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A N I N T E R N A T I O N A L J O U R N A L

The physiological and biochemical mechanism of nitrate-nitrogen removal by water hyacinth from agriculture eutrophic wastewater

WU Wenwei[1,2], LIU Ang[1], WU Konghuan[2], ZHAO Lei[2], BAI Xiaohua[2], LI Kun-zhi[1], Muhammad Aqeel ASHRAF[3], CHEN Limei[1*].

[1]Biotechnology Research Center, Kunming University of Science and Technology, Chenggong, Kunming 650500, China；[2]Yunnan Institute of Environmental Science, Kunming Yunnan650034; [3]Faculty of Science and Natural Resources, University Malaysia Sabah 88400 Kota Kinabalu, Sabah, Malaysia.

ABSTRACT

Large amount of agriculturl wastewater containing high level nitrate-nitrogen (NO_3^--N) is produced from modern intensive agricultural production management due to the excessive use of chemical fertilizers and livestock scale farming. The hydroponic experiment of water hyacinth was conducted for analyzing the content of NO_3^--N, soluble sugar content, N-transported the amino acid content and growth change in water hyacinth to explore its purification ability to remove NO_3^--N from agriculture eutrophic wastewater and physiological and biochemical mechanism of this plant to remove NO_3^--N. The results showed that the water hyacinth could effectively utilize the NO_3^--N from agriculture eutrophic wastewater. Compared with the control, the contents of NO_3^-change to NO_3^--N in the root, leaf petiole and leaf blade of water hyacinth after treatment in the wastewater for a week was significantly higher than that in the control plants treated with tap water, and also the biomass of water hyacinth increased significantly, indicating that the accumulation of biomass due to the rapid growth of water hyacinth could transfer some amount of NO_3^--N.^{13}C-NMR analysis confirmed that water hyacinth would convert the part nitrogen absorbed from agriculture eutrophic wastewater to ammonia nitrogen, which increased the content of aspartic acid and glutamic acid, decreased the content of soluble sugar, sucrose and fructose and the content of N-storaged asparagine and glutamine, lead to enhance the synthesis of plant amino acids and promote the growth of plants. These results indicate that the nitrate in agriculture eutrophic wastewater can be utilized by water hyacinth as nitrogen nutrition, and can promote plant growth by using soluble sugar and amide to synthesis amino acids and protein.

Key words：water hyacinth, nitrate-nitrogen removal, nitrate conversion, N-transported amino acids

[*]Authors for correspondence: chenlimeikm@126.com.

INTRODUCTION

Research in recent decades showed that artificial wetland water purification system built by aquatic plant has good purification effect, low cost, convenient operation and management, as well as good landscape and ecological benefits. Therefore, researchers around the world are paying more attention to the ecological restoration of polluted water by using aquatic plant(Zhang et al., 2014; Vymazal, 2013; Saeed and Sun, 2012). Water hyacinth (Pontederiaceae, EichhorniaKunth), one of the floating plants, has developed root system underwater. Its good growth in wastewater illustrated that it possess super water purification ability(Malik, 2007; Villamagna and Murphy, 2010; Wang et al., 2012), this makes it become the aquatic plant which is researched most in-depth, as well as widely used in practical ecological restoration engineering. Water hyacinth can purify eutrophic water and special material contaminated water, such as polymer organic wastewater, heavy metal wastewater, landfill leachate, etc(Zimmels et al.,2006; Schneider et al.1995; Guittonny et al., 2014). Applying in rivers, lakes, aquatic plant pond, surface flow constructed wetland is the primary application form of water hyacinth(Zhang et al., 2014; Vymazal, 2013; Saeed and Sun, 2012). The results of the experiments demonstrate that water hyacinth can not only absorb N and P (Zimmels et al., 2006; Zhang et al., 2006), but also remove heavy metal (Cd, Cu, Ni and Pb, etc)(Lin, 2013), toxic substance such as As (Alvarado et al., 2008), radioactive substance such as ^{137}Cs and ^{60}Co (Saleh, 2012) and organic pollutants (Saeed and Sun, 2012)in waste water.

It is reported (Zhang and Wang, 2007)that water hyacinth purifying water quality includes following approach: 1) overspreading the surface of water body with leaves, as a result the algae die because photosynthesis is blocked; 2) creating a favorable growth condition for microorganism by temperature adjustment; 3) supplying microorganism of carbon source which was composed of leaves; 4) supplying attached carrier, filtering pollutant, absorbing nutrition (N, P, some heavy metals and organic matters) for microorganism by developed roots; 5) degrading pollutant by root attached microorganism; 6) redistributing intracorporal nutrient substance and adjusting its own shape to achieve the best reproduction rate (Xie et al., 2004).

In recent, there are a large number of researchers pay attentions to decontamination mechanism of constructed wetland(Zhang et al., 2014; Vymazal, 2013; Saeed and Sun, 2012). However, these researchers more focus on microorganism purification, less on plant purification. Over the past ten years, farmers applied chemical fertilizers (especially nitrogenous) excessively in agriculture eutrophic wastewater for the purpose of high yield and high profits in China. As a result, serious salinization happened. Moreover, in order to reduce the content of salt in soil, these farmers needed to use large numbers of water to irrigation. Finally great quantities of agricultural wastewater which contained high concentration of NO_3^- (up to 10 mg/L some times) were produced. This wastewater has been discharged to adjacent rivers and lakes directly what caused eutrophication in water. Therefore, pollution improvement on NO_3^- in agricultural wastewater has become one of the important research directions of environmental science.

Researches and applications of water hyacinth in water ecological restoration are substantial. However, the purification mechanism of water hyacinth has not been studied deeply, even the opposite conclusions existed. This study try to explain physiological and biochemical mechanism that water hyacinth removes NO_3^- in agricultural wastewater by analyzing the changes of NO_3^- concentration in wastewater, root, petiole and leaf of water hyacinth after water hyacinth was used to wastewater treatment. Simultaneously, ^{13}C-NMR analyzing was also introduced in this study to detect the relative change of carbohydrate substance content and N-transport amino acids content in root, petiole and leaf of water hyacinth. Beyond that, this study can provide some theoretical basis for construction of constructed wetland where water hyacinth is applied to treat agricultural wastewater.

MATERIALS AND METHODS

Culture of water hyacinth and treatment of agricultural wastewater

Agriculture eutrophic wastewater in this study was collected from a vegetable cultivated area in north shore of Qilu lake, where located in Yuxi, Yunan province. The collected agriculture eutrophic wastewater was stored at 4℃. Water hyacinth which was collected from artificial wetland was culturedin tap water. The water hyacinth with same size was divided into two groups after they have been weighted. One of them was culturedin the agriculture eutrophic wastewater, and another was culturedin tap water as control group. Each group was set three groups of parallel sample.Cultivate time was a week. During this week, the concentration of NO_3- in water was detected every day. After this week, water hyacinth was wiped off and weighted. One gram of root, petiole and leaf were taken and stored respectively in liquid nitrogen in -80℃.

Determination of NO_3^-concentration

Fivemilliliter water from experimental group and the control group were collected every day during the cultivate time of water hyacinth. Then about 1-2 drops of aluminum hydroxide suspension were added in water. After flocculation, deposition and centrifugation, supernatant was selected to detecting concentration of NO_3^--N. Quartzose cuvette with optical path length of 10 mm was used to detect the concentration of NO_3^--N at wave length of 220 nm and 275 nm, and fresh deionized water was used as control. Adjusted value of absorbance of NO_3^--N is A_{220}-$2A_{275}$. Potassium nitrate solution which conclude0.25, 0.50, 1.00, 1.50 and 2.00 mg·$L^{-1}$$NO_3^-$-N, respectively, were prepared as standard solution. Their absorbance was detected with the same method as below. Subsequently, standard curve was drawn as X axis is adjusted absorbance of standard solution and Y axis is the concentration of NO_3^--N in solution. The concentration of NO_3^--N (unit: mg·L^{-1}) could be found out from this standard curve.

Determination of plant fresh weight growth rate

Fresh weight of every plant has been detected before they were processed, then these plants were processed by agriculture eutrophic wastewater and tap water for one week as described above. Subsequently, the processed plants were weighted. Growth rate of plant = (W_a –W_b) / W_a×100%, where W_a is fresh weight of plant after process, and W_b is fresh weight of plant before process.

Determination of NO_3^-concentration in water hyacinth tissue

The root, petiole and leaf of water hyacinth mentioned before were put in grinding bowl, respectively. Then a little liquid nitrogen was added in the grinding bowl. After root, petiole and leaf have been pulverized, five milliliter deionized water was added in and all of them were transferred into EP tube. Supernatant was transferred in new EP tube after centrifugation (3000r·min^{-1}, 20min). Subsequently, concentration of NO_3^- in different tissue of water hyacinth before and after processing was detected according to the method below.

Analysis of ^{13}C-NMR

The water hyacinth with same size was weighted and they were processed for one week according to the method below. After the processing, residuewhich produced from 50 ml sample of agriculture eutrophic wastewater after cryodesiccation was dissolved in 0.5 ml deionized water. The dissolved sample was transferred in 5 mm nuclear magnetic tube and analysed by ^{13}C-NMR after moderateformamide was added in the tube as internal reference (Ref). Surface water of water hyacinth was wiped out, and 5 g root, petiole and leaf of water hyacinth were collected and grinded in liquid nitrogen, respectively. Extraction was processed after 100 mM kalium potassium phosphate buffer(KPB, pH 7.4) has been added in. Extract was boiled in boiling water for 3 min to make enzyme loose activity and cell debris was removed after centrifugation (12000×g, 10 min). The supernatant was dissolved in 0.5 ml, 100 mM KPB after cryodesiccation. Subsequently, the dissolved samples were transferred into 5 mmnuclear magnetic tube and analysed by ^{13}C-NMR after moderateformamide was added in the tube as internal reference (Ref).

Data of NMR was obtained by BrukerNuclear Magnetic Resonance (DRX 500-MHz), and parameters were set as following: broadband proton decoupling, pulse was 5-μs (90°), spectral width was 37594 Hz, sampling time was 0.5 s, lag time was 1.2 s, sample temperature was 25 ℃, 32000 data points for per sample, number of scan was 1200, linewidth while data processing was 4 Hz. Chemical shift of sample according to formamide resonance peak (166.600 ppm). Adscription of resonance peak in NMRspectrum could be speculated through ^{13}C-NMR spectrum of known compound. Subsequently, relative amount of metabolites in different samples could be calculated by integrating to target resonance peak while the formamide has been set as internal reference (Ref).

Data processing

All analysis of physiological indexes repeated 3 times at least, and the mean value and error were calculated by Excel, statistically significant difference of the data ($P<0.05$) obtained was analyzed by DPS data system. Different lowercase letters indicated that there was statistically significant difference between two groups.

RESULTS

Purification efficiency of water hyacinth to NO_3^--N in agriculture eutrophic wastewater

Concentration of NO_3^- in agriculture eutrophic wastewater was 4.78 $mg \cdot L^{-1}$, 24 times higher than total nitrogen of Water Quality Standard I (TN, 0.2 $mg \cdot L^{-1}$,GB3838-2002),according to detection result. In order toevaluate the efficiency of water hyacinth purify NO_3^--N in agriculture eutrophic wastewater, the plant which processed by tap water was set to control (CK). Result indecated that concentration of NO_3^--N in agriculture eutrophic wastewater was decreasing obviously over time, and it reduced to 0.34 $mg \cdot L^{-1}$after 5 days (Fig. 1), and it was as same as the concentration of NO_3^--N in tap water. This indicated that the NO_3^-N purification efficiency of water hyacinth in agriculture eutrophic wastewater was highly significant. However, a slight increment of NO_3^--N concentration in agriculture eutrophic wastewater was observed in subsequent processing. This may be because that the NO_3^--N which has been absorbed by root of water hyacinth was released into water.

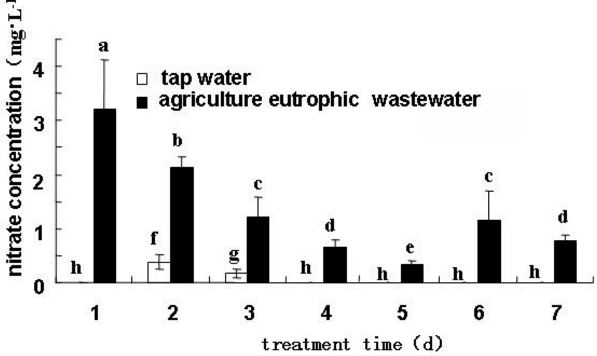


Figure 1 Changes of nitrate concentration in agriculture eutrophic wastewater and tap water after treatment with water hyacinth for a week

Changes of water hyacinth fresh weight and NO_3^- concentration in tissue

The growth rate of water hyacinth fresh weight was detected after water hyacinth has been processed in agriculture eutrophic wastewater for one week. The result showed that the fresh weight of water hyacinth has increased obviously while water hyacinth was processed in agriculture eutrophic wastewater for one week. Its growth rate was 2 times higher than what was processed in tap water. This indicated that water hyacinth has a fairly rapidgrowth rate in agriculture eutrophic wastewater. Concentrations of NO_3^- in root, leaf petiole and leaf blade of water hyacinth which was processed in agriculture eutrophic wastewater for one week were detected. The results showed that all of these concentrations were obvious higher than what in tap water (Fig. 2). Respectively, concentration of NO_3^- in root, leaf petiole and leaf bladeof water hyacinth which was processed in agriculture eutrophic wastewater were 40%, 10% and 20% higher compared with which was processed in tap water. This indicated that partial NO_3^- which has been absorbed by water hyacinth accumulated in the tissues of root, leaf petiole and leaf blade.

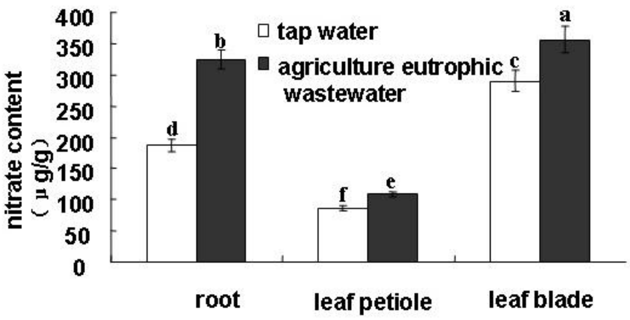


Figure 2 Changes of nitrate content in different tissues of water hyacinth after treatment with agriculture eutrophic wastewater and tap water

Changes of tissue metabolic product concentrations of agriculture eutrophic wastewater treated water hyacinth

The changes of tissue metabolic product concentrations of water hyacinth before and after agriculture eutrophic wastewater treated were detected by ^{13}C-NMR in order to analyze the organ metabolism that water hyacinth purify agriculture eutrophic wastewater at the metabolic level. The result showed that there was no obvious ^{13}C resonance signal peak was observed in original agriculture eutrophic wastewater before treatment (Fig. 3). This indicated that there was hardly any soluble carbon containing compounds in agriculture eutrophic wastewater. However, two obvious ^{13}C resonance signal peak were observed after water hyacinth has been processed. [5-^{13}C] and [1-^{13}C] are attribute to sucrose and acetic acid, respectively. This indicated that the root of water hyacinth can secreted some carbon-containing compound in processing.

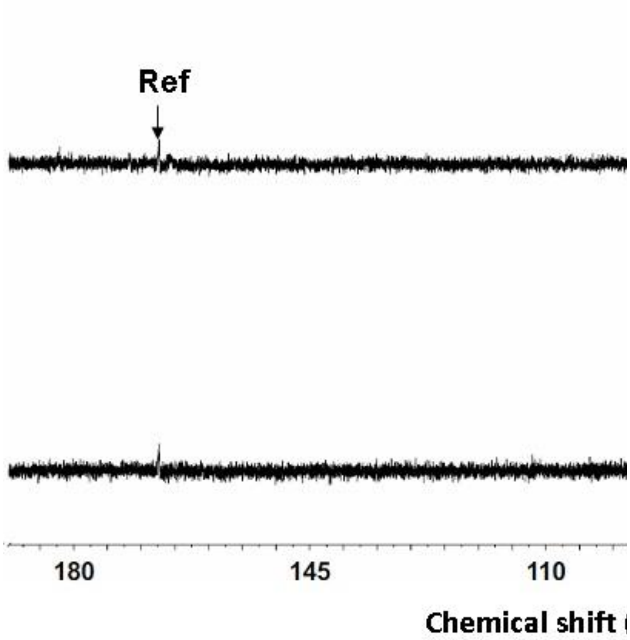


Figure 3 C^{13}-NMR analysis of agriculture eutrophic wastewater after treatment with water hyacinth for a week

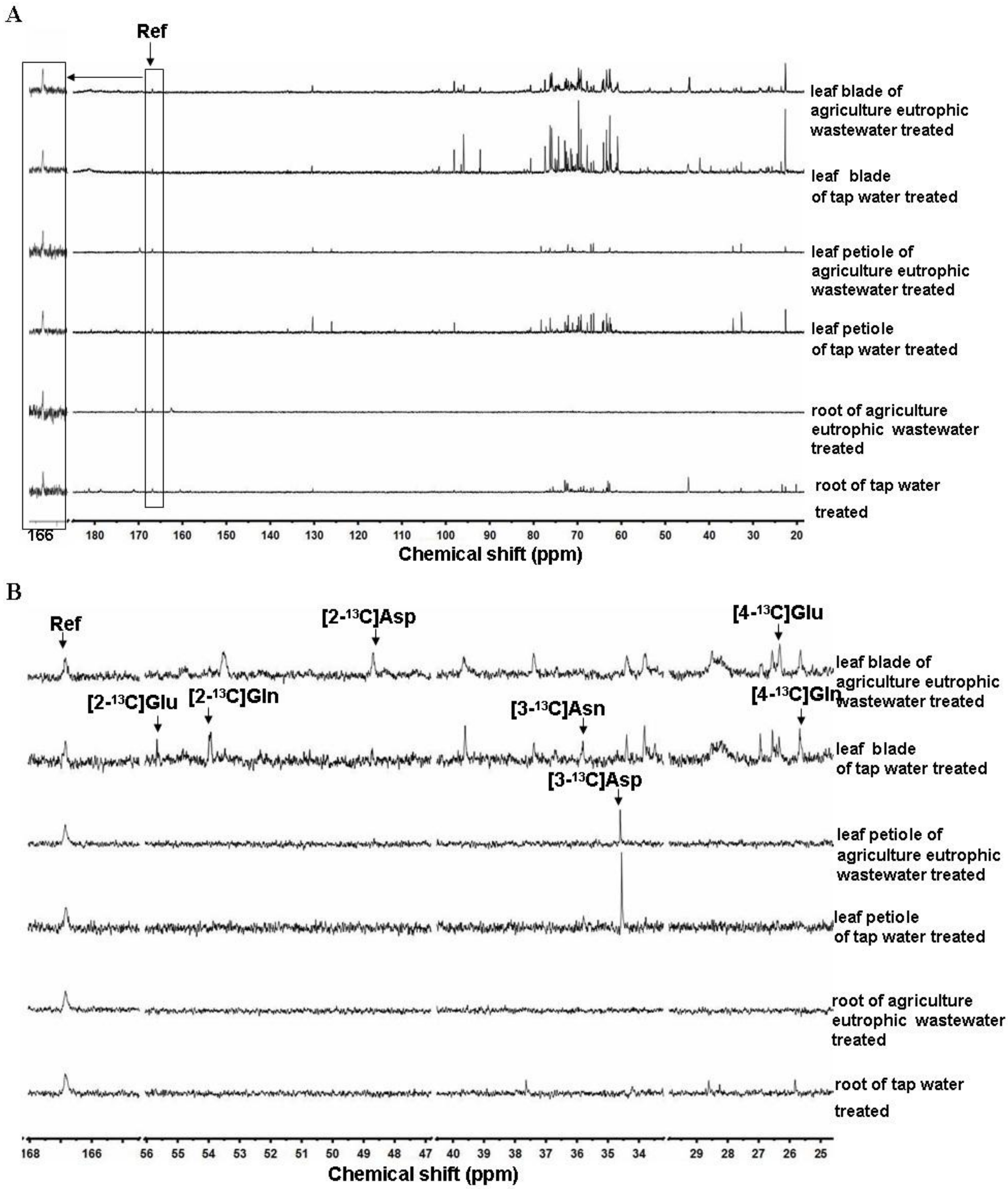
^{13}C-NMR spectrums of root, leaf petiole and leaf blade were analyzed after water hyacinth has been processed in agriculture eutrophic wastewater for one week, the results showed that all of the resonance signal peaks of N-transported amino acids (aspartic acid, Asp; asparagine, Asn; glutamate, Glu; glutamine, Gln) of water hyacinth root which processed by agriculture eutrophic wastewater were very weak (Fig. 4B). This indicated that there was very little N-transported amino acids stored in root of water hyacinth. The unique N-transported amino acids, [3-^{13}C]Asp, can be observed in root of tap water treated water hyacinth. However, its peak disappeared after water hyacinth has been processed by agriculture eutrophic wastewater (Fig. 4B). This indicated that wastewater treatment decreased Asp content of water hyacinth in its root (Fig. 4C).

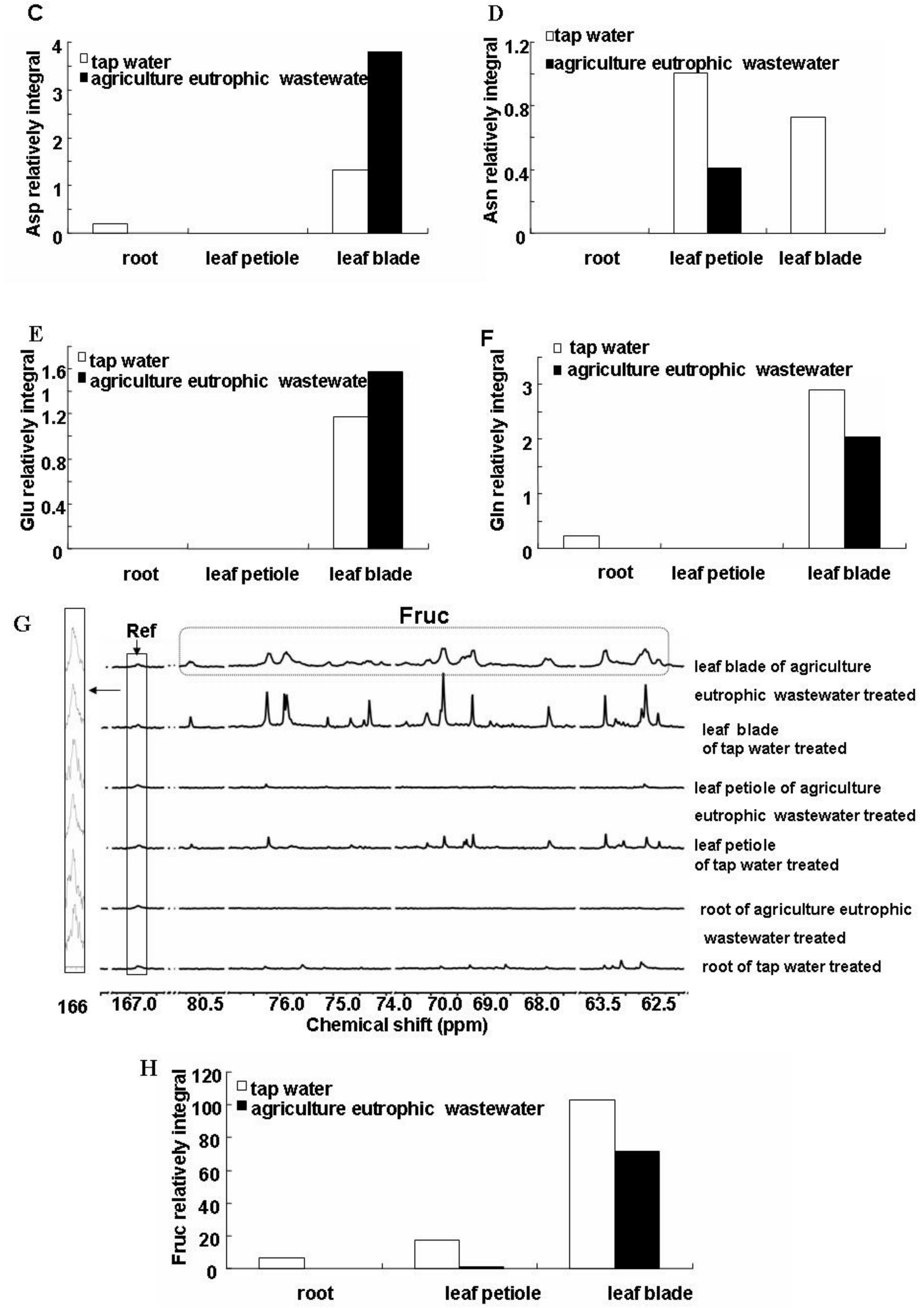
The signal of [3-^{13}C]Asp in petiole of tap water treated water hyacinth was very strong. This indicated that Asp were main N-transported amino acids which stored in petiole of water hyacinth. The signal of [3-^{13}C]Asp in petiole of agriculture eutrophic wastewater treated water hyacinth was very weak, and the relative amount of Asp in petiole of agriculture eutrophic wastewater treated water hyacinth was just 40% of what in petiole of tap water treated water hyacinth (Fig. 4C). This indicated that wastewater purification of water hyacinth can obviously reduce its own Asp content in petiole.

Above four resonance signal peaks of N-transported amino acids can be observed obviously in leaf of tap water treated water hyacinth (Fig. 4B). These signal peaks included [3-^{13}C]Asp, [2-^{13}C]Asp, [3-^{13}C]Asn, [4-^{13}C]Glu, [2-^{13}C]Glu, [4-^{13}C]Gln and [2-^{13}C]Gln. Among these, peak signals of [2-^{13}C]Aspand [4-^{13}C]Glu, peak signals of [3-^{13}C]Asn, [3-^{13}C]Asp, [2-^{13}C]Glu and [2-^{13}C]Gln, and peak signals of [4-^{13}C]Gln enhanced, disappeared and weakened, respectively, in leaf of wastewater treated water hyacinth.

The amounts of Asp (Fig. 4C) and Glu (Fig. 4E) in leaf of wastewater treated water hyacinth were relatively higher than that in leaf of tap water treated water hyacinth. However, the amounts of Asn (Fig. 4D) and Gln (Fig. 4F) in leaf were opposite. This indicated that the contents of Asp and Glu in leaf were increased in wastewater treatment of water hyacinth while the contents of Asn and Gln were decreased.

Signalsof [U-^{13}C]fructose (Fig. 4G) and [U-^{13}C]glucose (Fig. 4I) in root, petiole and leaf of tap water treated water hyacinth were very strong, especially in leaf. However, they were weakened obviously in petiole and leaf after water hyacinth has been treated by wastewater, even disappeared completely in root (Fig. 4G and 4I). The relative content of fructose and glucose in petiole of wastewater treated water hyacinth was just about 20% (Fig. 4H) and 10% (Fig. 4J) of what in petiole of tap water treated water hyacinth. The relative content of fructose and glucose in leaf of wastewater treated water hyacinth decreased to about 60% (Fig. 4H) and 40% (Fig. 4J) of what in leaf of tap water treated water hyacinth. This indicated that wastewater treatment can obviously reduce the content of soluble sugar in tissues of water hyacinth.


A
Ref
leaf blade of agriculture eutrophic wastewater treated
leaf blade of tap water treated
leaf petiole of agriculture eutrophic wastewater treated
leaf petiole of tap water treated
root of agriculture eutrophic wastewater treated
root of tap water treated
166
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
Chemical shift (ppm)
B
Ref
[2-13C]Asp
[4-13C]Glu
[2-13C]Glu
[2-13C]Gln
[3-13C]Asn
[4-13C]Gln
[3-13C]Asp
leaf blade of agriculture eutrophic wastewater treated
leaf blade of tap water treated
leaf petiole of agriculture eutrophic wastewater treated
leaf petiole of tap water treated
root of agriculture eutrophic wastewater treated
root of tap water treated
168 166 56 55 54 53 52 51 50 49 48 47 40 39 38 37 36 35 34 29 28 27 26 25
Chemical shift (ppm)


C
tap water
agriculture eutrophic wastewate
Asp relatively integral
0
1
2
3
4
root
leaf petiole
leaf blade
D
tap water
agriculture eutrophic wastewater
Asn relatively integral
0
0.4
0.8
1.2
root
leaf petiole
leaf blade
E
tap water
agriculture eutrophic wastewate
Glu relatively integral
0
0.4
0.8
1.2
1
1.6
root
leaf petiole
leaf blade
F
tap water
agriculture eutrophic wastewater
Gln relatively integral
0
1
2
3
root
leaf petiole
leaf blade
G
Fruc
Ref
leaf blade of agriculture eutrophic wastewater treated
leaf blade of tap water treated
leaf petiole of agriculture eutrophic wastewater treated
leaf petiole of tap water treated
root of agriculture eutrophic wastewater treated
root of tap water treated
166
167.0
80.5
76.0
75.0
74.0
70.0
69.0
68.0
63.5
62.5
Chemical shift (ppm)
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tap water
agriculture eutrophic wastewater
Fruc relatively integral
0
20
40
60
80
100
120
root
leaf petiole
leaf blade

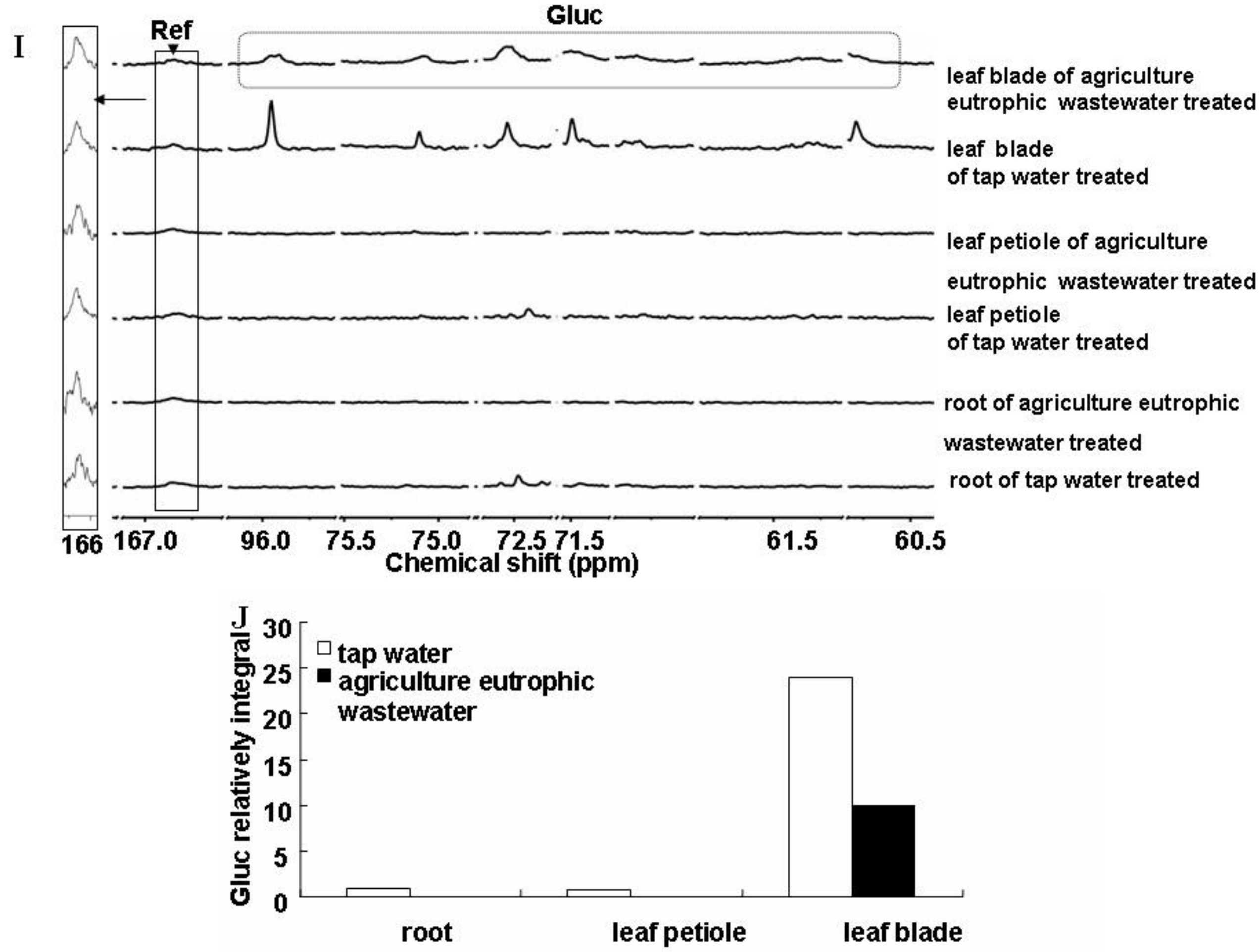

Figure 4 C^{13}-NMR analysis for different tissues of water hyacinth after treatment with agriculture eutrophic wastewater and tap water

DISCUSSION

Many researches considered that water hyacinth has very developed root system absorbing nitrogen form eutrophic water. This hastened the reproduction and growth of water hyacinth, and water hyacinth transferred a part of nitrogen from water (Xie et al., 2004). Simultaneously, nitrification and denitrification of microorganism which attached on root can also remove a part of nitrogen (Huang et al., 2005). This research confirmed obviously effect of water hyacinth absorbing NO_3^--N from agriculture eutrophic wastewater. It was consistent with previous researches. It observed that the increment of NO_3^--N concentration in root of water hyacinth was apparently higher than what in petiole and leaf. This indicated that the absorbed NO_3^--N were mainly stored in root, and partially in petiole and leaf. According to anatomic structure of NO_3—N(Schneider et al.,1995; Chen, 2011), it can be speculated that the large surface area of root and the parietal cell (Chen, 2011)of petiole and leaf were reasons for this. The concentration of NO_3^--N risen slightly when water hyacinth was treated in agriculture eutrophic wastewater for 6 or 7 day. This may be because the NO_3^--N which has been absorbed by root of water hyacinth was released into water again.

Many researchers considered that root of plant which growth in constructed wetland can secrete some organic matters enhancing denitrification of microorganism as carbon sourc(Vymazal, 2013). Consistent with this, ^{13}C-NMR analysis in this research also proved that the root of water hyacinthcan definitelysecrete some micromolecular carbohydrate, sucrose and acetic acid in the process of agriculture eutrophic wastewater treatment. These compounds maybe can supply carbon source to inorganic nitrogen which was assimilatedby microorganism. N-transported amino acid, mainly include Gln, Glu, Asp and Asn, was the primary materials stored by ammonium nitrogen in plant body. Ammonium nitrogen can be reduced to nitrite nitrogen by nitrate reductase after it has been absorbed by plant cell. Finally, ammonium nitrogen was assimilated to N-transported amino acid (Tian et al., 2009; Han et al., 2004).

^{13}C-NMR analysis result showed that contents of aspartate and glutamic acid in root and petiole of wastewater treated water hyacinth were markedly higher than whatin root and petiole of tap water treated water hyacinth. However, opposite phenomenon was observed in contents of asparagine and glutamine. This indicated that ammonium produced by assimilation of root and petiole tissue cell of water hyacinth was used to synthesize amino acid, this increased the content of aspartate and glutamic acidand promoted the growth of water hyacinth. Simultaneously, the contents of N-transported asparagine and glutamindue was decreased due to growth dilution effect.

Anaerobic respiration of plant under submerged condition can consume a lot of carbohydratein vivo. Beside this, many environmental stresses will also make plant consume a lot of carbohydrate. The carbohydrate may be used to produce or accumulate glycosylation of toxic substances decreasing toxicity of toxic substances(Li et al.,2001). Compared with tap water treated plant, wastewater treatment can significantly reduce the contents of glucose and fructose in root, petiole and leaf of water hyacinth. This indicated that the decrement of carbohydrate was not because anaerobic respiration, and wastewater treatment may produce a certain stress to water hyacinth. This stress can consume large amountsoriginal carbohydrate in root and leaf of water hyacinth. Moreover, it was also because the intermediate propagation of water hyacinth will consume large amounts carbohydrate to form its carbon skeleton and new tissue.

Conclusion: Water hyacinth has better ability to remove NO_3^--N in agriculture eutrophic wastewater. It assimilated NO_3^--N which was absorbed by agriculture eutrophic wastewater into amino nitrogen. This enhanced the utilization of soluble sugar and amide nitrogen in vivo, promoted the growth of plant and stored excess NO_3^--N in root, petioleand leaf. Ultimately, NO_3^--N can be removed from agriculture eutrophic wastewater by harvesting Water hyacinth.

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