RESEARCH ARTICLE

Isolation and Characterization of Heterotrophic and chemo-autotrophic bacteria (nitrifying) from Polluted Pond Water of Raipur District from India

Anjulata Suman Patre* and S. B. Gupta

Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

ABSTRACT

The present investigation was carried out at the Department of Agricultural Microbiology, College of Agriculture Raipur, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2017-2019 on bioremediation of polluted pond water for increasing fish production. In all, 8 treatments were replicated three times and used with Completed Randomized Design (CRD) was inoculation of the fish aquarium with the autotrophic and heterotrophic bacteria from the polluted fish ponds, i.e., T1 (Control), T2 Organic source of N (Urea 2 ppm) + No inoculums, T3 Organic source of N (Urea 2 ppm) + Composite culture of heterotrophic bacteria, T4 Organic source of N (Urea 2 ppm) + It was a mixture of the composite culture of autotrophic and heterotrophic bacteria, T5 Organic source of N (Urea 8 ppm) + No inoculums, T6 Organic source of N (Urea 8 ppm) + Composite culture of heterotrophic bacteria, T7 Organic source of N (Urea 8 ppm) + It was a mixture of the composite culture of autotrophic and heterotrophic bacteria and T8 Organic source of N (Urea 12 ppm) + Composite culture of heterotrophic bacteria. This study was carried out in twenty-four aquariums (volume 75x45x30 cm3) containing 70.0litre water under greenhouse conditions. The soil and water samples from polluted pond water were taken for isolation of autotrophic and heterotrophic bacterial isolates, twenty-five samples from different locations of the Raipur area were collected. In this study, the pond water and pond soil pH, EC (µhmos/cm) & TDS (mg/l) range was observed from 7.4 to 8.2, 17×10 to 38×10& 234 to 508, respectively and 7.6 to 8.4, 21×10 to 40×10 and 310 to 545, respectively. In the polluted pond water, inoculation of autotrophic and heterotrophic bacteria positively affected ammonia concentration and heterotrophic bacteria (Micrococcus luteus and Ochrobactrum pituitosum) PS5 and PS16 isolateare performing best results in decreasing the ammonia concentration and increasing the nitrate concentration, respectively.

Keywords: *Micrococcus lutes, Nitrosomonas, Nitrobacter, Ochrobacterum pitutisom*

INTRODUCTION

Chhattisgarh state of our country is blessed with various water bodies in the form of reservoirs (83,873 ha), ponds (70,000 ha), and rivers (3,573km). However, natural and artificial ponds constitute a significant source of fish culture. Due to tropical climate location, there is a considerable variation in environmental factors like temperature, rainfall, photoperiod, etc., which also physically affect the water bodies. Fish production is influenced by physical characteristics like temperature, pH, sunlight, and chemical factors like dissolved O_2 and CO_2 levels, and levels of inorganic nutrients [1]. For location-specific optimum fish production, it is necessary to know about the variations in environmental factors under local conditions, which can be matched with farmer managerial practices for maximizing fish production.

Water is the most essential component in the environment for life, 70% of the surface of the earth is covered by water, out of which only 3% is freshwater. Freshwater is essential for agriculture, industry, and even human existence; without freshwater of adequate quantity and quality, sustainable development will not be possible. 2.4% is trapped in polar icecaps and glaciers, from which icebergs break off and slowly melt at sea. Less than 1% of water is present in ponds, lakes, rivers, dams, etc., which is used for industrial, domestic and agricultural purposes [2]. Water is one of the abundantly available substances

OPEN ACCESS JOURNAL

Check for updates

Citation: Anjulata Suman Patre and S. B. Gupta (2021). Isolation and Characterization of Heterotrophic and chemo-autotrophic bacteria (nitrifying) from Polluted Pond Water of Raipur District from India. *Chemical Engineering.* v02i02, 01-07. http://dx.doi.org/10.53709/CHE.2021.v02i02.001

DOI: http://dx.doi.org/10.53709/CHE.2021.v02i02.001

Corresponding Author:
Anjulata Suman Patre
anjulatasuman90@gmail.com

Received on: December 8, 2020
Revised on: February 21, 2021
Accepted on: june 01, 2021

in nature, which man has exploited more than any resources for the sustenance of life. The water of good quality is required for living organisms. Physical, chemical, biological characteristics describe water quality. All these factors affect the quality of water and the lifecycle of different aquatic organisms like fish etc. Because, all living organisms can tolerate a specific range of these parameters. Large deviations of these parameters from their contents can seriously effect on body functions of aquatic organisms.

The recent approach to improve the quality of polluted water bodies in aquaculture was the application of beneficial eco-friendly microbes, enzymes, and other bio-chemicals to the ponds, known as 'bioremediation', which involves the manipulation of microbes in ponds to enhance mineralization, nitrification, and denitrification processes to minimize toxic effects to increase sustainable, eco-friendly fish production. Bacteriological nitrification was the most important practical method for the removal of excess ammonia from polluted urban water bodies and it can be achieved by setting of sand and gravel bio-filter through which water was allowed to circulate. The ammonia oxidizers for conversion of ammonia to nitrite are placed under five genera, *Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus,* and *Nitrospira,* and nitrite oxidizers for conversion of nitrite to nitrate, under three genera, *Nitrobacter, Nitrococcus* and *Nitrospira.* Mixed cultures of nitrifiers have been demonstrated to nitrify more efficiently. Nitrification produces nitrate and alters the pH slightly towards the acidic range, facilitating the availability of soluble materials. The vast majority of urban water bodies accumulate nitrate, as they do not contain suitable effective eco-friendly denitrifying filters. Denitrifying filters also helps to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas for increasing fish production in heavily polluted water bodies.

MATERIALS AND METHODS

A sampling of Soil and Water for Bacterial Isolation

Twenty-five soil samples (0-15 cm) were collected from the corner bottom of 25 fish ponds belonging to the Raipur district (Chhattisgarh). The samples were taken from five places of each pond bed. Just after collection of samples, they were kept in air tight polythene bags and used for isolation of heterotrophic bacteria; then, after muddy soil samples were kept for air drying for isolation of the autotrophic bacteria (nitrifying). Further, these bacterial isolates were tested as bio-remediators in vitro and in the set of fish aquariums containing polluted urea water to observe their efficiency, especially their effect on the rate of fish growth.

Physico-Chemical Analysis

The water samples were collected from each pond mentioned in Table 1 for physical and chemical characterization. Analysis was carried out for various water quality parameters such as pH value, EC and TDS, the results are mentioned in Table 1. Standard methods of pH were measured by pH meter. Electrical conductivity was measured by a conductivity meter, Elico-microprocessor based conductivity meter, model 1601. Electrical conductivity indicates the capacity of electrical current that passed through the water. TDS was measured with the help of a Digital TDS meter, E.I., model 651. The electrical conductivity of water samples, correlates with the concentration of TDS of water.

Isolation of autotrophic (nitrifying) bacteria

Autotrophic (nitrifying) bacteria were isolated from moist fish pond soil by means utilizing serial dilution and plating [3] using nitrifying agar media containing red phenol [4]. The agar plates were incubated for 4 weeks at 30 °C. For their multiplication and testing in nitrifying broth media, desirable colonies were picked up.

Culture media composition

*Nitrosomonas*culture medium

The chemical composition of ammonia broth medium for *Nitrosomonas* as follows: ammonium sulfate $(NH_4)2\ SO_4$- 2.00gm, magnesium sulfate ($MgSO_4$ $7H_2O$) - 0.50gm, Ferrous sulfate ($FeSO_4$ $7H_2O$)- 0.03gm, sodium chloride (NaCl)- 0.30gm, magnesium carbonate ($MgCO_3$) - 10.00gm, di-potassium hydrogen phosphate(K_2HPO_4) - 1.00gm, double distilled water- 1000ml

Nitrobacter culture media

The chemical composition of nitrite broth medium for *Nitrobacter* was as follows:

Sodium nitrite ($NaNO_2$) - 1.00g,magnesium sulfate ($MgSO_4.7H_2O$) - 0.50 gm, ferrous sulfate ($FeSO_4.\ 7H_2O$) - 0.03gm, sodium chloride (NaCl) - 0.30gm, sodium carbonate (Na_2CO_3)- 1.00gm, di-potassium hydrogen phosphate (K_2HPO_4)- 1.00gm, double distilled water - 1000ml

Isolation of heterotrophic (denitrifying) bacteria

Just after collecting samples, heterotrophic bacteria were isolated from the muddy moist fish pond soil using the serial dilution and plating process [3]. In this relation, for the isolation of the heterotrophic

http://dx.doi.org/10.53709/CHE.2021.v02i02.001

bacterium, PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609, its medium was used for its multiplication and testing as bio-remediators for contaminated water to increase fish growth rate, as defined by the writer/edit or handbook.

Using a sterilized dropper, roughly 1gram of the muddy soil was aseptically transferred to a sterile test tube containing 9ml of the diluents. This yielded a 10^{-1} dilution. Up to six-fold (10^{-6}) serial dilutions were subsequently prepared from the 10^{-1} dilution [5]. In addition, sufficient dilution of the aliquot was used for inoculation on its agar medium. The inoculated agar plates were incubated for up to 72 hrs at 28 ± 2oC. As Joel and Amajuoyi mentioned, the identified selected colonies were counted at an interval of 24 hours.

Culture media composition

The chemical composition of the heterotrophic medium for heterotrophic bacteria was as follows (composition for 1 liter): Agar -20.0gm, Na_2HPO_4 -7.9gm, KH_2PO_4-1.5gm, NH_4Cl -0.3gm, $MgSO_4.7H_2O$ -0.1gm, east extract solution -10.0ml, trace elements solution SL10 -1.0ml, pH- 7.5.

Determination of ammonium and nitrate

The concentration of ammonia and nitrate was determined by Kjeldhal method by using Devardaalloy. The Devardaalloy was used as a reducing catalyst that once the ammonium was driven off during the first part of the N-distillation, then after, it was added to the solution to help for conversion (reduce) nitrate to ammonium. The solution still has a high pH, so that the $NO_3$is converted to NH_4 quickly which can be again distilled off for analysis [6].

RESULT AND DISCUSSION

Physico-chemical characteristics of soil and water samples collected for isolation of autotrophic and heterotrophic bacterial isolates

The soil and water samples from polluted pond water were taken for isolation of autotrophic and heterotrophic bacterial isolates, twenty - five samples from different locations of Raipur area were collected, and the parameters viz., pH, Ec, TDS were measured in the samples as the data are presented in Table 1.

Pond water lowest Ec (17x10micro mhos/cm) was associated with Kankali para pond. While, the highest value of water Ec (38x10micro mhos/cm) recorded in Kailashpuri Kusalpur dabri pond. Similarly, the lowest TDS was also associated with Kailashpuri Kusalpur pahadi pond (235 mg/l) followed by Amlidih first pond (250mg/l). The highest TDS value was observed with water of Kailashpuri Kusalpur dabri pond (508 mg/l). Pond water pH was recorded towards the alkaline range,and the minimum value was 7.4 while, the highest pH was recorded at 8.2.

Pond soil lowest Ec(20x10 micro mhos/cm) was associated with Kailashpuri Kusalpur Pahadi pond and Math purena first Chiranjeevi pond. While, the highest value of soil Ec (40x10micro mhos/cm) recorded in Kailashpuri Kusalpur dabri pond followed by Kushalpur gitti khadan pond (37x10micro mhos/cm). Further, the lowest soil TDS was associated with Math purena first Chiranjeevi pond (256 mg/l) While, 545 mg/l value was considered the highest associated with Kailashpuri Kusalpur dabri pond. The most elevated pH of pond soil was recorded at 8.4 while, 7.6 remained lowest.

In this study, the pond soil pH range was observed from 7.6 to 8.4, which was almost similar to the findings of [7]. The acceptable range of the pH was 7-9.5, the desirable range was 6.5-9, and in stress conditions, it reached <4 and >11 as reported by [8].The pH of the water collected from different ponds ranged between 8.20 to 7.4. These observations are close to statements of [9].

Morphological and bio-chemical characteristics of autotrophic (nitrifying) and heterotrophic bacterial isolates

Nitrosomonas isolates were isolated from 25 soil and water samples of polluted fish ponds belonging to area of Raipur, Chhattisgarh. Further, these isolates were tested for their ability to convert ammonia into nitrite by using an ammonia broth medium. Results of weekly qualitative analysis clearly indicate that ammonia started to transform into nitrite after 15DAI *Nitrosomonas* isolates under controlled conditions. It was also observed that ammonia total disappeared after 30DAI. The selection of effective *Nitrosomonas* isolates was made on the basis of the rate of color change due to the conversion of ammonia into nitrite. During isolation of nitrifying bacteria of *Nitrosomonas* and *Nitrobacter*, both media become turbid and this further confirms the presence of nitrifying bacteria. Gram's staining and microscopic observation also confirmed the existence of nitrifying bacteria.

Further selected isolates of *Nitrosomonas* and *Nitrobacter* were multiplied to exploit-bioremediation in a simulated aquaculture system. *Nitrosomonas* and *Nitrobacter* strains were also isolated from drained fish ponds by [10]. They expressed similar views and reported that the soil of Chhattisgarh plains was exposed to an extremely dry and hot climate during summer resulting in drastic lots in the population of

http://dx.doi.org/10.53709/CHE.2021.v02i02.001

Table1: Physico-chemical characteristics of soil and water samples collected from polluted water pond for isolation of autotrophic (nitrifying) and heterotrophic bacterial isolates.

S.No.	Location Village /N.Nigam Ward Name	Pond Name	Water			Soil		
			pH	Ec µhmos/cm	TDS mg/l	pH	Ec µh-mos/cm	TDS mg/l
1.	Purena	1st Purena pond,	7.8	20×10	335	7.9	28×10	370
2.	Purena	2nd Purena pond,	7.8	25×10	325	8.0	28×10	387
3.	Amlidih	1st Amlidih pond	7.9	18×10	250	8.0	22×10	311
4.	Amlidih	2nd Amlidih pond	7.4	28×10	312	7.8	29×10	323
5.	Katora Talab	Katora Talab pond	7.6	19×10	290	7.7	25×10	320
6.	Telibandha	Marine drive pond	7.8	22×10	299	8.0	24×10	361
7.	Tikrapara	NariyaTikra para pond	7.6	28×10	335	7.9	30×10	376
8.	Math para	1st Dudhadhari pond	7.7	18×10	283	7.9	28×10	378
9.	Math para	2nd Dudhadhari pond	7.7	22×10	306	8.0	30×10	391
10.	Kankali para	Kankaliparapond	7.6	17×10	288	7.8	25×10	380
11.	Kalibadi	Budha Talab pond	8.2	19×10	323	8.3	29×10	374
12.	Mova	Mova pond	7.7	28×10	307	8.0	30×10	325
13.	Pandri	NariyaPandripond	7.8	29×10	356	8.0	30×10	371
14.	Raja para	Raja Talab pond	7.5	25×10	312	7.9	28×10	333
15.	KailashpuriKushal-pur	Dabri pond	7.5	38×10	508	8.4	40×10	545
16.	KailashpuriKushal-pur	Kho-Kho pond	8.0	22×10	356	8.2	25×10	394
17.	KailashpuriKushal-pur	Pahadi pond	7.6	17×10	235	7.9	20×10	310
18.	Kushalpur	Gittikhadan pond	7.8	30×10	425	8.2	37×10	491
19.	Kushalpur	Malsat pond	7.9	19×10	320	8.1	26×10	354
20.	Professor colony Kushalpur	BhaiyaTalabpond	7.9	20×10	364	8.2	29×10	396
21.	Purana basti	Maharaja pond	7.5	28×10	307	7.6	25×10	312
22.	Math purena	1st Chiranjiv pond	8.2	25×10	384	8.4	20×10	256
23.	Math purena	2nd Chiranjiv pond	8.1	19×10	320	8.2	23×10	378
24.	Kharun	Kharun	7.4	17×10	289	7.7	21×10	369
25.	Sarona	Sarona pond	7.7	22×10	456	8.1	28×10	366

mesophilic microbes, including nitrifying bacteria. A further similar view was also given by [3]. They suggested that 10^{-3} dilution can be considered appropriate dilution to isolate nitrifying bacteria from drained fish pond soil.

Ammonia and nitrate dynamics in the water of polluted pond as influenced by autotrophic and heterotrophic bacteria with different levels of organic N urea under controlled condition

In the present study, eight treatment combinations were used to reduce the ammonium concentration in polluted pond water under controlled conditions. Data presented in Table 2 about ammonium and nitrate concentration was measured at 15 DAI and 30 DAI. At the 15 DAI, the ammonia concentration varied between 0.29 to 9.17 ppm among different treatment combinations. Among the 7 treatments (T_2-T_8), the lowest ammonia concentration was detected in T_4 (1.74ppm) *i.e.*, 2 ppm urea + mixture of *Nitrosomans* (Isolate no.NS8), *Nitrobacter*(Isolate no. NB8) and *Ochrobactrium pituitosum* (Isolate no. PS5), *Micrococcus luteus* (Isolate no. PS16) and at 30 DAI ranged between 0.281 to 7.00 ppm. The ammonia concentration in the polluted pond water after 15

http://dx.doi.org/10.53709/CHE.2021.v02i02.001

Table 2: Morphological and bio-chemical characteristics of autotrophic (nitrifying) and heterotrophic bacterial isolates.

	Characteristics	Autotrophic bacteria		Heterotrophic bacteria	
		***Nitrosomonas* culture of NS8**	***Nitrobacter* culture of NB8**	***Ochrobactrum pituitosum* culture of PS5**	***Micrococcus luteus* culture of PS16**
Common of the charac-terized colony	Colony colour			Translucent and shiny	Yellowish brown
	Shape of bacteria	Straight rod	Straight rod	Straight or slightly curved	Coccus
	Gram reaction	(-ve)	(-ve)	(-ve)	(+)
	Cell shape	Long rod	Short rod	Short rod	Round
	Cell arrangement	Motile (polar flagella)	Non motile	Non motile	Non motile
Biochemical character-istics	Oxidase test	(+)	(+)	(+)	(+)
	Catalase test	(+)	(+)	(+)	(+)
	Ammonia utiliza-tion test	(+)	(-)	(-)	(+)
	Nitrate reduction test	(-)	(+)	(+)	(-)
	Urease test	(+)	(+)	(-)	(+)
	Motility test	(+)	(+)	(+)	(-)

(-ve)= Gram negative (+ve) = Gram positive

days showed maximum concentration, but after 30 DAI showed a decrease in ammonia concentration in polluted pond water mostly because of nitrification. The ammonia concentration acquired in T4, *i.e.*, 1.51 ppm, which was nearly 0.235 ppm of ammonia reduction, was noticed at 30 DAI over 15 DAI.

The nitrate concentration of the polluted pond water at 15 DAI ranged between 25.9 to 66.11 ppm. However acceptable range of nitrate was 0-100 ppm, but when the application of urea was made at 3 level combinations (urea concentration 2,8,12 ppm) as per Table 4.5, increased the concentration of nitrate in the T_4 treatment by about (3.01 ppm) as shown at 15 DAI (35.42 ppm) and 30 DAI (39.77 ppm), respectively. Nitrate concentration of the polluted pond water at 30 DAI of the treatment T_7 ranged between 25.7 to 72.83ppm. The concentration of nitrate in the T_7 treatment reduced 14.38 ppm as shown at 15 DAI (55.62 ppm) and 30 DAI (70 ppm), respectively, because of biological oxidation of ammonia by nitrifying microbes.

The ammonia concentration, the best result, was associated with the T_4 treatment as it helped to decrease the level of ammonia concentration from 15 DAI (1.74) to 30 DAI (1.51). A the level of 8-ppm organic N level, the best result was associated with T_7 treatment as it was shown the decrease in the ammonia concentration from 5.10 ppm (15 DAI) to 2.90 ppm (30 DAI). In the next urea concentration level, i.e. 12-ppm, it had the slightest effect upon lowering the ammonia concentration from 7.17 (15 DAI) to 7.00 ppm (30 DAI).

A similar type of observation was made by [8], who reported that the acceptable range of ammonia was up to 0.05 ppm and the desirable range was 0 - < 0.025 pp, and under stress conditions, the concentration ranges > 0.3ppm.The ammonia nitrogen was decreased from a maximum level 10 mg/l to a minimum level of 0.34 mg/l when inoculated with 2.5 μlL^{-1} as reported by [10].

Among 3 levels of the urea concentration as 2 ppm,8 ppm, 12 ppm in which under the 2-ppm urea concentration when comparing the treatments T_3 & T_4 with the treatment T_2 for the analysis of the nitrate concentration, the best result similar to that of ammonium level, the better result associated with T_4 treatment as it helped in increasing the level of nitrate concentration from 15 DAI (35.42 ppm) to 30 DAI (39.77 ppm) and when comparing between the T_5 with T_6& T_7treatment,got the best result due to T_7 among them as it was showing the increase in the nitrate concentration from 15 DAI (55.62 ppm) to 30 DAI (70.00 ppm) mostly because of microbial transformation ammonia to nitrate. In the next urea concentration level, i.e., 12-ppm, it had the most negligible effect upon increasing the nitrate concentration from 15 DAI (66.11 ppm) to 30 DAI (70.00 ppm). A similar type observation was made by [8]. The findings of the present investigation were identical to the scientist [6].

http://dx.doi.org/10.53709/CHE.2021.v02i02.001

Table 3: Ammonia and nitrate dynamics in the water of polluted pond as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled conditions.

Abbre.	Treatments	Ammonia concentration ppm(mg/l)		Nitrate concentration ppm(mg/l)	
		At 15 DAI	At30 DAI	At 15 DAI	At30 DAI
T_1	No inoculums and no chemical	0.289	0.281	26.1	26.0
T_2	Organic source of N (Urea 2 ppm) + No inoculums	2.20	2.16	25.9	25.7
T_3	Organic source of N (Urea 2 ppm) + I_1	2.05	1.95	26.2	26.6
T_4	Organic source of N (Urea 2 ppm) + I_2	1.74	1.51	35.42	39.77
T_5	Organic source of N (Urea 8 ppm) + No inoculums	7.08	6.33	36.99	40.0
T_6	Organic source of N (Urea 8 ppm) + I_1	6.23	5.00	44.00	50.15
T_7	Organic source of N (Urea 8 ppm) + I_2	5.10	2.90	55.62	70.00
T_8	Organic source of N (Urea 12 ppm) + I_1	9.17	7.00	66.11	72.83
	CD (0.05)	1.07	0.69	5.24	5.42

I_1 =Mixed culture of heterotrophic bacteria (PS5, PS16), I_2 = It was a mixture of the culture of NS8, NB8 and PS5, PS16

CONCLUSION

Upon collecting the water and soil samples from different 25 different pond of different village/n. nigam/ward the best performance of *Nitrosomonas* (soil-NS8S and water-NS8W) and *Nitrobacter* (soil-NB8S and water-NB8W) which was the autotrophic bacteria has been seen which was taken from the 1st Dudhadhari pond.

The heterotrophic bacteria *(Micrococcus luteus* and *Ochrobactrum pituitosum)* of isolate number PS5 and PS16 has the best result among the 7 best performing samples of different village/n. nigam/ward which was taken from the Katora talab pond and Budha talab pond respectively.

In the polluted pond water, the heterotrophic bacteria *(Micrococcus luteus* and *Ochrobactrum pituitosum)* PS5 and PS16isolateare performing best results in decreasing the ammonia concentration and increasing the nitrate concentration respectively.

REFERENCES

[1] Ruttner-Kolisko, A. 1963. The interrelationships of the Rotatoria. The Lower Metazoa. University of California Press, Berkeley. 263-272.

[2] Moses, B.S. 1983. Introduction to Tropical Fisheries, Ibadan University Press, UNESCO/ICSU, 1983; 102-105.

[3] Schmidt, E.L. and Belser, L.W. 1982. Nitrifying bacteria method of soil analysis, Part 2: chemical and microbiology properties. *Agronomy monograph*, 9:1027-1041.

[4] Ford, D.L. 1988. Design, operation and control of biological nitrification denitrification systems. International Workshop on waste water treatment technology, Danish Association of Consulting Engineers, *Copenhagan,* June 11-13.

[5] Joel, O.F. and Amajuoyi, C.A. 2010. Determination of the Concentration of Ammonia that 688 could have Lethal Effect on Fish Pond. Asian Research Publishing Network (ARPN) 689 *Journal of Engineering and Applied Sciences*, 1-5.

[6] Ogbonna, J. and Chinomso, A. 2010. Determination of the concentration of ammonia that could have lethal effect on fish pond. Journal of Engineering and Applied Sciences (Asian Research Publishing Network), 5: 1-5.

[7] Bajaj, S. 2017. Effect of environmental factors on fish growth. *Indian Journal of Science Research,* 12(2): 87-91.

[8] Bhatnagar, A. and Devi, P. 2013. Water quality guidelines

http://dx.doi.org/10.53709/CHE.2021.v02i02.001

for the management of pond fish culture. *International journal of environmental sciences,* 3(6): 1980-2009.

[9] Shrivastava, S.K., Vajpai, K., Shrivastava, D.K., Vajpai, S.K. 2008. Study of physicochemical quality of pond water in Bilaspur, Chhattisgarh. *Current World Environment,* 3(1): 97.

[10] Barik, P., Vardia, H.K. and Gupta, S.B. 2005. Exploiting nitrifying bacterial isolates (*Nitrosomonas* and *Nitrobacter*) as a bioremediator of ammonia and nitrite in fish ponds of rice ecosystems. *Journal of Agriculture*, 10(2): 49-52.

 http://dx.doi.org/10.53709/CHE.2021.v02i02.001