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Original Research

Rice Husk as Carbon Source and Biofilm Carrier for Water Denitrification

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Abstract

We investigated rice husk as the sole carbon source as well as biofilm carrier in the biological denitrification of wastewater in up-flow laboratory reactors. Fast startup of the reactor and high nitrate removal efficiency was observed. The highest denitrification rate (about 0.096kg/m^3·d) was achieved when flow rate and nitrate concentration were 41.4L/d and 25.0mg/L, respectively. Flow rate and nitrate concentration of the influent were observed to have a significant effect on nitrate removal efficiency. The reactor had the ability to accommodate a wide range of pH (6.5-8.5) and DO (1.5-4mg/L). A time-dependent decrease in nitrate removal efficiency was observed after 72 days of operation. And the addition of new rice husk brought about a rapid increase of nitrate removal efficiency. Results showed that rice husks can be an economical and effective carbon source for the nitrate removal process.

Keywords: rice husk, carbon source, biological denitrification, biofilm carrier

Introduction

Nitrogen contamination has become an environmental and public health problem in many developed and developing countries, and the removal of nitrogen compounds from wastewater before they are discharged into receiving bodies is of increasing importance. Nitrate pollution is caused by intensive use of nitrogen fertilizers, crop irrigation with domestic wastewater and uncontrolled on-land discharges of raw and treated wastewater [1-2].

Various treatment techniques such as reverse osmosis, ion exchange, chemical reduction, physical adsorption and biological denitrification have been employed to remove nitrates from wastewater [3-5]. Nevertheless, reverse osmosis and ion exchange are always used to solve a small quantity of nitrate-polluted water; moreover, they produce nitrate-concentrated waste streams that pose a disposal problem due to high salinity [6, 7]. The disadvantage of chemical reduction lies in the production of ammonia [7]. Physical adsorption is not fit for removing excess nitrate from wastewater, due partly to their high cost. On the other hand, many kinds of absorption materials are not fit to remove nitrate from wastewater because of the presence of numerous impurities in the wastewater [8-11]. However, Biological denitrification is the reduction of nitrate to nitrogen gas by facultative bacteria, which can occur naturally. As of now, biological denitrification is accepted as the best available technology for the reduction of nitrate from wastewater [12].

The majority of biological denitrification treatments rely on heterotrophic bacteria, which require organic carbon substrate. The traditional technique is to add an organic carbon source (e.g. methanol, ethanol, acetic acid) into a denitrification reactor [3, 13]. The disadvantages of this treatment process are the need of a close, rather sophisticated and costly process control, the risk of overdosing and deep knowledge about the operation of this biological system. Moreover, traditional liquid carbon sources (such as

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methanol, ethanol, and acetic acid) are always expensive and potentially hazardous. Hence, a global search is on for a new and viable alternative. Studies in recent years have indicated that some natural organic substances can be used as efficient carbon sources to remove nitrate from wastewater [12, 14-16]. In this research, we chose rice husk as a carbon source and the only physical support for microorganisms. In contrast to conventional treatment units, denitrification with rice husk presented here is a simple process. Rice husk can be used as both the carrier and carbon source for denitrification.

As the main by-product of rice, rice husk accounts for approximately 20% of all rice products [17]. The focus of the study was to determine whether rice husk can serve as the sole carbon for the denitrification of wastewater and the sole physical support for bacterial attaching. The use of unmodified or modified rice husk as an adsorbent for removing pollutants has attracted considerable attention in recent years [18-22]. However, currently little information is available on the behaviors of nitrogen removal from wastewater by use of natural rice husk.

Materials and Methods

Pretreatment of Rice Husk

Natural rice husk with a length of 4-7mm and width of 2-3mm was collected from a local village of Chongming county in Shanghai, and the same material was used in all experiments. Rice husk was washed by tap water prior to oven drying (30ºC). The material was preserved at room temperature (25ºC) and kept in a moisture-free container. The weight and filling height of the substances were measured to determine the percentage loss of material during the experiment. In the beginning, the weight and filling height of rice husk were 1,000g and 60cm, respectively.

Characteristic of Rice husk Lixivium

The experiments on characteristic of rice husk lixivium were conducted in 500-ml glass vessels with distilled water (400ml) and 10g of rice husk were packed in each reactors. A small outlet at the bottom of the glass vessel was used for taking water samples. The glass reaction vessels were placed in an incubator (30ºC). After 30 days, lixivium of rice husk was analyzed by Inductively Coupled Plasma Optical Emission Spectrometer (Perkin-Elmer Company, USA). The above process was repeated three times.

Inoculum

Fresh activated sludge was taken from a local municipal wastewater treatment plant (QuYang wastewater treatment plant, Shanghai, P. R. China). The sludge was cultured for 3 days in a liquid medium (KNO_3 2g/L; K_2PO_4 0.5g/L; $MgSO_4 \cdot 7H_2O$ 0.2g/L; $C_4H_4KNaO_6 \cdot 4H_2O$ 20g/L) and then used as inoculum seeding in the denitrification reactor.

Experimental Apparatus

PVC columns used as reactors were 70cm high with an inner diameter of 14.5cm. Rice husk was placed into the reactor after pretreatment as described above in section 2.1. A thin layer of glass wool was placed at both ends of the packing material and the upper glass wool was fixed with wire netting. After rice husk was packed, medium and inoculum were dosed into the column. The inoculated reactor was allowed to stand for 3 days before flow was initiated. The flow created an up-flow through the column with a rate regulated by a peristaltic pump (BT01-100). All experiments were conducted in dark condition by cover of aluminium paper (Fig. 1).

Water Medium

Medium solution was prepared daily by tap water supplemented with KNO_3 (A. R., Shanghai Chemicals Plant, China) as N source and K_2PO_4 (A. R., Shanghai Chemicals Plant, China) as P source. To establish different Dissolved Oxygen (DO) conditions in the feed vessel, the media was swept by nitrogen gas.

Analytical Methods

Samples were collected daily from the inlet and outlet of the reactor. NO_3^-, NO_2^-, COD, pH, DO, flow rate and temperature were measured daily. The pH value was measured with a Model pHS-25 acidity meter (Shanghai Precise Science Instruments Co., China). The temperature and the oxygen concentration were measured with a standard electrode (HACH). NO_3^-, NO_2^- and COD concentrations were measured using standard methods (Chinese NEPA Standard Methods); COD concentration was analyzed using the $K_2Cr_2O_7$ oxidization method; NO_3^- concentration was analyzed using the UV-spetrophotometry method ($A = A_{220} - 2A_{275}$); NO_2^- concentration was analyzed using the spectrophotometric method with N-(1-naphthyl) ethylenediamine [23].

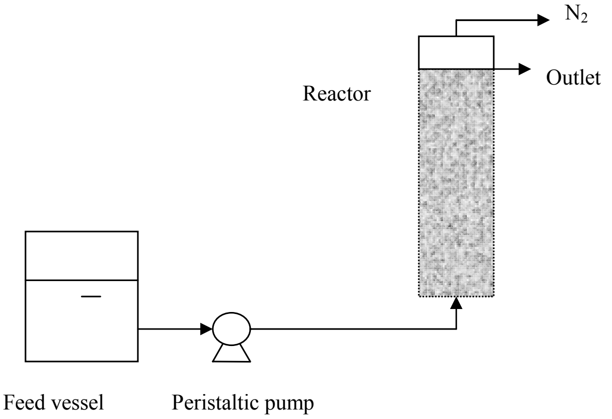

Fig. 1. Schematic of a laboratory-scale experiment.

Table 1. Analysis of lixivium.

Element	Ca	K	Mg	Na	Si	Ba	Cu	Pb	Cd	Cr	Zn	P
Concentration (mg/L)	4.125 ±0.009	139.050 ± 0.063	5.688 ±0.017	8.781 ±0.021	27.126 ±0.045	0.594 ±0.014	-	-	-	-	0.216 ±0.011	20.076 ±0.024

The denitrification rate r_D in $mgNO_3$-N/ (L·d) of the reactor was given by the equation:

$$r_D=Q \times (C_I-C_E)/V$$

...where C_I is the inlet nitrate concentration ($mgNO_3$-N/L) and C_E is the effluent nitrate concentration ($mgNO_3$-N/L). Q is the flow rate (L/d) and V is the reactor volume.

Results and Discussion

Characteristics of Rice Husk Lixivium

The characteristics of rice husk lixivium were studied before starting the experiments. As shown in Table 1, high concentrations of Ca, K, Mg, Na, Si, P were measured in the lixivium. These "major elements" were needed by microorganisms. Trace elements such as Ba and Zn were also observed in the lixivium. On the other hand, heavy metals such as Cu, Pb, Cr, and Cd, which negatively affect the metabolism of microorganisms, were not detected. The literature has reported that metal elements in the lixivium can have positive effects on denitrification rate due to metal ion used as an active center in the denitrification process [24].

The above results indicate that it is safer to use rice husk as a substrate in wastewater denitrification compared with traditional liquid carbon sources (such as methanol).

Startup and Water Quality of the Reactor

Plant operating parameters of this experiment were shown as follows: pH (inlet), 7.5-8.5; DO (inlet), 2-4mg/L; temperature, 29-30ºC. As can be seen from Fig. 2, high nitrate removal efficiency was observed during the beginning of the operation, when little nitrate washed out from the reactor. The percentage of N eliminated to N applied (nitrate removal efficiency in other words) was usually within a range of 90.6%-97.8%. One week of continuous system operation was needed to establish a steady-state condition in this experiment. More time could be saved when compared to the use of cotton or newspaper as the substrate [25-26]. A larger bacterial population always results in faster reactor startup. Rice husk has a large external specific surface area which makes it easy for bacteria to attach to the surface. Moreover, the soluble fraction of carbon presenting in the fresh rice husk allowed rapid microbial growth. Therefore, startup of a reactor with rice husk as the carbon source is quicker than others. On the other hand, ammonia and nitrite concentration in the treated water never exceeded 2mg/L and 0.1mg/L, respectively.

The changes of COD and pH in the treated water are shown in Fig. 2. At the beginning of the investigation, significant amounts of COD in the effluent water were observed due to the readily biodegradable organic matter in the rice husk. After a few days, the concentration of COD decreased rapidly and stabilized at about 30mg/L, which could be explained as:

(1) In the first days of operation, the number of microorganisms in the reactor was limited and only a part of the soluble fraction of rice husk presenting in the fresh rice husk was used. Most of the soluble carbon produced by rice husk was washed out in the effluent. As time went by, the quantity of microorganism increased and a more soluble fraction of rice husk was used.

(2) The relative percentage of soluble components decreased. It can be presumed that as time went by, all soluble components and a majority of the cellulose and hemicellulose had been lost while lignin and mineral components remained. Therefore, the COD concentration in the effluent decreased after some days.

Volokita (1996) reported that during denitrification, using newspaper or cotton as carbon source, there were usually three phases of DOC change [14]. In the first phase, a high concentration of DOC was observed. In the second phase, rapid decrease of DOC was observed, and during the final phase the concentration always stabilized at low level.

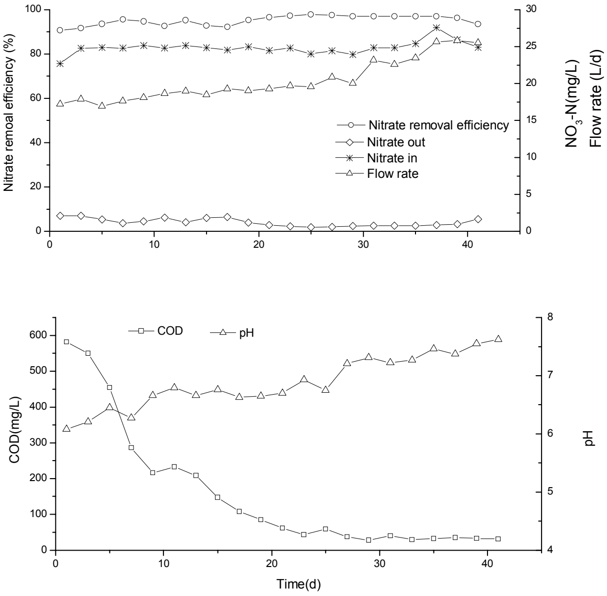

Fig. 2. Nitrate removal efficiency of the system and change of COD (mg/L) and pH according to time (d) in the effluent of a reactor packed with rice husk as physical substrate as well as sole carbon source (Temperature 29-30ºC; Flow rate 16-23L/d).

These observations are similar to our results described above.

An increase in the pH of the treated water was also observed. Denitrification induced an increase in pH, whereas degradation of that readily degradable in the rice husk led to a decrease in pH. At the beginning of the process, pH value of the treated water was lower than influent water. It was indicated that ready degradation in the rice husk was very intensive, which led the way at the beginning of the investigation. As time went by, there was an increase in the pH of the treated water, which showed that the effect of denitrification came to emerge. Recent research also showed increases in the pH of the treated water in denitrification reactors [15-16, 27].

Effect of Flow Rate

The effect of flow rate on denitrification was studied in a reactor packed with rice husk. Plant operating parameters of this experiment were shown as follows: pH (inlet), 7.6-8.3; DO (inlet), 2-3.5mg/L; temperature, 30-32ºC. Inlet nitrate concentration was kept at 24.0-25.5mg/L and the flow rate varied from 7.2L/d-41.0L/d (Fig. 3). A correspondingly steady state of nitrate removal efficiency of higher than 90% was observed when the flow rate changed from 7.2L/d to 30L/d. And the nitrate concentration in effluent remained less than 3mg/L. Breakthrough of nitrate starting at flow rate was higher than 30L/d. When the flow rate was up to 41L/d, the nitrate removal efficiency decreased to 83% (Fig. 3).

Denitrification rates, however, increased when flow rate ranged from 7.2L/d to 41.0L/d (Fig. 3). Higher flow rate was not studied because of limits of the peristaltic pump. And the highest rate of denitrification in this system (about 84.3g/m^3·d) was achieved under conditions in which flow rate and nitrate concentration were 41L/d and 25.3mg/L, respectively. Usually, complete removal of nitrate is not required in a denitrification system, and the system can be operated at flow rates allowing the highest efficiency while maintaining acceptable concentration of nitrate and nitrite. In this system studied, the flow rate could be increased to 41L/d.

In sum, flow rate plays an important role in denitrification performance of the system and the reasons for the decrease in nitrate removal efficiency at the higher velocities may result from washout of microorganism and solubilized substrate.

Effect of Nitrate Loading on Denitrification

Effect of NO_3-N loading on nitrate removal was studied in this research. Plant operating parameters of this experiment were shown as follows: pH (inlet), 7.5-8.5; DO (inlet), 2-4mg/L; temperature, 28-29ºC. The nitrate concentration used here ranged from a low level (15 mgNL^{-1}) to a high level (40 mgNL^{-1}). As shown in Fig. 4, nitrate removal efficiency was markedly affected by nitrate concentration in inflow, which suggested that high nitrate concentrations reduced nitrate removal efficiency in the reactor. The decrease in nitrate removal efficiency at the higher concentration may result from the lack of soluble carbon compared to high NO_3-N loading. In other words, the C/N ratio was too low in the medium. In addition, the negative effect was likely induced by an increase in nitrate concentration beyond the tolerance range of the bacteria. Therefore, microorganism metabolism was inhibited and low nitrate removal efficiency was observed at the higher nitrate concentration. Hou Hong-juan (2005) reported that the reduction of C/N ratio in the influent resulted in higher nitrate concentration in the effluent [28].

Effect of DO on Denitrification

The effect of dissolved oxygen (DO) in the influent water was measured with rice husk as carbon source and

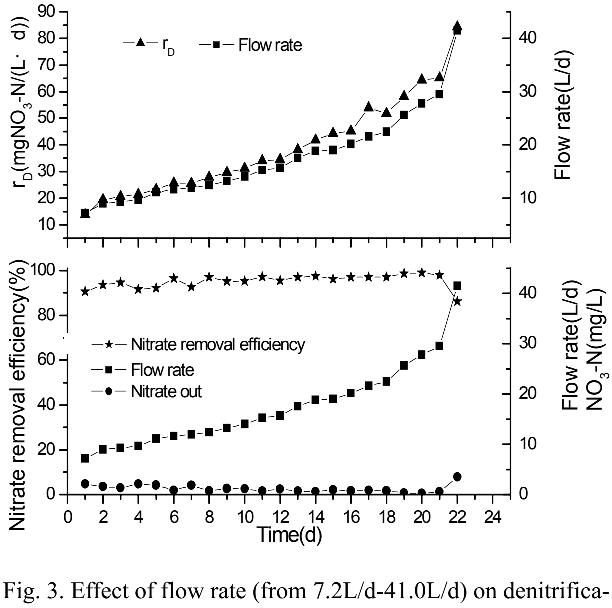

Fig. 3. Effect of flow rate (from 7.2L/d-41.0L/d) on denitrification rate and nitrate removal efficiency in a reactor packed with rice husk.

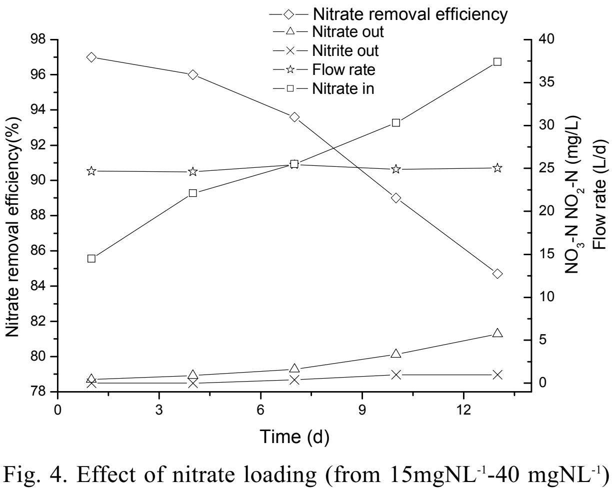

Fig. 4. Effect of nitrate loading (from 15mgNL^{-1}-40 mgNL^{-1}) on nitrate removal efficiency in a reactor packed with rice husk.

biofilm carrier. To establish different dissolved oxygen conditions in the feed vessel, the media was swept by nitrogen gas. Results showed that no significant difference was observed when DO changed from 1.2mg/L to 4.2mg/L, and that the denitrification rate of the reactor was always higher than 95% (Fig. 5). Only when the concentration of DO in the influent water was higher than 4.2mg/L did the presence of oxygen decrease the removal efficiency of nitrate and cause an increase of nitrate concentration in the treated water. However, the removal efficiency of nitrate was still higher than 70%. In sum, DO change in the influent has little effect on nitrate removal efficiency when rice husk was used as a carbon source and biofilm carrier.

It has always been reported that DO negatively affects biological denitrification. Generally, denitrification was believed to be a strictly anoxic process because many researchers have found that very low DO concentrations could cause complete cessation of denitrifying activity [29, 30]. However, no significant difference was observed in this experiment with DO in the range of 1.2mg/L - 4.2mg/L, which can be explained as follows: inner denitrifying bacteria were protected by microorganisms in the outer space. Oxygen, which has to be transported into the biofilm by diffusion, was almost completely used before it got to the inner zone, and it was still anoxic in the inner place. On the other hand, increasing DO may have consumed media volume and hydraulic retention time for aerobic metabolism of the carbon, leaving less volume for denitrification until breakthrough occurred. In other words, there was enough anoxic bed available to denitrify the nitrates until the DO reached 5 mg/L, at which time there was insufficient bed left. Besides, a higher concentration of carbon source was present in the inner zone and more electron acceptors were needed. Thus, in the inner zone, NO_3-N must be reduced even in the presence of oxygen. Therefore, the negative effect of oxygen on denitrification was minimized.

Gómez also reported that nitrogen removal was almost constant under conditions in which DO concentration was less than 4.5mg/L [31]. According to the results presented above, rice husk used as a carbon source and biofilm carrier in denitrification offers a viable alternative, as it is less affected by DO concentration.

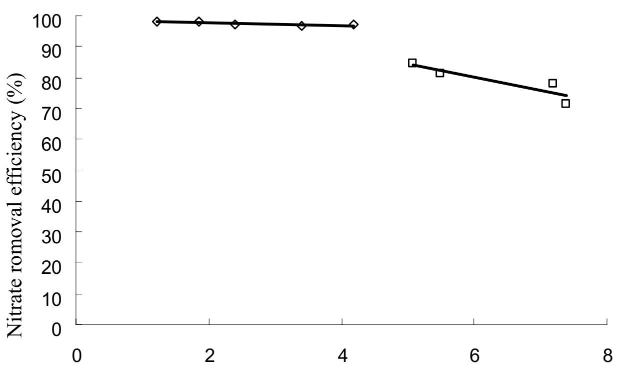

Fig. 5 Effect of dissolved oxygen on denitrification experiment using rice husk as carbon source and physical support.

Effect of pH on Denitrification

Experimental results showed that pH (influent) in the range of 6.5-8.5 did not significantly affect biological denitrification. Almost the same denitrification performance was achieved. It was reported that pH did not make a statistically distinguishable effect on nitrate removal in the range of 6.0-9.0 [1, 7, 15, 16, 32], which meant pH in the range of 6.5-8.5 did not go beyond the tolerance range of the bacteria and the bacteria activity in the reactor was stable. The result demonstrated that the reactor was able to accommodate a wide range of pH.

Long-Term Operation

The nitrate concentration in effluent started to increase after 72d of operation due to the exhaustion of carbon sources. During the operation, packing height of rice husk in the reactor changed from 60cm to 35cm and the COD decreased to 20mg/L, almost the same as COD in the influent. Fresh rice husk was filled into the reactor when nitrate removal efficiency of the reactor decreased to 60%. Then a rapid improvement of nitrate removal efficiency was observed, which increased to 95%. Therefore, it is necessary to pack new substrate into the reactor periodically in order to keep high nitrate removal efficiency.

Denitrification supported by solid substrates has been studied by several researchers. Della Rocca et al. reported that the use of cotton induced very good nitrate removal performance [33]; however, process performances decreased during the experiment because of compression and the subsequent loss of permeability. In other experiments, it needed to be back-flushed periodically to remove the excess biomass under conditions in which synthetic substrates such as polystyrene packaging or inert materials such as expanded clay was used as substrate. However, the natural organic substrate used in this experiment did not need to be back-flushed because rice husk could be consumed in the process and no clogging was found.

Conclusions

The results suggest that rice husk is effective in wastewater treatment as the sole chemical and physical substrate for the denitrifying microorganism. Moreover, it is safer to use rice husk as substrate in wastewater denitrification when compared with traditional liquid carbon sources. The natural organic substrate described here not only avoids the use of expensive carbon sources (e.g. ethanol, acetic acid, PHB) but also offers an alternative way to reuse agricultural waste.

Flow rate and nitrate concentration of the influent significantly affects denitrification. However, pH and DO had little effect on denitrification: the efficiency of nitrate removal was not changed at pH 6.5-8.5, or DO of influent in the range of 1.5-4mg/L. The system worked well within a wide range of pH and DO.

Table 2. Comparison of highest denitrification rate among different solid carbon supported denitrification systems.

Substrate	Highest denitrification rate mgNO$_3$-N/(L·d)	Reference	Price US$/t
Wheat straw	53	Soares M. IM., 1998 [12]	29-32
Newspaper	37	Volokita et al. 1996 [34]	125-143
Cotton	81	Volokita et al. 1996 [14]	1,200-1,300
PHB	528	Schick V., 1998 [35]	10,463-16,520
PCL	312	Schick V., 1998 [35]	2,325-2,657
Ethanol	750	Kim Y., 2002 [36]	600-1,000
Rice husk	**96**	**THIS WORK**	**12-14**

The addition of fresh rice husk brought about a rapid increase of nitrate removal efficiency. The highest rate of denitrification (about 0.096kg/m^3·d) was achieved with flow rate and nitrate concentration of 41.4L/d and 25.0mg/L, respectively. The denitrification rate based on the use of rice husk was not as good as that of the classical treatment with a liquid carbon source. But it had a much higher efficiency (96mgNO$_3$-N/ (L·d)) than the other studies (37-81mgNO$_3$-N/ (L·d)) (Table 2).

The high denitrification rate, easy operation and low expense of the running reactor made the application of the system possible in the nitrate removal from wastewater. Rice husk is therefore recommended as an economical and effective external carbon source for nitrate removal.

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