Performance of Two-Stage A/O Process on High-Strength Ammonia Nitrogen Wastewater

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Abstract. In order to understand the two-stage A/O process which contained two independent A/O (Anoxic/Oxic) subsystems when applied to treat high-strength ammonia nitrogen wastewater, connection mode of the two A/O subsystems and DO concentration of oxic phase were selected as the influencing factors in this paper. The connection modes of the two independent A/O subsystems were parallel-connected and tandem-connected. Initially, the performance of two-stage A/O process in different connection modes were investigated by comparing the ability to remove COD, NH4+-N and TN. It was observed that tandem-connected two-stage A/O system was suitable for the treatment of high-strength ammonia nitrogen wastewater, and two-stage A/O system which was operated in parallel-connected mode failed to meet the direct emission standard of Discharge Standard of Water Pollutants for Ammonia Industry in China (GB13458-2013) with remanent COD, NH4+-N and final effluent TN concentration of 234.2mg/L, 25mg/L and 74.2mg/L, respectively. Following the above observation, five different levels of DO concentration (2, 3, 4, 5 and 6 mg/L) of stage-one and stage-two were varied to figure out the optimal DO concentration in tandem-connected two-stage A/O system. Examination of effluent quality indicated that 5.0mg/L was determined to be the optimal DO concentration for Stage-one and 2.0mg/L for Stage-two. In this case, the quality of effluent was perfectly satisfied the direct emission standard.

1. Introduction

High-strength ammonia nitrogen wastewater have been triggering severe water pollution problem like Red tide in the past decades. Commonly, it is originated from petroleum chemical industry, chemical fertilizer, landfill leachate and so on [1]. This kind of wastewater containing excessive nitrogen compounds in the form of ammonia (NH4+-N), nitrite (NO2--N), nitrate (NO3--N) and organic bound N as well as a great deal of organic matters may be toxic to aquatic life, affect the suitability of wastewater for reuse, increase in the chloride dose for drinking water disinfection due to the present of ammonia, cause low dissolved oxygen levels and eutrophication in receiving waters [2-4]. Nitrogen, known as a kind of extraordinarily important pollutant, accounts for approximately 20% of all the pollutants in the wastewater [5]. Organic matters existing in wastewater serve as carbon source and provide energy for algae growth, which contributes a lot to causing water eutrophication. Annual report of Chinese environment statistics reported that the amount of ammonia nitrogen in China in the
year of 2014 reached 2.385 million tons, equating 7.8 times as much as the Environmental capacity of receiving waters. Thus high-efficient removal of ammonia-nitrogen and organics before their direct discharge to waters is an urgent task for the moment.

To treat high-strength ammonia nitrogen wastewater drained from synthetic chemical ammonia industry, various physicochemical processes such as chemical precipitation, ammonia stripping and breakpoint chlorination method [6-10] have been numerous studied before. However, these methods were unfortunately proved to be developed and applied with huge operational cost which was the critical limitation of their wide usage. Traditional biochemical processes like activated sludge is favorable for large-scale wastewater treatment, but it is unadaptable for application of most common synthetic ammonia industries. Additionally, many new biochemical processes including anaerobic ammonium oxidation, shortcut nitrification-denitrification, modified SBR seemed to be suitable to remove nitrogen. Nevertheless, it is tough for the start-up of ammonium process and slow for bacterial growth though anaerobic ammonium oxidation was traditionally thought to be promising according to [11]. Study [12] showed that the operating conditions such as pH and SRT of shortcut nitrification-denitrification and modified SBR were hard to control. Traditional A/O process could obtain good treating effect, but it is unable to guarantee the stability of effluent quality under sudden burden changes in raw water quality and weaken in adapting changeable water quality and quantity due to single Anoxic/Oxic treating unit. As an original process developed in recent years, two-stage A/O process have been demonstrated to be high-efficient for removal of high concentrations of COD, nitrogen and toxic matters in mixed chemical wastewater, and independent COD oxidation and nitrification that carried out by two different microorganisms could be completely accomplished using two-stage A/O process [13].

[14] stated the nice treating capacity of two-stage A/O process of blackwater [15]. Yet so far few reports have been found aiming at the treatment of wastewater with high ammonia-nitrogen content using this process, and the relevant operational conditions optimization and the principles of main influencing factors such as DO were rarely seen in these literatures.

Different connection mode of two-stage A/O process will undoubtedly lead to the differences of influent diffusion and processing efficiency between tandem-connected and parallel-connected systems. Therefore, exploration of the effect of connection mode on treating ability for high-strength ammonia nitrogen wastewater is desperately in need. In addition, despite the fact that dissolved oxygen (DO) level in reactor had been frequently explored since it has significant influence on the behavior and activity of the heterotrophic and autotrophic microorganisms in various biological systems [17], it is of great necessity to offer much more investigation on the optimum DO levels in different stages of two-stage A/O system. Hence, the influence of connection mode and DO concentration were studied in this study.

2. Materials and Methods

2.1. Two-stage A/O system

Each two-stage A/O process consisted of two A/O subsystems (Fig.1), and every subsystem actually was one A/O reactor, which was operated utilizing time control to create anoxic and oxic environment. Fig.1 (a) showed the schematic diagram of tandem-connected two-stage A/O system, which meant that the two A/O reactors were linked together. Fig.1. (b) displayed the schematic diagram of parallel-connected two-stage A/O process, which indicated that two A/O reactors were paralleled. Each subsystem was a whole system with an anoxic phase and an oxic phase by distributing the operating cycle length in accordance with [13]. All reactors were made of cylindrical organic glass with a working volume of 3L. A plastic mat was equipped in each reactor to seal the top of the reactors where there were a few holes arranged for sample collection and measurement of pH, temperature and DO value. Besides, a mechanical stirrer was installed in the middle to stir the mixed liquor. The reactors were agitated using a blasting aeration controlled by air pump in anoxic phase to adjust the DO concentration to the expected level. Thermostatic controlled water circulation surrounded the reactors.
was adopted to maintain the temperature of reacting solution at 25℃.

2.2. Synthetic wastewater
The synthetic experiment wastewater was prepared with 75% distilled water and 25% wastewater drawn from the gasification concentrated wastewater of a chemical gasification plant in Huaqiang Chemical Industry Group Co., Ltd. in Yichang, P.R China. Methanol was added as exogenetic carbon source for nitrate denitrification which required a C/N ratio greater than 4. The characteristics of the raw wastewater from Huaqiang Chemical Industry Group Co, Ltd and the emission standard of indicators in Discharge Standard of Water Pollutants for Ammonia Industry in China (GB13458-2013) were given in Table 1.

After synthesing, influent COD, NH$_4^+$-N and TN was 1600mg/L, 250mg/L and 400mg/L, respectively.

Table 1. Characteristics of raw gasification concentrated wastewater collected from Huaqiang Chemical Industry Group Co, Ltd and the emission standard of various indicators

| Indicator  | pH  | T(℃) | TN (mg/L) | COD (mg/L) | Sulfide (mg/L) | Cyanide (mg/L) | SS (mg/L) |
|------------|-----|------|-----------|------------|----------------|----------------|-----------|
| Raw value  | 8.5 | 45   | 1500      | 1000       | 3000           | 40             | 20        | 900       |
| Standard   | 6-9 | —    | ≤50       | ≤40        | ≤100           | ≤0.5           | ≤0.2      | ≤0.5      |

2.3. Inoculation sludge
The seeding sludge of two-stage A/O process systems in this study was collected from a practical aerobic tank in Huaqiang chemical plant. It had stronger resistance to the biotoxicity of complex compositions and shorter cultivating time compared with other traditional activated sludge. The sludge
inoculation procedures were shown as follow: (1) The seeding sludge was settled statically for 30min and the supernatant was then removed to obtain the precipitated sludge; (2) 0.6L newly obtained sludge coupled with 2L synthetic experimental wastewater was fed to each reactor by pump suction, and then the reactors were sealed for a three-day continuous aeration; (3) After aeration, the mixed solution was statically settled for another 30min, and the supernatant was drained to get the desired mature sludge. The procedures of (2) and (3) were repeated all along until the concentration of effluent COD, NH4+-N, TN satisfied the related emission standard and SV, SVI and MLSS of sludge achieved the desired cultivating levels shown in Table 2.

The sludge inoculation was conducted under initial conditions that parameters C/N ratio, COD, ammonia nitrogen and total nitrogen were controlled at 4, 1600mg/L, 250mg/L and 400mg/L, respectively. DO concentration was kept around 4.0 mg/L in aerobic phase and 0.5mg/L or less in anoxic phase in each reactor. In addition, the reactors were operated with total hydraulic retention time of 32h, among which 24h designed for aerobic phase and 8h for anoxic phase. Reaction temperature was maintained around 25°C by thermostatic circulating water, and pH was adjusted to 7.5 which was optimum for simultaneous nitrification and denitrification.

| Table 2. The main parameters of raw sludge and desired mature sludge after domestication and cultivation |
|-----------------------------------------------|
| **Index** | **color** | state | MLSS (mg/L) | SVI (mL/g) | SV30(%) |
| Raw sludge | black brown | aerobic sludge | 2796 | 182.4 | 51 |
| Mature sludge | deep yellow | flocculent sludge | Stage-one 4285 | Stage-two 3876 | Stage-one 121.4 | Stage-two 116.1 | Stage-one 51 | Stage-two 45 |

2.4. Analysis methods

All influent and effluent samples were collected on a daily basis and analyzed immediately. COD was determined using standard potassium dichromate method. NH4+-N was measured via Nessler’s reagent spectrophotometry. TN was analyzed by the potassium super-sulphate oxidation-ultraviolet spectrophotometric. DO and PH were monitored using a dissolved oxygen meter and a PH meter. MLSS was analyzed by weighing method using electronic analytical balance after sludge being dried at 105°C for two hours.

The COD removal ratio of stage-one (R1), stage-two (R2) and total removal ratio (R) of COD were calculated according to Eq. (1):

\[
R_1 = \frac{(C_0 - C_1)}{C_0};
\]

\[
R_2 = \frac{(C_1 - C_2)}{C_0};
\]

\[
R = R_1 + R_2 = \frac{(C_0 - C_2)}{C_0};
\]

Where C0 (mg/L) was influent COD concentration, C1 (mg/L) was effluent COD concentration of stage-one, C2 (mg/L) was effluent COD concentration of stage-two. Removal calculation of NH4+-N and TN was the same as that of COD.

3. Results and discussions

Connection mode of two A/O subsystems and DO concentration of oxic phase were selected as the influencing factors in this paper to investigate the performance of two-stage A/O process on high-strength ammonia nitrogen wastewater. Initially, the performance of two-stage A/O process in different connection modes were investigated by comparing the ability to remove COD, NH4+-N and TN. Subsequently, five different levels of DO concentration (2, 3, 4, 5 and 6 mg/L) of stage-one and stage-two were varied to figure out the optimal DO concentration of oxic phase in the two A/O subsystems of the system with better performance.
3.1. The effects of two-stage A/O process systems in different connection modes on high-strength ammonia nitrogen wastewater treatment

In this part, investigation of both tandem-connected and parallel-connected two-stage A/O process systems were operated under the same conditions as sludge inoculation described in Section 2.2. 2L mixed experimental wastewater was fed to the first reactor at the beginning and then fed to the second reactor in tandem-connected system as shown in Fig.1 (a). The difference of parallel-connected two-stage A/O system from tandem-connected two-stage A/O system lay in that the 2L mixed wastewater was divided apart averagely to simultaneously feed the two parallel installed A/O reactors and the wastewater was discharged directly after treated only by single A/O reactor. Seven samples were consecutively collected under each stable operating condition, and the results were shown in Fig.2.

3.1.1. COD removal

![Figure 2. The effects of two-stage A/O process systems in different connection modes on COD removal](image)

According to Fig.2a, the average removal ratio of COD reached as high as 97.49% with effluent concentration of 39.8mg/L, indicating a good treating efficiency of tandem-connected two-stage A/O system. Comparatively, COD removal ratio and effluent concentration in parallel-connected two-stage A/O system were 85.43% and 234.2mg/L from Fig.2b, which obviously failed to satisfy the direct emission standard. These data declared that tandem-connected two-stage A/O system obtained better performance when ammonia nitrogen concentration was approximately provided 250mg/L. Possible biological explanations of better performance of tandem-connected two-stage A/O system were shown below: (1) both systems was totally equal in wastewater flow defined as Q, but the wastewater was averagely divided into two share defined as Q/2 in each reactor of parallel-connected two-stage A/O system. The relationship between COD load and wastewater flow was shown as Eq (2):

\[
XV = \frac{Q_{S_a}}{S_a} \tag{2}
\]

Where Q is the wastewater flow, S_a represents the influent COD concentration, X represents MLSS of activated sludge, V is the volume of reactors. Theoretically, influent COD concentration, volume and MLSS were equal in both systems. Calculation based on Eq (2) reflected that COD load of tandem-connected two-stage A/O system was twice as that of parallel-connected two-stage A/O system. COD removal rate was enhanced with the increase of COD load [13], because higher COD load could enhance the valid contacts between organic pollutants and zoogloea and promote the adsorption of organic particles by zoogloea. Thus tandem-connected two-stage A/O system got a higher COD removal ratio during the same reacting time. (2) Organics in tandem-connected two-stage
A/O system was degraded double time. It was well known that easy-biodegradable organics in wastewater were principally eliminated through the adsorption of zoogloea, while hard-biodegradable organics were removed via concurrent anabolism and catabolism of microorganism. The microorganisms in stage-two was domesticated to adsorb the organics which was in the low levels couldn’t be consumed by microorganisms in stage-one. As a result, these organic matters were more completely decomposed by corresponding enzymes of microorganisms in activated sludge with achieving a higher COD ratio and generating more energy.

Fig.2a and Fig.2b also revealed the good stability of effluent COD for system in tandem-connected, but COD concentration of effluent for system in parallel-connected system fluctuated irregularly, demonstrating the stronger adaptability and buffering capacity.

3.1.2. NH₄⁺-N removal

Seen from Fig.3, average NH₄⁺-N removal ratio of the samples were 98.19% for tandem-connected two-stage A/O system and 90.38% for parallel-connected two-stage A/O system. All ammonia nitrogen concentrations of influent were about 250mg/L, but effluent NH₄⁺-N were 25 mg/L and 4.5 mg/L respectively. Effluent NH₄⁺-N of 25 mg/L had obviously surpassed the 15mg/L specified in direct emission standard. These might be due to: (1) organics had been mostly eliminated owing to the higher COD load in stage-one of tandem-connected system, so inhibition of organic matters on nitrification was pretty low, making it possible for nitrobacteria to be the dominant bacteria in stage-two and to more adequately accomplish the nitrification converted NH₄⁺-N to NO₂⁻ and NO₃⁻ using oxygen as electron acceptors. In comparison, the organic matters remained in parallel-connected system was too much and possibly excess for the growth of heterotrophic microorganisms, which unfortunately produced more competition between organics and NH₄⁺-N for oxygen and resulted in inhibition of nitrobacteria metabolism. (2) Ammonia nitrogen was firstly removed in stage-one and then stage-two in tandem-connected system, while only treated in single A/O subsystem in parallel-connected system. Provided E was the removal ratio of one A/O subsystem, then removal ratio of tandem-connected system was [18]:

\[ E₁= 100\% - (100\%-E)(100\%-E) \]

Removal ratio of parallel-connected system was:

\[ E₂=100\%-E \]

\( E₁>E₂ \) was mathematically occurred, demonstrating the better performance of tandem-connected system. (3) Sludge concentration in tandem-connected system was relatively higher, which would
offer more surface area for attachment of microorganism, thereby increasing the possibilities of oxygen capture and NH₄⁺-N removal ratio was arised. Additionally, if nitrifying bacteria was located in inferior sludge, it would be easily taken away along with wastewater drainage in parallel-connected system, which whereas would less likely happen in tandem-connected system.

It also could be observed from Fig.3 that the tandem-connected system was more resistant to the varying water quality and changing water quantity. Therefore, tandem-connected system was much more favorable for treatment of irregular wastewater.

3.1.3. TN removal

### Figure 4.
The effects of two-stage A/O process systems in different connection modes on TN removal (Removal ratio of TN was calculated as R in Eq (1), DO=5±0.5mg/L for oxic phase and 0.3±0.2mg/L for anoxic phase, pH=7.5±0.5)

As was shown in Fig.4, the average TN removal rate of tandem-connected system reached 94.86%, and TN of effluent was reduced to 20.8mg/L. By contrast, average TN removal rate of parallel-connected system was only 84.88%, and average effluent TN was 74.2mg/L exceeding the 25mg/L in emission standard when initial TN of influent was controlled nearly 400mg/L in all systems. TN removal was a process mostly related to conversion of NO₃⁻ to N₂ depicted as Eq (3):

\[
\text{NO}_3^- + 5\text{H(Organics)} \rightarrow \frac{1}{2}\text{N}_2 + 2\text{H}_2\text{O} + \text{OH}^- \tag{3}
\]

Organics served as electron donors here, undoubtedly caused the competition between oxygen and NO₃⁻. Causes that responsible for the better TN treating efficiency of tandem-connected system were: (1) stage-one and stage-two in tandem-connected system created an "anoxic→oxic→anoxic→oxic" cycled alternating operation environment for growth of activated sludge. The formed alternate environment would stimulate the microbial potential to accelerate degradation rate of pollutants and to improve the removing efficiency in accordance with unsteady theory of Wang Tao [18] and other relevant studies [19-20]. (2) Though COD removal ratio of tandem-connected system was higher, effluent COD concentration of stage-two was higher than that of parallel-connected system. Eq (3) illustrated that higher content organic carbon used as electronic donors would strengthen dinitrification and enlarge TN removal ratio. Therefore, parallel-connected system had a lower removal efficiency due to relatively insufficient organic carbon in wastewater for complete denitrification. (3) TN removal of high ammonia nitrogen wastewater was principally conducted as Eq (4):

\[
\text{NH}_4^+ - \text{N} + 0.75\text{O}_2 \rightarrow \text{N}_2 + 1.5\text{H}_2\text{O} + \text{H}^+ \tag{4}
\]

According to Section 3.1.2, NH₄⁺-N removal of tandem-connected system was higher than that of parallel-connected system. TN removal was ascended by the higher NH₄⁺-N removal in line with the ammonia conversion described as Eq(4). In addition, microbial optimum adaptability range of NH₄⁺-N
concentration changes in stage-one and stage-two were different in tandem-connected system, giving another advantage of better independent growth environment.

3.2. The effect of DO concentration on COD, NH$_4^+$-N and TN removal in tandem-connected system

Based on the conclusion in Section 3.1, tandem-connected two-stage A/O system was chosen to evaluate the effect of DO on ammonia nitrogen wastewater treatment and to accomplish exploration of optimal DO concentration in this part. The experiment was conducted under the same conditions as sludge inoculation process except DO concentration. Five different DO levels of 2.0mg/L, 3.0mg/L, 4.0mg/L, 5.0mg/L and 6.0mg/L were controlled in oxic phase in five independent experiment. Procedures performed in this part were identical to that of the tandem-connected two-stage A/O system applied in Section 3.1. Fig.3.a, b, c showed the COD, NH$_4^+$-N and TN removal efficiencies under different DO concentrations.

**Figure 5.** The COD, NH$_4^+$-N and TN removal efficiencies of tandem-connected two-stage A/O system under different DO concentrations (Total removal ratio of COD, NH$_4^+$-N and TN was calculated as R in Eq (1), removal ratio of stage-one was calculated as R$_1$ in Eq (1) and stage-one was R$_2$; DO concentration here was referred to oxic phase in each subsystem; pH=7.5±0.5)
As was shown in Fig.3.a, COD removal ratio of the stage-one exhibited a gentle growth from 65.8% to 73.9% with the increase of DO concentration from 2mg/L to 6 mg/L. It could be concluded that DO concentration had little influence on the stage-one, and DO concentration of 5mg/L was enough for COD removal in this case. The reason might be that a large part of the carbon source in this experiment was methanol, which was facile to degrade by microorganism. The addition of methanol increased the C/N ratio and biodegradability of the wastewater, so DO concentration played little part in COD removing. Furthermore, no apparent variation, only a slight reduction from 21.6% to 17.7%, of the removal ratio appeared when DO concentration was raised from 2mg/L to 6 mg/L in stage-two system. After digested by microbial activities in stage-one, organics entered in stage-two was in small amount and little oxygen was required for respiration of microorganism. It seemed that the lower the DO concentration was, the less the cost would be. In general, DO concentration of 2mg/L was the least level to maintain the regular microbial activities based on representation in. Additionally, total ratio (R) of COD removal was increased from 87.4% to 95% and the effluent COD of stage-two was 80.4mg/L, which undoubtedly demonstrated the better treating effect of tandem-connected two-stage A/O process.

Variation of NH₄⁺-N removal in Fig.3.b showed a similar trend as the observation of COD removal. When DO concentration increased from 2mg/L to 5mg/L in oxic phase, the NH₄⁺-N removal ratio of stage-one enhanced from 35.2% to 69.8% while that of stage-two was mildly declined, suggesting that DO concentration was a crucial factor for stage-one but not for stage-two. It may due to that in the stage-one, higher DO concentration would strengthen the microbiological activity of nitrobacteria and accelerate the update of formed water film that located on the new floc surface during the up-floating of the boomed bubble in liquid which enhanced the valid collisions with microbial floc. Meanwhile, higher DO concentration would speed the removal ratio of organic matters, thereby weakening the inhibition of heterotrophic bacteria on nitrifying bacteria. However, there was no improvement of NH₄⁺-N removal when DO concentration was further rised up to 6mg/L. DO concentration of 5mg/L was enough for the reduction of organics and NH₄⁺-N in the stage-one. If DO concentration was supplied overdose, the proportion of valid oxygen used for the elimination of pollutants was dropped down [21]. As what had discussed about COD removal, 2mg/L also was determined to be the desired DO levels for NH₄⁺-N removal. Simultaneously, effluent NH₄⁺-N of stage-two was as low as 4.2mg/L, indicating a good NH₄⁺-N elimination performance of two-stage A/O process.

TN removal mainly consisted of the two process of NH₄⁺-N oxidization and NO₃⁻ reduction. It could be clearly observed from the Fig.3.c that total TN removal ratio was enhanced from 75.95% to 95.04% with effluent TN of 11.1mg/L as DO concentration raised from 2mg/L to 5mg/L. NH₄⁺-N removal ratio would be in low level when oxygen was in low dissolution, which would provide less and inadequate NO₃⁻ for denitrification. Yet the further increase of DO concentration to 6mg/L resulted in a deterioration of TN removal ratio from 95.04% to 92.03%. This could be explained by the fact that NO₃⁻ removal was excess for organics and NH₄⁺-N removal, making it possible for liquid reflux to bring dissolved oxygen from oxic phase to anoxic phase and destroy the desired anoxic environment(≤0.5mg/L) which lead to a serious restriction of nitration reaction [22]. The backflowed oxygen enlarge the ability of oxygen to compete for electrons donated from organics, and decreased the available organic carbon for NO₃⁻ removal [21]. In addition, it was pretty easy for excess DO to penetrate sludge floc, and this could break the anoxic/oxic circumstance in anoxic phase. Moreover, excessive dissolved oxygen could also promote the cell aging, stop the growth of activated sludge microorganisms and lower the microbial activity as well as cause bacteriolysis. Hence, DO concentration of 5mg/L was the optimum for stage-one. Moreover, DO concentration had little effect on TN removal in stage-two according to Fig.3.c, and the removal ratio under different DO concentration ranged from 35.6% to 27.8%. Consequently, 2mg/L was the perfect DO concentration for TN removal in stage-two. Effluent TN was perfect with concentration of 12mg/L.

Based on the discussions above, it was finally summarized that DO concentration of 5mg/L was the most suitable for stage-one and 2mg/L for stage-two. High-efficient could be achieved and good quality was obtained under the circumstances. A lot of energy was saved at the same time.
4. Conclusion
1. The treatment efficiency of tandem-connected two-stage A/O process for high ammonia nitrogen wastewater was far better than that of parallel-connected two-stage A/O process, especially for the ammonia nitrogen removal, showing that the tandem-connected two-stage A/O process was favorable to deal with the synthetic ammonia wastewater two-stage A/O process when concentration of COD, NH$_4^+$-N and TN was about 1600mg/L, 250 mg/L and 400 mg/L respectively.  
2. It was finally summarized that DO concentration of 5mg/L was the optimal concentration for stage-one and 2 mg/L for stage-two in tandem-connected two-stage A/O process. All the indicators of effluent could well meet the relevant emission standard and the removal efficiency of COD, ammonia nitrogen and total nitrogen achieved 80.04%, 94.2%, 95.04%, respectively. In this manner a lot of operating cost was saved and much wastage to facilities was immensely avoided.

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