A Comparative Study on Removal of Ammonical Nitrogen using Chemical and Biological Processes

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Abstract—*Nutrient compound such as ammonical nitrogen (NH_4-N), often present in different types of waters and wastewaters, can find their way to lakes, rivers and drinking water reservoirs and is becoming more important in the alleviation of environmental problems including Eutrophication, corrosion and fouling. For this (1)Study of MAP Process at laboratory scale for the removal of NH_4-N has been carried out along with, (2)The biological treatment using microbial growth of bacterial sppi for treating pharmaceutical wastewater. MAP process is one of the innovative Physico-chemical processes which can majorly contribute for the removal of NH_4-N by forming the struvite complex under required condition. For this we evaluated the effort of feeding sequence of precipitating reagent (alum, flocculent, magnesium and phosphate) for NH_4-N removal by forming MAP i. e. struvite deposit And its comparative study with biological process in which different sppi of bacterias such as Pseudomonas, staphylococcus werneri and nitrosomonas were carried out for removal of NH_4-N. Different sppi gives different percentage of removal of ammonical Nitrogen and their removal efficiencies were compared with MAP Process Results.*

Keywords: *Ammonical Nitrogen, struvite precipitation, MAP, Pseudomonas, Nitrosomonas, staphylococcus werneri, Nitrification-denitrification*

1. INTRODUCTION

Nitrate contamination of water resources is becoming a serious environmental problem worldwide. High nitrate levels in water can cause methemoglobinemia, a condition found especially in infants of age under 6 months. Severe methemoglobinemia can result in brain damage and even death. Also, animals are affected by nitrates in the same way as human babies. Ammonical nitrogen (unionized ammonia, NH_3, and ammonium ion, NH_{4+}) has been found to exist in various types of agricultural, municipal (domestic) and many industrial wastewaters. [2] The presence of nitrogen excess in the aquatic environment has caused serious distortions of the natural nutrient cycle between the living world and the water and soil.

Up to now, the main NH_4^+-N removal processes involve biological nitrification, denitrification, air stripping, [19, 6]chemical treatment and selective ion exchange. [9] Also, ammonical nitrogen has a contribution to corrosion of certain metals and to reduce the amount of dissolved oxygen in water due to nitrification process. High concentration of ammonium in surface water makes it unsuitable as drinking water; ammonium can reduce disinfection efficiency, conduct to nitrate formation, and cause taste and odor problems. The maximum level for drinking water set by the Council of the European Community is of 0. 5 mg NH^{4+}/L. Ammonia is also known to be very toxic for fish; concentrations as low as 0. 2mg/l to 0. 5mg/l can be fatal . Ammoniacal nitrogen also contributes to BOD in water. Nitrifying bacteria require a large amount of dissolved oxygen to convert NH_3 into NO_3.(4. 3mg of O_2 for every 1. 0mg of NH_3). Dissolved oxygen levels are commonly 8-10mg/l and fish require at least 5mg/l. Therefore not only is NH_3 toxic but its presence also significantly reduces the level of dissolved oxygen in slow flow rivers and lakes. In turbulent waters this is less of a problem. If NH_4+ is released into waterways it will be degraded into nitrites and nitrates (NO_2-, NO_3-). Nitrates stimulate algal growth in waterways.

Most of the toxic effluent from dyes and fertilizer industries having high NH_4-N is acidic and this in general are neutralized using lime slurry which brings calcium interference in the picture. As an alternative to eliminate high level of NH_4-N in leachate, the precipitation of NH_4-N by forming magnesium ammonium phosphate (Struvite) has been studied, demonstrating that struvite precipitation is an excellent pretreatment process [3]. Struvite crystallizes as a white orthorhombic crystalline structure, which is composed of magnesium, ammonium, and phosphate in equal molar concentrations[13]. Struvite precipitation is controlled by pH, supersaturation, temperature, and impurities such as calcium. Struvite solubility decreases with increasing pH, while above a pH of 9 its solubility begins to increase[6]. Struvite precipitation will not occur without a nucleation in itself. Struvite precipitation is principally based on the thermodynamic equilibrium of constituent ions in the solution, this study was conducted in the assumption that a performance of struvite precipitation might vary according to feeding sequence of chemicals of magnesium and phosphate source [11]. The formation of struvite crystals significantly reduced

gaseous loss of ammonia and resulted in substantial increase in the ammonia content in the compost, attaining 1. 5%. [8] In this context, the present study was conducted to determine the optimal doses of Mg and P salts for struvite crystallization keeping calcium interference in mind. Various molar ratios are defined in different literature i. e. maximum of 88% ammonia removal at a pH of 9. 5 with added magnesium and phosphate to achieve an ammonium: magnesium: phosphate molar ratio of 1:1. 25:1. [14] Hence it becomes essential to optimize the dosage.

Biological nitrogen removal is performed in nature by bacteria which can only thrive under very specific environmental conditions. In order to make these bacteria perform these functions in the biological treatment process of a WWTP, an understanding of the processes and careful control of process conditions is required. As untreated wastewater can contain nitrogen in the form of organic nitrogen, ammonia (NH_3-N), nitrite (NO_2-N) and nitrate (NO_3-N). Biological nitrogen removal involves initial conversion of nitrogen contained in the wastewater to nitrate (NO_3-N) and then converting the NO_3-N to inert nitrogen gas (N_2) which is released from the wastewater to the atmosphere. Biological treatment of wastewater for removal of nitrogen occurs in 3 steps:

• Ammonification (breakdown of organic N to NH_3-N);

• Nitrification (oxidation of NH_3-N to NO_3-N); and

• Denitrification (conversion of NO_3-N to N_2).

The oxidized nitrogen compounds (NO_2^- and NO_3^-) are reduced to gaseous nitrogen by heterotrophic microorganisms that use nitrite and/or nitrate instead of oxygen as electron acceptors and organic matter as a carbon and energy source. Denitrifiers are common among the Gram negative bacteria such as *Pseudomonas, Alcaligenes, Paracoccus,* and *Thiobacillus.* Some Gram-positive bacteria (such as Bacillus) and a few halophilic archaeal microorganisms (e. g. Haloferax denitrificans) are able to denitrify. [10, 5] Unlike some contaminants which are in need for a certain microbe to be treated, denitrifying bacteria are ubiquitous in nature[18] and numerous researchers cultivated them using mixed cultures taken from wastewater treatment plants as seeds. Dong Wei(2013) showed biological nitrogen removal (BNR) is commonly used in wastewater treatment plants involving two processes: NH_4-N is oxidized to NO_3^--N during aerobic nitrification process and NO_3^--N to molecular nitrogen during anoxic denitrification. Compared to conventional nitrification–denitrification processes, shortcut nitrification–denitrification process via NO_2^--N (full nitritation, 100% ammonium conversion to nitrite) [7]

For the nitrogen biological removal it is required the occurrence of two biological processes, which include the nitrification process, conducted under aerobic conditions and consists of two consecutive phases; in the first one, the ammonium is oxidized to nitrite (NO_2^-), which is carried out by bacteria of the genera *Nitrosomonas*, *Nitrosococcus*,

Nitrosospira and *Nitrosolobus.* Subsequently, nitrite is oxidized to

nitrate (NO_3^-), process performed by bacteria *Nitrobacter.* Finally the process of denitrification occurs under anoxic conditions; it is essentially a sequential process in which nitrates are gradually transformed to nitrite, nitric oxide, nitrous oxide, and finally molecular nitrogen, which is released into the atmosphere. [16]The microorganisms involved in denitrification are *facultative heterotrophic* and are usually abundant in domestic sewage; examples are *Pseudomonas*, *Micrococcus* and others (Arceivala, 1981). [1] (Schmidt et al, 2003) review those processes that make use of new concepts in microbiology: partial nitrification, nitrifier denitrification and anaerobic ammonia oxidation (the anammox process). [17] Under two condition of treatment (aerobic and anaerobic) environment in SBR which were applied and three isolated bacteria from worm tea have been used in the semiconductor wastewater treatment; there are *Bacillus pumilus, Micrococcus lutues* and *Staphylococcus warnaeri*(Nur Farehah Z et al , 2014) The results showed efficiencies of removal of ammoniacal nitrogen by Staphylococcus warneri is 97. 2% which remained NH_3N 30 mg/L from initial NH_3N 1000 mg/L, Bacillus pumilus is 60. 7% and Micrococcus lutues is 79. 2% respectively[15]

2.   MATERIALS AND METHODS

2. 1. Raw Wastewater

The raw wastewater used in this experiments was collected from the outlet of the Effluent Treatment Plant (ETP) of a pharmaceutical company located near Vapi, Gujarat. The sample was stored at 4° C immediately after collection. Its initial Characterization are as shown in Table 1

2.2. Chemicals for struvite Formation

Wastewater considered here contain negligible magnesium and concentration of phosphate in comparison to ammonia(NH_3-N)(see Table 1). For the effective removal of ammonia, some phosphate and magnesium salts are required to be added. In this study, $MgCl_2. 6H_2O$ was with 11. 8 % Mg content was used as a source of magnesium in struvite precipitation. As for the phosphate source, Na_2HPO_4 was used with 21. 8 % P content. In addition, $MgCl_2. 6H_2O$, and Na_2HPO_4 of analytical grade were used in this study.

Table 1: Characteristic of Raw Wastewater

Sr. No	Parameter	Values
1.	pH	7. 8-8. 1
2.	TDS	15, 248 mg/L
3.	TSS	48 mg/L
4.	NH4-N	1, 050 mg/L
5.	COD	5, 747 mg/L

2.3. Experimental Procedure for MAP Process

It is a two step process. In the first step precipitants are mixed with wastewater allowing the formation of insoluble metal precipitants. Initially all the parameters are estimated that are listed in table-3. The experiment is carried out in a 1 L of beaker with moderate speed agitator. If the sample is acidic, it has to be neutralized using 10% lime slurry. The sample considered here is alkaline hence direct treatment can be done. Once all the parameters are estimated, calculative amount of precipitating reagents can be added. Other than the chemicals, other important thing in chemical precipitation is pH. Metal hydroxides are amphoteric in nature and can react chemically as acids or bases and their solubility increases towards higher or lower pH.

Here pH should be maintained at about 8. 5 for struvite precipitation. The sequential addition of reagent should be magnesium source followed by phosphate and then allowing the mixture to agitate for about and 15 minutes at moderate speed and estimated the results. Degree of agitation along with pH plays an important role in efficient formation of struvite. After 15 min of continuous stirring, precipitant is allowed to settle down the container. The supernatant is collected and again all the parameters are estimated to find out the parentage reduction in NH_4-N.

2. 3. 1. Stoichiometric Calculation: It is necessary to optimize the dosage. The sample considered here has NH_4-N of about 1050mg/L after pretreatment. Different pretreated and the optimum ratio considered is 1:1:1. The calculation is shown for one sample and rest are calculated as the same manner.

Table 2: Calculation Of Precipitating Reagent for Ratio 1:1:1

NH_4-N present in the sample	1. 050 /14 = 0. 075 gmol
Mg required to treat the sample,	0. 075 x 1 = 0. 075 gmol
Mg(24) content in MgCl2. 6H2O (203)	=11. 82 %
Mg Required	0. 075 x1x24/0. 118 = 15. 254 g/l
Na2HPO4 required	=(0. 075 x1x31) / 0. 218 = 10. 66 g/l

2. 4. Experimental Procedure for Biological Process

Take 100 ml sample in each set of 3 sterile flask after washing it with distillate water. Inoculate the sample with Nitrosomonas (10 ml) which was cultivated before and keep them on shaker mechanism for 5 days. Process of Nitrification takes place under aerobic condition only. Now add denitrifying bacterias such as *Pseudomonas, Staphylococcos Werneri,* and *Anaerobic* Mixed bacterial Culture into three different samples in anaerobic condition, as the process of denitrification takes place most efficiently in anaerobic condition. Label them properly. Analyse the result after next 5 days for reduction in NH_4-N. For Biological Nitrogen removal, 10 days were given for simultaneous nitrification and denitrification process to take place.

3.   RESULTS AND DISCUSSION

To carry out MAP Process for struvite formation pH should be raised upto 8. 5. After raising pH, MAP Process is Carried out keeping the ratio of Mg:N:P = 1:1:1 constant and changing time period chemical reaction.

3.1. Reduction By MAP Process:

- Mg:N:P= 1:1:1
- pH- 8. 5

Table 3: Reduction of NH_4-N By MAP Process

Sr. No	Time period (min)	pH	NH4-N after treatment (mg/L)	COD after treatment (mg/L)
1.	0	8. 5	1050	5747
2.	15	4. 5	426	4990
3.	30	4. 3	434	4736
4.	60	4. 2	420	4854
5.	90	4. 2	426	4786
6.	120	4. 2	426	4748

It is clear from the above table that the above MAP process is not time based and its moles to moles reaction. It is quick chemical reaction process. And the reduction in ammonical nitrogen is obtained upto 426 mg/L. Reduction in COD was obtained upto 4748 mg/L.
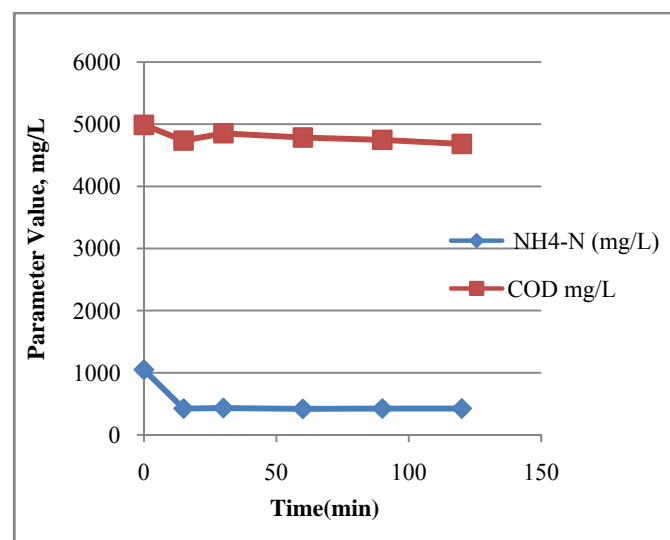

Fig. 1: Graphical presentation of % Removal of NH_4-N

3.1.1. Varying different molar ratios of Mg and P

For maximum removal of NH_4-N by varying magnesium chloride and Di-sodium Hydrogen Phosphate ratios, we get the following results for reduction in ammonical nitrogen in form of struvite formation. The table below shows the different ratios with mixing time 15min, the molar ratio range of (a) Mg:N:P=(0. 8-1. 3 : 1 : 1); (b) Mg:N:P= (1 : 1 : 0. 8-1. 3)

Table 4(a): Percentage removal of NH4-N for Mg:N:P=(0. 8-1. 3 : 1 : 1)

- pH=8. 01
- Initial NH$_4$-N- 1050 mg/L

Sr no	Ratio Mg:N:P	Final NH4-N (mg/L)	% Removal of NH4-N
1.	0. 8:1:1	378	64
2.	0. 9:1:1	364	65. 33
3.	1:1:1	336	68
4.	1. 1:1:1	322	69. 3
5.	1. 2:1:1	322	69. 3
6.	1. 3:1:1	329	68. 6

3.1.2 Graphical representation of NH$_4$-N removal for Mg:N:P=(0. 8-1. 3:1:1)
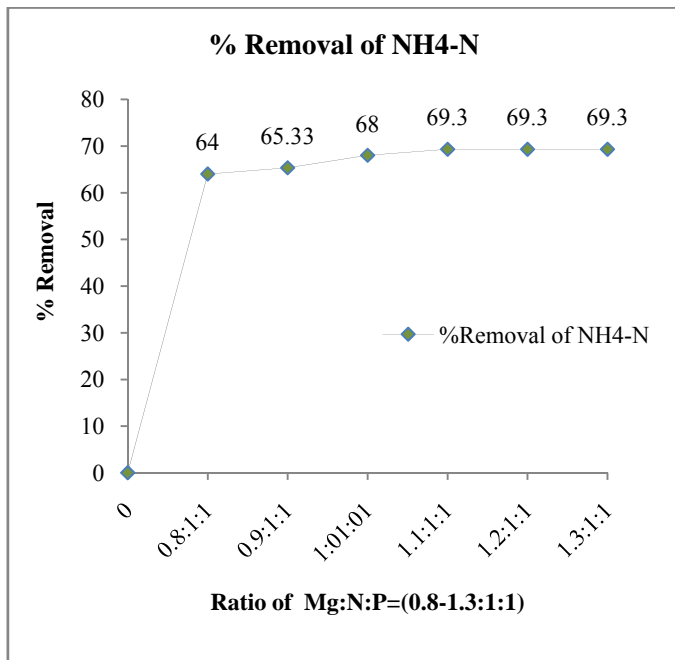

% Removal of NH4-N

Fig. 2: Graphical Representation of %removal of NH$_4$-N

Table 4(b): Percentage removal of NH$_4$-N for Mg:N:P=(01 : 1 : 0. 8-1. 3)

- pH=7. 5
- NH$_4$-N=1050 mg/L

Sr. No	Ratio Mg:N:P	NH4-N after treatment (mg/L)	% Removal of NH4-N
1.	1:1:0. 8	266	74. 47
2.	1:1:0. 9	308	70. 6
3.	1:1:1	336	68
4.	1:1:1. 1	350	66
5.	1:1:1. 2	364	65. 33
6.	1:1:1. 3	364	65. 33

1.3 Graphical representation of NH$_4$-N removal for Mg:N:P=(1:1:0. 8-1. 3)

The Experimental results showed that NH$_4$-N removal reached nearly the maximum values at the stoichiometric ratio, when Mg:N:P was of the molar ratio 1:1:0. 8. Molar Ratio of 1:1:0. 8 was determined to be sufficient for the removal of NH$_4$N from Pharmaceutical wastewater by struvite precipitation.
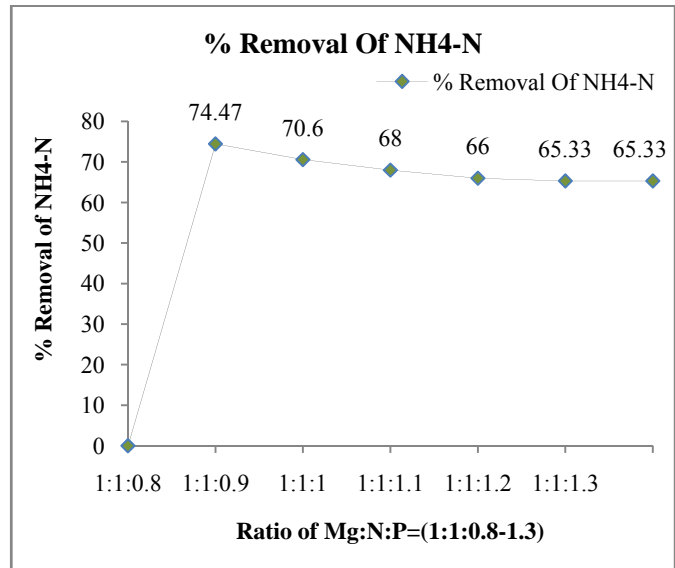

% Removal Of NH4-N

Fig. 3: Graphical representation of NH$_4$-N removal for Mg:N:P=(1:1:0. 8-1. 3)

3.2 Biological Process Results

As bacteria are easily available and their reduction in ammonical nitrogen have also been noticed, different species of bacterial culture along with their mixed combination have been studied. Waste sample is treated by Nitrosomonas bacteria upto 5 days for nitrification and after that denitrifiers such as *Pseudomonas, Staphylococcus werneri*, and *Anaerobic Mixed Culture* are added and again kept in incubator for other 5 days. Their results are shown below table 5.

Table 5: Percentage Removal Of NH$_4$-N by bacterial *Species*

- Initial NH$_4$-N - 1050 mg/L
- Initial COD - 4990 mg/L

Sr. No	Bacterial Species	Reduction in NH4-N (mg/L)	% Reduction
1.	Pseudomonas	226	78. 47
2.	Staphylo coccus Werneri	126	88
3.	Anaerobic Mixed Culture	406	61. 3

From above results it is clear that *Staphylo coccus Werneri* bacteria gives the maximum reduction in NH$_4$-N from all the other bacterial species. Reduction in NH$_4$-N is obtained upto 88% and along with it reduction in COD is about 10. 6 % only. *Pseudomonas Species* give maximum COD reduction . i. e 63%, that was checked during experimental work. But for a comparative study against removal of ammonical nitrogen using chemical and biological processes, biological process proved to be more effectively and efficiently. It is non polluting as well as do not generate sludge that needs to be

discarded. Only the problem exists in biological processes are they take much time to give final results and species used needs much favourable condition to grow.

Here in this study, Chemical process such as MAP Process gives maximum reduction up to 73. 3 % whereas biological process using *Staphylo coccus Werneri* bacteria gives upto 88 % reduction in ammonical Nitrogen and noticable reduction in COD upto 21. 44% by MAP Process and 49. 93% by Biological Process. Also the sludge in form of struvite complex formed here can be sold out to fertilizer making industries. So, chemical process can now also be more favourable for most effective removal of NH_4-N from waste stream.

4. CONCLUSION

From Chemical Process, if MAP process is used for NH_4-N reduction, depending on the waste parameters, it can not only reduce ammonical nitrogen but also the complex formed in this process is in a form of struvite which can be used for many other purposes such as horticulture, agriculture and for recovery of N and P. Thus, using Chemical Process also is more convenient for any industries to treat their waste, as no industries will be able to consume their time for biological process to take place for more than 1 to 2 days. And for reduction of NH_4-N, there will be atleast 10 days given for their biological process to carry out.

5. ACKNOWLEDGEMENTS

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