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Original Research

Comparing Three Carbon Substrates with Cow Dung Liquid for Denitrification of Agricultural Drainage Water

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Abstract

Nitrate in agricultural drainage water has been one of the most contributing sources of nitrate contamination of surface water in China. Denitrifying bioreactors are a promising technology to reduce the amount of nitrate, in which an external carbon is necessary to maintain continuous denitrification. Corn cob, wheat straw and woodchips, amended with different amount of cow dung liquid, were mixed with soil and incubated anaerobically for 90 days. KNO_3 was added periodically to maintain NO_3-N concentrations between 50 and 200 mg/L. All the substrates stimulated NO_3-N removal, and cumulative NO_3-N removed from highest to lowest was: corn cob, wheat straw and woodchips. Substrates with cow dung liquid stimulated more NO_3-N removed than those without. Concentrations of ammonium and organic nitrogen in systems with cow dung liquid were higher than without. Corn cob and wheat straw lead to higher NO_3-N removal rates than woodchips, but these two substrates degraded more rapidly, which could not maintain long term denitrification. The addition of cow dung liquid to woodchips significantly increased NO_3-N removal rates over woodchips alone, but the rates were still much less than that of corn cob and wheat straw.

Keywords: denitrification, carbon substrate, agricultural drainage

Introduction

Agricultural drainage water containing excess nitrogen is a contributing factor to eutrophication of surface water in China. For the low fertilizer utilization ratio of N, over 15 million tons of N are discharged to rivers, lakes and other water body each year. Some strategies are being implemented to reduce the nitrate load to aquatic ecosystems. In agricultural ecosystems, a number of land management approaches have been proposed to reduce N losses, such as controlling N inputs, improving N use efficiency in crops, controlled irrigation and drainage [1-6]. Many physical, chemical and biological technologies have also been used to reduce nitrate contamination [7-14]. Heterotrophic denitrification, which converts nitrate to nitrogen

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Table 1. Organic C and N contents of soil, C sources and cow dung liquid.

	Organic C	Ammonium	Nitrate	Total Nitrogen	C to N ratio
Soil (g/kg)	4.72	0.02	0.003	0.44	11.75
Corn cob (g/kg)	923.5	0.27	0.11	8.53	55.51
Woodchips (g/kg)	801.7	0.14	0.36	1.86	264.89
Wheat straw (g/kg)	794.1	0.75	0.38	12.36	55.36

Note: Moisture content of soil was 12.29%; corn cob, woodchips and wheat straw were dried.

gases, can remove nitrate from aquatic ecosystems irreversibly [15-18]. Nitrate and available carbon source concentrations are key controlling factors of heterotrophic denitrification. The availability of carbon source becomes the limitation of denitrification while nitrate reaches a relatively high level, which may happen in agricultural drainage settings [19, 20].

A number of studies focused on the selection and optimization of carbon sources. Liquid carbon sources, such as methanol, ethanol and methanoic acid, could sustain relatively high denitrification rates. But for the chance of secondary pollution and costs, liquid carbon sources haven't been widely used to reducing nitrate in surface and subsurface water [21-25]. Woody materials with low cost and high permeability can support steady but relatively low denitrification rates [26-30]. Wheat straw, rice hulk and newspaper can sustain higher denitrification rates, but they degraded much more rapidly, which may not sustain long-term denitrification [31-34]. A number of polymer materials were also used for denitrification, but more studies are needed to lower their costs [35-38]. To select carbon substrates reaching a balance among availability, cost, denitrification rate and longevity will be significant to reduce nitrate in surface and subsurface water.

Studies on soil amendments had found that co-composting of cattle manure with organic waste can efficiently enhance lignocellulose degradation [39-41]. The role of cow dung liquid was evaluated in acidification of lignocelluloses materials, which indicated that lignocellulose decomposing microorganisms contained in cow dung liquid may stimulate the acidification and increase dissolved organic concentration in the system [42]. Following these evidence, this study evaluated the influences of cow dung liquid with different carbon sources to stimulate denitrification in laboratory.

Materials and Methods

Three different carbon sources used in this study were: corn cob, wood chips (predominately *Populus* spp.) and wheat straw. These carbon sources were ground to 1 to 2 mm size to avoid the effect of particle size on rates of nitrate removal. The C and N contents of these materials are listed in Table 1.

The influence of cow dung liquid with different carbon sources on denitrification was determined in anaerobic incubations where 5 g carbon material (dry weight basis) was mixed with 5 g of field moist subsoil and certain amount of cow dung liquid (0, 10 or 20 mL) in 250-mL glass jars. All jars received 1 mL of a nitrate solution that contained 20 mg NO_3–N mL^{-1} and distilled water to keep the liquid volume at about 200 mL. The mixture of field moist subsoil, nitrate solution and distilled water resembles conditions in agricultural drainage water.

The cow dung used was collected in Dangyang City, China. 200 g fresh cow dung was fully mixed with distilled water and filtrated by 0.5 mm strainer, and the dissolved part was diluted to 1000 mL. The soil used to inoculate the microcosms was subsoil taken 50 cm below the surface of loamy sand soil located in a cotton field in Wuhan, China and the C and N contents were listed in Table 1. Jars prepared as described above without any carbon source (subsoil only) served as the contrast. Jars were incubated for 90 days at 24.5 to 25.5ºC in an anaerobic growth chamber with a N_2 gas atmosphere. A sufficient number of jars were prepared so that three replications could be destructively sampled on 6 dates spaced 15 days apart. Initial substances added in different treatments was listed in Table 2.

Throughout the experiments NO_3–N and NH_4–N concentrations as well as chemical oxygen demand (COD) in the jars were monitored every 2 or 3 days and re-treated with certain amount of the nitrate solution to sustain nitrate concentrations between 50 to 200 mg/L. Jars were shaken for 30 min before a sample was taken and after the NO_3–N solution was added. At 15 days intervals, three replicates of each treatment were sacrificed for accurately aqueous-phase analysis of NO_3–N, NH_4–N and COD, as well as solid-phase analysis of weight. Contents in jars were mixed thoroughly and poured into 500 mL high-density polyethylene bottles, then centrifuged for 30 min at 6000 rpm.

The supernatant was decanted, filtered, and used for analysis. The remaining soil and solid carbon substrates were rinsed two more times to remove remaining NO_3–N and NH_4–N in the moist pellet by adding 100 mL distilled water, mixing thoroughly, and centrifuging as described above. These rinse water

Table 2. Initial substances added in different culture bottles.

Number	Carbon source	Carbon source added (g)	Cow dung liquid added (mL)	Soil added (g)	NO_3-N (mg/jar)
Contrast	Null	0.0	0	5.0	20.0
CC–0	Corn cob	5.0	0	5.0	20.0
CC–10	Corn cob	5.0	10	5.0	20.0
CC–20	Corn cob	5.0	20	5.0	20.0
W–0	Woodchips	5.0	0	5.0	20.0
W–10	Woodchips	5.0	10	5.0	20.0
W–20	Woodchips	5.0	20	5.0	20.0
WS–0	Wheat straw	5.0	0	5.0	20.0
WS–10	Wheat straw	5.0	10	5.0	20.0
WS–20	Wheat straw	5.0	20	5.0	20.0

Note: CC stands for corn cob, W stands for woodchips, WS stands for wheat straw; 0, 10 and 20 stand for the amount of cow dung liquid.

supernatants were pipetted into the supernatant. The ultimate supernatant was diluted into 1000 mL. The rinsed solid materials were dried at 60ºC for 72 h.

Aqueous-phase extracts were analyzed for NO_3–N and NH_4–N on a CleverChem 200 (DeChem–Tech. GmbH, Germany) auto-discrete analyzer using colorimetric reaction. Chemical oxygen demand contained in aqueous-phase extracts was detected using potassium dichromate. The pH was measured using a pH electrode.

Evidence in both laboratory and field studies indicated denitrification is the dominant mechanism of NO_3^- removal in heterotrophic denitrification with solid carbon sources [19, 30, 43-46]. The mechanism analysis wasn't included in this study, but the influences of concentrations of NH_4–N and TN resulted from cow dung liquid were studied as the indirect influence of the mechanism in these laboratory incubation systems.

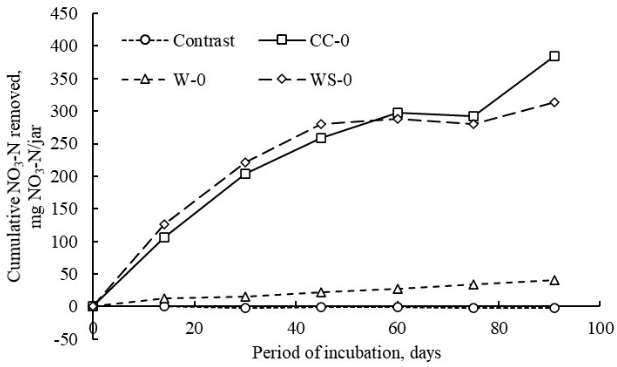

Fig. 1. Cumulative NO_3–N removed during incubation of three carbon substrates.

Results and Discussion

Nitrate Removal

Performance of Three Carbon Substrates

The nitrate removal represented the difference between the total NO_3–N added to the microcosms and the NO_3–N recovered at each sampling date. Without adding cow dung liquid, all three carbon substrates stimulated nitrate removal from the microcosms (Fig. 1). In the woodchips treatment, NO_3-N removal carried on at lower but steadier rates than the other two carbons. For the first 45 days, wheat straw treatment stimulated more nitrate removed than corn cob. But from 60^{th} to 90^{th} day, corn cob treatment stimulated more nitrate removed. The cumulative NO_3-N removed on 90^{th} day from highest to lowest was: corn cob with 384.9 mg/jar, wheat straw with 313.8 mg/jar and woodchips with 40.8 mg/jar. The cumulative NO_3-N removed of subsoil without carbon source was nearly zero.

The Influence of Cow Dung Liquid for Corn Cob Treatment

Different amounts (10 and 20 mL) of cow dung liquid were added in corn cob treatment systems to the investigate the influences of cow dung liquid for nitrate removal. Cumulative NO_3–N removed during incubation of corn cob with different amount of cow dung liquid was shown in Fig. 2. For the first 15 days, CC-0, CC-10 and CC-20 stimulated nearly the same amount of NO_3–N reduction. The differentials became obvious on the 30^{th} day and reached a peak on the 45^{th} day. NO_3–N reduction rate decreased in CC-20 treatment on the 45^{th} day. CC-0 treatment had a decline in NO_3–N removal rate on the 60^{th} day and the rate rose on the 75^{th} day. On the 90th day, cumulative NO_3–N removed

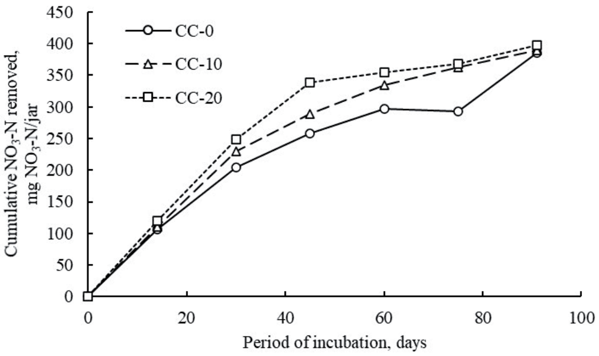

Fig. 2. Cumulative NO_3–N removed during incubation of corn cob with different amount of cow dung liquid.

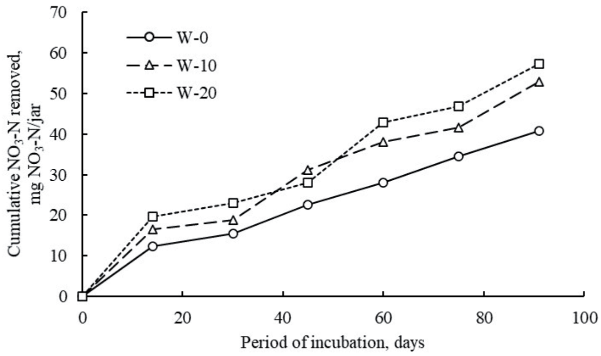

Fig. 3. Cumulative NO_3–N removed during incubation of woodchips with different amount of cow dung liquid.

of CC-0, CC-10 and CC-20 reached a close level, and one-way analysis of variance of cumulative NO_3–N removed indicated that no obvious differential existed (95% confidence level). However, the addition of cow dung liquid gets more NO_3–N removed when corn cob was adequate. The decline after the 45th day has mainly resulted from the rapid degradation of corn cob (Table 5).

The Influence of Cow Dung Liquid for Woodchips Treatment

Different amount (10 and 20 mL) of cow dung liquid were added in woodchips treatment systems to investigate influences of cow dung liquid for nitrate removal. Cumulative NO_3–N removed during incubation of woodchips with different amount of cow dung liquid was shown in Fig. 3. The nitrate-removal differentials between W-0, W-10 and W-20 became obvious on the 15th day and kept till the 90th day. On the 90th day, the cumulative NO_3–N removed of W-10 and W-20 treatments were respectively 29.5% and 40.3% higher than W-0 treatment. One-way analysis of variance of cumulative NO_3–N removed between three treatments indicated that obvious differential existed (95% confidence level). One-way analysis of variance of cumulative NO_3–N removed between any two treatments suggested the same conclusion (95% confidence level). As shown in Fig. 3, the addition of cow dung liquid can drive the NO_3–N removal to a relatively higher and steady status.

The Influence of Cow Dung Liquid for Wheat Straw Treatment

Different amount (10 and 20 mL) of cow dung liquid were added in corn cob treatment systems to investigate influences of cow dung liquid for nitrate removal. Cumulative NO_3–N removed during incubation of corn cob with different amount of cow dung liquid was shown in Fig. 4. For the first 45 days WS-0, WS-10 and WS-20 stimulated nearly same amount of NO_3–N reduction. NO_3–N reduction rate of WS-0 treatment started to decrease on the 45th day and began to rise

Table 3. Changes of ammonium and organic nitrogen concentrations in different culture bottles.

Carbon sources and the amount of cow dung liquid added	Initial ammonium concentration (mg N/jar)	Ammonium concentration at 90th d (mg N/jar)	Increase of ammonium concentration (mg N/jar)	Initial organic nitrogen concentration (mg/jar)	Organic nitrogen concentration at 90th d (mg/jar)	Increase of organic nitrogen concentration (mg/jar)	Increase of ammonium and organic nitrogen to NO_3–N removed
CC–0	1.002	1.303	0.301	0.480	2.286	1.806	0.55%
CC–10	1.013	1.417	0.404	0.679	7.443	6.764	1.84%
CC–20	1.326	4.964	3.638	1.060	14.018	12.958	4.17%
W–0	1.525	1.548	0.023	0.678	2.318	1.640	4.07%
W–10	1.676	1.773	0.097	1.003	3.459	2.456	4.83%
W–20	1.772	2.064	0.292	1.156	4.198	3.042	5.82%
WS–0	2.053	2.386	0.333	0.573	11.133	10.560	3.47%
WS–10	1.576	4.229	2.653	0.853	16.473	15.620	5.98%
WS–20	2.181	4.251	2.070	1.138	16.126	14.988	5.11%

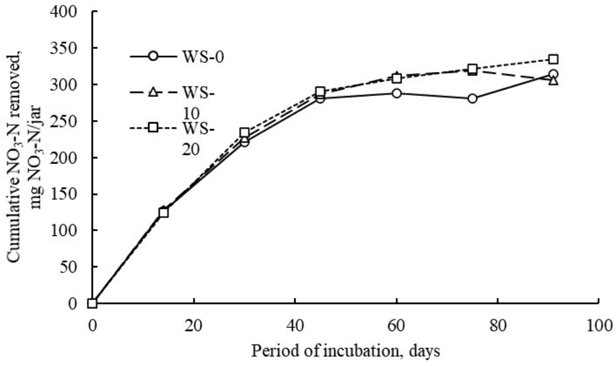

Fig. 4. Cumulative NO_3–N removed during incubation of wheat straw with different amount of cow dung liquid.

on the 45th day. On the 90th day, cumulative NO_3–N removed of WS-0, WS-10 and WS-20 reached close level, and one-way analysis of variance of cumulative NO_3–N removed indicated that no obvious differential existed (95% confidence level).

Ammonia Nitrogen and Organic Nitrogen

Monitoring of ammonia nitrogen and organic nitrogen of each treatment indicated that both the concentrations stayed at a relatively low level (<10 mg N/L) and rose slowly. The initial and 90th-day concentrations of ammonia nitrogen and organic nitrogen of each treatment have been shown in Table 3. In each treatment, the ratio of increase of ammonium and organic nitrogen to NO_3–N removed was less than 6%, which can partly prove denitrification was the dominant mechanism of NO_3–N removal. The addition of cow dung liquid made ratio higher, which may be owing to complex microbial communities in cow dung liquid.

COD

The availability of carbon source is one of the controlling factors of denitrification. Chemical oxygen demand (COD) was used as an indicator of

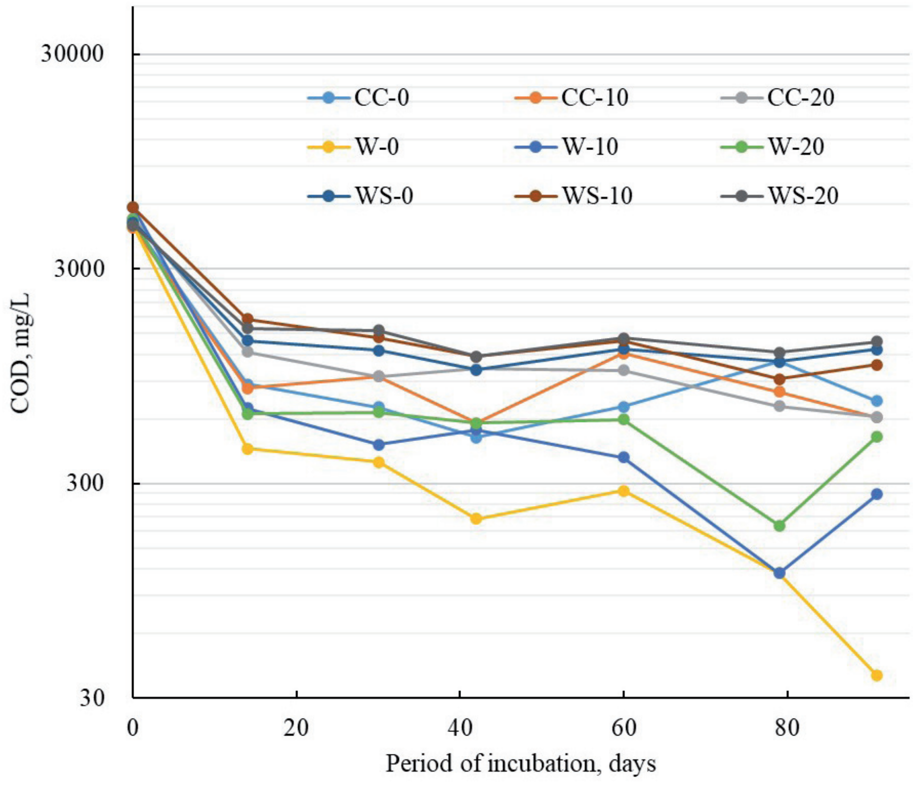

Fig. 5. Changes of COD during incubation in different culture bottles. Note: In order to obtain clear figure, Y-axis is set as logarithmic scale.

Table 4. Correlation between COD and NO_3–N removal rates in different culture bottles.

	CC–0	CC–10	CC–20	W–0	W–10	W–20	WS–0	WS–10	WS–20
Correlation	0	0	0	1	1	1	0	0	0

Note: 0 stands for consistency failed test, 1 stands for consistency passed test.

Table 5. Mass reduction of solid remains in 90 days (unit: g).

Days	CC-0	CC-10	CC-20	W-0	W-10	W-20	WS-0	WS-10	WS-20
15	1.841	1.800	2.019	0.477	0.256	0.570	1.927	2.126	2.229
30	2.011	2.115	2.182	0.510	0.489	0.612	2.015	2.228	2.338
45	2.208	2.421	2.345	0.511	0.514	0.641	2.221	2.287	2.394
60	2.270	2.425	2.537	0.556	0.519	0.699	2.246	2.369	2.532
75	2.350	2.568	2.638	0.611	0.535	0.705	2.357	2.428	2.762
90	2.563	2.654	2.735	0.656	0.563	0.760	2.428	2.596	3.018

the availability of carbon sources in our study. COD had also been chosen as a key factor in studies on denitrification kinetics [47, 48]. The analysis of COD at 15-d intervals of each treatment has been shown in Fig. 5.

Initial COD of each incubation system was high and COD decreased sharply in the first 15 days. COD decreased steadily and slowly from the 15th day to the 90th day. COD of all treatments from highest to lowest was: wheat straw, corn cob and woodchips. Treatments with cow dung liquid added had higher COD than those using the same carbon substrates without cow dung liquid. 20 mL of cow dung liquid can result in a higher COD level than 10 mL. As one controlling factor of denitrification, COD can influence nitrate removal rates. The correlation analysis of COD and average nitrate removal rates of each sampling was listed in Table 4 (99% confidence level).

In woodchips treatments, there was an obvious correlation between COD and nitrate removal rates. But for corn cob and wheat straw treatments, no obvious correlation can be found. The differentials were determined by the three carbon substrates themselves: Corn cob and wheat straw contain some polysaccharose and protein, which degraded rapidly and lead to a high COD level. Woodchips degraded much more slowly owing to its stable structure, so low COD levels existed in woodchips treatments. Relatively lower COD could limit the nitrate removal rates, as a result, an obvious correlation between COD and nitrate removal rates existed in woodchips treatments.

Mass Decrease of Remaining Solid

Analysis of the mass decrease of remaining rinsed solid materials showed a certain relationship with cumulative NO_3–N removed and COD (Table 5). On the 15th day, the mass decrease proportions of corn cob and wheat straw treatments were 35% to 45%, which revealed that easily degradable component of corn cob and wheat straw weighed at least 30% of the total mass. Masses of woodchips treatments decreased 5% to 11% proportioned to initial mass. Greater mass decrease of carbon substrates resulted in a higher COD level in relevant treatments. On the 90th day, 51%-55% of corn cob, 48%-60% wheat straw and 11%-15% woodchips have degraded. The decrease proportions of corn cob and wheat straw were close to the organic carbon contents of these two materials, which resulted in the decline of nitrate removal rates in the later period of incubation. The degrading rates of woodchips were low, for which woodchips may sustain long-term denitrification.

Conclusions

This study chose corn cob, woodchips and wheat straw as carbon sources for denitrification. The mixture of field moist subsoil, nitrate solution and distilled water in glass jars resembled conditions in agricultural drainage water. The study included the stimulation of carbon sources and the influences of cow dung liquid added. The results indicate:

1) Corn cobs, wood chips and wheat straw can stimulate denitrification and sustain continuous denitrification for 90 days.

2) In the woodchips treatment, NO_3-N removal was carried on at lower rates than the other two carbon sources. Corn cob and wheat straw contain some polysaccharose and protein, which were more easily utilized by denitrifiers, while woodchips degraded slowly.

3) Mixed with cow dung liquid, further denitrification was stimulated in all three carbon treatments. In woodchips treatments, adding 20 mL cow dung liquid remove more NO_3-N removed than 10 mL. In corn cob and wheat straw treatments, the addition of cow dung liquid got more NO_3–N removed when carbon sources were adequate.

4) Monitoring of ammonia nitrogen and organic nitrogen of each treatment indicated that both the concentrations stayed at a relatively low level and denitrification was the dominant mechanism of NO_3–N removal.

5) In woodchips treatments, correlation analysis indicated an obvious correlation between COD and nitrate removal rates. Analysis of the mass decrease of remaining rinsed solid materials revealed a greater mass decrease of carbon substrates resulted in higher COD levels in relevant treatments.

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Conflict of Interest

The authors declare no conflict of interest.

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