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Effect of Nutrients on Biological Nitrification

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Authors' contributions

This work was carried out in collaboration between both authors. Author PBNLD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YPS managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Biological nitrification is the most commonly used process for nitrogen removal from wastewater. Nitrification is carried out in two steps. First ammonia is converted to nitrite by ammonia oxidizing bacteria. In the second step nitrite oxidizing bacteria convert nitrite to nitrate. The study involves the effect of nutrients (both organic and inorganic components) on biological nitrification and the optimum concentrations of di-sodium hydrogen phosphate, potassium di-hydrogen phosphate, sodium hydrogen phosphate, sucrose and ferric chloride were observed over ammonium ion removal. The effect of dissolved oxygen also was studied and maximum percentage removal of ammonium ion was found to be 89.2%.

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Keywords: Dissolved oxygen; nitrification; Nitrosomonas; Nitrobacter; nutrients; liquid-solid bioreactor; wastewater treatment; ammonia removal.

1. INTRODUCTION

Removing nitrogen, as one of the most common and abundant pollutant of ground and surface waters is very important. Nitrification is a biological process during which nitrifying bacteria convert toxic ammonia to less harmful nitrate. All we need to do is provide the right conditions for the nitrifying bacteria to thrive. It is important to bear in mind the potential threat to fish health if nitrification is affected. Though nitrification is one of the critical processes in the nitrogen cycle, unrestricted and rapid nitrification in agricultural systems can result in major losses of nitrogen from the plant-soil system. This nitrogen loss is due to the leaching of nitrate out of the rooting zone and emission of gaseous oxides of nitrogen to the atmosphere, where it causes serious pollution problems. Biological systems for nitrogen removal can be improved by separate treatment of highly concentrated waters, such as effluents from the fertilizer industry and fish canning industry etc. Nitrification is an autotrophic aerobic process that converts ammonium into nitrate. Ammonium is oxidized to nitrite during the first step by ammonium-oxidizing bacteria (AOB), and nitrite is oxidized to nitrate during the second step by nitrite oxidizing bacteria (NOB) [1,2].

Jin [3] studied the partial nitrification process for the treatment of monosodium glutamate waste-water using airlift bioreactor fed with wastewater. They reported the nitrite yield of up to 87.0% and an effluent NO_2-N/NH_4-N ratio of 1.33, suitable for the influent of the subsequent ANAMMOX process. Miladinovic and Weatherley [4] performed the combination of nitrification and ion-exchange process in a packed bed system using the natural zeolitic ion-exchangers, clinoptilolite, and mordenite on which colonies of nitrifying bacteria are cultivated. Xia [5] studied three different C/N ratios (C/N=10:1, 5:1 and 3:1) in a suspended carrier biofilm reactor and reported, 90% removal of chemical oxygen demand (COD) and over 83.3% of simultaneous nitrification and denitrification (SND) efficiency. Zhang [6] studied high strength ammonium wastewater in an aerobic submerged membrane bioreactor and an anaerobic packed-bed biofilm reactor. They reported that membrane bioreactor showed nitrification efficiency higher than 98% at hydraulic retention time of 24h and packed-bed biofilm reactor showed satisfactory denitrification efficiency with very low effluent nitrite and nitrate concentration. Zhu and Chen [7] studied the effect of sucrose carbon on the nitrification rate of biofilters which was evaluated under steady-state conditions using a reactor series experimental system and reported the experimental solution with a carbon/nitrogen ratio of C/N=1.0 or 2.0 that resulted in approximately a 70% reduction of total ammonia nitrogen and removal rate as compared with a solution that has a similar nitrogen level, but without carbon (C/N=0).

The application of biofilm reactors in wastewater treatment systems is popular in view of their high volumetric productivity. These reactors are especially useful for slow-growing organisms. The present work involves the effect of nutrients such as di-sodium hydrogen phosphate, potassium di-hydrogen phosphate, sodium hydrogen phosphate, sucrose and ferric chloride on biological nitrification. The concentration of nutrients cited above is necessary to have proper activity of microorganisms. There is no reported work on determination of optimum concentration of nutrients for biological nitrification of ammonia as observed from literature. Hence the work is taken up to know the optimum concentration of these nutrients. Based on the concentration of any particular nutrient, the rate of nitrification changes due to change in the activity of microorganisms. The concentration corresponding to maximum ammonium ion removal is taken as optimum concentration of nutrient.

2. MATERIALS AND METHODS

2.1 Culture Preparation

Nitrosomonas (NCIM No-5076) and *Nitrobacter* (NCIM No-5062) were obtained from National Chemical Laboratory, Pune, India. The subcultures were prepared according to the procedure given by NCL, Pune. The culture was preserved in a refrigerator at a temperature of 4℃ by periodic subcultures.

2.2 Preparation of Inoculum

The liquid broth having the composition $(NH_4)_2CO_3$ 0.303 g, $NaHCO_3$ 25g, $MgSO_4.7H_2O$ 0.257g, Na_2HPO_4 1.135g, KH_2PO_4 1.092g, $FeCl_3.6H_2O$ 0.035g, Sucrose 6.174g per liter [7] has been prepared. The composition was adjusted to initial ammonium concentration of 100ppm by varying the amount of ammonium carbonate proportionately. The pH of the solution was adjusted to a value of 7.0 using HCl and NaOH. In order to kill the undesirable microorganisms, the broth was sterilized in an autoclave for 15 minutes at 15 psi pressure and 120℃. The broth was cooled to room temperature (28℃) and then medium of *Nitrosomonas* and *Nitrobacter* were introduced into the broth. Further, the medium was kept for the growth in an incubator for 24 h for the formation of biofilm over polypropylene beads for attached growth of microorganisms where the temperature was maintained at 30℃. 20mL of the product was collected at an interval of 2h and was used for the analysis of final ammonium ion and dissolved oxygen concentration. The analysis for ammonium ion and dissolved oxygen (DO) are carried out with Orion Ion Selective Electrode [8]. The effect of various nutrients on biological nitrification of ammonia in wastewater has been determined.

2.3 Effect of Various Nutrients on Biological Nitrification of Ammonia in Wastewater

To study the effect of various nutrients on biological nitrification of wastewater, the concentration of nutrients was varied. The concentration ranges for various nutrients is as: di-sodium hydrogen phosphate -1 to 5g/l; potassium di-hydrogen phosphate -1 to 5g/l; sodium hydrogen carbonate - 20 to 40g/l; sucrose - 5 to 9g/l; ferric chloride - 0.01 to 0.05g/l. Samples were prepared for all parameters as per the composition discussed in section 2.2. The conditions for all the samples were maintained at a temperature of 30^oC, pH of 7 and initial concentration of ammonium ion at 100ppm in incubator shaker with two phase (liquid-solid bioreactor) system. The work related to the effect of magnesium sulphate and ammonium carbonate on biological nitrification of wastewater has been reported elsewhere [9].

The concentration of ammonium ion and DO were plotted with time to determine the %removal of ammonium ion.

3. RESULTS AND DISCUSSION

The concentration of each nutrient has its own effect on rate of nitrification and on microorganisms. Sodium and potassium increase oxygen solubility in the cells. Sodium gives higher solubility than potassium [10]. Variation in phosphorous load results in change in N_2O emission rate which also depends on NO_2 accumulation [11]. The carbon source helps the cells to store energy in the form of PHA under limited oxygen conditions [12]. Ferric chloride

acts as an oxidizing agent. The discussion about the effect of concentration of various nutrients on ammonium ion concentration is given below.

3.1 Effect of Di-sodium Hydrogen Phosphate on Ammonium Ion Concentration

It was observed from Fig. 1 that the optimal concentration of di-sodium hydrogen phosphate is 2g/l with maximum ammonium removal of 89.2%. It was observed that the rate of removal of ammonium ion decreased at higher concentration of di-sodium hydrogen phosphate. Di-sodium hydrogen phosphate is mainly required for enzyme digestion which was used in the medium. If the concentration increases the activity of microorganisms decreases which tends to decrease in ammonium removal. The concentration of DO decreases with time which is due to the oxidation process of ammonium ion. This applies for every parameter. In the absence of external air supply, phosphorous is used to form Phosphorous Accumulating Organisms (PAOs) which are able to take up organic substrates from the medium and store them as polyhydroxyalkanoates (PHA) using the energy obtained partly from the sucrose utilization and hydrolysis of the intracellular stored polyphosphate (polyP) [12].

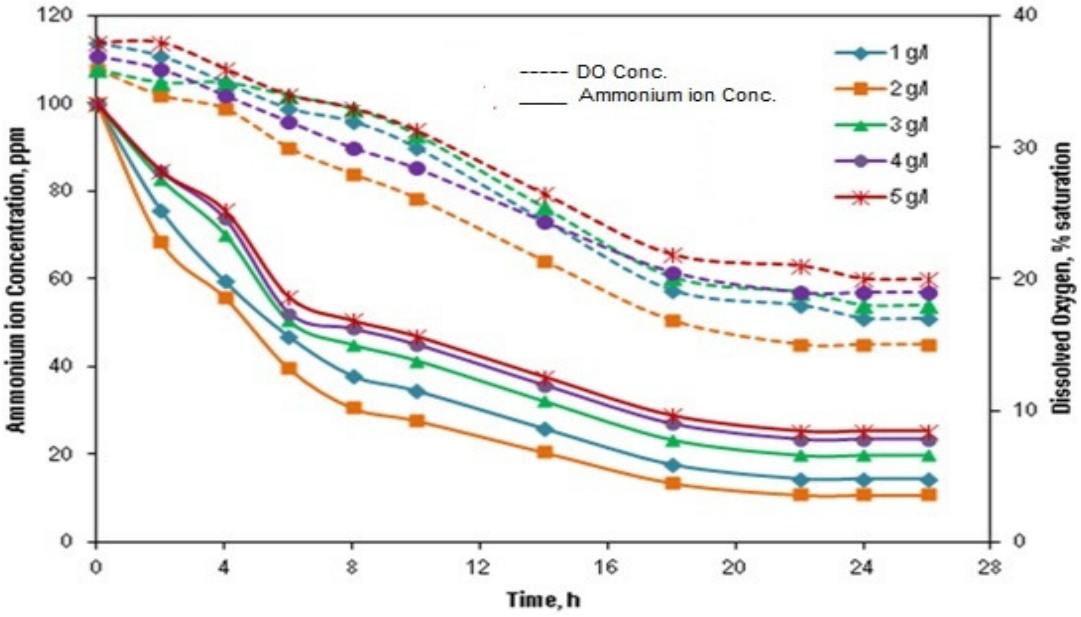

Fig. 1. Effect of Na_2HPO_4 on ammonium ion concentration and DO

3.2 Effect of Potassium Di-hydrogen Phosphate on Ammonium Ion Concentration

It was observed from Fig. 2 that the optimal concentration of potassium di-hydrogen phosphate is 1g/l and maximum ammonium concentration removed is up to 10.8ppm. It was also observed that as concentration of potassium di-hydrogen phosphate increases the removal rate of ammonium ion decreases. If the concentration increases the activity of microorganisms decreases which tends to decrease in ammonium removal.

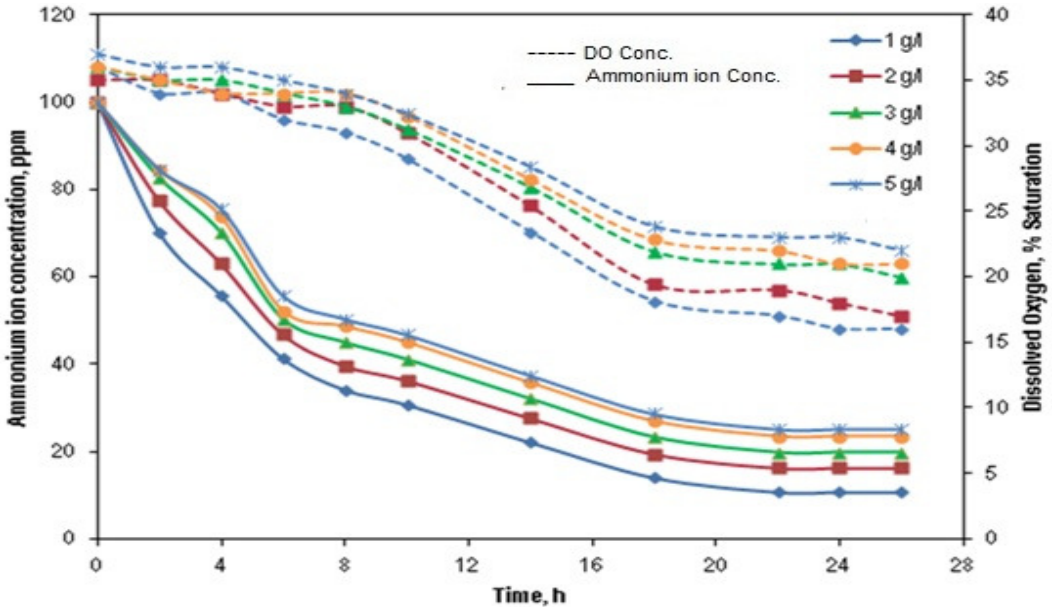

Fig. 2. Effect of KH_2PO_4 on ammonium ion concentration and DO

3.3 Effect of Sodium Hydrogen Carbonate on Ammonium Ion Concentration

It was observed from Fig. 3 that the optimal concentration of sodium hydrogen carbonate is 30 g/l and maximum ammonium concentration is removed up to 10.8ppm. It was observed that the rate of removal of ammonium ion decreased at higher concentration of sodium hydrogen carbonate which may be due to formation of bicarbonate.

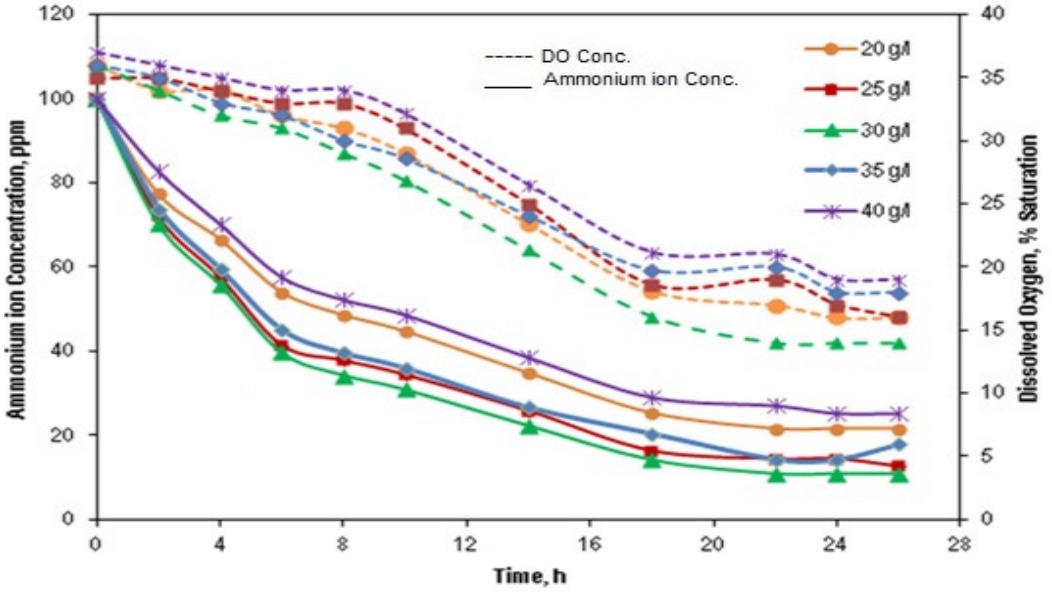

Fig. 3. Effect of $NaHCO_3$ on ammonium ion concentration and DO

3.4 Effect of Sucrose on Ammonium Ion Concentration

It was observed from Fig. 4 that the optimal concentration of sucrose is 7g/l and maximum ammonium concentration removed is up to 10.8ppm from 100ppm. It was also observed that as concentration of sucrose increases the removal rate of ammonium ion increases up to optimal concentration and then decreases with increase in sucrose concentration.

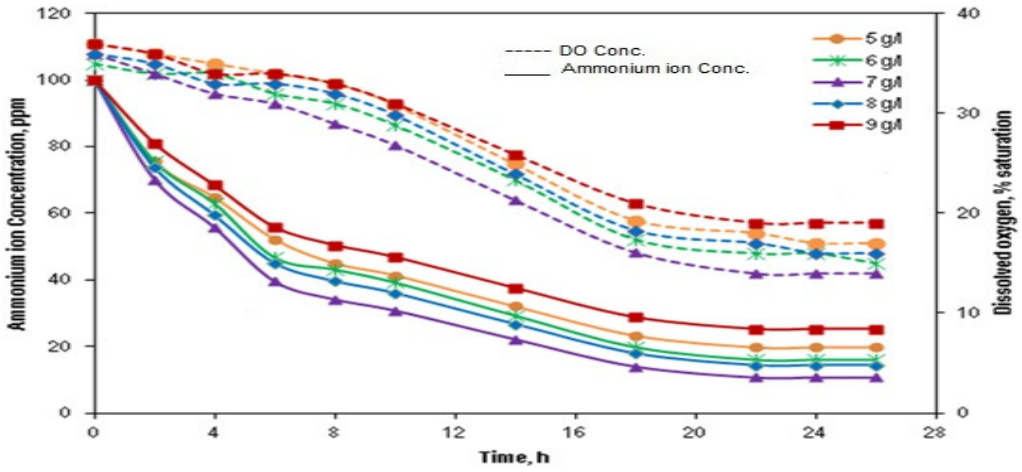

Fig. 4. Effect of sucrose on ammonium ion concentration and DO

3.5 Effect of Ferric Chloride on Ammonium Ion Concentration

It was observed from Fig. 5 that the optimal concentration of ferric chloride is 0.04g/l and maximum ammonium concentration removed is up to 10.8ppm. It is observed that the rate of removal of ammonium ion increases with increase in concentration of ferric chloride and then decreases with increase in concentration of ferric chloride due to excess chlorine in the medium which inhibits the oxidation process.

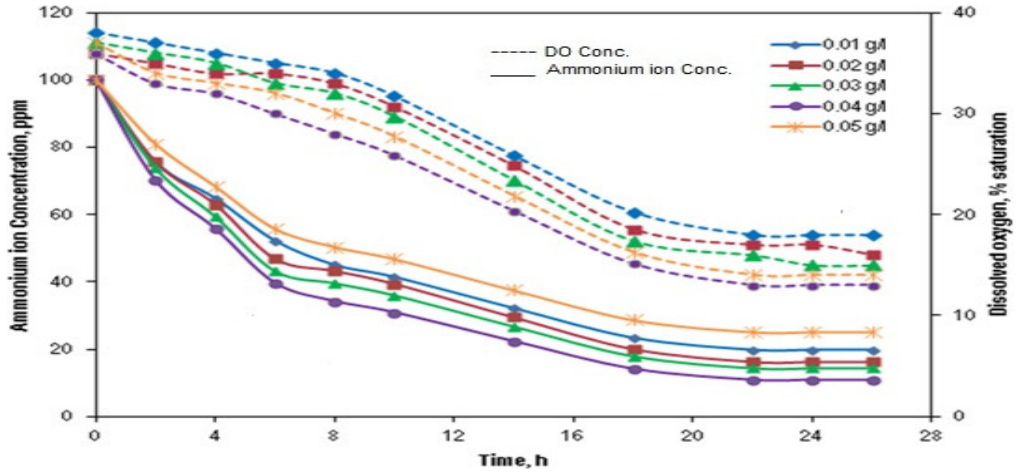

Fig. 5. Effect of $FeCl_3$ on ammonium ion concentration and DO

4. CONCLUSION

Biological nitrification is the most possible way to remove nitrogen compounds in wastewater by two successive reactions: To convert ammonia into nitrite and nitrite to nitrate using ammonia oxidizing bacteria and nitrite oxidizing bacteria. In the present study, the influence of minor nutrients such as di-sodium hydrogen phosphate, potassium di-hydrogen phosphate, sodium hydrogen phosphate, sucrose and ferric chloride were carried for different concentrations over ammonium ion removal and optimum values obtained are 2g/l, 1g/l, 30g/l, 7g/l and 0.04g/l respectively with maximum removal of 89.2%. The influence of dissolved oxygen was also reported for every component. The optimum values of parameters giving maximum removal of ammonium ion were determined experimentally and reported.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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