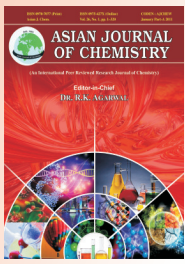
Asian Journal of Chemistry; Vol. 26, No. 21 (2014), 7207-7213

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16567



Isolation of Nitrification-Denitrification Fungus for Removal of Ammonium-Nitrogen

YONGKANG LV[1,*], FEILONG NIU[1], YUXIANG LIU[2] and XIAOHUA YANG[1]

[1]Key Laboratory of Coal Science and Technology, Ministry of Education and Shanxi Province, Taiyuan University of Technology, Taiyuan, Shanxi Province, P.R. China
[2]College of Environmental Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, Shanxi Province, P.R. China

*Corresponding author: Tel: +86 18234127247; E-mail: yongkanglv@163.com

Received: 23 October 2013; *Accepted*: 16 April 2014; *Published online*: 30 September 2014; AJC-16104

A filamentous fungus, which exhibited an ability to remove high-strength ammonium nitrogen and had nitrification-denitrification characteristics, was isolated from the activated sludge of a cokes wastewater treatment facility and named A60. It was provisionally identified as *Fusarium sp*, according to its colonial morphology, microscopic photos and 26S rDNA-ITS sequences analysis. In order to optimum its ammonium removal conditions, several experiments were designed at different carbon sources, nitrogen sources, C/N ratios (mol/mol) and pH. Under optimum conditions, an initial concentration of 450 mg/L NH_4^+-N was removed by 94.97 %, while total nitrogen was reduced by 88.35 %, however, with a few accumulation of hydroxylamine, nitrite and nitrate during nitrification process. Ammonium or the nitrification products such as hydroxylamine, nitrite and nitrate was used as the only source of nitrogen in aerobic denitrification experiment, respectively. The results indicated that all the kinds of nitrogen source could be utilized and converted to nitrogen gas (N_2) about 35-45 %. A60 performed powerful simultaneous nitrification-denitrification ability.

Keywords: Fungus, High-strength ammonium, Optimization of condition, Nitrification, Denitrification.

INTRODUCTION

Coking wastewater contains high concentration of nitrogen compounds and carbon materials[1]. Especially, with increasing of ammonium-nitrogen concentration in cokes wastewater, numerous environmental and health problems will be serious if not treated effectively[2]. Nitrogen removal is an important aspect of present wastewater treatment system. The most common, efficient and cost effective methods involve heterotrophic nitrifiers and aerobic denitrifiers to remove ammonium-nitrogen[3]. Recently, studies have highlighted the existence of some bacteria such as *Paracoccus denitrificans*[4], *Pseudomonas denitrificans*[5], *Bacterium*[6] and *Alcaligenes faecalis*[7]. However, studies on these bacteria have focused on a low ammonium concentration while research on the treatment of high-strength ammonium wastewater is rare[8].

Compared with bacteria, filamentous fungi are adsorbed with each other, easy to be got and immobilized after cultivating[9]. Fungi, such as *Phanerochaete chrysosporium*[10] and *Penicillium*, have made contribution to remove metalion, organic compound and pigments in wastewater. But available fungi used to remove high-strength ammonium-nitrogen are few[11]. Based on above, further research is focused on screening for fungi with high-strength ammonium removal efficiency. In this study, a *Fusarium sp* was isolated from cokes wastewater and firstly showed effective ability to remove high-strength ammonium-nitrogen. It was hoped to have potential applications in future nitrogen bio-treatment systems.

EXPERIMENTAL

Filamentous fungus, which exhibited an ability to remove high-strength ammonium and had nitrification-denitrification characteristics, were obtained from Shanxi Coal Gasification Company Wastewater Treatment Facility, Taiyuan China. Medium: Basal medium: 3 g sucrose, 0.21 g $(NH)_4SO_4$, 0.1 g K_2HPO_4, 0.05 g KCl, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.001 g $FeSO_4{\cdot}7H_2O$, pH 7.0-7.4, 100 mL of distilled water. Optimum medium: 1.09 g sucrose, 0.21 g $(NH)_4SO_4$, 0.1 g K_2HPO_4, 0.05 g KCl, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.001 g $FeSO_4{\cdot}7H_2O$, pH 6, 100 mL of distilled water.

General procedure

Enrichment and isolation: Basal medium was autoclaved for 20 min at 121 °C. In a 250 mL conical flask including 100 mL sterile medium and 0.3 mL of 1 % streptomycin, 2 mL of fresh activated sludge from a cokes wastewater treatment facility was inoculated and incubated at 30 °C and 150 rpm. Every 4 days, 1 mL medium was spot-tested for the consumption of ammonium using Nessler's reagent. When the test proved positive, the microorganism was further purified by

repeated streaking on fresh agar plates (liquid basal medium with 2 % agar). A fungus with effective ammonium removal ability was obtained and named A60.

Identification of the isolated fungus: Colonial morphology and microscopic photos of A60 was analyzed. The 26S rDNA-ITS sequences of the isolate was identified by TaKaRa and compared with those of similar sequences from NCBI Blast online tool.

Shaking culture experiment: A60 placed on fresh agar plates was incubated at 30 °C for seven days. Then its spores was scraped into sterile physiological saline and cultured for 24 h at the same condition as a preculture suspension.

Influence of culture conditions on ammonium removal efficiency of strain A60

Carbon sources experiment: Seven carbon compounds, glucose, fructose, sucrose, farina, sodium acetate, sodium citrate, calcium carbonate, were used as a sole carbon source, respectively, in basal medium without changing other compositions. In a 250 mL conical flask including 120 mL sterile medium, 2 % preculture suspension of A60 was inoculated and incubated at 30 °C and 150 rpm. The optimum carbon source was decided by ammonium removal efficiency after 4 days.

Nitrogen sources experiment: Based on the optimal carbon source, ammonium sulfate, ammonium nitrate, ammonium chloride, ammonium acetate, ethanamide, urea, beef extract, peptone and yeast extract, were employed as a sole nitrogen source, respectively, instead of ammonium sulfate in basic medium. The method of experiment was same as above and the optimum nitrogen source was decided after 4 days.

C/N ratio experiment: In basic medium with optimum carbon and nitrogen sources, the amount of carbon was changed to adjust the C/N ratio by fixing the amount of ammonium sulfate as the nitrogen source. Ammonium was measured after 4 days with the aforementioned method and the best C/N ratio was certain.

pH Experiment: Using the optimal carbon source, nitrogen source and C/N ratio, initial pH of the medium was adjusted from 2 to 10 with 0.1 mol/L HCl or 0.1 mol/L NaOH solutions. The method of experiment was described as above. Ammonium removal efficiency and change of final pH were investigated after 4 days. Then, the optimum pH was selected.

Comparative study on nitrification characteristics of strain A60 in basal and optimum medium: 2 % Preculture suspension of A60 was inoculated into basal and optimum medium, respectively. Under the same experiment condition described above, NH_4^+-N, NH_2OH-N, NO_2^--N, NO_3^--N and total nitrogen were tracked every 12 h for 6 days. Nitrogen removal and nitrification properties were analyzed by compared results between them.

Study on nitrification characteristics of strain A60 under different ammonium concentration: In optimum medium, the initial ammonium concentration was adjusted to approximately 100, 450, 800 and 1000 mg/L on the basis of N content and four concentrations represented low, intermediate, high and higher ammonium loads, respectively. Ammonium removal ability was tracked every 24 h for 5 days with aforementioned method.

Study on denitrification characteristics of strain A60: Four nitrogen compounds, ammonium sulfate and products possibly tested during nitrification such as hydroxylamine, nitrite and nitrate, were used as a sole nitrogen source in optimum medium and adjusted to the concentration of 400, 100, 100 and 100 mg/L N, respectively, by fixing the optimum C/N ratio[12]. 2 % preculture suspension of strain A60 was inoculated into a 250 mL conical flask including 120 mL sterile medium, then, the flask was filled with pure oxygen and sealed. It was incubated at 30 °C and 150 rpm for 4 days. Then, it elucidated the denitrification mechanism of A60 to consume the nitrogen source and nitrifying products and gaseous in flask were detested at the beginning and fourth day to explain the denitrification performance.

Detection methods: The concentrations of ammonium (NH_4^+-N), hydroxylamine (NH_2OH), nitrite (NO_2^-), nitrate (NO_3^-) and denitrification product (N_2) were determined as follows. Ammonium, NH_2OH and NO_2^- were measured using Nessler's reagent photometry, spectrophotometer and N-(1-naphthalene)-diaminoethane photometry, respectively[13]. NO_3^- and nitrogen (N_2) were detested using ion chromatography (PIC-10, PUREN, Qingdao) and gas chromatography, respectively. Total nitrogen (TN) was analyzed by TOC-TN analyzer (CN200, Shimadzu, Kyoto).

RESULTS AND DISCUSSION

Identification of strain A60: Isolated filamentous fungus was firstly analyzed through its colonial morphology and microscopic photos. On agar plates of PDA, colonies of strain A60 grow fast as white, opaque. Its mycelium is well-developed and transparent and spore is oval[14]. Based on the result of 26S rDNA-ITS sequences, A60 revealed 100 % sequence similarity with *Fusarium sp* or Gibberella.

However, *Fusarium sp* belongs to Gibberella during its sexual period[15]. Then, it was provisionally identified as *Fusarium sp*, named *Fusarium sp* A60.

Influence of culture conditions on ammonium removal efficiency of A60

Carbon sources experiment: As shown in Fig. 1 strain A60 grew well and removed ammonium in different degree under all kinds of carbon sources. Especially, when sucrose was added as a sole carbon source, dry biomass and removal of ammonium reached a maximum. It shows that strain A60 can acclimatize itself to complex coal wastewater without restriction of organic carbon or inorganic carbon and has the best ammonium removal ability in sucrose medium. It reported that the ammonium removal ability of microorganism was strongly influenced by carbon sources[16]. Then, sucrose was chosen to the optimum carbon source.

Nitrogen sources experiment: A60 was adaptable to medium contained inorganic nitrogen source as well as organic nitrogen source. When nitrogen source, such as ethanamide, ammonium acetate, peptone, urea, ammonium sulfate, ammonium chloride and ammonium nitrate, was added, dry weight of mycelia was on the decline. But in ammonium sulfate medium, total nitrogen and NH_4^+-N were decreased by maximum removal rate and the fastest unit removal speed. Then, ammonium sulfate was the optimum nitrogen source. Furthermore,

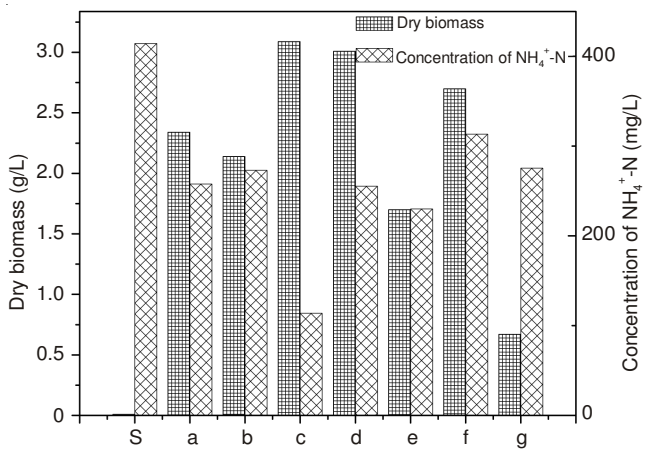


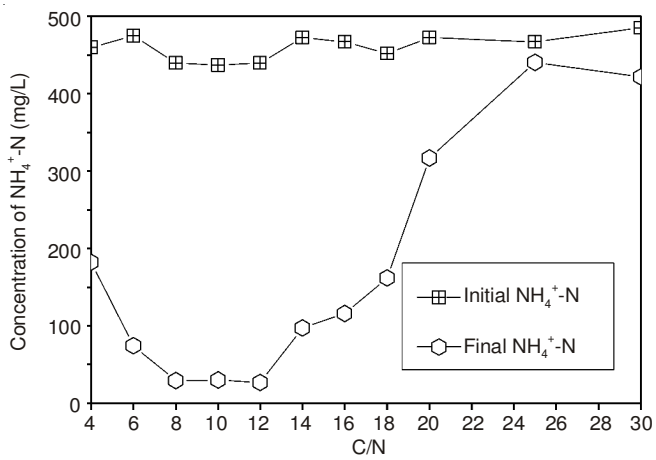
Fig. 1. Growth and ammonium removal efficiency of A60 under different carbon source S: blank control a: glucose b: fructose c: sucrose d:starch e: sodium acetate f: sodium citrate g: calcium carbonate

when ammonium nitrate was used as a sole nitrogen source, Decrease of total nitrogen or NH_4^+-N was far more than the accumulation of nitrite, hydroxylamine and nitrate (data not shown)[17]. Based on this result, it inferred that strain A60 may had denitrification ability which would be tested in later experiments. Data were analyzed from Table-1.

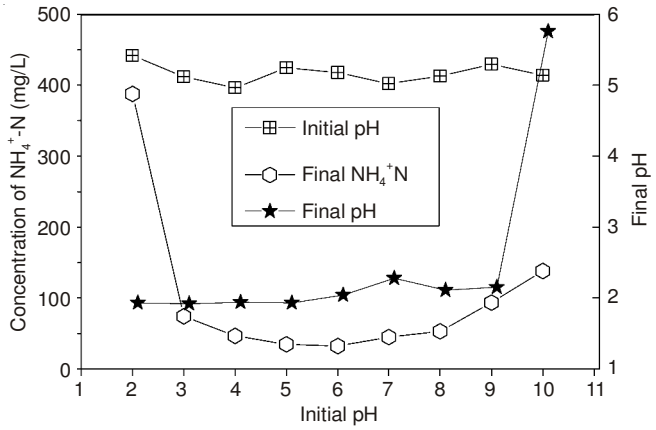
C/N ratio experiment: Influence of C/N ratio on ammonium removal was investigated in shaking culture as shown in Fig. 2(a). When sucrose and ammonium sulfate were used as optimum carbon and nitrogen sources in medium, initial NH_4^+-N concentration of 450 mg/L was consumed at C/N ranging from 4 to 30.

The best ammonium removal efficiency occurred at C/N 8 (93.39 %), C/N 10 (93.12 %) and C/N 12 (93.91 %) remaining final NH_4^+-N concentration of 29.05 mg/L, 30.08 mg/L and 26.79 mg/L, respectively. At C/N of 12, the final NH_4^+-N concentration was mininum, though other results were almost similarly close to it. The consumption rates of carbon and NH_4^+-N were balanced and both compounds were exhausted almost simultaneously under these conditions.

The final concentration of NH_4^+-N presented the trend of decrease from C/N 4 to 8, was reduced to 182.37 mg/L, 74.29 mg/L and 29.05 mg/L, respectively[18]. The consumption of NH_4^+-N was away from that at C/N 12 mainly due to the exhaustion of the carbon source used for growing. The trend of increase began from C/N 12, indicating that the increase of organic matter restrained growth of A60, possibly[19]. Results showed that A60 always held effective ammonium removal



(a) Removal under different C/N ratio



(b) Removal under different pH

Fig. 2. Ammonium removal efficiency of A60 under different C/N ratio and pH

ability at different C/N ratios, even when carbon source was not enough and organic matter was over.

It showed that C/N ratio was one of the most important factors for ammonium removal and C/N 12 was optimum C/N ratio in this study.

pH Experiment: After selecting the optimum carbon and nitrogen sources fixing C/N ratio of 12, the influence on adaption and ammonium removal of A60 under different initial pH are shown in Fig. 2 (b). When the initial pH value was adjusted to 2, A60 grew slowly because it could not adapt to peracid condition and ammonium was almost not utilized.

TABLE-1
NITROGEN REMOVAL EFFICIENCY OF STRAIN A60 UNDER DIFFERENT NITROGEN SOURCES

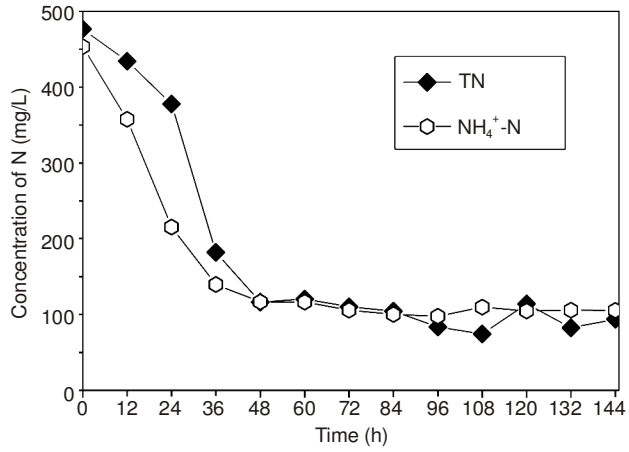
Nitrogen source	Dry biomass (g/L)	Initial total nitrogen (mg/L)	Final total nitrogen (mg/L)	Initial NH_4^+-N (mg/L)	Final NH_4^+-N (mg/L)	Removal rate (%)
Ammonium sulfate	3.05	449.75	145.13	435.71	111.66	74.37
Ammonium nitrate	2.14	435.75	161.25	225.01	63.66	71.71
Ammonium chloride	2.82	475.75	311.75	437.12	195.83	55.20
Ammonium acetate	3.22	445.50	153.38	427.02	127.32	70.18
Ethanamide	3.67	453.25	200.83	416.91	204.14	51.03
Urea	3.15	407.25	159.88	276.63	92.87	66.43
Beef extract	2.04	265.25	118.45	266.53	75.19	71.79
Peptone	3.19	274.25	127.75	217.30	122.27	43.73
Yeast extract	0.85	244.83	145.13	231.18	130.85	43.40

Except for pH 2, increasing the initial pH from 3 to 6, environment was changed from peracid to weak acid and the ammonium removal efficiency was enhanced with final NH_4^+-N concentration from 74.26 mg/L to 29.05 mg/L. At initial pH of 6, ammonium removal reached 93.05 %. When the initial pH value was 7, 8, 9 and 10, the final NH_4^+-N concentration was slightly increased with increasing alkaline. Within initial pH of 3 to 8, final concentrations of NH_4^+-N were all maintained below 74.26 mg/L and ammonium removal rates were over 81.97 %. Then, the initial pH of 6 was the best choice.

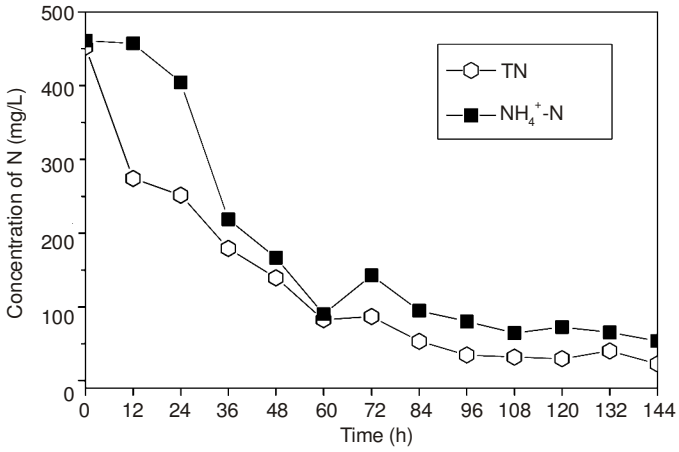
By analyzing final pH in medium, A60 created acid environment for itself in spite of not adapting to initial peracid medium. It was guessed that peracid condition just restrained its growth at adaptive phase but did not affect its ammonium removal ability at later stages. Results indicated that A60 had good adaptation to environment[20] and could remove ammonium in acid, neutral as well as alkaline cultures. Compared with many nitrifies which grow only in weak acid or alkaline condition[21], A60 will be more positive.

Comparative study on nitrification characteristics of A60 in basal and optimum medium: The NH_4^+-N and total nitrogen concentrations were traced in basal and optimum medium every 12 h for 6 days. Results were showed in Fig. 3 (a) and (b). In optimum medium, the concentration of NH_4^+-N (about 450 mg/L) was decreased to 82.60 mg/L after 60 h and continued to be reduced in later time, the ultimate NH_4^+-N concentration was just 22.75 mg/L (the removal rate of 94.75 %) and the fastest removal rate reached to 14.83 mg/L/h. while, in basal medium, the NH_4^+ -N was removed stopping at about 100 mg/L after 48 h with final removal rate of 78.44 %. The total nitrogen was removed by 88.35 % in optimum medium at this time. It was concluded A60 exhibited stronger ability of nitrogen removal in optimum medium.

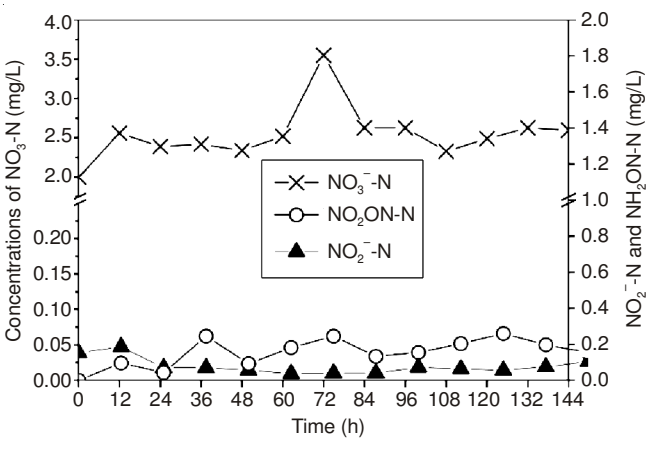
Intermediates of nitrite, hydroxylamine and nitrate were tested during ammonium removal process, shown in Fig. 3 (c) and (d). In basal optimum, the accumulation of NO_2^--N was always remained at a low level without changing; NH_2OH-N was accumulated like waves with about 0.05 mg/L; concentration of NO_3^--N turned up a higher spot of 0.355 mg/L after 72 h. In optimum medium, the changing trend of NO_2^--N and NH_2OH-N were similar to that in basal optimum, but the concentration of NH_2OH-N was accumulated about 0.1 mg/L at the later time and concentration of NO_3^--N turned up a same high spot after 24 h. In sum, strain A60 produced hydroxylamine and nitrate during ammonium removal process and had faster nitrification in optimum medium. Because decrease of total nitrogen or removal of NH_4^+-N was far more than accumulation of nitrite, nitrate and hydroxylamine, strain A60 likely had ability of denitrification[22]. But the way of denitrification would be further discussed.



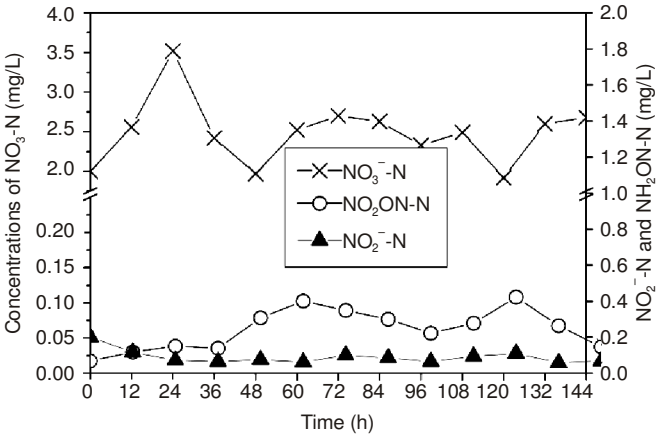
(a) Removal in basal medium



(b) Removal in optimum medium



(c) Accumulation in basal medium



(d) Accumulation in optimum medium

Fig. 3. Nitrification characteristics of A60 in basal and optimum medium

Study on nitrification characteristics of A60 under different ammonium concentration: In optimum medium with ammonium sulfate as sole nitrogen source, concentrations of 100, 450, 800 and 1000 mg/L NH_4^+-N were observed during ammonium removal process every day for 5 days. Results were showed in Fig. 4.

In medium of low ammonium concentration, an initial concentration of 111.66 mg/L NH_4^+-N was reduced by 74 % (28.93 mg/L) and 100 % by A60 within 24 and 48 h, respectively. Total nitrogen was decreased by 99 % with final concentration of 1.52 mg/L after 48 h. Nitrifying products NH_2OH-N and NO_3^--N were detected during nitrogen removal process without accumulation of NO_2^--N.

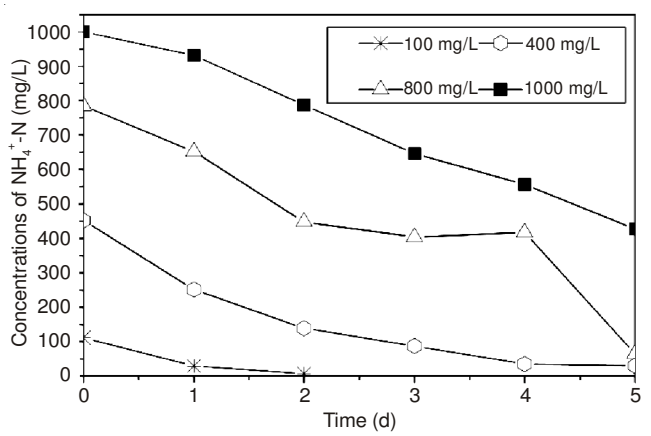
In medium of intermediate and high ammonium concentration, NH_4^+-N was finally removed by 95 % (22.73 mg/L) and 92 % (64.70 mg/L), respectively. Final total nitrogen was reduced to 23.98 mg/L by 95 % when initial concentrations of NH_4^+-N was about 450 mg/L. The concentrations of hydroxylamine and nitrate increased to a maximum after one day and two days, respectively, then decreased and repeated the similar pattern in either intermediate or high ammonium concentration medium. These results showed that the nitrogen removal process was not inhibited by high concentrations of ammonium.

Even when medium contained higher initial NH_4^+-N concentration of 1000 mg/L, NH_4^+-N was continued to decrease until the 5th day, total nitrogen and nitrifying products were changed by the similar way to that in 400 mg/L and 800 mg/L NH_4^+-N medium.

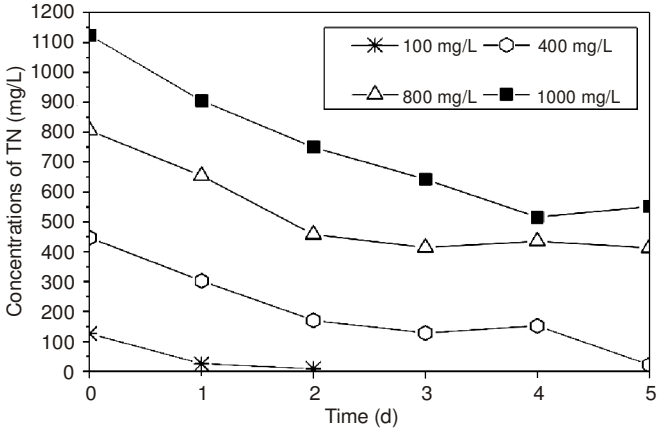
These indicated that A60 manifested the ability not only to remove high concentration ammonium but also to reduce total nitrogen effectively and it could be a meaningful feature for applications in coal wastewater treatment systems where ammonium concentrations tend to be high.

Study on denitrification characteristics of strain A60: The decrease of total nitrogen or removal of NH_4^+-N was far more than the accumulation of nitrite, hydroxylamine and nitrate during the ammonium removal process. This suggested NH_4^+-N was possibly converted to gas by the process of aerobic denitrification. In order to study denitrification characteristic of A60, the consumption of nitrogen sources shown in Table-2 and products during experiment process were detected at the 4th day.

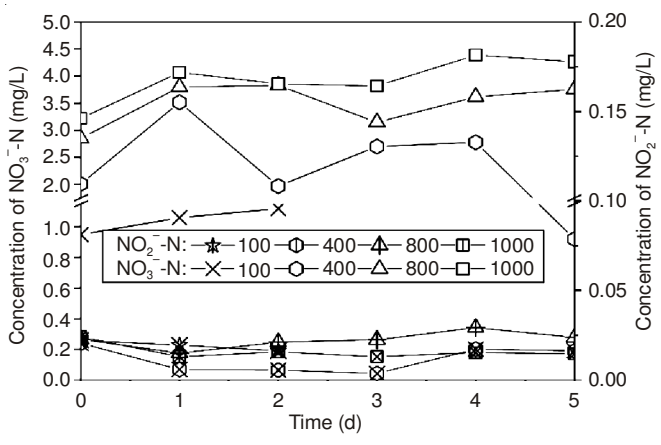
When 400 mg/L NH_4^+-N was added in optimum medium, 331.49 mg/L NH_4^+-N was consumed. Some of which were converted to NH_2OH-N, 5.90 mg/L NO_3^--N and 158 mg/L N_2. This indicated that A60 could utilize the NH_4^+ and convert it to N_2 through mediums with denitrification ratio of 40.10 %. When 100 mg/L NO_2^--N, NO_3^--N and NH_2ON-N were added, respectively, A60 could grow well and consume these nitrogen sources. During this time, NH_4^+-N (about 20 %) was found in $NaNO_2$ medium, NH_4^+-N (about 20 %) and 0.09 mg/L NO_2^--N were increased in $NaNO_3$ medium and 7.12 mg/L NH_4^+-N



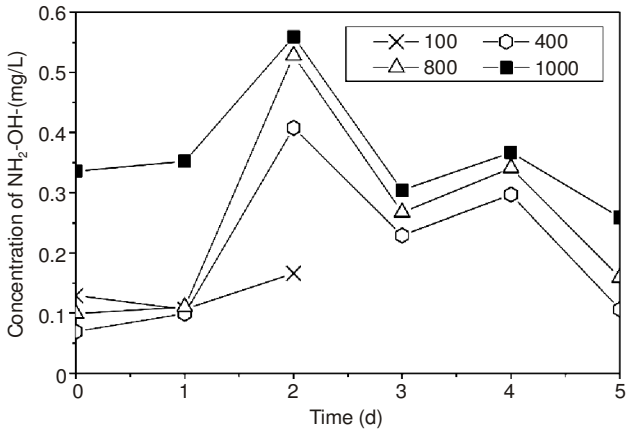
(a) Removal of NH_4^+-N of A60



(b) Removal of TN of A60



(c) Accumulation of NO_2^--N and NO_3^--N



(d) Accumulation of NH_2OH-N

Fig. 4. Nitrification characteristics of A60 under different ammonium concentration

TABLE-2
NITROGEN ANALYSIS IN AERATED DENITRIFICATION EXPERIMENT BY A60

Nitrogen source	NH_4^+-N (mg/L)		NO_2^--N (mg/L)		NO_3^--N (mg/L)		NH_2OH-N (mg/L)		Final TN (mg/L)	N_2 (mg/L) (Denitrification %)
	Initial	Final	Initial	Final	Initial	Final	Initial	Final		
$(NH_4)_2SO_4$	395.01	63.52	0.02	0.01	0	5.90	0.10	0.31	72.67	158.4 (40.10)
$NaNO_2$	0.26	19.80	113.17	0.33	0.97	0	0.08	0.09	23.44	37.6 (33.22)
$NaNO_3$	10.36	21.67	0.02	0.11	107.64	20.81	0.14	0.11	49.70	37.07 (34.44)
$NH_2O{\cdot}HCl$	0.92	8.04	0.02	0.02	0.87	26.40	94.50	9.90	46.95	31.13 (32.94)

was accumulated in $NH_2O{\cdot}HCl$ medium, while N_2 was detected about 32-35 % of the concentration of total nitrogen. Based on the nitrogen balance[23], other nitrogen was utilized as intracellular nitrogen. These indicated that A60 could utilize four nitrogen sources and converted them to N_2, performed powerful simultaneous nitrification-denitrification ability.

Results were concluded by analyzing data above as follows: firstly, when $NaNO_2$ was used as a sole nitrogen source, N_2 and NH_4^+-N were detected. Combining accumulation of NH_2OH-N and NO_3^--N in above and Fig. 4 (B), it was inferred that NO_2^- as the medium of nitrification and denitrification was converted to NH_4^+ and N_2 and also converted few to NO_3^- in NH_4^+ medium. Secondly, because the changing pattern of NH_2OH-N was in accord with but ahead of that of NO_3^--N and NO_3^--N and NH_4^+-N were produced in $NH_2O{\cdot}HCl$ medium, it was explained that NO_2^- was likely converted to NO_3^-, NH_4^+ and N_2 quickly. Thirdly, just a few NO_2^--N was found in $NaNO_3$ medium. Combining the accumulation of NO_3^--N in ammonium sulfate medium, it was clarified that NO_3^- was converted to NH_4^+ and N_2. Based on conclusion above and denitrification experiment in ammonium sulfate medium, it was suggested as follows: when product possibly tested during nitrification, such as hydroxylamine, nitrite, or nitrate, was used as a sole nitrogen source, it was firstly converted to NH_4^+ and then continued to utilize by A60. The suggested sequence of nitrification and denitrification is illustrated in Fig. 5. However, further research is still needed to confirm the precise nitrogen removal pathway by A60.

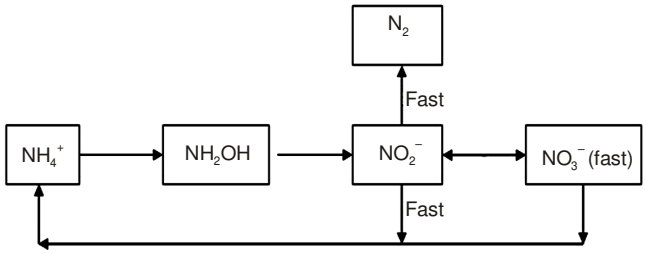


Fig. 5. Possible pathway of nitrification and denitrification

Conclusion

A filamentous fungus which could remove high-strength ammonium, named A60, was isolated from the activated sludge of a cokes wastewater treatment facility with the common method. It was provisionally identified as *Fusarium sp*, according to its colonial morphology, microscopic photos and rDNA-ITS sequences analysis.

A60 could grow and remove high-strength ammonium in medium with various kinds of carbon and nitrogen sources, adapt to not only acid but alkaline cultures. While the best carbon and nitrogen sources were sucrose and ammonium sulfate, the optimum C/N ratio and pH were 12 and 6, respectively.

When the initial NH_4^+-N concentration was adjusted to about 450 mg/L in optimum medium, NH_4^+-N was removed by 94.97 % with the fastest removal rate of 14.83 mg/L/h. At this time, total nitrogen was decreased by 88.35 %. During the whole nitrification, the accumulation of NO_2^--N, NH_2OH-N and NO_3^--N was far less than the decrease of NH_4^+-N or total nitrogen. Even in initial NH_4^+-N concentration of 800 and 1000 mg/L medium, A60 still maintained effective nitrogen removal ability with similar nitrifying pattern.

Results of aerated denitrification experiment of A60 showed that the ammonium sulfate and other three nitrogen sources possibly produced in nitrification such as hydroxylamine, nitrite and nitrate, were utilized well by A60. Nitrogen was detected in every medium, which indicated that A60 held denitrification characteristic with the maximum denitrification ratio of 40.10 %. The pathway of nitrification and denitrification was guessed by analyzing the consumption of nitrogen source and accumulation of all kinds products.

Based on the powerful simultaneous nitrification-denitrification ability of A60, it was believed to be a meaningful for applications in coal wastewater treatment systems where ammonium concentrations tend to be high.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 51078252), Internation cooperation projects of Shanxi Province (2010081018) and Natural Science Foundation of Shanxi, China (2010011016-1).

REFERENCES

1. Y.K. Lv, J.Y. Yang and Y.X. Liu, *Asian J. Chem.*, **25**, 7243 (2013).
2. J.K. Kim, K.J. Park, K.S. Cho, S.-W. Nam, T.-J. Park and R. Bajpai, *Bioresour. Technol.*, **96**, 1897 (2005).
3. J. Wang, Y.S. Pei, Z.F. Yang and C.J. Lin, *Fresenius Environ. Bull.*, **20**, 114 (2011).
4. A.H. Stouthamer, A.P.N. de Boer, J. van der Oost and R.J.M. van Spanning, *Antonie van Leeuwenhoek*, **71**, 33 (1997).
5. J.J. Su, B.Y. Liu and C.Y. Liu, *Appl. Microbiol.*, **90**, 457 (2001).
6. X.-P. Yang, S.-M. Wang, D.-W. Zhang and L.-X. Zhou, *Bioresour. Technol.*, **102**, 854 (2011).
7. T. Nishio, T. Yoshikura, K. Chiba and Z. Inouye, *Biosci. Biotechnol. Biochem.*, **58**, 1574 (1994).
8. H.S. Joo, M. Hirai and M. Shoda, *Biotechnol. Lett.*, **27**, 773 (2005).
9. P. Li, S. Zhang, Y.L. Zhang, Y. Jiang, S.L. Chen, Y.P. Zou and D.L. Liu, *Environ. Sci. Technol.*, **29**, 9 (2006).
10. Y. Lu, H. Yan, Wang, S. Zhou, J. Fu and J. Zhang, *J. Hazard. Mater.*, **165**, 1091 (2009).
11. Y. Wen, C. Xu, G. Liu, Y. Chen and Q. Zhou, *Frontiers Environ. Sci. Eng.*, **6**, 140 (2012).

12. S. Tang, Q. Yang, H.T. Shang and T. Sun, *Fresenius Environ. Bull*., **19**, 3193 (2010).
13. G. Koch, J.R. Vander Meer, H. Siegrist and K. Egli, *Water Sci. Technol*., **41**, 191 (2000).
14. J.J. Xing, H. Xiao and S.H. Deng, *Chinese J. Environ. Eng*., **4**, 1541 (2010).
15. C. Booth, The genus fusarium, Agricultural Press, Beijing, pp. 1-12 (1988).
16. D.S. Arora and P.K. Gill, *Bioresour. Technol.*, **77**, 89 (2001).
17. A.R. Lesley, E.W.J. Van Niel, R.A.M. Torremans and J.G. Kuenen, *Appl. Environ. Microbiol*., **54**, 2812 (1988).
18. Z.X. Peng, Y.Z. Peng, Z.B. Yu, X.L. Liu, X.L. Li and R.D. Wang, *Frontiers Environ. Sci. Eng*., **6**, 884 (2012).
19. C. Tang, P. Zheng, C. Wang and Q. Mahmood, *Bioresour. Technol*., **101**, 1762 (2010).
20. P. Klangduen and K. Jurg, *Water Sci. Technol*., **39**, 235 (1999).
21. L.A. Robertson and J.G. Kuenen, *J. Gen. Microbiol*., **129**, 2847 (1983).
22. C. Glass, J. Silverstein and J. Oh, *Water Environ. Res*., **69**, 1086 (1997).
23. Y.X. Zhang, J.T. Zhou, J.S. Zhang and S.Z. Yuan, *J. Environ. Sci. (China)*, **21**, 568 (2009).