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Removal of Ammonical Nitrogen using Biological Bacterial process.

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***Abstract-* The study culminates in the development of a hybrid process in abatement of one environmental problem of the industries generating ammonical wastewater. The removal of NH4-N from Pharmaceutical wastewater was conducted successfully on a laboratory sale. The Scope of this research or experiment was to study the Biological Process by the use of mixed bacterial culture for the treatment of Pharma wastewater. Mixed bacterial culture with different concentration were used for biological nitrification and denitrification process for a comparative study. In biological process, different concentration of bacterial inoculum were innoculated in anaerobic condition. The results shows around 90% reduction in COD and Ammonical nitrogen by this process.**

***Key words:* Ammonical Nitrogen, Pharmaceutical Wastewater, Bacterial Process, Inoculation, Incubation.**

I. INTRODUCTION

The Indian pharmaceutical industry is estimated to grow at 20 % compound annual growth rate (CAGR) over next five years, as per India Ratings.[I] Pharmaceutical industry is one of the major industries causing water pollution. In India, Pharmaceutical industry generates about Gallons of wastewater processed depending upon the process employed and product manufactured. As per latest statistics available, there are total of 6631 units engaged in the production and formulation of drugs and pharmaceuticals including fine chemicals in the country.Treatment processes reduces the concentrations of pharmaceuticals in water, but the degree of efficiency is often a function of: a)chemical structure, b)cost, and c)energy. Life-cycle analyses should be undertaken to ensure that the solutions for environmental control are not more risky than the problem. (**Shane Snyder,2008)** Source control of contaminants to wastewater treatment plants should always be considered when unknown or questionable occurrence in effluents is predicted or observed.[36] Biological nitrogen removal is performed in nature by bacteria which can only survive under specific environmental conditions. 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), in order to make these bacteria perform these functions in the biological treatment process of a Waste Water Treatment Plant, their proper understanding of processes and careful control of process conditions is required. Biological nitrogen removal involves three steps in which initial conversion of nitrogen contained in 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. Biological treatment for removal of nitrogen of wastewater occurs in 3 steps:

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

Equations describing the oxidation of NH_3-N to NO_2-N and oxidation of NO_2-N to NO_3-N are presented as follows:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{New Cells}$$

$$2NO_2 + O_2 \longrightarrow 2NO_3^- + \text{New Cells}$$

- Nitrification (oxidation of NH_3-N to NO_3-N)
- Denitrification (conversion of NO_3-N to N_2)

$$6NO_3^- + 5CH_30H \rightarrow 3N_2 + 5C0_2 + 7H_20 + 6\ 0H^- + \text{New Cells}$$

The oxidized nitrogen compounds (NO2 − and NO3−) 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.[16,8] Unlike some contaminants which are in need for a certain microbe to be treated, denitrifying bacteria are ubiquitous in nature [27] and numerous researchers cultivated them using mixed cultures taken from wastewater treatment plants as seeds. In recent years, new biological nitrogen removal processes are wildly investigated due to their superiority to traditional BNR. Compared to conventional nitrification–denitrification processes, shortcut nitrification–denitrification process via NO2--N (full nitritation,100% ammonium conversion to nitrite). For the nitrogen biological removal it is required the occurrence of two biological processes, which include the nitrification process, conducted under aerobic conditions and

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consists of two consecutive phases; in the first one, the ammonium is oxidized to nitrite (NO2−), which is carried out by bacteria of the genera Nitrosomonas, Nitrosococcus, Nitrosospira and Nitrosolobus. Subsequently, nitrite is oxidized to nitrate (NO3−), 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.31 The microorganisms involved in denitrification are facultative heterotrophic and are usually abundant in domestic sewage; examples are Pseudomonas, Micrococcus, Rizobium, Nitrobacter, etc (Arceivala, 1981).[3]

Schmidt,(2003) review those processes that make use of new concepts in microbiology: partial nitrification, nitrifier denitrification and the anammox process. The review addresses the microbiology, its consequences for their application, the current status regarding application, and the future developments.[26] Li Y Z, et al (2004) during their study at laboratory scale experiments investigate the nitrogen removal from pharmaceutical manufacturing wastewater. The results indicate that by selective inhibition of free ammonia on oxidizers, nitrogen removal can be achieved by nitrification and denitrification process. The nitrite ratio was above 98% in the aerobic stage and the nitrogen removal efficiency was about 99%. The complete ammonia removal corresponded exactly to the "Ammonia Valley" in the pH versus time graphic and the anoxic reaction was completed when the "Nitrite Knee" appeared in the ORP versus time graphic. Peng Y Z, et al (2006) in their study showed laboratory scale experiments which were conducted by applying a Sequencing Batch Reactor (SBR) activated sludge process to a wastewater stream from a pharmaceutical factory. Their studies concluded that average accumulation rate of nitrite was much higher than that of nitrate.[29] Cai-Hong Yu, et al (2012) through his experiments showed the simulation of ammonia nitrogen wastewater, set up with different environment conditions, including the time, inoculums volume, temperature, pH value, ammonia nitrogen concentration, carbon addition and shaking revolution. Nur Farehah Z. A , Norli I, et al (2014) has shown 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. They are are Bacillus pumilus, Micrococcus lutues and Staphylococcus warnaeri.[26]

MATERIAL AND METHODOLOGY

Wastewater collection: The treated wastewater 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.

Chemicals used: Chemicals used for the various treatments are as follows: Alum, NaOH, Potassium dichromate (K2Cr2O7), Sulfuric acid (H2SO4) about 98%, Mercuric sulfate (HgSO4), Silver Sulphate (AgSO4), Ferroin indicator, Boric Acid Indicator, Borate Buffer Solution, Peptone Water.

Instruments Used: pH Meter, Analytical Balance, Chemical Oxygen Demand(COD) Apparatus, Jar Test apparatus, Kjeldahl analytical Sampler, Magnetic Stirrer, Biochemical Oxygen Demand(BOD) Apparatus, Gas Chromatograph(GC), Incubator, Bacterial Incubator.

Experimental setup and Methodology:

Figure 1. Incubation by Shaker Mechanism

Figure 2. Fungal incubation

Figure 3. Bacterial Incubation

Procedure for Fungal Inoculation: Take 100 ml sample in sterile flask after washing it with distillate water. Innoculate the sample with Aspergillus Niger (2 to 3 loopful) which was cultivated before as shown in figure 4.

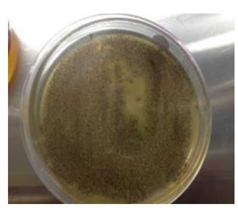

Figure 4. Aspergillus Niger

Figure 5. Fungal Incubation

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Incubate at 22-25 0C in Fungal Incubator. Take another 100 ml of sample in sterile flask and follow the same procedure. Put it on Shaker Mechanism. Both the samples are kept for 5 days and calculate the results for analysis. Peptone water was added for fungal growth of Aspergillus to be activated in the sample throughout their biological cycle.Analyse the result and estimate percentage reduction. Carry Out the process in bio-safety room where the process of inoculation is carried out at lowest temperature as shown in figure 4.

Procedure for Bacterial Inoculation: Mixed bacterial culture of different concentrations were inoculated within the sample and the same above procedure was followed in each set of 3 different sterile flasks and kept in bacterial incubator for 5 days. The mixed bacterial culture posses all the nitrifying and denitrifying bacterias such as Nitrosomonas, Nitrobacter Pseudomonas, Methtanomonas, Achromobacter, S Werneri, Bacillius. Lable them properly. Analyse the results at 72 hrs(3 days), 96 hrs(4 days), 120 hrs(5 days) for reduction in NH4-N and COD. For Biological Nitrogen removal, 5 days were given for simultaneous nitrification and denitrification process to take place. It should be noted that during biological process, colour change was observed after the period of 5 days from brown to light greenish which is shown below in figure

Figure 6. Inoculation of Anaerobic Mixed bacterial sppi

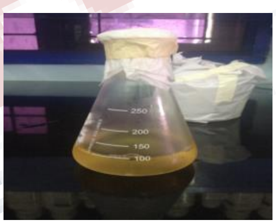

Figure 7. Sample Inoculated with Anaerobic Mixed bacterial sppi

Figure 8. Sample collected after 5 days for Analysis

Figure 9. Sample before Treatment

Figure 10. Sample After Treatment

RESULT ANALYSIS

Characteristics of raw pharmaceutical waste water are carried out to know the nature of the wastewater. To select the proper treatment process the characterization of sample are useful. 40 Lit sample was collected from a Pharma company from the discharge point of treated effluent and the appearance of sample was Dark Brown. Full Characterization of waste before treatment and after treatment were done as shown in table below.

Table 1. Characteristic of Raw Wastewater

Sr. No	Parameter	Method Specification	Method Used	Values
1.	pH	Standard Method by APHA Ed.22nd .2012,4500 - H+B	Glass Electrode	7.8-8.1
2.	TDS	Standard Method by APHA Ed.22nd .2012,2540 - C	Gravimetric Method	15,248 mg/L
3.	TSS	Standard Method by APHA Ed.22nd .2012,4500 - D	Gravimetric Method	22 mg/L
4.	NH_4-N	Standard Method by APHA Ed.22nd .2012,4500 NH_3-B & C	Kjeldal method	1,710 mg/L
5.	COD	Standard Method by APHA Ed.22nd .2012,5220 - B	Open Reflux	5,747 mg/L
6.	BOD (3 Days at 27°C)	IS 3025(Part 44)1993Amd.01	-	1170 mg/L

Table 2. Full Characterization of Raw Wastewater

Sr.no	Parameters	Values mg/L	Permissible Limit mg/L

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1.	Cadmium as Cd	0.168	1
2.	Chlorine as Cl	513.13	600
3.	Hexavelent Chromium	N.A	0.1
4.	Copper as Cu	0.078	-
5.	Cynide as CN	N.A	0.2
6.	Iron as Fe	0.232	3
7.	Magnesium as Mg	N.A	-
8.	Nickel as Ni	1.267	2
9.	Oil & Grease	25.1	10
10.	Phenolic Compounds	N.A	1
11.	Potassium as K	15	-
12.	Sodium as Na	489	-
13.	Sulphate as SO_4^{-2}	90	100
14.	Sulphide as S^{-2}	18.11	2
15.	Zins as Zn	0.916	1
16.	Poly Aeromatic Hydrocarbon	N.A	-

Pre-Treatment By Coagulation Studies: Coagulation treatment of raw waste: Different Dosage of Alum as coagulation process was used initially as a pre-treatment to decrease the Concentration of Ammonical Nitrogen and also in COD values. Their results are being listed in Table-5.

Table: 3 Coagulation of raw wastewater (Pre-Treatment) at pH – 8.02 and Settling Time - 2 Hours

Sr. No.	Alum Dose, gm/L	NH_4-N of Treated Waste, mg/L	% Removal	COD, mg/L	% Removal
1	1	1,510	11.69	5488	4.50
2	2	1,448	15.32	5331.2	7.23
3	3	1,120	34.50	5174.4	9.96
4	4	1,050	38.5	5017.5	12.69
5	5	1,120	34.50	5017.5	12.69

Biological Process Results: While using Aspergillus Niger fungi for checking the results of reduction in ammonical nitrogen, reduction notices were as follows:

Table: 4 Results Of Biological Process Using Fungi Initial NH4-N - 1050 mg/L, Initial COD - 4990 mg/L

Sr. No	Method	Reduction in NH_4-N (mg/L)	% Reduction	Reduction in COD (mg/L)	% Reduction
1.	Incubation	966	8	1110	77.7
2.	Shaker Mechanism	966	8	1110	77.7
3.	Anaerobic Treatment	893	14.9	3452	30.82

Above results states that Aspergillus Niger fungi does not give much reduction in ammonical nitrogen but it gives upto 77.7% of reduction in COD values in five days. Fungi helps reduction in COD values but not in NH4-N removal.

Percentage Removal of NH4-N by Biological Process using Bacteria.

As bacteria are easily available and their reduction in ammonical nitrogen have also been noticed, different species of mixed bacterial culture along with their mixed combination have been studied. Waste sample is treated by upto 5 days for simultaneous nitrification and denitrification processes that takes place within the mixed bacteria seeded waste sample and kept in incubator at 350C under anaerobic conditions for results and analysis. Their results are shown below. Three different samples of 100 ml each were inoculated with three different concentration of mixed bacterial culture as sample A- 5 ml inoculum, sample B- 10 ml and sample C- 15 ml inoculum respectively.

Table: 5 % Removal by Mixed bacterial Species of Sample A
Initial NH_4-N - 1050 mg/L, Initial COD - 4860.8 mg/ L

Sr. No	**Time, hr**	**NH_4-N (mg/L)**	**% Reduction in NH_4-N**	**COD (mg/L)**	**% Reduction in COD**
1.	0	1050	-	4860.8	-
2.	72	966	8	3606.4	25.8

3.	96	532	49.3	1881.6	61.29
4.	120	280	73.3	784	83.87

Below results are shown of sample B with 10 ml inoculum of mixed bacterial culture.

Above results shows highest reduction in NH4-N and COD as well at time period of 120 hours(5 days) which gives values of NH4-N as 280 mg/L i.e 73.3 % reduction and COD values of 748 mg/L i.e. 83.87 % reduction. Below results are shown of sample B with 10 ml inoculum of mixed bacterial culture.

Table: 6 % Removal by Mixed bacterial Species of Sample B

Initial NH4-N - 1050 mg/L, Initial COD - 4860.8 mg/L

Sr. No	Time, hr	NH_4-N (mg/L)	% Reduction in NH_4-N	COD (mg/L)	% Reduction in COD
1.	0	1050	-	4860.8	-
2.	72	938	10.6	3449.6	29.03
3.	96	518	50.6	1568	67.74
4.	120	448	57.3	627.2	87.09

Table:7 % Removal by Mixed bacterial Species of Sample C

Initial NH4-N - 1050 mg/L, Initial COD - 4860.8 mg/L

Sr.No	Time, hr	NH_4-N (mg/L)	% Reduction in NH_4-N	COD (mg/L)	% Reduction in COD
1.	0	1050	-	4860.8	-
2.	72	826	21.3	3136	35.4
3.	96	448	57.3	1254.4	74.19
4.	120	70	92	313	93.56

From above results it is clear that maximum reduction n NH_4-N as well as COD values using mixed bacterial species is achieved upto 92 and 93.53 %. 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 becomes problem of disposal. Only the problem exists in biological processes are that, they take much time to give final results and species used needs much favourable condition to grow. Given table below shows all the final estimated parameter values of the sample treated with mixed bacterial culture by biological process performed.

Table: 8 Characterization of Treated Sample by Biological Process

Sr.No	Parameter	Values before treatment	Values After treatment
1.	pH	7.8-8.1	7.4
2.	TDS	15,248 mg/L	17,250 mg/L
3.	TSS	22 mg/L	22 mg/L
4.	NH_4-N	1,710 mg/L	70 mg/L
5.	COD	5,747 mg/L	313 mg/L
6.	BOD(3 Days at 27^{o}C)	1170 mg/L	350 mg/L
7.	Cadmium as Cd	0.168	0.162 mg/L
8.	Chlorine as Cl	513.13	512 mg/L
9.	Hexavelent Chromium	N.A	N.A
10.	Copper as Cu	0.078	0.06 mg/L
11.	Cynide as CN	N.A	N.A
12.	Iron as Fe	0.232	0.232 mg/L
13.	Magnesium as Mg	N.A	N.A
14.	Nickel as Ni	1.267	1.152 mg/L
15.	Oil & Grease	25.1	25 mg/L
16.	Phenolic Compounds	N.A	N.A
17.	Potassium as K	15	11 mg/L
18.	Sodium as Na	489	420 mg/L
19.	Sulphate as SO_4^{-2}	90	90 mg/L
20.	Sulphide as S^{-2}	18.11	18.11 mg/L
21.	Zinc as Zn	0.916	N.A
22.	Poly Aeromatic Hydrocarbon	N.A	N.A

Here in this study, biological process using bacterias gives upto 92 % reduction in ammonical Nitrogen and noticable reduction in COD upto 93.56% by Biological Process.

CONCLUSION

Based on the results of the experimental tests, the following conclusions could be drawn: pH was important parameter in the removal of NH4-N from particular sample that was used during research work. The optimum pH was observed at pH 10.5.But at the end of the process treated waste comes out to be in pH range of neutral i.e. 7-7.5. Biological process using bacterias gives upto 92 % NH4-N reduction. Process reduces COD in biological processes which gives 93.56% reduction which is more efficient. Biological process takes 5 days to reduce the concentration of ammonical nitrogen and COD as well. Inspite of all, though biological Process are time consuming and generate sludge, they are cost effective and eco-friendly.

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