

Original Research

Synthetic Black Water Treatment by Aeroponic Cultivation of Water Spinach: Effect of the Pump Run Time

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Received: 14 December 2019

Accepted: 31 July 2020

Abstract

In this study, we report the treatment of simulated black water using a decentralised aeroponic system for the cultivation of water spinach. The effect of the pump run time on the treatment efficiency of black water at low temperature ($15^{\circ}\text{C}\pm 5^{\circ}\text{C}$) in winter was systematically investigated. The results show that aeroponic cultivation process yields average chemical oxygen demand (COD) removal of 90.04%, ammonia nitrogen ($\text{NH}_4^+\text{-N}$) reduction of 35.02%, total nitrogen (TN) abatement of 28.14%, and total phosphorus (TP) elimination of 14.71%. The increase in the pump run time from stop time (min) to spray time (s) ration of 45:30 (QW3) to 15:30 (QW1) significantly promotes the efficiency of the COD, $\text{NH}_4^+\text{-N}$, TN, and TP removals. The sequence of the removal efficiency is as follows: QW1 (15:30)>QW2 (30:30)>QW3 (45:30). The higher efficiency of the COD and $\text{NH}_4^+\text{-N}$ removals in QW1 is due to the enhanced action of aerobic microorganisms because of the increased amount of dissolved oxygen. We believe that this study provides a simple and potential option for decentralised sewage.

Keywords: aeroponic cultivation system, black water, low temperature, pump run time, water spinach

Introduction

Water shortages significantly affect the livelihoods of human beings. China is one of 13 countries with severe water shortages [1]. Recently, the urbanisation in China has accelerated, resulting in the direct

discharge of considerable amounts of industrial and domestic sewage into surface- and ground-water. This causes an enormous economic loss and waste of resources, threatens the human health, and degrades the environment [2]. Thus, the problems associated with the water conservation and utilisation of wastewater must be solved [3].

Currently, domestic wastewater is treated using end collection and subsequent centralised treatment

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in most cities in China, which is expensive due to the high costs associated with the construction and maintenance of the collection network. This treatment method leads to excessive amounts of sludge and the recovery of nitrogen, phosphorus, and recycled water is difficult. The application of the centralised process in rural areas is limited because of the complex terrain and low financial status. An alternative strategy for the treatment of rural wastewater is decentralised wastewater treatment, which has many benefits, for example, there is no need for a large-scale pipe network, the construction costs are low, the implementation and operation are convenient, and the recovery and utilisation of water are simple [4].

Approximately 90% of the total nitrogen (TN), 50% of the chemical oxygen demand (COD), and 80% of the total phosphorus (TP) in the domestic wastewater originate from toilet-flushing sewage (black water), accounting for approximately one-third of the total water [5]. Therefore, the separation of the water containing feces and urine (black water) from other drainage water (grey water) and the application of decentralised ecological black water treatment technologies, such as constructed wetlands, drip irrigation, and aquatic plant remediation technologies, would greatly reduce the cost of the sewage treatment and improve the treatment efficiency. Moreover, those processes favour the recirculation and reuse of black water for agricultural production and reduce the physical footprint of sewage treatment facilities, which has many advantages including low cost and energy consumption, high efficiency, smaller disturbance of the environment, no secondary pollution, and circulation of nutrients such as nitrogen and phosphorus [6-10].

Aeroponic cultivation is a new type of soilless culture technology. The nutrient solution is atomized to meet the water and fertilizer demand for plant growth. It does not require an implant matrix; therefore, the cultivation facility is suitable for roofs or balconies of buildings due to its light weight. In addition, aeroponic cultivation has strong reaeration ability because of atomization of the solution. At present, this technology is mostly used for agricultural production to enhance crop yields and reduce the water and fertiliser volumes [11]. However, little research has been carried out on the purification and reuse of black water.

The pump run time is a key parameter in aeroponic cultivation. The effect of the pump run time on the treatment of black water was investigated in this study. The efficiencies of the removals of COD, $\text{NH}_4^+\text{-N}$, TN, and TP and transformation of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were analysed to interpret the process. The variance analyses of the water quality indexes were compared using SPSS 14.0. Finally, the biomass obtained from the aeroponic cultivation system was analysed.

Table 1. Water quality of the simulated black water (SBW) and tested black water (TBW).

Component	SBW (mg/L)	TBW (mg/L)
COD	200	183–237
$\text{NH}_4^+\text{-N}$	29.0	29.57–34.01
TN	30.0	30.89–36.54
TP	18.2	13.57–19.59
SO_4^{2-}	32	—
Ca^{2+}	10.8	—

Material and Methods

Test Plant

A test plant, water spinach (*Ipomoea aquatica*), was purchased from the market. All old leaves were removed and a plant length of ~10 cm was retained prior to the experiment to ensure the plant survival and growth.

Test Black Water

The COD and total nitrogen concentration of black water are very high, with values reaching 1000 and 300 mg/L, respectively [11]. Real black water with high COD and ammonia nitrogen content is disadvantageous for the survival of the test plants in the aerosol system [5, 12-14]. Therefore, synthetic black water comparable to five-fold diluted black water was used for the experiments. The water quality is shown in Table 1 and the composition is illustrated in Tables S1 and S2.

Aeroponic Cultivation Devices and Experimental Procedures

The aeroponic experiments were carried out in polypropylene (PP) plastic devices ($0.41 \times 0.2 \times 0.33$ m), with 15 plants in an array (5 columns and 3 rows; Fig. 1). The planting holes were evenly arranged in the lid of the box containing four atomising nozzles. Four water spinach plants were planted in each basket (the planting density of the water spinach is 732 trees/m²) and then fixed and packed with cylindrical activated carbon. The roots of the plants were not submerged in black water but suspended above it such that the cells of the roots were not affected by sewage, even if the BOD or COD were high. The sewage volume of each device was maintained at 11 L, including 10 L fresh prepared black water and 1 L sludge-rich stock solution. The test sewage was atomised and sprayed regularly with a booster pump (Deng Yuan TYP2500) controlled by a timing controller. Based on the ratio of the stop time (min) to the spray time (s), the run time was set as follows: spraying for 30 s every 15 min (15:30), 30 min (30:30), and 45 min (45:30).

Three parallel devices were included in this test, that is, QW1, QW2, and QW3, and continuously worked for 24 hours per day in interval of 15, 30 and 45 min, respectively. The average evaporation capacities of black water in QW1, QW2, and QW3 were 150, 180, and 350 ml/24 h, respectively. The proportion of the evaporated water was less than 3.5%; therefore, the effect of evaporation induced by spraying on the test results was negligible. The devices started working at the same time and with the same influent quality. Every device contained a booster pump and timing controller; thus, there was no influence or interference between the devices.

After the aeroponic cultivation devices were prepared, the water spinach was allowed to adjust to the new environment for at least a week. The adjustment stage lasted from 25-31 October and the measurements began on 1 November. Measurements of the COD, $\text{NH}_4^+\text{-N}$, TN, TP, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, dissolved oxygen (DO), water temperature, and pH were performed twice a week. Every parameter was measured 15 times. On the day before testing, the black water stored for 24 hours in the devices was poured out, leaving behind ~ 1 L of stock solution containing a large amount of activated sludge. Subsequently, 10 L new synthetic black water was added to the devices. At the same time, raw synthetic black water was used to determine the initial concentrations of pollutants and other indicators. After 24 hours, the treated black water was retrieved to analyse the same indicators. Subsequently,

200 ml black water from the septic tank was added to the devices every day during the test interval to ensure the biological activity of the microorganisms inside the devices. The addition of real black water led to a small variation in the water quality of the actual tested black water, as shown in Table 1.

Methods

The detection methods used for various parameters are as follows. The COD was measured using the HACH reagent digestion method and a HACH DR3900 bench-top spectrophotometer. The $\text{NH}_4^+\text{-N}$ was detected using Nessler's reagent spectrophotometry. The TN was analysed using alkaline potassium persulfate digestion spectrophotometry. The TP was determined with potassium persulfate digestion spectrophotometry. The $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were separately measured using ultraviolet (UV) spectrophotometry and N-(1-naphthyl)-ethylenediamine spectrophotometry. The DO, water temperature, and pH were measured with a HACH DO/pH meter.

At the end of the experiment, the water spinach above the surface of the filler was collected, dried in an oven at 105°C , until a constant weight was obtained, and weighed with an electronic balance. The variance analysis of the experimental results and comparison between different samples were performed using SPSS 14.0.

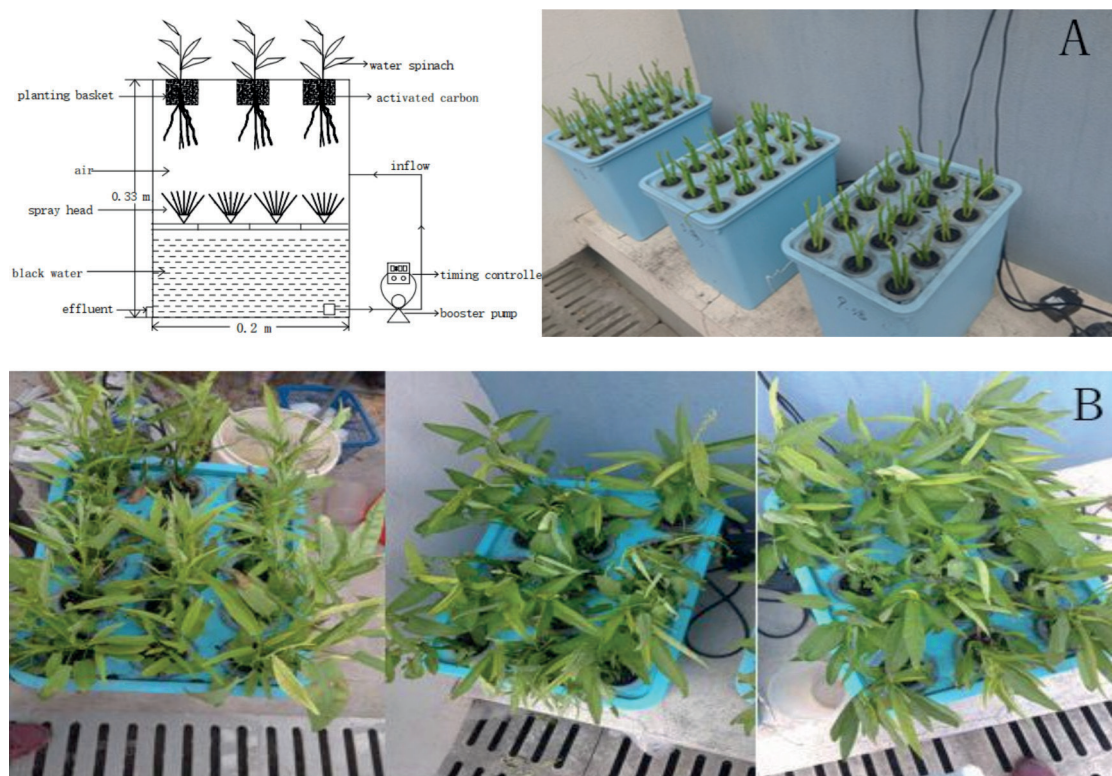


Fig. 1. Schematic diagram of the aeroponic cultivation devices and photography of the actual devices (A: initial-stage test; B: mid-stage test).

Results and Discussion

Effect of the Run Time on the COD Removal Efficiency

Throughout the entire process, the COD removal rate of QW1 was significantly higher than that of QW2 and QW3, with a maximum of 98.54% (Fig. 2a). The removal rate of QW2 was higher than that of QW3, with

a maximum of 88.07%. The removal rate of QW3 was lower than that of QW1 and QW2, with a peak value of 81.97%. During the 50-day trial period, the influent COD concentration ranged from 183.00 to 237.00 mg/L. After treatment for 24 hours, the concentrations in QW1, QW2, and QW3 varied from 3.00 to 30.00, 24.00 to 66.00, and 33.00 to 95.00 mg/L, respectively, with an average removal rate of 90.40%, 81.27%, and 71.66%, respectively. The removal in the three devices can be

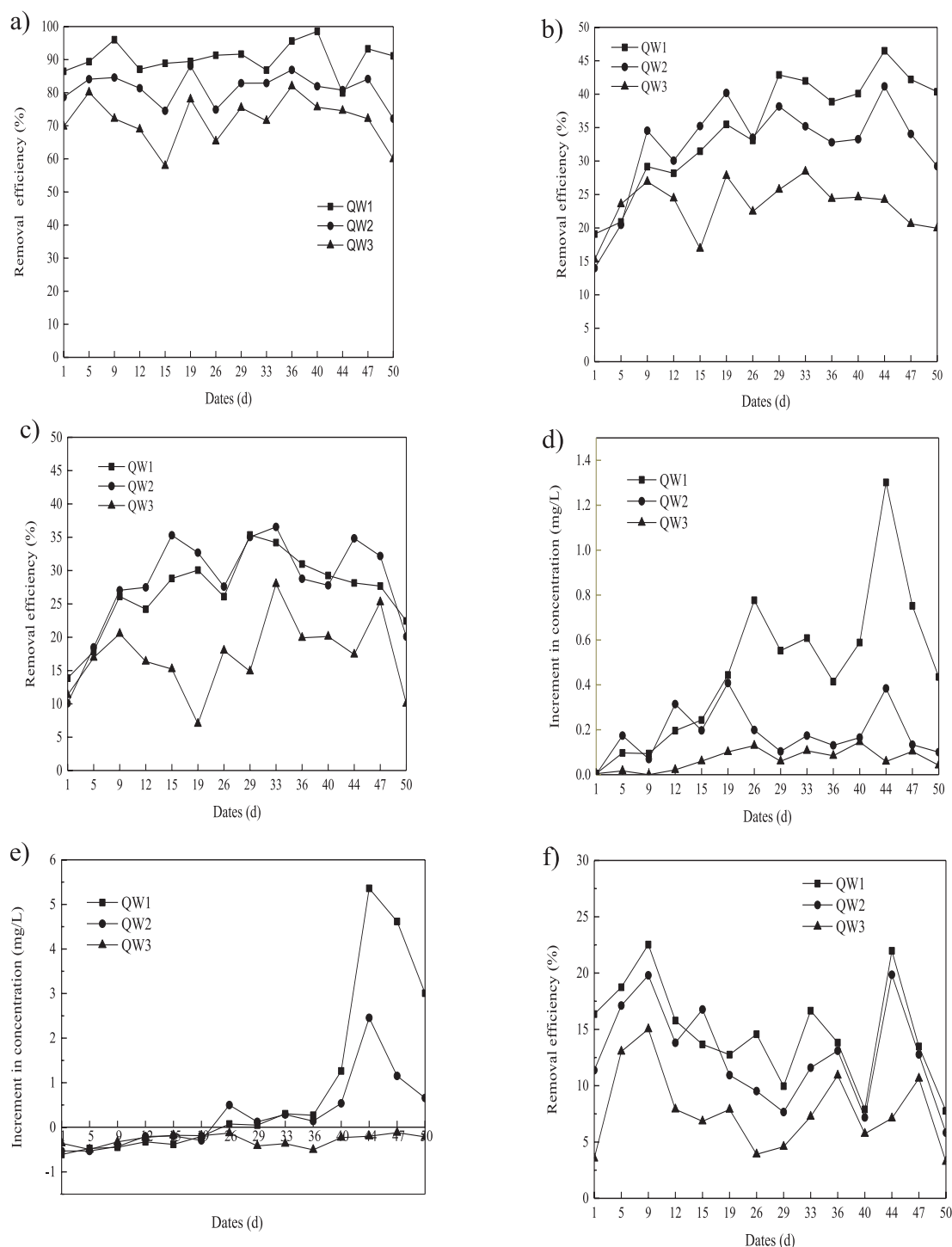


Fig. 2. Efficiency of the removal of COD a), NH_4^+ -N b), TN c), and TP f) and concentration variation of NO_2^- -N d) and NO_3^- -N e) in the aeroponic devices.

divided into three stages, that is, small-scale fluctuation in the first period, slight increase in the middle period, and gradual decline in the later period. The removal rates of the three devices reached their maxima in the middle and later stages.

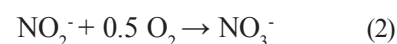
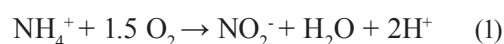
During the purification of wastewater by plants, the COD removal can be mainly attributed to the adsorption of the filler in the planting basket, degradation of the microbial membrane that formed on the surface of the filter media, self-sedimentation of organic pollutants, and absorption and degradation of plants during the growth progress [15]. The filler selected for this test was columnar activated carbon, which has a large specific surface area and high porosity. It does not only absorb organic substances from black water but also provides an excellent living environment for microorganisms to degrade pollutants. The activated carbon in the aeroponic system without plants approached adsorption saturation after a run time of approximately five days, with a COD removal of 51.00% (Table S3). In the early stage of the test, more than 50.00% of the COD was removed by the adsorption and interception of activated carbon because of the slow growth of the water spinach and incomplete development of the microbial community. Because the run interval was shorter, the contact between black water and air and activated carbon was increased, which was conducive to the formation of aerobic microbial communities and absorption of pollutants by activated carbon. Therefore, the COD removal rate of the three devices in the initial stage of the test was ranked as follows: QW1>QW2>QW3. With the increase in the growth rate, the water spinach roots increasingly developed, as a vast living space for microorganisms and adsorbing more pollutants. Thus, the COD removal rate reached the maximum in the intermediate stage of the test, with an amount of growing activated sludge accumulated and the microbial community attached on plant roots formed gradually. In this case, the removal mainly depended on the assimilation of plants and degradation of microorganisms. At the end of the test, the growth of the water spinach almost stopped and the removal mainly depended on the complete microbial community. Therefore, the removal rates of the three devices showed small decreases in the later period of the test.

The synthetic black water was in contact with air in the form of droplets because of intermittent atomisation of the devices, which greatly promoted the mass transfer between gas and liquid. The DO content of the synthetic black water in QW1, QW2, and QW3 differs because of the different run times. The DO concentration in QW1, QW2, and QW3 varies from 4.52 to 7.35, 3.19 to 6.54, and 0.82 to 4.30 mg/L, respectively. The COD removal in the aeroponic system positively correlates with the DO variation. Thus, it can be concluded that the respiration of aerobic microorganisms plays a critical role in the removal of organic pollutants in the aeroponic system.

Effect of the Run Time on the $\text{NH}_4^+\text{-N}$ Removal Efficiency

In the first five days of the experiment, the removal of $\text{NH}_4^+\text{-N}$ in the three devices was similar (Fig. 2b). However, major differences were observed after the ninth day. From the 9th to the 26th day of the test, the removal rate of $\text{NH}_4^+\text{-N}$ in QW2 was higher than that in QW1 and QW3. However, after the 29th day, the removal rate in QW1 started to significantly increase and eventually exceeded that of QW2. Starting on the 9th day of the trial, the treatment performance of QW3 was worse than that of QW1 and QW2. During the whole test, the influent $\text{NH}_4^+\text{-N}$ concentration ranged from 29.57 to 34.01 mg/L. After treatment for 24 hours, the $\text{NH}_4^+\text{-N}$ concentration in QW1, QW2, and QW3 varied from 16.25 to 26.51, 17.87 to 28.18, and 22.35 to 27.76 mg/L, respectively. In QW1, the highest $\text{NH}_4^+\text{-N}$ removal rate was observed on the 44th day, with a peak of 46.49%. The removal rate of QW2 was highly variable and reached maxima on the 19th and 44th day, respectively, with peak values of 40.19% and 41.17%, respectively. The removal rate of QW3 fluctuated in the early stage and then peaked on the 19th and 33rd day respectively, with a maximum of 28.43%.

The removal of $\text{NH}_4^+\text{-N}$ in an aeroponic cultivation system possibly depends to the adsorption onto activated carbon and physical, chemical, and biological effects between the root system of water spinach and microorganisms. The adsorption of $\text{NH}_4^+\text{-N}$ onto activated carbon alone increased with increasing run time. A total of 45.00% of $\text{NH}_4^+\text{-N}$ was finally removed within 3 days (Table S3), which is basically consistent with the $\text{NH}_4^+\text{-N}$ removal in the initial stage of the experiment. Therefore the $\text{NH}_4^+\text{-N}$ of black water was mainly removed by the adsorption onto activated carbon in the initial stage. At this point, the formation of the microbial community was incomplete and thus had no effect on the removal. With increasing growth rate of the water spinach, the uptake of $\text{NH}_4^+\text{-N}$ began to increase. The higher removal efficiency in QW2 compared with QW1 in the first 26 days is due to the better growth status of the water spinach in QW2. At the same time, the nitrifying bacteria that grew on the surface of the activated carbon filler and among the roots of the water spinach and the activated sludge that formed at the bottom of the devices converted $\text{NH}_4^+\text{-N}$ into nitrate or nitrite nitrogen [16]. The nitrification process can be described with the following equation [17]:



Therefore, using activated carbon as the filler enhances the microbial activity within the aeroponic system and promotes the absorption of rhizosphere microorganisms, root retention, and nitrification and

denitrification around the rhizosphere, resulting in improved efficiency of the $\text{NH}_4^+\text{-N}$ removal.

In the middle and later stages of the experiment, microorganisms began to dominate. The interval of QW1 was shorter and thus the black water was more frequently exposed to air, which enhanced the DO concentration in the black water. As a result, the growth and reproduction of nitrifying bacteria were promoted and the $\text{NH}_4^+\text{-N}$ removal was accelerated.

The device QW3 was in an anoxic environment for a longer time. This was not conducive to the activity of aerobic nitrobacteria and resulted in a low $\text{NH}_4^+\text{-N}$ removal rate. Because the trial was carried out in winter, the low outdoor temperature ($\sim 15^\circ\text{C} \pm 5^\circ\text{C}$) and the large temperature difference between day and night severely inhibited the activity of the nitrifying bacteria [18, 19]. Therefore, the $\text{NH}_4^+\text{-N}$ removal efficiency of the three devices was overtly confined, ranging from 15.26% to 46.49%.

Effect of the Run Time on the TN Removal Efficiency

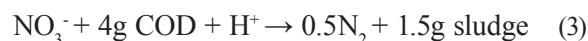
During the whole experiment, the TN concentration of the influent varied from 30.89 to 36.54 mg/L. After a run time of 24 hours, the TN concentration in QW1, QW2, and QW3 ranged from 21.33 to 27.92, 20.18 to 29.14, and 24.06 to 32.19 mg/L, respectively. The average TN removal rate in QW1, QW2, and QW3 was 26.79%, 28.14%, and 17.21%, respectively (Fig. 2c).

During the whole test, the TN removal of QW1 was similar to that of QW2, but that of QW3 was worse than that of QW1 and QW2. The TN removal efficiency of the three devices significantly varied in the early stage of the trial and then gradually decreased in the end. The maximum removal rates in QW1 were obtained on the 9th, 19th and 29th day, respectively. In QW2, it reached peaks on the 15th, 33rd and 44th day, respectively. In QW3, the highest rates were achieved on the 9th, 33rd and 47th day, respectively. The maximum TN removal occurred at different times in the three devices, which all exhibited fluctuations. This may be due to the sudden increase or decrease in temperature. Overall, the microbial activity is susceptible to changes in the temperature.

In the phytoremediation process, the nitrogen removal from sewage is due to the adsorption onto activated carbon filler, assimilation of plants, and nitrification or denitrification due to microbial organisms in the water and roots [20]. The plants had a smaller nitrogen demand because of the initially slow growth of the water spinach. The main removal of nitrogen in three devices mainly depended on the physical adsorption onto activated carbon such that the removal efficiency insignificantly changed. In the middle period of the test, it was necessary to absorb more inorganic nitrogen as nutrient for the synthesis of organic nitrogen, such as vegetable protein, because the water spinach thrived. At the same

time, the aerobic-anoxic-anaerobic microenvironment formed near the roots of the water spinach, which was conducive to the coexistence of nitrifying and denitrifying bacteria. Thus, the efficiency of the nitrogen removal from sewage significantly improved due to the enhanced nitrification and denitrification [21]. In addition, the microorganisms in the activated sludge also removed parts of the nitrogen from the water by nitrification and denitrification.

Denitrification is a result the reduction of $\text{NO}_3^-\text{-N}$ to N_2 or N_2O by denitrifying bacteria under anaerobic conditions [17]. The reaction equation is as follows [22]:



The spray interval time in QW3 was the longest such that the anaerobic reaction time was sufficient. However, the TN removal rate was the lowest because of the shorter aerobic reaction time, which was not conducive to the formation of $\text{NO}_3^-\text{-N}$. The devices QW1 and QW2 had an adequate aerobic reaction time but lacked an anaerobic reaction time. This may also explain the similar TN removal efficiency of QW1 and QW2. In the later period of the test, the growth of the water spinach most stopped, which resulted in a decreased nitrogen demand.

Effect of the Run Time on the $\text{NO}_2^-\text{-N}$ Removal Efficiency

Throughout the whole experiment, the concentration of $\text{NO}_2^-\text{-N}$ in the three devices was higher than the influent concentration (Fig. 2d). In other words, the reduction of $\text{NO}_2^-\text{-N}$ was lower than the production during the experiment. The increase in the concentrations of QW1 and QW2 in the early stage was similar and the concentrations were higher than that of QW3. Later, the concentration in QW1 was significantly higher than that of QW2 and QW3. During the entire experiment, the influent $\text{NO}_2^-\text{-N}$ concentration was negligible. After treatment for 24 hours, the $\text{NO}_2^-\text{-N}$ concentration in QW1, QW2, and QW3 varied from 0.005 to 1.302, 0.002 to 0.416, and 0.005 to 0.147 mg/L, respectively. The peak values of the $\text{NO}_2^-\text{-N}$ concentration in QW1 were observed on the 26th and 44th day, respectively, which roughly corresponded to the time with the lowest TN and highest $\text{NH}_4^+\text{-N}$ removal rates. The $\text{NO}_2^-\text{-N}$ concentration in QW2 first increased and then remained constant. In QW3, it imperceptibly increased in the early period and then remained low.

The $\text{NO}_2^-\text{-N}$ produced in the three devices is mostly due to the nitrification of $\text{NH}_4^+\text{-N}$. Under aerobic conditions, the nitrification process can be mainly divided into two stages. In the first stage, ammonia oxidation bacteria (AOB) oxidise ammonia nitrogen to nitrite (NO_2^-) catalysed by ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). In the second stage, nitrite oxidising bacteria (NOB) oxidise NO_2^- to NO_3^- [23]. The concentrations of

the two most significant microorganisms in the nitrification process, AOB and NOB, are limited at low temperatures ($\leq 15^{\circ}\text{C}$) [24, 25], leading to the accumulation of NO_2^- -N in the later stage of QW1, which is consistent with the accumulation of NO_2^- -N at 12°C - 14°C reported by Yuan and Peng [26]. Similarly, the NO_2^- -N concentration in QW2 was higher than that in QW3. The reason for the great fluctuation may be a distinct temperature change, which has a significant impact on the activity of nitrifying bacteria. The elevated temperature promoted the nitrification reaction, especially in the later period.

Effect of the Run Time on the NO_3^- -N Removal Efficiency

Throughout the test, the influent NO_3^- -N concentration ranged from 0.48 to 0.86 mg/L (Fig. 2e). After treatment for 24 hours, the NO_3^- -N concentration in QW1, QW2, and QW3 varied from 0.07 to 6.09, 0.09 to 3.18, and 0.12 to 0.62 mg/L, respectively. As shown in Fig. 2e, the NO_3^- -N content in QW1 and QW2 began to increase on the 26th day and reached the maximum on the 44th day. The maximum concentration was 5.36 and 2.46 mg/L, respectively. However, the NO_3^- -N content of QW3 was always lower than that of raw water and the average removal rate was 44.03%. The NO_2^- -N and NO_3^- -N concentrations in the three devices first increased and then decreased during the experiment. The devices QW1 and QW2 both reached peaks on the 44th day, probably because of the simultaneous occurrence of the two nitrification stages.

Nitrogen absorbed by plants mainly involves nitrate nitrogen and ammonia nitrogen of water as well as urea and amino acids, which can be converted into parts of their tissues through assimilation [27, 28]. In the initial stage of the test, the water spinach thrived; thus, the NO_3^- -N demanded was greater. At the same time, the removal NO_3^- -N from black water was better due to the adsorption onto activated carbon. Based on Fig. 2e), it can be concluded that the vigorous growth period of water spinach lasted 26 days, which is similar to the research results of Li et al. [29]. After the 26th day, the water spinach entered a declining phase and the absorption of nitrogen decreased.

Moreover, NH_4^+ -N entered plant cells in an active process, while NO_3^- -N passively entered the cells; therefore, the absorption of NO_3^- -N stopped in the end of this study [30]. At this time, the NO_3^- -N removal in the three devices mainly depended on the denitrification by denitrifying bacteria. However, the run time interval of QW1 and QW2 was smaller than that of QW3, leading to a shorter anaerobic reaction, which was not conducive to denitrification. In summary, NO_3^- -N began to accumulate, and the accumulated concentration in QW1 was higher than that in QW2. A better NO_3^- -N removal was achieved in QW3 because the anaerobic reaction time was long enough. In the later stage of the test, the NO_3^- -N concentration

in QW1 and QW2 decreased, possibly because the increased temperature promoted the nitrification and denitrification.

Effect of the Run Time on the TP Removal Efficiency

During the experiment, the concentration of influent TP varied from 13.57 to 19.59 mg/L. After treatment for 24 hours, the TP concentration in QW1, QW2, and QW3 varied from 11.36 to 16.74, 11.67 to 17.73, and 12.54 to 18.82 mg/L, respectively. The TP removal efficiency of QW1 and QW2 lightly differed during the entire test (Fig. 2f). The removal efficiency of QW3 was lower and the average removal rate of TP for QW1, QW2, and QW3 was 14.71%, 12.66%, and 7.69%, respectively.

The phosphorus could be removed from the black water by the absorption by the water spinach and adsorption onto activated carbon. Because of the formation of activated sludge at the bottoms of the devices and alternating anaerobic and aerobic operating conditions, parts of the phosphorus could be removed from the black water by phosphorus release and excessive phosphorus uptake processes. Because QW3 had the longest run time interval, the device was exposed to an anaerobic environment for a longer time such that the phosphorus absorbed by activated sludge under aerobic conditions was released. This may explain that the TP removal of QW3 is the worst. The run time intervals of QW1 and QW2 were shorter and such the exposure time of the activated sludge to anaerobic conditions was shorter. Phosphorus could be removed with the discharge of the activated sludge. However, most of the activated sludge must be retained in the devices to ensure the efficient removal of other pollutants. Therefore, the TP removal rate in the three devices began to decrease after the ninth day. The phosphorus removal in the end of the test possibly increased because the increase in the temperature promoted the phosphorus uptake by phosphate-accumulating organisms [31-34].

Difference Analysis of Different Models

Based on the test results obtained for the black water treatment, the removal rates of the water quality indicators (e.g. NH_4^+ -N, TN, TP, COD, NO_3^- -N, and NO_2^- -N) were used to analyse the different experimental treatments (Table 2). The efficiency of the NH_4^+ -N removal in QW3 was markedly different from that of QW1 or QW2, while the efficiencies of the TN, TP, and COD removals of QW1, QW2, and QW3 significantly differed. The increase in the NO_3^- -N and NO_2^- -N concentrations in QW1, QW2, and QW3 noticeably differed. With respect to nitrification and denitrification, the DO concentration in the black water significantly affects the transformation of N including NH_4^+ -N, NO_2^- -N, and NO_3^- -N. The notable difference of the NH_4^+ -N, NO_2^- -N, and NO_3^- -N

Table 2. F test analysis

Detection indicators	Samples	F	P	Significant difference
NH ₄ ⁺ -N	QW1QW2	1.319	0.312	
Removal	QW1QW3	4.687	0.005	*
Rate	QW2QW3	3.553	0.015	*
TN	QW1QW2	0.600	0.184	
Removal	QW1QW3	1.083	0.444	
Rate	QW2QW3	1.806	0.150	
TP	QW1QW2	1.015	0.489	
Removal	QW1QW3	1.567	0.215	
Rate	QW2QW3	1.543	0.222	
COD	QW1QW2	1.011	0.493	
Removal	QW1QW3	0.456	0.085	
Rate	QW2QW3	0.451	0.082	
NO ₃ ⁻ -N	QW1QW2	6.045	0.000	*
Increment	QW1QW3	236.092	0.000	*
In concentration	QW2QW3	39.054	0.000	*
NO ₂ ⁻ -N	QW1QW2	8.832	0.000	*
Increment	QW1QW3	54.346	0.000	*
In concentration	QW2QW3	6.153	0.001	*

(P≤0.05 is considered to be the significant difference)

concentrations in the three devices can be ascribed to their different reoxygenation capacities. The TN and TP removal was not notable among the three devices. However, the NH₄⁺-N removal in QW1 and QW3, QW2 and QW3 notably differed.

Analysis of the Water Spinach Growth under Low Temperature

After an operation for more than two months, the dry weight of the water spinach in three devices, that is, QW1, QW2, and QW3, was 13.50, 21.50 and 20.00 g, respectively. The device QW1 exhibited the lowest biomass because it had the shortest spraying interval and the roots were exposed to low temperature for a longer time, resulting in the withering of the water spinach in the later stage. The devices QW2 and QW3 had a longer interval and thus the adverse effect of the low temperature on the water spinach growth was smaller. Therefore, the growth status in both QW2 and QW3 was better than that in QW1.

Conclusions

(1) The efficiencies of the COD, NH₄⁺-N, and TP removals in the three devices were ranked as follows:

QW1>QW2>QW3. The average removal rates were 90.40%, 35.02%, and 14.71% in QW1; 81.27%, 32.27%, and 12.66% in QW2; and 71.66%, 23.23%, and 7.69% in QW3; respectively.

The TN removal efficiencies of the three devices have the following order: QW1 ≈ QW2>QW3. The average TN removal rates were 26.79%, 28.14%, and 17.21%, respectively.

(2) The NO₂⁻-N content in QW1, QW2, and QW3 increased, with QW1>QW2>QW3, and the average content was 0.465, 0.183, and 0.067 mg/L, respectively. The nitrate removal rate of QW1 and QW2 gradually decreased with the increase of running time, and finally showed an increasing state, and the decrease and increase rate of QW1 was higher than QW2. However, the nitrate of QW3 was reduced throughout the whole experiment.

(3) The NH₄⁺-N removals in QW1QW3 and QW2QW3 significantly differed. The removals of TN, TP, and COD in QW1, QW2, and QW3 were similar. The increase in the concentrations of NO₃⁻-N and NO₂⁻-N concentrations in QW1QW2, QW1QW3, and QW2QW3 also significantly differed.

(4) The dry weights of the water spinach in QW1, QW2, and QW3 after a run of more than two months were 13.50, 21.50, and 20.00 g, respectively. The inhibition of the growth of the water spinach was

stronger in QW1 than that in QW2 and QW3.

Acknowledgements

This work was supported by the National Science and Technology Major Project (No. 2013ZX0731200101), Major Project of Science and Technology in Zhejiang Province (No. 2015C03016), National Natural Science Foundation of China (Grant No. 51808406) and Wenzhou Science and Technology Innovation Project in the Control of the Contaminated Water (No. W2017004).

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Material

Synthetic Black Water Treatment by Aeroponic Cultivation of Water Spinach: Effect of the Pump Run Time



Fig. S1. The actual devices of aeroponic cultivation (the vegetation with leaves).

Table S1. Ingredients of simulated black water (30 L).

Reagents	Dosing amount	Index	Concentration (mg/L)
$C_6H_{12}O_6$	6 g	COD	200.00
NH_4Cl	2.75 g	NH_4^+-N	29.00
KH_2PO_4	2.4 g	TP	18.20
$MgSO_4$	1.2 g	SO_4^{2-}	32.00
$CaCl_2$	0.9 g	Ca^{2+}	10.80
Trace element solution	30 ml	-	-

Table S2. Ingredients of simulated black water.

Element	Reagent	Dosing amount (g/L)
Fe	$FeSO_4 \cdot 7H_2O$	1.54
B	H_3BO_3	0.15
Co	$Co(NO_3)_2 \cdot 6H_2O$	0.183
Zn	$ZnCl_2$	0.102
I	KI	0.03
Mn	$MnSO_4 \cdot H_2O$	0.013
Mo	$Na_2MoO_4 \cdot 2H_2O$	0.06
Cu	$CuSO_4 \cdot 5H_2O$	0.03

Table S3. The removal of COD and NH_4^+-N by the absorption of activated carbon in aeroponic system without water spinach.

Time (d)	COD removal rate (%)	NH_4^+-N removal rate (%)
1	26.8	38.5
3	41.1	45.1
5	51.2	47.2
9	55.2	-

