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De-Ammonification Of Ammonical Effluent Using Dregs Microflora Of Mangrove

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Abstract:
Effluents released from industries are the day-to-day firm for environment especially marine aquatic life. The removal of ammonical nitrogen from effluent has become an important role of the treatment process due to its significant impacts on the environment, human health, and the stringent legislation on waste water discharges. Because of the wide range of technologies and their drawbacks, the novel and cost-effective biological treatment processes take the advantages of the ability of micro-organisms to use diverse waste water constituents to provide the energy for microbial metabolism and building blocks for cell synthesis. The study emphasize is focused to removal of ammonical nitrogen using dregs nitrifying microflora and improve the waste water quality. There are numerous natural sources that contain nitrifying bacteria that will facilitate the conversion of toxic ammonia to non-toxic nitrate. The experimental system used is based on aerobic ammonia oxidation and system treated real industrial waste water for 6 hours cycle for 15 days within every sequential batch process. The high strength ammonia was brought upto the limit within 18 hours of bacterial activity. During this period, it showed the capacity for oxidizing all ammonium at a rate of 99.97% with the Nitrification efficiency of 95.22%. This paper will benefit researchers and industrialists in planning and designing an appropriate enrichment of nitrifying microflora and widen its application in biological ammonia removal system for nitrogenous effluent.

Keywords: ammonical nitrogen, dregs nitrifying microflora, aerobic ammonia oxidation, nitrogenouseffluent.

1. Introduction:

Effluents generated by industrial process activities are a major concern in most countries. Industrial effluents often contain nitrogenous compounds (ammonia, nitrite, nitrate) and phosphorous, which cause environmental deterioration at high concentration [1]. The excess amount of ammonia in a fertilizer manufacturing effluent is a potential hazard to the environment. The discharge of untreated waste water with a high ammonia concentration in the receiving water can harm aquatic life because the presence of the substance may lead to serious problems including oxygen depletion, eutrophication and red tidal phenomena. Many waste water treatment plants are subject to discharge permits, which limit the amount of ammonia that can be lawfully discharged. Government permits and regulations influence levels of ammonia and nitrite that may be discharged into streams [2] [3]. The discharge of ammonia or nitrite into a stream, lake or river may cause oxygen to be consumed thereby lowering the dissolved oxygen concentration and endangering the aquatic ecosystem [1].

Ammonia (NH_3) is the product of fish respiration and decomposition of excess pf organic matter. Chemoautotrophic bacteria (Nitrosomonas and Nitrobacter) tend to oxidize ammonium ions (NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-) ions [4] [5]. Nevertheless, these ions are removed by aquatic plants, algae and bacteria, since they assimilate them as a source of nitrogen. These nitrogen compounds are nutrients for generating eutrophication which disrupt aquatic ecosystem in a severe manner as shown in **Fig.1.** [1]

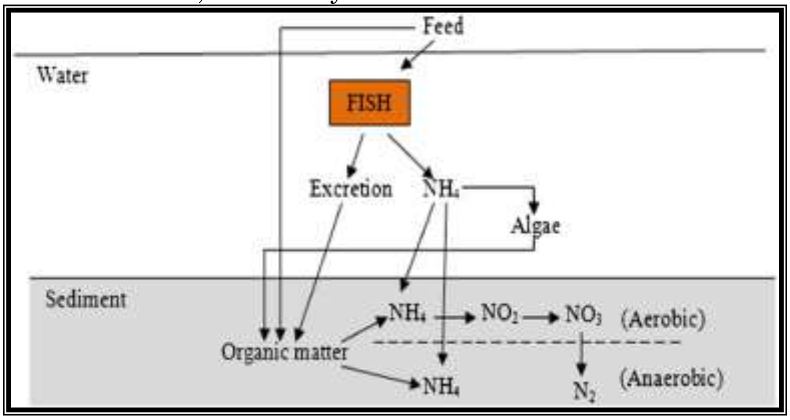


Figure 1: Nitrogen transformation in fishpond [1]

Several studies have been conducted on the toxicity of nitrate on aquatic animals and results indicate that nitrate reacts with haemoglobin causing shortage of oxygen in their body (methaemoglobin) and finally death. When nitrate enters human intestines, it is also converted into nitrite under anaerobic conditions and these may lead to methaemoglobinaemia in infants. Besides that, formation of nitrosamines from nitrite can give rise to cancers of the digestive tract, since nitrosamines are the most efficacious carcinogens in mammals. Therefore, World Health Organization (WHO) established the limit for nitrate in drinking water to 10 mg NO_3-N/L. So, treating waste water by removing ammonia is considered to be an environmentally crucial step before the water can be safely discharged [2] [3].

There are few methods for the ammonical nitrogen removal which are chemical, physical and biological treatment like adsorption, chemical precipitation, membrane filtration, reverse osmosis, ion-exchange, air stripping, breakpoint chlorination and biological nitrification and de-nitrification [6]. However, chemical and physical methods of treatment produced secondary pollutants which are form through chemical and photochemical reactions and toxic to humans. Waste water is added to sequencing batch bioreactor to remove undesirable components, prior to discharge. This reactor works by loads repeating sequences such as filling, aeration, recycling and emptying in a set time period. Each treatment length and both sequences depend on the type of water to be treated and the organic matter or nutrient wanted to be removed.

Nitrification is the biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of these nitrites into nitrates. Nitrification is an important step in the nitrogen cycle. The oxidation of ammonia into nitrite, and the subsequent oxidation to nitrate is performed by two different bacteria. The first step is done by bacteria of (amongst others) the genus Nitrosomonas. The second step (oxidation of nitrite into nitrate) is (mainly) done by bacteria of the genus Nitrobacter. All organisms are autotroph, which means that they take carbon dioxide as their carbon source for growth. Nitrification also plays an important role in the removal of nitrogen from industrial wastewater. In most environments both organisms are found together, yielding nitrate as the final product. Nitrification is a process of nitrogen compound reduction:

$$NH_3 + O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 3H^+ + 2e^-$$

$$NO_2^- + H_2O \xrightarrow{\text{Nitrobacter}} NO_3^- + 2H^+ + 2e^-$$

Nitrifying bacteria are classified as obligate chemolithotrophs. This simply means that they must use inorganic salts as an energy source and generally cannot utilize organic materials. They must oxidize ammonia and nitrites for their energy needs and fix inorganic carbon dioxide (CO_2) to fulfill their carbon requirements. They are largely non-motile and must colonize a surface (gravel, sand, synthetic bio-media, etc.) for optimum growth. They secrete a sticky slime matrix which they use to attach themselves. Species of Nitrosomonas and Nitrobacter are gram negative, mostly rod shaped, microbes ranging between 0.6-4.0 microns in length. They are obligate aerobes and cannot multiply or convert ammonia or nitrites in the absence of oxygen. The temperature for optimum growth of nitrifying bacteria is between 25-30°C while the pH is between 6.5 and 8.5. Maximum nitrification rates will exist if dissolved oxygen levels exceed 80% Saturation. Nitrification will not occur if dissolved oxygen concentrations drop to 2.0 mg/l (ppm) or less [2].

Thus, many studies have focused on developing techniques for culturing the bacteria with the shorter startup period to establish a successful nitrification culture. The duration of the startup period of the aerobic ammonia oxidation process is influenced by the proper selection of sludge, reactor type and optimal operational conditions [7].

The present study aimed for removal of ammonical nitrogen of fertilizer manufacturing discharge effluent containing high concentration of ammonical nitrogen by nitrification process and improves the quality of water discharge to the sea for betterment of aquatic life.

2. Methodology:

2.1. Source of Microflora

Natural sediment (leguminous roots) containing nitrifying bacteria that facilitate the conversion of toxic ammonia to non-toxic nitrate was collected from green belt plantations within the premises of 800 Acres out of which 35 Acres is natural marshy wetlands.

Mangrove Belt

North Drain

Figure 2: Microflora collection from green belt

2.2. Feasibility study of Industrial and Synthetic waste water

The high-strength ammonium waste water (N-wastewater) was collected from effluent treatment plant (ETP) of R.C.F Ltd, Alibag. In order to investigate the effect of ammonium, 1000 mg/L of synthetic waste water was prepared using NH_4Cl which is same with fertilizer manufacturing industrial wastewater in terms of high concentration of ammonical nitrogen.

Figure 3: N-Wastewater Collection from balancing pond

2.3. Analysis of effluent

Various physicochemical parameters were evaluated daily after every 24 hours such as pH, Free ammonia, Total Ammonical Nitrogen (TAN), Total Kejadal Nitrogen (TKN), Nitrate, Total Suspended Solids, M-Alkalinity, Turbidity, Conductivity, Total Hardness, Ca-Hardness, Mg-Hardness, Iron, Phosphate, etc.

2.4. Experimental setup and its operational strategy

The Sequencing Batch Reactor (SBR) was filled with high concentrated ammonium waste water, inoculated with culture of nitrifying organisms, and was operated batchwise with aeration and mixing. The reactor operation was carried out within 30 days for 24 hours' aerobic process. The process of nitrification was carried out by the microorganisms in the SBR and resulted in oxidation of ammonical nitrogen and generation of nitrate. Some volume of this nitrate containing water was fed to further phycoremediation process while rest was recycled.

Top View **Front View**

Figure 4: Sequencing Batch Reactor (SBR)

2.5. Determination of Nitrifying bacteria

Samples were taken during aeration to determine the population density (O.D) of nitrifying bacteria present in the reactor. Growth curve for nitrifying bacteria was detected by using nitrate analyzer. Also, morphological characterization of bacteria was done using gram staining technique. Finally, Bactaslyde analyses involved the determination of Total Bacterial Count (TBC) using Microbe Detection device.

2.6. Hydroponic Analysis

Epipremnum aureum (Money plant) were hydroponically grown on 3 different samples of industrial waste water in a round bottom flask. The ammonia waste water was labeled W1, nitrate water as W2 and diluted nitrate water was labeled as W3. Thus, the wastewater with hydroponic system were labeled and samples were taken before and after for analysis.

3. Results and Discussion

3.1. Factors affecting optimum growth of Microflora.

Various parameters required for successful nitrification process was checked for leguminous sediment containing roots.

Table 1: pH, Temperature, Dissolved Oxygen compared with Optimum Range.

Parameters	Values	Optimum Range
pH	7.5	6.5-8.5
Temperature	28ºC	25-30ºC
Dissolved Oxygen	4 ppm	Not less than 2 ppm

3.2. Comparision of Synthetic wastewater with N-Industrial waste water.

The feasibility study of synthetic wastewater along with N-wastewater produced the following applicable parameters.

Table 2: Comparative Study of Synthetic and N-Industrial waste water.

Parameters	Synthetic waste water	N-Industrial effluent
Free ammonia	1000	1440
pH	8.7	9.31
Nitrate	35	21.7
Phosphate	0.041	0.069

Figure 5: Comparison of effluent and synthetic waste-water.

3.3. Physico-chemical analysis before and after 24 hours of treatment.

Table 3 shows some of the physico-chemical characteristics of raw effluent and treated water under experimental study, at Effluent Treatment Plant

.**Table 3:** Physico-chemical analysis of Raw and treated effluent.

Parameters (units)	Raw Effluents	Treated water	% change	
			% Increase	% Decrease
pH	9.3	6	-	35.48
Free ammonia (ppm)	1440	22	-	98.47
TAN (ppm)	303	0.074	-	99.97
Nitrate (ppm)	149	3122	95.22	-
M-Alkalinity (ppm)	1100	20	-	98.18
Turbidity (NTU)	68	60	-	11.76
Conductivity(µs or ms)	2.85 ms	3.56 ms	19.94	-
Total Hardness (ppm)	100	305	67.21	-
Ca-hardness (ppm)	60	186	67.74	-
Mg-hardness(ppm)	40	124	67.74	-
Iron (ppm)	0.052	1.183	95.60	-
Phosphate (ppm)	2.8	15.884	82.37	-

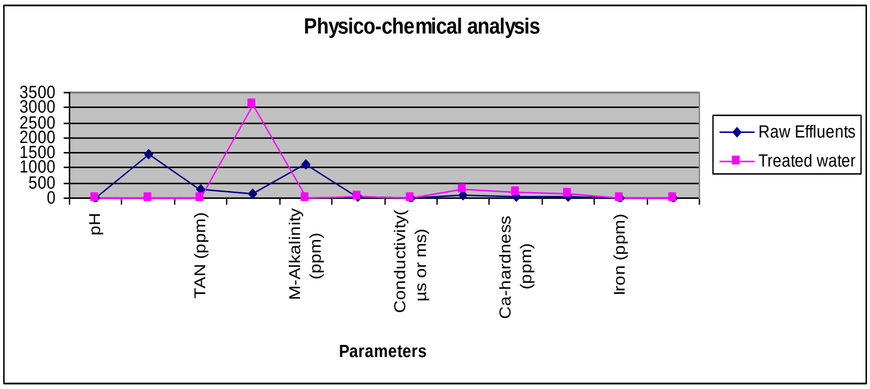


Figure 6: Comparison of various physico-chemical parameters.

3.4. Ammonia removal, pH reduction and Nitrifying bacteria growth curve in SBR for 48 hours.

Ammonia removal and growth of nitrifying bacteria have been determined using SBR. The influent ammonia concentration in industrial and synthetic waste water was initially 1440 ppm and 1000 ppm respectively. The high strength ammonia was brought within the limit within 18 hours of bacterial activity (the prescribed MPCB limit for ammonia is 100 ppm). In 48 hours, the concentration of ammonia was reduced to a range between 22-28 ppm with decreasing pH upto 6. The graph obtained is shown in Figure 7, 8

Table 4: High efficiency of Ammonia Removal with Nitrifying bacteria for Industrial Effluent

Time (Hrs)	Industrial Effluent			Nitrate (ppm)	% Nitrification efficiency
	Total ammonia (ppm)	% Removal of Ammonia	pH		
0	1440	0	9.31	149	10.34
6	802	44.30	8.5	268	33.41
12	361	74.93	8	385	106.64
18	87	93.95	7.2	504	579.31
24	70	95.13	7	725	1035.71
30	52	96.38	6.8	1641	3155.76
36	40	97.22	6.5	1983	4957.5
42	35	97.56	6.2	2027	5791.42
48	22	98.47	6	3122	14190.90

Table.5: High efficiency of Ammonia Removal with Nitrifying bacteria for Synthetic waste water

Time (Hrs)	Synthetic wastewater (Ammonium Chloride)			Nitrate (ppm)	% Nitrification efficiency
	Total ammonia (ppm)	% Removal of Ammonia	pH		
0	1000	0	8.7	98	9.8
6	720	28	8.5	189	26.25
12	511	48.9	8.2	246	48.14
18	381	61.9	7.5	383	100.52
24	175	82.5	7.2	501	286.28
30	98	90.2	7	673	686.73
36	66	93.4	6.8	891	1350
42	43	95.7	6.5	1052	2446.51
48	28	97.2	6.2	1788	6385.71

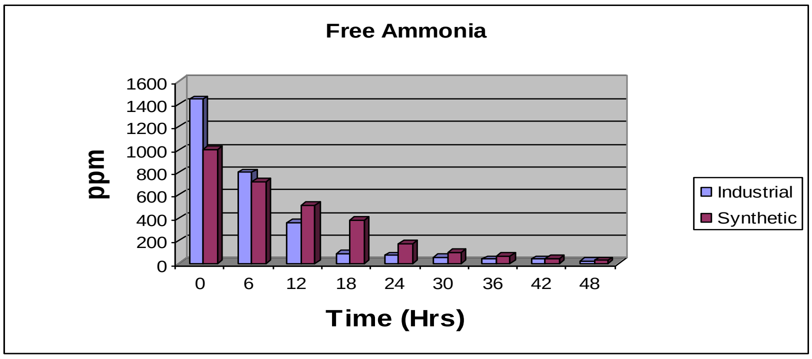


Figure 7: Ammonia removal efficiency in Industrial and Synthetic waste water.

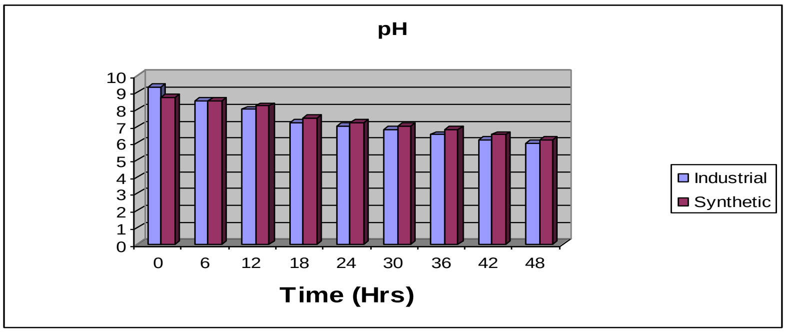


Figure 8: pH reduction in Industrial and Synthetic waste water.

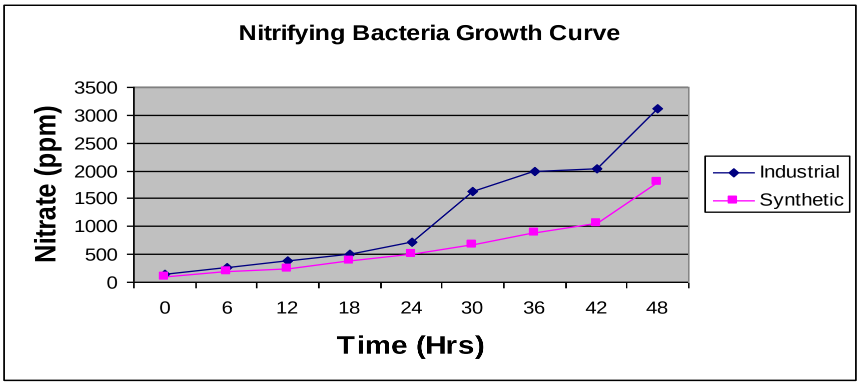
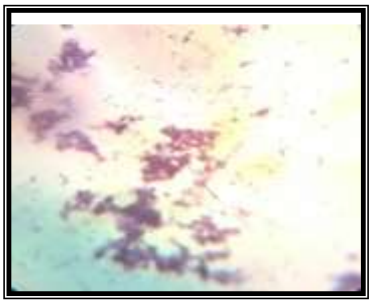


Figure 9: Nitrifying Bacteria Growth Curve.

The % Nitrification efficiency was found to be 95.22% which shows that average nitrification rate is very high (Figure 9).

3.5. Total Bacterial Count and Morphological characterization of Microflora



Total Bacterial Count (TBC) Gram Staining

Figure 10: Morphological Characterization of dregs microflora

Figure 10 Confirms Nitrifying Bacteria as Gram negative with Gram Staining test and Brown Colonies on Total Bacterial Count.

3.6. NH_4-N concentration / Cell density profile

The removal of NH_4-N was found due to the growth of nitrifying bacteria and thus nitrification step was carried out quickly and efficiently without inhibition and accumulation in any stage **(**NH_4 to NO_2^- and NO_2^- to NO_3^-).

The density of bacteria as shown in Figure 10 has a direct proportional relationship to the remaining of NH_4-N. The NH_4-N removal rate was found to be 99.97 %. This showed that when the density of bacteria increased; the remaining NH_4-N drastically decreased from 303 ppm to 99.67 ppm and then eventually lowered to 0.074 ppm, which means the reduction of Ammonical Nitrogen increased. This is because a large population of nitrifying bacteria transforms NH_4-N to NO_2-N faster. The results of ammonical removal and density of bacteria down to the balance between these two parameters are adequate to promote the growth of nitrifying bacteria, thus achieving efficient removal of ammonical nitrogen.

Table 6: Relation of Percent Ammonical Nitrogen with Cell density.

Days	NH_4-N concentration	% Removal of NH_4-N	Cell density (O.D)
1	303	0	2.8
2	99.67	67.10	9.881
3	74.1	75.54	22.221
4	51	83.16	28.5413
5	40.3	86.69	32.5264
6	32	89.43	35.9112
7	18	94.05	36.005
8	13.8	95.44	38.1712
9	9.09	97	39.952
10	5.89	98.05	40.313
11	2.75	99.09	40.989

12	0.873	99.71	42.4143
13	0.397	99.86	43.119
14	0.105	99.96	45.5264
15	0.074	99.97	48.826

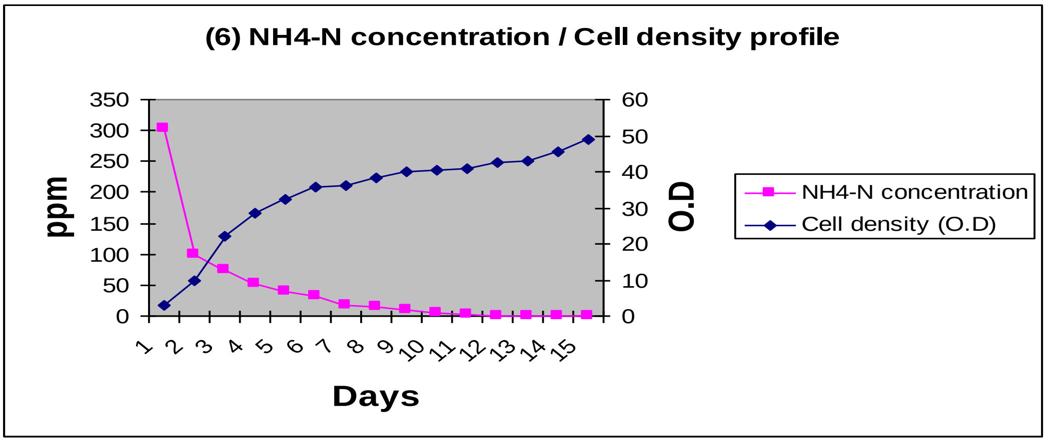


Figure 11: Ammonical Nitrogen Concentration/ Cell density Profile

3.7. Impact of untreated and treated N-Industrial waste water on plant.

Day 1

Day 3

Day 6

Day 8

Figure 12: Hydroponic Analysis using untreated and untreated ammonical waste water

Epipremnum aureum (Money Plant) survived in Nitrate Wastewater (W2) till Eighth day of experiment as compared to ammonia effluent (W1) and Diluted Nitrate water (W3).

4. Conclusion

The biological treatment for the fertilizer manufacturing industrial waste water is one of the Best methods to treat the industrial effluent. This method concerns with biological oxidation using Nitrifying Bacteria which is responsible for reduction of ammonia in nitrification step. The effluent Treatment by Physic-Chemical methods is energy and cost intensive, often resulting in secondary pollutants. Thus, SBR proved to be the Best Alternative for removal of ammonical Nitrogen from Waste water. Complete nitrification can be achieved in the SBR within a period of 18 hours provided with alkaline condition and the effluent will be free of ammonia but rich in nitrate. This natural system reduced ammonia by 99.97% with percent nitrification efficiency of 95.22% respectively.

5. Discussion

Effluents coming from various plants contain high concentrated ammonia which is needed to be treated before it is discharged to the sea, because the high strength Ammonia not only affects marine aquatic life but also the entire ecosystem. For this reason, De-ammonification using Biological method such as nitrification process where biological oxidation of Ammonia takes place along with oxygen into nitrite and then by oxidation process into nitrates. Biotechnological method is a process to have gain very much importance to mitigate in environmental pollution faced by various industries having the process for the Ammonia removal which is carried out in industries such as Dilution with raw water and with Homegenization process by Aeration with the use of floating surface aerators which helps in two way process D.O level malignance and proper mixing of different types of Effluents of high concentration to the level of limited lower concentration. Nowadays many Industries requires lots of Raw water for production and dilution purposes, thus are facing through consequent water shortage problems. These problems can be overcome by using this eco-friendly biological treatment method.

Deammonified water containing large concentration of nitrates can be used in Hydroponics cultivation without soil and can be applicable for cultivation purpose in cities where Agricultural land is not ample. In draught areas, this type of treated water available can be used for Agriculture.

Biological Treatment of ammonical waste water is not only cost effective but also environmentally friendly particularly for aquatic life. This Reaserch work will benefit industrialists and farmers in effective treatment of waste water and to utilize it in order to overcome water shortage problem.

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