the bottom to the top. Biomass in the filter was associated with pollutant load. The quantity of substrate at the bottom of the filter (organic matter and urea.) was relatively great with plenty of dissolved oxygen providing the conditions for the

growth and reproduction of microbes. As the height of filter increased, the concentration of substrate gradually decreased, and the materials that can be used by microbes reduced and the biomass of unit carrier reduced. Biological activity in the test was defined as oxygen consumption of unit carrier, so the decreasing of biomass of unit carrier led to the reducing of biological activity.



a Biomass



b Activity

Fig. 4: Variation of biomass and biological activity at different filter height

In order to do more intuitively study on the characteristics of microorganisms in BAF, the biofilm structure at different positions of filter layer was observed by scanning electron microscope and high optical microscope, the results were shown in Fig. 5 a, b, c.

Activated carbon with biofilm formation

could be seen and a lot of obvious micro-organisms attached to the surface of packing compared with before immobilization (As shown in Fig. 5b and Fig. 5c). At the same time, the change characteristics of biofilm morphological structure consistent with variation of biomass along the height of BAF could be seen. The thickness of biofilm decreased along the flow direction, degrees of rough surface and stretch ups and downs decreased. There existed a significant difference between morphological structure of biofilm surface of inflow and effluent.

Compositions of biofilm were complex at the forefront of reactor. Surface of biofilm staggered. It could be clearly seen short rod-shaped bacteria tight coupled with the carrier surface; the biofilm surface was relatively flat at the back end of reactor, and there were a lot of irregular particles, rarely rod-shaped bacteria and less biomass.

The Dominant Flora of Hydrolyzing Urea was Initially Defined

According to previous results of test, urea of wastewater was mainly removed by the biological effect. In order to determine the metabolic types of hydrolysis bacteria for urea, the method of adding inhibitors into the samples was used in the test initially.

ATU and NaClO₃ were added into the samples in the test to inhibit the activities of nitrosobacteria and nitrobacteria, respectively, at the same time control test was used to distinguish the metabolic types of hydrolysis bacteria for urea.

As it could be seen from Table-2, whether the inhibitors existed or not, the difference of the hydrolysis effect of urea in the beakers was less, showing that decrease of urea concentration and increase of ammonium nitrogen concentration were very close. The existence of inhibitors did not have a big influence on hydrolysis of urea, showing that inhibition ratio of hydrolyzing urea was below 10%. These indicated that the hydrolysis bacteria of urea would be mainly heterotrophic bacteria (Activities of nitrosobacteria and nitrobacteria were inhibited). The reason why there still some microbial activity was inhibited at the height of 10cm was that organic matter concentration of wastewater quality in experiment was lower so that there was phenomenon of a certain nitrifying bacteria existing of inflow and there was not condition of heterotrophic bacteria and autotrophic bacteria stratifying; while as the

increasing of the height of the sampling connection, the inhibition ratio of hydrolyzing urea increased gradually. The inhibition ratios of hydrolyzing urea were respectively 1.41%, 6.39% and 7.69% of biologic activated carbon at the bottom of 10cm, 110cm and 210cm of the filter. At the same time we could also see from Table-2, under the conditions that urea concentrations were the same in the test wastewater samples, after the two corresponding wastewater samples reacting for 30min, there were a certain differences among the remaining urea concentrations. Under the conditions of the presence of inhibitors, the remaining urea concentrations were higher than those without inhibitors at the three corresponding sampling ports. The reason may be the autotrophic bacteria (nitrosobacteria and nitrobacteria) could hydrolyze urea at the central section of the BAF reactor (The activities of nitrobacteria were inhibited fundamentally, and nitrate nitrogen was not detected). Parts of nitrosobacteria and nitrobacteria could take life activities using urea as nitrogen source. Under the experimental conditions, the urea removal was the result of the combined action of heterotrophic bacteria and autotrophic bacteria. The

dominant flora belonged to heterotrophic bacteria.

Experimental

Test Equipment

Experiments were performed in an organic glass column reactor with the height of 3700mm and the diameter of 150mm and working volume of 39.74 l[9]. The reactor was packed with ZJ-15-type granular activated carbon (Taixi Activated Carbon Factory, Ningxia in China). A sampling port was set up every 250mm interval along the height of BAF, perforated pipe was adopted for aeration in the bottom of BAF, and gas - water combined backwash was adopted for backwash. Up-flow operation mode was used in the tests, namely, the wastewater to be treated was pumped from the bottom of the filter, and the treated wastewater was discharged from the top of the filter. The flows of water and gas were controlled by the rotameters. Water flow of the treatment of experiment was 50 l / h, ratio of gas and water was 3:1.

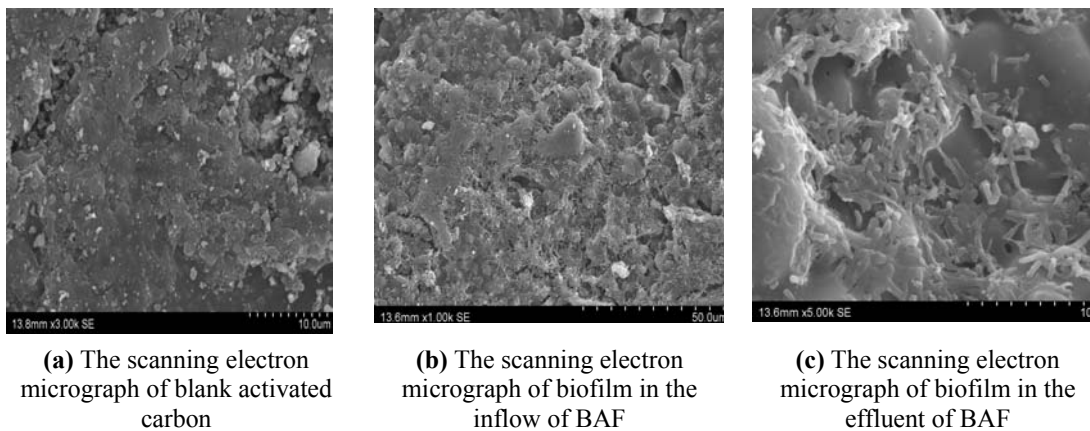


Fig. 5: The scanning electron micrograph of blank activated carbon and biofilm.

Table-1: Characteristics of experiment wastewater.

Items	COD /mg/L	Urea/mg/L	NH ₄ ⁺ -N /mg/L	NO ₂ ⁻ -N /mg/L	NO ₃ ⁻ -N /mg/L	Temperature/°C	pH
Max	45.30	1118.50	1.53	3.26	0	27	8.52
Min	7.97	94.00	0.06	0	5.26	23	7.20
Average	35.83	449.47	0.54	1.28	2.73	25	8.26

Table-2: Effects of inhibitor on urea hydrolysis.

Items	Inhibitors	BAF Height(cm)		
		10	110	210
Remaining urea/mg/L	No	89.76±0.17	91.65±0.19	94.25±0.13
	Yes	90.00±0.15	92.60±0.22	95.19±0.14
Inhibition ratio/%	—	1.41	6.39	7.69

Test Wastewater Quality

Experimental wastewater was from the sewage outlet of a urea production plant in China. The characteristics of wastewater quality were showed in Table-1.

Test Analysis Methods and Test Items

Parameters such as urea, ammonium, nitrate, nitrite, and Chemical Oxygen Demand (COD), were carried out according to Standard Methods for the Examination of Water and Wastewater [29]. The pH was measured by using a precision pH meter of PHS-3C. The DO was measured by DO meter of DO-14P type. Biomass and biological activity procedure were carried out according to the procedure described by Bai Yu [30].

Adsorption Experiments

Activated carbon soaked with distilled water for a few days to elute residual impurities that could be present, and the qualities of the activated carbon above-mentioned were respectively 0g, 5g, 10g, 20g and 40g (dry weight). The activated carbon above-mentioned was placed in series of conical flasks with plugs containing 400ml urea solution. The adsorption studies were carried out when urea concentration was 187.34mg/L, pH value was 7.8, the temperature was 23 °C, and agitation speed was 150rpm. The mixtures were then filtered and urea concentration of sample was determined after 120 minutes, and equilibrium adsorption quantity was calculated according to the formula (5). Isotherms of activated carbon adsorbing urea were determined according to the same method, and Freundlich isothermal adsorption equation was for analysis. For specific, see the formula (6).

$$q_e = \frac{V \times (C_0 - C_e)}{M}$$

In formula, q_e is equilibrium adsorption quantity (mg/g), C_0 is initial concentration of solution (mg/L), C_e is equilibrium concentration (mg/L), V is the volume of solution (mL), and M is the mass of activated carbon (g).

$$q_e = kC_e^{\frac{1}{n}} \quad (6)$$

In formula, q_e and C_e are the same as former

ones, k and n are constants.

Preliminary Confirmed Experiments of the Hydrolysis Bacteria of Urea

Hydrolysis bacteria of urea tests were carried out using biological activated carbon particles that were collected from different heights of BAF. A certain amount of biological activated carbon particles (2.0g) were put into clean conical flasks with plugs, and a certain amount of pre-config solution (wastewater quality of sample: urea was 106.52 mg/L, and pH was 7.58) was added. Then a pulse of NaClO_3 and ATU were added in the beginning of the experiment to reach 0.02mol/L and 5mg/L respectively. The Hydrolysis bacteria of urea tests were carried out when the temperature was 23 °C, and agitation speed was 150rpm. All of the results of each test were repeated three or more times. Inhibition ratio was calculated according to formula (7):

$$\text{Inhibition ratio} = \frac{(C_N - C_Y) \times 100}{C_N} \quad (7)$$

In formula, C_N is the variation of urea concentration without inhibitor, mg/L; C_Y is the variation of urea concentration with inhibitor, mg/L.

Conclusions

Efficient urea removal in the wastewater by BAF related to the adsorption characters of carrier and the biological effects on the carrier. The adsorption process of the activated carbon for urea demonstrated was weaker with only 1.69mgCO(NH₂)₂ / (g activated carbon). The ammonium nitrogen concentration of BAF effluent and the theoretical urea transformation demonstrated that microorganisms were the key factors for urea removal in the BAF. Both biomass of the unit carrier and total bioactivity decreased gradually along the height of filter layer, biomasses and bioactivities at three intervals from inflow to effluent were respectively 10.99mgSS/g, 4.86mgSS/g, 3.31mg SS / g activated carbon, and 4.09mgO₂ / (L·h·g activated carbon), 1.20 mgO₂/(L·h·g activated carbon), 0.71 mgO₂/(L·h·g activated carbon). Selective microbial activity inhibition demonstrated that the dominant floras of hydrolyzing urea were heterotrophic bacteria. Parts of nitrosobacteria and nitrobacteria also had a certain capacity of hydrolyzing urea.

Acknowledgments

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