Article

Catalytic Performance of Cow-Dung Sludge in Water Treatment Mitigation and Conversion of Ammonia Nitrogen into Nitrate

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Abstract: The present research study was performed to find a solution for the mitigation of ammonia nitrogen in municipally treated sewage effluent (MTSE) using two natural bio-resources. One was composted cow-dung sludge that had catalytic enzymes and nitrifying bacterial mass, and the second was *Yucca* extract (a desert plant). MTSE samples put in one-liter beakers/jars having initial ammonia nitrogen content of 34.78 mg/L, when treated with 0.0 g/L (control Sample), 1 g/L, 5 g/L (cowdung) and 10 mg/L, 50 mg/L (*Yucca* extract), respectively, reported depletion of ammonia nitrogen to about 0.00 mg/L ammonia (NH₃) as N. It (NH₃) transformed to 17.8, 0.18, 0.09, 18.65, and 18.85 mg/L nitrite as N. Ammonia converted to 21.8, 110.1, 133.5, 20.5, 20.8 mg/L nitrate as NO₃, respectively. After eight days of treatment, the jar test apparatus reported the results at 35 rounds per minute (RPM) and a temperature of 32 °C. It was found that digested cowdung acted catalytically in eliminating the ammonia nitrogen by converting it to nitrate in a short period of nearly eight days, leading to almost 100% ammonia conversion.

Keywords: municipal sewage; biological treatment; ammonia nitrogen; cow-dung sludge; *yucca* extract

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1. Introduction

Today, globally, drinking water sources have either been polluted or are under continuous threat of contamination by wastewater intrusion. Ammonia pollution in water has become a significant concern for environmentalists, chemists, and biologists due to the health hazards. There is a necessity for an appropriate technology that can accelerate nitrification by which ammonia nitrogen can be converted to stable compounds such that its adverse effects could be neutralized. Mere aeration in wastewater for biological stabilization of biological oxygen demand (BOD) and nitrification takes an extended time [1,2]. The elevated nitrogen concentrations in the surface waters have primarily resulted from modern agricultural practices, mainly due to nitrogen fertilizers [3–5]. However, the nitrogen discharge from the point sources, such as sewage treatment plants and industries, also contributes significantly to riverine nitrogen loading. Ammonia exists in the water in both organic and inorganic nitrogen. Organic nitrogen (ON) includes a wide variety of compounds derived from natural and anthropogenic sources. The natural sources are principally nitrogenous end products of biological metabolism. Agricultural fertilizers also aid in the anthropogenic cause of organic nitrogen. Many bacteria, such as species of *Nitrosomonas*, possess the enzyme urease, which catalyzes the conversion of urea to inorganic nitrogen (IN) forms, like Ammonia (NH₃) or ammonium ion (NH₄⁺), that further oxidize to nitrite by the process of nitrification [6–8]. The wastewater rich in ammonia nitrogen harms the environment significantly as the ammonia nitrogen may inhibit natural nitrification, cause water hypoxia, result in fish poisoning, and decrease the water purifica-

tion capacity [4,5,8]. Composted cowdung was studied to analyze its catalysis properties in accelerating nitrification.

2. Ammonia Nitrogen in the Water and Discharge Limit

Ammonia nitrogen exists in water in two forms, free or unionized ammonia (NH_3) and ionized ammonium (NH_4^+) [9], and both these forms are pH and temperature dependent [10]. In aqueous ammonia solutions, the un-ionized ammonia exists in equilibrium with the ammonium and hydroxide ions [10]. Central Pollution Control Board (CPCB), a statutory organization of the Indian Government, has amended its discharge standards for sewage under the Water (Prevention and Control of Pollution) Act, 1974, vide order No. A-10014/43/06-MON/709 dated 21 April 2015, and further proceedings of the 199th Board meeting held on the 8 September 2015. The new standard for ammonia nitrogen in the treated sewage has stipulated that it is not more than 5mg/L and that total nitrogen is not more than 10mg/L. Previously the standard values for ammonia nitrogen were limited to the extent of 50 mg/L and that of total nitrogen or total Kjeldahl nitrogen 100 mg/L [11].

3. Available Techniques and Demerits on Mass-Scale Treatment

There are many techniques for removing ammonia nitrogen from waste water, such as nitrification–denitrification, ammonia stripping, ion exchange, zeolite process, breakpoint chlorination, and algae ponds. Still, none are free from the operational complications [1,12–16]. Moreover, the available techniques are not feasible for large water masses, as are required by municipal sewage disposal works in large cities [17–21]. Secondary treated sewage effluents require treatment so that the ammonia nitrogen can be converted into nitrate without any limitations/demerits of the process. In some cases, the growth of algae is also a problem. The ammonia nitrogen contributes to the development of the algal bloom in the presence of bluish-white light, as it accumulates in the algae's moiety. The algal growth increases the pH of the water. It produces cyanotoxins [22]. When disinfected with chlorine, it creates a problem of filter bed choking in the water treatment utilities and hinders the water treatment process by making coagulants ineffective at water treatment plants. Higher pH of water (>8.2), as sometimes faced at municipal waterworks of Delhi (India), creates hindrance in the conventional water treatment processes, as it makes the chlorine ineffective. Furthermore, the coagulant poly aluminum chloride is inefficient at higher pH [23].

4. Focus on New Research: Catalytic Effect of Cow-Dung Sludge

The present studies have been conducted to combat the ammonia nitrogen in the secondary treated municipal sewage water by using a desert plant extract, *Yucca schidigera*, and cowdung. However, *Yucca* extract has been reported in some previous studies for its capability of removing ammonia nitrogen from the water [24–27]. The experimental studies conducted using the cowdung on treated sewage yielded better results for combating the ammonia nitrogen and have shown its potential to be applied in real life for large masses of wastewater. In addition to cowdung, Blue-buck dung and Buffalo dung, both fresh and digested, were also employed for the studies on removing ammonia nitrogen, but the digested cowdung was found to be effective.

5. CowDung as a Bioresource of Catalytic Enzymes and Nitrifying Bacterial Mass

The cowdung has been used successfully in previous studies for industrial wastewater treatment for combating heavy metals, pharmaceuticals, pesticides, and petrochemicals [28–32]. cowdung harbors a rich microbial diversity, containing different bacteria, protozoa, and yeast. Godambe and Fulekar [32] separated the species of bacteria: *Enterobacter cloacae*, *Pseudomonas pseudoalcaligenes*, *Archromobacter* sp., *Aeromonashydrophila*, *Bacillus pumilis* belonging to the genus of *Pseudomonas* sp., *Escherichia* sp., *Staphylococcus* sp., *Aeromonas* sp., *Bacillus* sp., *Enterobacter* sp. and yeast *Rhizopus* sp. In addition to protozoa and yeasts (*Saccharomyces* and

Candida), a beneficial bacterial mass of *Citrobacter koseri*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Kluyvera* spp., *Morganella morganii*, *Pasteurella* spp., *Providencia alcaligenes*, *Providencia stuartii*, and *Bacillus* spp., *Corynebacterium* spp., and *Lactobacillus* spp. are present in cowdung [31,33]. Randhawa and Kullar (2011) found that the indigenous Indian cowdung contains more calcium, phosphorus, zinc, and copper than the cross-breed cowdung. Teo and Teoh (2011) studied five different isolates from the cowdung. They found that all isolates were positive for the presence of esterase and esterase lipase. All strains were able to produce protease, esterase, and esterase lipase. The protease is an enzyme that catalyzes proteolysis, which breaks down the proteins to their respective amino acids. Furthermore, they have a variety of applications, mainly in the detergent and food industries. Esterase and esterase lipase belonging to the hydrolase enzymes split esters into acids and alcohols, as was observed in a chemical reaction of the hydrolysis process involving the addition of water molecules [34]. Cowdung, containing a diverse group of microorganisms such as *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Alcaligenes* spp., makes them suitable for microbial degradation of the pollutants [29,35,36]. The cowdung slurry maintained in the ratio of 1:10 or 1:25 can degrade the rural, urban, and hospital wastes, including oil spillage, to five essential elements [31].

Quaraishi et al. (2018) evaluated the performance of the cowdung as microbial inoculums in industrial wastewater treatment and found a significant reduction in chemical oxygen demand (COD). They observed a 45.7, 62.3 and 84.04% reduction on the 5th, 10th, and 15th day of treatment. The bacterial mass found in the cowdung offers practical bioremediation for treating hydrocarbon-benzene. In a study, benzene was degraded entirely from 50 and 100 mg/L concentration after 96 and 144 h, respectively, whereas for 200 mg/L, about 99 percent degradation was observed after 240 and for 400 mg/L up to 69.2% degradation observed. As per the study of Godambe and Fulekar, cowdung possesses a huge microbial load, Table 1 [32].

Table 1. Characteristics of Cow Dung.

Microbial Enumeration of the cow dung Sample	
Parameters	Results
Total viable count	2.29×10^8 cell/ml
Total Fungal count	1.16×10^7 cell/ml
Yeast count	7.5×10^6 cfu/ml
Physico-Chemical Characterization of a Cow Dung Sample	
pH	7.3
Dissolved Oxygen	6.4 mg/L
Temperature	25.9 °C
% Organic Carbon	0.67%
Biological Oxygen Demand	19.83 mg/L
Chemical Oxygen Demand	195.2 mg/L
Phosphorous	0.23 mg/L
Sulphate	38.5 mg/L

The bold from the title it's important because it distinguishes between microbial and physico-chemical properties.

The cow dung consortium (bacteria, fungi, and actinomycetes) has also helped degrade phenol ranging from 100 to 1000 mg/L Table 2. The phenolic toxicant is converted to nutrients, biomass, and CO₂ via biodegradation through their intermediates. Thus, cow dung can potentially be used in the petrochemical and chemical industries, generating waste-containing phenol compounds [31,37].

However, there are no reports of cow dung being used in wastewater treatment specifically to combat ammonia nitrogen. India's government provides enormous funds on Scientific Validation and Research on Panchgavya (a concoction of cowdung, cow urine, milk, curd, and ghee). The present research presents a comparative study of *Yucca* extract and cow dung use for ammonia combat in wastewater.

Table 2. Degradation of phenol with cow-dung slurry.

Concentration of Phenol	Degradation %	Time Duration
100 mg/L	98.59	24 h
250 mg/L	99.4	72 h
500 mg/L	99.6	96 h
1000 mg/L	Not degraded	up-to 168 h

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6. Materials and Methods

6.1. Collection of Samples and Testing

The 25 L, secondary treated sewage effluent samples were collected from Sewage Disposal Works (SDW), Delhi, India. This SDW treats about 40 MGD municipal sewage using the activated sludge method. The collected sample was immediately tested for the initial parametric values of ammonia nitrogen, nitrite, nitrate, pH value, total dissolved solids, electrical conductivity, temperature, etc. The ammonia nitrogen, nitrite, and nitrate tests were performed on Eppendorf Bio Spectrometer model 2019 (Eppendorf AG, Hamburg, Germany). The ammonia nitrogen was tested as per the Nesslerization method, 4500-NH₃ C, Nitrite by 4500-NO₂[−] B, and Nitrate by 4500-NO₃[−] B, prescribed in the standard methods for the examination of water and waste water [38]. The pH values, total dissolved solids, and electrical conductivity were measured by a Hach HQ 440 d Multi Ion Analyser, Loveland, Colorado, United State The authors prepared standard solutions for ammonia nitrogen, nitrite, and nitrate tests designed from analytical-grade reagents for calibration purposes.

6.2. Preparation of Yucca Extract

Yucca schidigera, a desert plant, was collected from the Forest Research Institute, Dehradun, Uttarakhand State, India. The Yucca plant's stem (about 250 g) was cut and chopped with a knife into small pieces. Then, the chopped pieces were ground in a grinder mixture, dried in the open sun for three days, and again dropped to make a fine powder. This Yucca powder was sieved through a 150-mesh sieve and stored in an airtight container. In the present study, the *Yucca* extract was used in the form of a solution. *Yucca* solution was prepared by mixing and dissolving 50 g of *Yucca* powder in 700 mL distilled water in a 1 L beaker, stirred on jar test apparatus for seven days (about 250 mL water was evaporated in the air) so that its contents could dissolve well, filtered through ordinary filter paper and made up to 500 mL with distilled water in a measuring flask. Thus, 1 mL of *Yucca* extract produces 100 mg solution.

6.3. Preparation of Indian Cow Dung

The cow dung was collected locally from a heap dumped by a farmer in a village of Delhi. The age of the cow-dung pile was about one year old. The digested wet dark-brown colored cow dung, about 500 g, was taken from a depth of 6 inches inside the heap in a clean glass bottle. Collected cow dung was ready to be used. A total of 1 g of cow dung was weighed in an electronic balance three times and poured into three different beakers containing 1 L of municipally treated sewage effluent (MTSE) in each. The experiments were performed in triplicate, and the average of all was taken for calculation purposes. Similarly, other operations were performed with a higher amount of cow-dung sludge (2–5 g).

6.4. Experimental Setup and Oxidation of Ammonia Nitrogen

The jar test apparatus (Figure 1) was used to observe the biomass's effectiveness (*Yucca* extract and cow dung) with a stirring speed of 35 RPM. The experiments were carried out in triplicate for more precise results. Because of the jar test apparatus's limitations, the triplicate samples were treated separately because it had only a six-beaker arrangement. The reported results are the final valid values extracted after several experiments under varying experimental conditions. The collected MTSE were taken into the six one-liter beakers.

Figure 1. Experimental Setup—Jar Test Apparatus.

Parameters for ammonia nitrogen, nitrite, nitrate, pH, electrical conductivity, total dissolved solids, color, odor, and temperature were tested immediately in the laboratory after sample collection. We kept beakers 1 and 2 for reference. Beaker 1 served as a control to measure the amount of water evaporated during the experiment. Beaker 2 served as a control to measure the change in parameters naturally. We added 1 and 5 g of cow dung into the beakers 3 and 4 while 0.1 and 0.5 mL of *Yucca* extract was added to beakers 5 and 6, respectively. Four 15-watt tungsten bulbs were hung over the jar test apparatus to illuminate the system round the clock. The stirrers of the equipment are set at 35 RPM. Every 48 h, a 100 mL sample was taken from the jars numbered from 2 to 6 and tested for changes in parameters. We used the content of beaker 1 for measuring the quantity of water evaporated during the processing period of 48 (2 days), 96 (4 days), 144 (6 days), and 192 (8 days) h, respectively.

7. Results and Discussions

Initially, *Yucca schidigera* extract was used with varying concentrations in the presence and absence of lights. We measured initial levels of ammonia nitrogen and other parameters of MTSE, and the experiments were performed at room temperature with pristine samples. It was observed that, even with multiple experimental studies using the different dosages of *Yucca* extract, there was no significant difference in the concentration of ammonia nitrogen, nitrite, and nitrate with the control sample. Instead, the ammonia nitrogen found increased in the *Yucca* extract dosed samples after the 8th day. An increase in ammonia content indicated that the *Yucca* extract started fermenting after the 6th day. The *Yucca* extract did not help combat ammonia nitrogen in municipal sewage water. After this, the experiments were conducted using the excreta of the bovine animals such as Blue-buck, Buffalo, and Cow, as a potential bioresource to combat the ammonia nitrogen from wastewater. Among all these, the cow dung was found most effective against removing ammonia nitrogen. As per the previously reported studies, the cow dung was very effective against reducing the

effluent's chemical oxygen demand (COD) [30]. COD is the amount of oxygen required to stabilize organic and inorganic (ammonia and nitrite) pollutants in water under heat and acidic conditions, using a highly oxidizing agent, potassium dichromate, corrected for the influence of chloride. The cow dung, in this case, acted as a bio-catalyst.

7.1. Biological Oxidation of Ammonia Nitrogen

We experimented with digested cow dung, and it was found that catalytical enzymes and nitrifying bacteria present in cow dung reduced ammonia significantly. The results of the present study are depicted in Figure 1, wherein an initial amount of 34.780 mg/L ammonia nitrogen (as NH_3) in municipally treated sewage effluent (MTSE) was reduced to 23.210 (in control sample), 22.590 (with 1 g/L cow dung), 18.390 mg/L (with five g/L cow dung), 23.580 mg/L (with 10 mg/L *Yucca* extract) and 24.705 mg/L (with 50 mg/L *Yucca* extract) in 48 h or the 2nd day of the process at 32°C temperature and 35 RPM. In 96 h, or the 4th day of the experimental set-up, ammonia nitrogen was reduced to 2.040, 0.960, 1.390, 2.735 and 7.135 mg/L, respectively. In 144 h, or the 6th day, ammonia nitrogen was reduced to 0.032, 0.00, 0.00, 0.036 and 0.154 mg/L. In 192 h, or the 8th day, ammonia nitrogen had been reduced to 0.00, 0.00, 0.00, 0.00 and 0.88 mg/L, respectively (Figure 2).

Figure 2. Biological Oxidation of Ammonia Nitrogen. (In Control, with Cow Dung and *Yucca* extract).

7.2. Biological Oxidation of Nitrite to Nitrate

Figure 2 shows the oxidation of nitrite (NO_2) into nitrate (NO_3). The initial content of ammonia nitrogen in municipally treated sewage effluent (MTSE) is converted to nitrite and nitrate simultaneously. The conversion of ammonia into nitrite and nitrate in biological oxidation is a continuous process. Experimental study explored that the effluent with an initial amount of 34.789 mg/L ammonia nitrogen and 0.150 mg/L nitrite nitrogen has converted or oxidized to 0.210 mg/L (in blank), 2.880 mg/L (with 1 g/L cow dung), 3.265 mg/L (with 5 g/L cow dung), 0.545 mg/L (with 10 mg/L *Yucca* extract) and 0.575 mg/L (with 50 mg/L *Yucca* extract) into nitrite (NO_2) in 48 h, or the 2nd day of the process, at 32 °C temperature and 35 RPM. In 96 h, or the 4th day of the experimental set-up, ammonia nitrogen oxidized

to 11.70, 16.15, 11.45, 13.525 and 9.60 mg/L nitrite (NO_2), respectively. In 144 h, or the 6th day, ammonia nitrogen was converted to nitrite (which further kept oxidizing to nitrate) as 14.70, 8.80, 0.30, 15.525 and 14.375 mg/L. In 192 h, or the 8th day, ammonia nitrogen oxidized to 17.80, 0.18, 0.09, 18.65 and 18.85 mg/L nitrite (NO_2), respectively (Figure 3).

Figure 3. Formation of nitrite nitrogen during oxidation of ammonia nitrogen (in control, with cow dung and *Yucca* extract).

7.3. Oxidation of Nitrite (NO_2) into Nitrate (NO_3)

Ammonia nitrogen is readily converted to nitrite biologically, but its further oxidation to nitrate (a stable form) is typical. It has been observed that the oxidation of nitrite into nitrate is recalcitrant. Cow dung accelerated the process of nitrification. Figure 4 shows that the initial amount of 34.789 mg/L ammonia nitrogen and 0.45 mg/L nitrate as NO_3 converted or oxidized to 2.25 mg/L (In blank), 3.80 mg/L (with 1 g/L cow dung), 11.70 mg/L (with 5 g/L cow dung), 1.60 mg/L (with 10 mg/L *Yucca* extract) and 2.35 mg/L (with 50 mg/L *Yucca* extract) into nitrate (NO_3) in 48 h, or the 2nd day of the process, at 32 °C temperature and 35 RPM. In 96 h, or the 4th day of the experimental set-up, ammonia nitrogen oxidized to 12.90, 22.25, 59.30, 11.75 and 8.05 mg/L nitrate (NO_3), respectively. In 144 h, or the 6th day, ammonia nitrogen was converted to nitrate as 16.60, 67.30, 109.0, 17.80 and 17.0 mg/L. In 192 h, or the 8th day, ammonia nitrogen oxidized to 21.80, 110.10, 133.5, 20.50 and 20.80 mg/L nitrate (NO_3), respectively (Figure 4).

The ammonia nitrogen is completely converted to its final and stable product nitrate in 6 to 8 days, depending on the amount of cow dung used. However, the samples' total dissolved solids have been found to increase due to water evaporation during ammonia nitrogen oxidation. Because of evaporation, there was 5.7, 10.2, 15.7 and 21.9% water loss on the 2nd, 4th, 6th, and 8th day of the experimental process.

We also determined the effect of the cow dung and *Yucca* extract dosing on different parameters such as ammonia, nitrite, nitrate, pH, electrical conductivity, and Total Dissolved

Solids (TDS). We found both light and temperature to affect ammonia removal significantly. During the initial experimental studies, it has been seen that, in the absence of light and at a low temperature (below 30 °C), the nitrite (intermediate product) could not be converted to nitrate substantially. In some cases, intense white light illuminated by LED bulbs resulted in algal growth. The development of algae in the samples hindered the conversion of nitrite into nitrate, increased high pH to about ten, and algae grew out into large masses. However, the total dissolved solids decreased substantially. The tungsten bulbs were found fit for illuminations, as they did not support the algal formation in the samples.

Figure 4. Conversion of ammonia nitrogen into nitrate (in control, with cow dung and *Yucca* extract).

The conversion of ammonia nitrogen into the stable nitrate form is a very typical mechanism. In general practice, the treated effluent's mechanical stirring oxidizes the ammonia nitrogen to nitrite in the presence of nitrifying bacteria and natural oxidation. However, nitrite is also as harmful as ammonia regarding the production of disinfection by-products. In the present experimental study, the conversion of ammonia nitrogen into nitrite and nitrite into nitrate is simultaneous. However, the cow dung (digested) contributes to adding an insignificantly small amount of ammonia nitrogen to the samples. This negligible amount has not been counted for the sake of convenience. The authors analyzed the exact maximum amount of nitrite and nitrate formation.

Based on the current experimental studies, the addition of the cow dung and a few additional modifications in the existing conventional sewage treatment plants' existing infrastructure can give better final effluent quality, free from ammonia nitrogen and nitrite pollution. The proposed design of a modified sewage treatment plant working on the activated sludge process has been suggested in Figure 5, including tertiary treatment units. Here, Hydraulic Retention Time (HRT) in the Flocculator chamber with RPM 35 is eight days for the biological oxidation of ammonia nitrogen ($\text{NH}_3\text{-N}$ into nitrate (NO_3)).

Cow dung is abundantly available in India. Using cow dung as a bio-resource in wastewater treatment plants is safe and environmentally friendly because cow dung and cow urine are used to treat several human diseases in Indian herbal and natural treatment systems [39–41]. Activated sludge from the final settling tank has to take to feed the aeration

tank. It would be required to maintain a temperature of 32 to 38 °C of the mesophilic temperature range as microbiological activities are more prominent at higher temperatures.

Figure 5. Proposed Sewage Treatment Plant with Biological Tertiary Treatment Units.

Cow dung has successfully proved to play an important role in wastewater treatment. Even cow dung ash has been found active in water disinfection [42]. The dried powder form of cow dung is also helpful as a green adsorbent material to remove carcinogenic and heavy metal water pollutants [28,43]. FAO, 1998 mentioned that cow dung and cow urine possess complex degrading substances [44].

8. Conclusions

The digested cow dung is an excellent biological remedy for the complete treatment of wastewater for combating ammonia nitrogen. The worldwide water scarcity can be mitigated by reusing properly treated municipal sewage and industrial effluents to augment water resources and a circular economy. In the current study, 1g cow dung has transformed 34.78 mg/L ammonia nitrogen in a municipally treated sewage effluent to 110.1 mg/L nitrate on the 8th day under aerobic conditions and a temperature of 32 °C performed with jar test apparatus at 35 RPM. At the same experimental conditions, the higher amount of cow dung of 5 mg/L mitigated ammonia nitrogen rapidly and yielded 133.5 mg/L nitrates. Most importantly, cow dung proved to be capable of transforming to the extent of 99.4% an intermediate product nitrite, as hazardous as ammonia. The presence of numerous microbiological organisms and biocatalysts in cow dung makes it a faster and more efficient way to treat ammonia nitrogen-contaminated wastewater. Secondly, cow dung may be rich in *Nitrosospira*, *Nitrosomonas*, *Nitrospira*, and *Nitrobacter*, which may assist in converting ammonia nitrogen to nitrite and nitrate. Cow dung can also be an excellent bio-resource for the biological treatment of sewage effluent, municipal, hospital, pharmaceutical, and industrial solid/ liquid wastes. For further research, a case study is recommended in the

existing activated sludge plant (SDW) using digested cow-dung slurry to study ammonia nitrogen conversion at the aeration chamber so that the present research can be utilized in real-life projects.

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