# “BIOREMEDIATION OF POLLUTED POND WATER FOR INCREASING FISH PRODUCTION”

**Ph. D. (Agri.) Thesis**

**by**

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**INDIRA GANDHI KRISHI VISHWAVIDYALAYA**
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# "BIOREMEDIATION OF POLLUTED POND WATER FOR INCREASING FISH PRODUCTION"

**Thesis**

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**Indira Gandhi Krishi Vishwavidyalaya, Raipur**

**by**

**Anjulata Suman Patre**

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**FOR THE DEGREE OF**

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**in**

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**(Agricultural Microbiology)**

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## CERTIFICATE - I

This is to certify that the thesis entitled **"Bioremediation of polluted pond water for increasing fish production"** submitted in partial fulfillment of the requirements for the degree of **"Doctor of Philosophy in Agriculture (Agril. Microbiology)"** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Anjulata Suman Patre** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or certificate course. All the assistance and help received during the course of the investigations have been duly acknowledged.

Date: 23.6.21

Dr. S.B. Gupta
(Major Advisor and Chairman)

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

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# CERTIFICATE – II

This is to certify that the thesis entitled "**Bioremediation of polluted pond water for increasing fish production**" submitted by **Anjulata Suman Patre** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture** in the **Department of Agricultural Microbiology** has been approved by the external examiner and Student's Advisory Committee after oral examination.

30/11/2021

Signature External Examiner

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**Date:** 30/11/2021

**Major Advisor** 30.11.21

**Head of the Department/Section** 24.12.21

**Faculty Dean**

Approved/Not approved

Director of Instructions

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*"Education plays fundamental role in personal and social development and teacher plays a fundamental role in imparting education. Teachers have crucial role in preparing young people not only to face the future with confident but also build up it with purpose and responsibility. There is no substitute for teacher pupil relationship". I start in the name of God-who has bestowed upon me all the physical and mental attributes that I possess and skills to cut through and heal a fellow human.*

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**I.G.K.V. Raipur (C.G.)**

**ANJULATA SUMAN PATRE**

**Date: 23.06.2021**

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# LIST OF ABBREVIATIONS

| ABBREVIATION | DISCRIPTION |
|---|---|
| % | Per cent |
| @ | at the rate |
| ˚C | Degree Celsius |
| CD | critical difference |
| Cm | Centimeter |
| DAI | days after inocultion |
| EC | electrical conductivity |
| *et al.* | and co- worker/ and other |
| g. | Gram |
| SEm | Standard error of mean |
| i.e. | that is |
| Kg | Kilogram |
| Mg | Milligram |
| Ml | Milliliter |
| Cfu | Colony forming unit |
| Viz | For examples |
| pH | potentiality of hydrogen |

## THESIS ABSTRACT

| | | | |
|---|---|---|---|
| a) | Title of the Thesis | : | "Bioremediation of polluted pond water for increasing fish production" |
| b) | Full Name of the Student | : | Anjulata Suman Patre |
| c) | Major Subject | : | Agricultural Microbiology |
| d) | Name and Address of the Major Advisor | : | Dr. S. B. Gupta, Prof. & Head<br>Department of Agricultural Microbiology,<br>College of Agriculture, IGKV Raipur, (C.G.) |
| e) | Degree to be Awarded | : | Doctor of Philosophy in Agriculture, (Agricultural Microbiology). |

Signature of the Student

23.6.21
Signature of Major Advisor

Date: 23.6.21

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Signature of Head of the Department


### ABSTRACT

Keeping in view that excess amount of ammonium in water bodies is harmful to fish cultivation and must be removed or converted into harmless or less harmful (like nitrate) substances before it builds up to lethal levels in the polluted water bodies. The present study entitled "Bioremediation of polluted pond water for increasing fish production" was planned to conduct at the Department of Agricultural Microbiology, College of Agriculture Raipur, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2017-2019. In this connection, 25 soil and water samples were collected from polluted ponds of capital city Raipur area for isolation of autotrophic (nitrifying) and heterotrophic local bacterial isolates especially in order to exploit them as bio remediators for ammonia polluted urban water bodies. Further, the collected samples were analyzed for the different quality parameters like pH, EC and TDS and isolation of autotrophic and heterotrophic bacterial isolates. The isolated bacterial isolates were selected on the basis of growth performance and other parameters to examine their effect on N




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transformations especially to decrease ammonia concentration with and without molasses and skimmed milk amendment and fish culture. The observations of organic N transformations were recorded properly in microbial growth room and green house aquarium environment at 15 days time intervals. The observations related to growth rate in terms of fish weight and length were also recorded properly in green house aquarium and deep cemented tank environment also at 15 days time intervals.

Both combinations of inoculants i.e. $I_1$ (mixed culture of local bacterial isolates PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum*AM490609) and $I_2$ (The mixed culture of local bacterial isolates N*itrosomonas* NS8 and *Nitrobacter* NB8 and PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum*AM490609) were used and got better result of the $I_2$ in the aquatic ecosystem.

It can be concluded from the study that in green house aquarium conditions, highest fish growth rate was associated with Treatment $T_4$ ($I_2$ + 2ppm organic N) while $T_8$ ($I_1$ + 12ppm organic N) gave lowest fish growth rate. The growth rate was remained statistically at par due to treatment $T_7$, $T_4$, $T_2$ and $T_1$ but differ significantly due to $T_1$-$T_4$, $T_6$-$T_7$ over $T_5$ and T8. Similarly, more fish growth rate was associated with T3 over T6. It can also be concluded from the data of present investigation that better fish growth rate can be obtained in deep cemented tank (WxLxH: 42.5x67x95 $cm^3$) over green house aquarium environment. It can also be concluded that bacterial bio remediators can help to obtain desirable fish growth rate up to 8 ppm organic N level by faster rate of microbial nitrification. It is significant finding of this investigation that almost same fish growth rate at up to 8 ppm level of organic N can be obtained statistically similar to that of fish growth rate at 2 ppm level of organic N because of inoculation of effective eco-friendly location specific mixed culture of desirable autotrophic and heterotrophic local bacterial isolates.

Hence, these bio remediators can be proved as low cost eco-friendly biological tools for enhancing sustainable fish cultivation in moderately polluted urban water bodies.

## शोध ग्रन्थ सारांश

| | | |
|---|---|---|
| अ) शोधग्रन्थ का शीर्षक | : | "मत्स्य उत्पादन बढ़ाने हेतु प्रदूषित तालाब के जल का जैविक उपचार" |
| ब) छात्रा का नाम | : | अंजूलता सुमन पात्रे |
| स) प्रमुख विषय | : | कृषि सूक्ष्म जीव विज्ञान |
| द) मुख्य सलाहकार का नाम एवं पता | : | डॉ. एस. बी. गुप्ता प्राध्यापक एवं विभागाध्यक्ष, कृषि सूक्ष्म जीव विज्ञान कृषि, महाविद्यालय, रायपुर (छ.ग.) |
| ई) प्रदान की जाने वाली उपाधि | : | पीएच.डी. कृषि (कृषि सूक्ष्म जीव विज्ञान) |

23.6.21

मुख्य सलाहकार के हस्ताक्षर

छात्रा के हस्ताक्षर

दिनांक: 23.6.21

23.6.21

विभागाध्यक्ष के हस्ताक्षर


## सारांश

वर्तमान शोध सन् 2017–2019 के दौरान कृषि सूक्ष्म जीवविज्ञान विभाग, कृषि महाविद्यालय, इंदिरा गांधी कृषि विश्वविद्यालय, रायपुर में किया गया। इस अनुसंधान का मुख्य उद्देश्य उन तालाबों में विशेषकर उन शहरी तालाबों में जिनमे अमोनिया का स्तर अत्यधिक होने के कारण मछली उत्पादन बुरी तरह गिर गया है, उन तालाबों के लिये अमोनिया के स्तर को कम करना या अमोनिया को मछली के लिए कम नुकसान दायक पदार्थ जैसे नाइट्रेट में पर्यावरणीय अनुकूल सुक्ष्मजीवो द्वारा बदल कर कम लागत से मछली उत्पादन बढ़ाना है। इस संदर्भ में 25 मृदा एवं जल नमूनों को स्वपोषी (नाइट्रीफाईंग) एवं परपोषी स्थानीय जीवाणु प्रभेदों को एकत्रित करने हेतु संग्रहीत किया गया ताकि इन सूक्ष्म जीवों का उपयोग शहर में उपस्थित अमोनिया से प्रदूषित जल के स्त्रोतों को शुद्ध करने के लिए किया जा सके।

इस अध्ययन में संग्रहित कि गई जल नमूनों का pH, EC और TDS मानों के अध्ययन के साथ स्वपोषी एवं परपोषी जिवाणु प्रभेदों के एकत्रिकरण किया गया, इन जिवाणु प्रभेदों का चयन उनके वृद्धि एवं नत्रजन के स्वरुप परिवर्तन की आधार पर किया गया तथा शोरा (molasses) एवं स्किम्ड मिल्क (skimmed milk) समिश्रत एवं समिश्रतहीन जल में अमोनिया के स्तर को कम करने के क्षमता के आधार पर किया गया। 15 दिवस के अंतराल पर सूक्ष्मजीवी वृद्धि रुम एवं ग्रीन हाउस एक्वेरियम में कार्बनिक नत्रजन जैविक रुप परिवर्तन का अध्ययन किया गया तथा मत्स्य के वृद्धि दर जानने के लिए मत्स्य भार एवं लम्बाई संबंधि अवलोकन ग्रीन हाउस एक्वेरियम एवं सिमेंट की बनी हुई टंकीयों में इसी अंतराल में किया गया।

इस अध्ययन में स्थानीय माइक्रोकोकस लूटीयस PS5 (CP00123) एवं आक्रोबैक्टरम पिटयूटोसूम PS16 (AM490609) जीवाणुओं का मिश्रित कल्चर ($I_1$) एवं स्थानीय नाइट्रोसोमोनास NS8 एवं नाइट्रोबैक्टर NB8 एवं माइक्रोकोकस लूटीयस PS5 (CP00123) एवं आक्रोबैक्टरम पिटयूटोसूम PS16 (AM490609) ($I_2$) का जीवाणुओं का मिश्रित कल्चर का तुलनात्मक अध्ययन किया गया। इस शोध में जलीय परिस्थितक तंत्र हेतु $I_2$ से प्राप्त नतीजे उत्तम पाये गये।

इस शोध से ये निष्कर्ष निकाला गया कि ग्रीन हाउस एक्वेरियम परिस्थिति में मत्स्य के सर्वोत्तम वृद्धि हेतु $T_4$ ($I_2$ + 2 पीपीएम कार्बनिक नत्रजन) उपचार सर्वोत्तम पाया गया जबकि न्यूनतम वृद्धि $T_8$ ($I_2$ + 12 पीपीएम कार्बनिक नत्रजन) उपचार से पाया गया। मत्स्य का वृद्धि दर सांख्यिकीय रुप से $T_7$, $T_4$, $T_2$ एवं $T_1$ उपचार में समान पाया गया परन्तु $T_1$एवं $T_4$ ,$T_6$ एवं $T_7$ उपचार $T_5$ एवं $T_8$ के तुलना में भिन्न पाई गई। इसी तरह मत्स्य वृद्धि दर $T_3$ उपचार में $T_6$ की तुलना में अधिक पाई गई।

इस अध्ययन से यह भी निष्कर्ष प्राप्त हुआ कि गहरी सिमेंटकृत टंकी में (ल.चौ.ऊॅ. 42.5×67×95 $cm^3$) मछली का वृद्धि दर ग्रीन हाउस ऐक्वेरियम पर्यावरण स्थिति से उत्तम पाया गया। इस अनुसंधान से यह भी जानकारी प्राप्त हुई कि जीवाणु जैव उपचार मत्स्य वृद्धि दर 8पीपीएम तक के कार्बनिक नत्रजन के शीघ्र सूक्ष्म जैविक नाइट्रीफिकेशन के लिए सहयोगी होता है

इस अनुसंधान की सबसे महत्वपूर्ण उपलब्धी यह है कि 8 पीपीएम तक कार्बनिक नत्रजन कि उपस्थित में प्राप्त मत्स्य वृद्धि दर प्रभावी परिस्थिति अनुकुल स्थानीय विशेष स्वपोषी एवं परपोषी जीवाणु प्रभेदों के (नाइट्रोसोमोनास NS8 एवं नाइट्रोबैक्टर NB8 एवं माइक्रोकोकस लूटोयस PS5 एवं आक्रोबैक्टरम पिटयूटोसूम PS16) मिश्रित उपचार से अमोनिया दुशः प्रभाव लगभग 2 पीपीएम कार्बनिक नत्रजन स्तर तक कम हुआ जिससे 8 पीपीएम एवं 2 पीपीएम नत्रजन पर सांख्यकीय समतुल्य मत्स्य वृद्धि पाई गई।

अतः ये जैविक उपचार न्यूनतम लागत वाली पर्यावरण अनुकूल जैविक सामग्री है, जो कि माध्यम प्रदूषित शहरी जल निकायों में सतत् मत्स्य उत्पादन में वृद्धि हेतु अति सहायक है।

## CHAPTER- I

# INTRODUCTION

Global aquaculture is changing day by day from extensive to intensive system. There is a tendency to increase the inputs *i.e*. over feeding, more use of fertilizers and various agro-chemicals (antibiotics, herbicides, pesticides etc.). These inputs may change the soil–water-air environment and lead to negative impact on living organisms resulting into adversely affecting soil water air quality. Agricultural production including fish population is severely affected due to such pollution specially in urban areas. Fish production is reduced significantly in urban water bodies due to heavy water pollution. The major changes in water quality are increasing the biochemical oxygen demand and chemical oxygen demand, increase in ammonia nitrogen ($NH_3$-N), nitrite-nitrogen ($NO_2^{-1}$-N), increase in available phosphate ($H_2PO_4^-$), accumulation of hydrogen sulfide ($H_2S$) at bottom of water bodies specially in urban water bodies because urban ponds are under the pressure of growing population adding tons of industrial waste, sewage, kitchen and other urban waste including detergents etc. in to the water bodies.

The recent approach to improve quality of polluted water bodies in aquaculture is the application of beneficial eco-friendly microbes, enzymes and other bio-chemicals to the ponds, known as ‘bioremediation’ which involves manipulation of microbes in ponds to enhance mineralization, nitrification and denitrification processes to minimize toxic effects in order to increase sustainable eco friendly fish production. Bacteriological nitrification is 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 is allowed to circulate. The ammonia oxidizers for conversion of ammonia to nitrite, are placed under five genera, *Nitrosomonas*, *Nitrosovibrio, Nitrosococcus, Nitrosolobus 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 not only produces nitrate but also alters the pH slightly towards the acidic range, facilitating the availability of soluble materials. The vast majority of urban water bodies accumulate ammonia, as they do not contain a suitable effective eco-friendly nitrifying filters. Denitrifying filters also help 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 (Barik *et al*., 2018).

Therefore, to nullify or eliminate or minimize the pollutant/toxicants from the polluted water bodies in order to increase fish production, the ecosystem needs eco-friendly remediation. Nitrification and denitrification both are natural processes, which occur in pond ecosystems but in some cases of heavily polluted urban water bodies, the results of these natural processes cannot be reaching a higher order of magnitude. Artificial growth and application of nitrifying and denitrifying bacterial cultures to enhance their population in the polluted water bodies in order to reduce toxicity specially ammonia in the aquatic system was used in this present investigation as a tool for bioremediation. Several bioremediators are developed by scientists as mentioned here.

| **Bioremediators Developed Identity of the bioremediator** | **Used on culturable species** | **Method of application** | **References** |
|---|---|---|---|
| *Bacillus sp.* | *Centropomus undecimalis* | Added to water; reduced salinity | Blain *et al*., 1998 |
| *Bacillus sp.* | *Penaeids* | Spread in pond water | Moriarty 1998 |
| *Aeromonas* media | *Crassostrea gigas* | Spread in pond water | Gibson *et al*., 1998 |
| *Aeromonas* CA2 | *Crassostrea gigas* | Spread in pond water | Douillet and Langdon 1994 |
| *Nitrosomonas* and *Nitrobacter* | *Cyprinus carpio communis* | Simulated condition | Barik *et al*., 2005 |
| *Roseobacter sp. BS 107* | *Oncorhynchus mykiss* | Spread in pond water | Ruiz-Ponte *et al*., 1998 |

Chhattisgarh state of our country is blessed with varied water bodies in the form of reservoirs (83,873 ha), ponds (70,000 ha) and rivers (3,573km). However, natural and man-made ponds are a major 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. The fish production is influenced by physical factors like temperature, pH, sun light and chemical factors like dissolved $O_2$ and $CO_2$ levels, and levels of inorganic nutrients (Ruttner-Kolisko, 1963). 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.

The Chhattisgarh state is situated in sub-humid tropics of Central India and spreads between $17^{o}$ 4'to $24^{o}$ N latitude and $79^{o}$ 30' to $84^{o}$16' E longitudes and covers an area of about 13.5 million ha. The region is divided into three agro-climatic zones *viz*. Chhattisgarh plain, Northern hills and Bastar plateau with average annual rainfall ranging from 1200 to 1600 mm. In addition to this, major part of Chhattisgarh state lies in catchments of the river Mahanadi and tributaries of the river Godavari.

About 35 thousand water tanks/ponds are available in Chhattisgarh. Some of them are heavily polluted by organic wastes and ammonia due to open drainage of cites, run off from industries etc. Hence efforts are needed to use properly screened especially native nitrifying and denitrifying microbes as bioremediators to maintain quality of pond water for enhancing fish production. In this connection an investigation was planned with following objectives:

**Objectives:**

1. Isolation of heterotrophic and chemo autotrophic bacteria (nitrifying) from polluted pond water.
2. Testing and screening of heterotrophic and chemo autotrophic bacteria for their ability to transform nitrogen.

3. Influence of inoculation of heterotrophic bacteria alone and in combination with chemo autotrophic bacteria on physico-chemical parameters of aquaculture system.
4. Influence of inoculation of heterotrophic bacteria alone and in combination with chemo autotrophic bacteria on fish growth in aquaculture system.

# CHAPTER-II
# REVIEW AND LITRATURE

The present investigation was undertaken on **“Bioremediation of polluted pond water for increasing fish production’’.** Few works were conducted on microbial bioremediation of polluted pond water. Some research findings show the heterotrophic and autotrophic bacterial inoculations were effective for bioremediation. Keeping this point in mind, efforts were made to collect and study various pertinent literatures on following different aspects of present investigation which are as follows:

2.1. Importance of heterotrophic and chemo-autotrophic bacteria and their isolation from polluted water

2.4. Ability of heterotrophic and chemoautotrophic bacteria for transformation of nitrogen

2.3. Co-inoculation of heterotrophic and autotrophic bacteria for improvement of physico-chemical parameters of aquaculture system

2.4. Inoculation of heterotrophic bacteria alone and combination of chemo-autotrophic bacterial on fish growth in aquaculture system

## 2.1. Importance of heterotrophic and chemo-autotrophic bacteria and their isolation from polluted water pond.

Heterotrophic bacteria *(Ochrobactrum pituitosum)* and chemoautotrophic bacteria (*Nitrosomonas and Nitrobacter)* isolated from the different polluted pond to assess their potential for conversion of ammonical nitrogen to nitrate and from nitrate to free elemental nitrogen by group of chemoautotrophic bacteria and heterotrophic bacteria, respectively. Chemoatotrophic spp present in water and soil are *Ntrosomonas* and *Nitrobacter* which responsible for the oxidation of ammonia to nitrate.

As now a days the water bodies present everywhere has severe polluted condition due to many causes like mixing of various chemical substances in the water bodies which are of man-made origin, whether it was due to the use of excessive insecticides, effluents or through the sewage waste of cities. The waste upon mixing with the water bodies leads to many problems like increasing the ammonia concentration in the water which further has the harsh effects on the different underlying organisms but specially on fishes and others. In such a way it is very important to reduce the ammonia level to such a lower level so as to make a much safe environment to the development and growth for the fishes under water. As related to overcome this problem several studies have been conducted from the different scientists for the ways to reduce the ammonical concentration in the water bodies. Some of them were described as below:

The chemoautotrophic nitrifiers have been isolated from the Moroccan soil where the culture enrichment and soil enrichment techniques were described for the isolation of *Nitrosomonas and Nitrobacter* as per the study conducted by Schmidt and Belser, 1982.

An organism was isolated by scientists which involved in the second phase of nitrification *i.e. Nitrobacter winogrudskyibuch*. The organism reproduced by polar budding and possessd a distinct and easily demonstrated nucleus. The life cycle included alternation of motile and non-motile stages. Motile cells possessd a single flagellum which was 20 times longer than the cell body (Zavarzin and Legunkova, 1959).

Sorokin *et al.* (1998) isolated five strains of lithotrophic, nitrite-oxidizing bacteria (AN1-AN5) from sediments of three soda lakes (Kunkur Steppe, Siberia; Crater Lake and Lake Nakuru, Kenya) and from a soda soil (Kunkur Steppe, Siberia) after enrichment at pH 10 with nitrite as sole electron source.

The isolation and detection of ammonia-oxidizing bacteria in soils collected from Chile was studied by Longeri and Moroni (1986) in which the ten (10) soil samples were analyzed from 8 separate soil series and soil enrichment cultures lead the isolation. Meincke *et al.* (1988) also isolated nitrifying bacterial population from four distinct historic buildings in Germany. In order to obtain the most abundant nitrifying organisms, the isolation protocol began at the maximum dilution steps from positive MPN test tubes. Enumeration and isolation of ammonia-oxidizing bacteria from 34 activated sludge's and ten ammonia oxidizers, all identified as *Nitrosomonas* spp., were isolated under the study of Suwa *et al.* (1994).

Prosser and Embley (2002) reported that increased awareness of metabolic diversity within autotrophic nitrifying bacteria has resulted in a reassessment of their role in nitrogen cycling in terrestrial and aquatic ecosystems. Wu *et al.* (2000) did the bioassay of the nitrification inhibition (NI) through guided fractionation of the methanol extract of lyophilized and milled *Leucaena leucocephala* roots and resulted in the isolation of four compounds, as confirmed from their spectral 1H and 13C NMR.

The isolated microorganisms DE2007 strain was able to show the capability of micro coleus consortium able to degrade crude oil and detoxification capacity. High toxic pollutant concentrations are a continually intensifying factor in the current soil and marine ecosystems. This strain easily growing at the substance where present many type of high toxic pollutant like crude oil and heavy metal (Cu and Pb) which was earlier observed by the Diestra (2007).

Huber *et al.* (2010) experimented on *Ochrobactrum pituitosum* in isolated condition at Sweden and found that this specific bacterium was Gram negative, rod-shaped, non-spore-forming and motile in nature. Patricia *et al.* (1995) studied on *Thiosphaera pantotropha* and reported that they denitrify aerobically and nitrify heterotrophically. However, recent evidence has indicated that these properties (particularly aerobic denitrification) have been lost. The occurrence and levels of aerobic denitrification and heterotrophic nitrification by *Parztntrophu* in chemostat cultures have therefore been re-evaluated. Only low nitrate reduction rates were observed, and the apparent nitrogen loss was of the same order of magnitude as the combined error in the calculated nitrogen consumption.

Similarly Sunitha and Padmavathi (2013) isolated beneficial bacteria (*Nitrosomonas* and *Nitrobacter* species) and pathogenic bacteria *Pseudomonas* and find out their effect on zooplankton and fish yields, which indicates that the concentrations of ammonia, nitrite and orthophosphates were higher in control ponds than in the treated ponds.

Lakshmipriya and Sivakumar (2012) studied the heterotrophic bacteria and their role in distribution, production, abundance and their involvement in nutrient cycling. Some isolates of microorganisms was selected on the basis of the morphology of the colony and of colonies identified by biochemical and phenotypic character. All the isolates *Bacillus subtilis, Streptococcus sp., Staphylococcus sp., Pseudomonas sp., Photobacterium sp., Enterobacteriaceae sp., Escherichia coli* and *Azotobacter* sp. were screened for producing Exopolysaccharide (EPS). Among all the *Azotobacter* sp. and *Pseudomonas* sp. produced high considerable amount of EPS. Also some chemoautotrophs species of *Nitrosomonas* and *Nitrobacter* were traced to be behind nitrosification and nitrification in this heavily fertilized soil zone. These soils have shown higher nitrification potentials due to the presence of nitrifying bacteria.

*Nitrosomonas eutropha*, a betaproteo-oxidizing bacterium found in areas with high levels of ammonium, such as waste water treatment plants. Under toxic and anoxic conditions, the effects of $NO_2$ on gene and protein expression of bacteria were determined by maintaining *N. Eutrophic* C91 strain as studied by Kartal *et al.* (2012) by then Phirke (2014) mentioned that ammonia-oxidizing bacteria (AOB), oxidize ammonia to nitrite in the first step of nitrification, play an important role in biological wastewater treatment systems. *Nitrosomonas mobilis* was an important and dominant AOB in various wastewater treatment systems. Park *et al.* (2017) had studied on *Nitrospira* spp., a chemo litho autotrophic nitrite-oxidizing bacteria (NOB) which are present everywhere, both in natural and engineered environments.

Reddy *et al.* (2015) isolated and characterized autotrophic ammonia oxidizing bacteria from a fish processing effluent treatment plant. The results obtained after sequencing of 16S rRNA gene showed AOB affiliation to

*Nitrosomonas marina*, *Nitrosomonas nitrosa* and *Nitrosospira lineages* of betaproteo bacteria.

Thandar *et al.* (2016) reported that the isolated ammonia-oxidizing bacteria, which oxidizes ammonia to nitrite in the first nitrification step plays an important role in biological waste water treatment systems. *Nitrosomonas mobilis* is a major and prevalent AOB in various waste water treatment systems.

Hoang *et al.* (2016) found that the cells of any group of microorganism msona bio-filmsticks to each other and adhere to a surface by excreting a matrix of extracellular polymeric substances (EPS). But the chemoautotrophic nitrifying bacteria hardly form bio-films due to their extremely low growth rate.

Abe *et al.* (2017) emphasised on nitrification and mentioned that it was an important reaction in waste water treatment plants (WWTPs) for the process of biological nitrogen removal. Since ammonia-oxidizing microbes are slow to grow and are prone to environmental factors such as free ammonia, it was difficult to obtain pure strains.

Barik *et al.* (2018) mentioned that ammonical nitrogen was an important limiting factor for intensive aquaculture system. Removal of unionized ammonia ($NH_3$) and nitrite ($NO_2^-$) through biological activity was therefore an important instrument for changing such ecosystems.

## 2.2. Ability of heterotrophic and chemoautotrophic bacteria for transformation of nitrogen.

After the process of isolation of both the heterotrophic and chemoautotrophic bacteria it is very important to check their effectiveness and capacity for transformation of nitrogen and to measure their efficiency under the same artificial environment. To see the results under various conditions many scientists obtained different results which are described as follows:

Bower and Turner (1981) found that there was an increase in ammonia and nitrite concentration after six and twenty-four hours of disconnection of air supply respectively in closed sea water aquarium. In an experiment Mevel and Chambroux (1981) found that in closed marine system, nitrification increases and becomes intense at high oxygen concentrations. At lower oxygen levels, nitrification was low and nitrite reduction occurs.

Sohier and Bianchi (1983) reported that ammonia oxidation occurred after ten days of experimentation whereas, nitrite oxidation needed twenty days. There was a decrease in heterotrophy at the same time. Boyd *et al.* (1987) researched that non-aerated treatments remain at very low oxygen levels (around 1.0 mg $l^{-1}$). All continuously aerated treatments had sufficient oxygen levels (around 6.0 mg $l^{-1}$). Nitrogen transformations were recorded to be different under non-aerated and aerated conditions. Rate of nitrification was so fast in the aerated aquaria that almost all ammonia nitrogen was oxidized within 3 days.

Barik *et al.* (2018) applied selected isolates of nitrifying bacteria viz., *Nitrosomonas* sp. and *Nitrobacter* sp. @ 2.5µ$l^{-1}$ and @ 5.0 µ $l^{-1}$ in simulated aquaculture system. Decrease of ammonical nitrogen concentration from 10mg $l^{-1}$ to below the minimum limit (0.300 mg $l^{-1}$) was observed within 3 days after inoculation (microbial inoculums with aeration). Aeration enhanced nitrification rate, which resulted in improvement of water quality in such ecosystems.

Feng *et al.* (2013) isolated a strain of autotrophic ammonia-oxidizing bacteria (AOB) CM-NRO14 from the aerobic activated sludge of a domestic wastewater treatment plant using enrichment, isolation and purification technique. Van *et al.* (1984) studied two strains of nitrifying bacteria named HA1 and HD5 which were selected from Tolich river in Hanoi, Vietnam. The optimum growing conditions for both strains and their nitrification activities were examined in which their experiment. Their experiment results showed that the nitrogenous compounds in the waste water decreased rapidly.

Studied were conducted on the community structures of planktonic ammonia-oxidizing *archaea* (AOA) and bacteria (AOB) for five high-altitude of Tibetan lakes, which could be classified as freshwater, oligosaline or mesosaline, to develop a general view of the AOA and AOB in lakes on the Tibetan Plateau Hu *et al.* (2010) and Satoh *et al.* (2004) studied two kinds of ammonia-oxidizing bacteria ( *Nitrosomonas sp.* IWT202 and *Nitrosomonas sp.* IWT514) isolated from biologically deodorizing plants in cold districts in Japan. They found the optimum pH for their growth was 8.0 (IWT202) and 7.5 (IWT514).

Li *et al.* (2001) isolated 331 bacterial strains from mud of shrimp ponds in Shandong province, China and selected ten functional bacteria for bioremediation of shrimp culture environment and they were capable of utilizing shrimp feed as the whole source of carbon and nitrogen. They found that these strains have no disease-causing ability and six of them can increase survival rate of shrimp larva. They suggested that the ten functional bacteria include nitrifiers (*Nitrosomonas* and *Nitrobacter*), which can rapidly eliminate organic and inorganic pollutants from bottom of the pond and water. In another study Hu *et al.* (2002) collected activated sludge from a wastewater treatment station of a pig farm and subjected to nitrifying bacteria enrichment and isolation. It was found that when the enrichment media contains 300 mg $l^{-1}$ of ammonia nitrogen, a high-efficient culture of *Nitrosomonas* was obtained.

Liu *et al.* (2012) isolated a nitrobacteria union called A2-6-3 which uses ammonia nitrogen as the only energy source and does efficient nitrification from substrate sludge of Xihu Lake in Wuhan by the separation of most probable number and solid plate. Made study to Spieck and Lipski (2011) on litho autotrophic nitrite-oxidizing bacteria (NOB), known as fastidious microorganisms, which are hard to maintain and not many groups are trained to keep them in culture convert nitrite stoichiometrically to nitrate and found growth was slow due to the poor energy balance.

Grommen *et al.* (2002) showed that, in an improved nitrifying enrichment broth containing suspended nitrifying cells @ 5 mg $l^{-1}$,decreased the ammonia concentration from 10 $mgl^{-1}$ to below the detection limit within 4 days. However, in present studies it took 3- 5 days when inoculated @ 5.0 µl $l^{-1}$ with or without aeration. Another scientist Park *et al.* (2017) studied on *Nitrospira* spp., chemo litho-autotrophic nitrite-oxidizing bacteria (NOB), which was found ubiquitous in natural and engineered environments.

Water probiotics/bioremediators consisting of nitrifiers have been used to remove excess ammonical nitrogen from aquaculture system by Prabhu *et al.* (1999), Sharif *et al.* (2001) and Sambasivam *et al.* (2002). Here both the bacteria have the different ability to convert/ transform the nitrogen and having the different ability for transformation as the chemoautotrophic bacteria has an ability to convert the ammonia into nitrite and nitrite into nitrate. Whereas, the heterotrophic bacteria have an ability to convert nitrate into nitrogen gas. Both these bacteria's have the ability to transfer the nitrate into nitrogen gas also, these chemoautotrophic bacteria convert ammonia into nitrite.

Yang *et al.* (2013) studied for the novel heterotrophic bacterial strains under laboratory condition in which they found that all the strains having the efficient heterotrophic nitrification-aerobic denitrification capabilities and they were able to utilize the nitrite and nitrate as a sole nitrogen source. Then seen that the total amount of nitrogen was converted into intra-cellular nitrogen and the remaining was seen to be denitrified. Here the optimal condition for all the heterotrophic ammonium oxidation strains were as follows: C/N ratio 10, pH-7.0, temperature at 30 °C and shaking speed of 160 rpm. To confirm that the aerobic denitrification was involved in the nitrogen removal process then tested the aerobic denitrification capacity of the isolates using the nitrite and nitrate respectively as a sole source of nitrogen and their study they observed a significant decrease in nitrate after 48 hours with 84.85 ± 3.58 %, 92.74 ± 1.62 % and 95.63 ±1.37 % removal efficiency for all the three strains respectively. The corresponding maximum removal rates were 4.37, 5.77 and 7.92 mg $l^{-1}$ $h^{-1}$ (6.24, 7.40 and 9.91 mg $g^{-1}$ $h^{-1}$).

Nitrifying bacteria when inoculated @2.5 ul $l^{-1}$ decreased ammonia nitrogen from 10.0 mg $l^{-1}$ to minimum level (0.34 mg $l^{-1}$) within 5 days after inoculation (DAI) shown under the study conducted by Barik *et al*. (2018).

Ogbonna and Chinomso (2010) observed microbiological activity in different water samples collected from 4 different sources in which they that the ammonia concentration level was highest with sample 4 (0.50 mg $l^{-1}$) followed by sample 2 (0.35 mg $l^{-1}$) and lest concentration was seen in sample 1 and 3 (0.25 mg $l^{-1}$ and 0.25 mg $l^{-1}$) respectively.

When molasses was added in the waste water then this was very helpful in the process of bacterial attachment on the carrier and the degradation of the ammonium in the submerged system, particularly in the case of low initial concentrations of ammonium as studied by Muter *et al.* (2013)

High stock density and intensive feeding in aquaculture system leads to accumulation of organic waste which result in an increase in the ammonia, nitrite and nitrate concentration in water bodies. Under this study the effect of different biofloc starters on ammonia, nitrate and nitrite concentration was seen. The mean concentration of ammonia ranged from 0.02 mg $l^{-1}$ to 0.07 mg $l^{-1}$, nitrite ranged from 0.20 mg $l^{-1}$ to 0.43 mg $l^{-1}$ whereas the nitrate ranged from 0.64 mg $l^{-1}$ to 3.20 mg $l^{-1}$ (Putra *et al.* 2020).

Ojeda *et al.* (2018) reported that nitrogen compounds (ammonia and nitrite in particular) are important constraints in aquaculture because they are toxic metabolites for fish and other culture organisms. The growth for aquatic products demand has generated the need to increase production, trying to minimize environmental damage, which has begun to be achieved with the recent development of new systems such as biofloc technology (BFT), which is based on the recycling of nutrients by bacteria and maintaining water quality conditions.

### 2.3. Co-inoculation of heterotrophic and autotrophic bacteria for improvement of physico-chemical parameters of aquaculture system.

Different parameters were seen in the aquaculture system like the pH, EC, total hardness, TDC etc. which usually leads to the show the pollution level in any water bodies and thus by using the different bacteria (single in combination) we could find the capacity of the bacteria to lower the pollution concentration in the aquarium condition, which could be beneficial for the fish to grow well under the aquatic condition as well. Along with this they are also capable of changing the various physico-chemical factors like pH, BOD,COD,DO. etc.

The role of two brands of probiotic, 'Environ AC' in pond water treatment and 'Biotech (CP) in gut microbial population enhancement in *Penaeus monodon* culture ponds. Basically, the probiotic 'Environ AC' contains the beneficial bacteria such as *Bacillus* spp., *Pseudomonas* spp. and *Nitrosomonas* spp. Scientists concerned observed that ammonia level was reduced in experimental ponds while it increased in control ponds (Sambasivam *et al.,* 2002). Thus, it was evident that nitrification in pond environment established itself and similarly Joseph *et al.* (2002) studied the effect of commercially available bioaugmentors on the removal of ammonia and hydrogen sulphide from brackish water system. In this experiment health stone, BN-10, and zeolite were used. BN-10 was a typical bacterial augmentation material containing *Nitrobacter, Nitrosomonas,* S*ulphobacteria* and organic analytical material.

A mixture of sodium bicarbonate and sodium acetate selected as the appropriate carbon source for cell growth and nitrogen removal Du *et al.* (2003) mentioned where ammonia could be oxidized aerobically to nitrite by the mixed culture and the intermediate nitrite was then reduced to nitrogen gas. No nitrite was detected during the process. 0.212 g $l^{-1}$ of ammonia could be removed in 30 hours and nitrate could not be utilized aerobically by the mixed culture. Nitrite could be degraded aerobically as well as an aerobically. However, very little ammonia was degraded an aerobically, but the ability to degrade ammonia could be recovered even after oxygen had been supplied for 42 hours.

Bhatnagar and Devi (2013) found that the optimum fish production was totally dependent on the physical, chemical and biological qualities of water to most of the extent. Water quality was determined by variables like temperature, transparency, turbidity, water colour, carbon dioxide, pH, alkalinity, hardness, unionized ammonia, nitrite, nitrate, primary productivity, BOD, plankton population etc.

The age and developmental stages are related to the effect of pH water on fishes in which the larval stages were more sensitive to pH change (Lloyd and Jodan, 1964).

Verma and Khan (2015) reported the physico-chemical parameters of fateh sagar talab in bagar of Jhunjhunu district in Rajasthan. The study was carried out for a period of one year *i.e.* July 2012 to June 2013. Water samples were collected monthly from surface water and analyzed for temp, pH, electric conductivity, total hardness, Alkalinity, TDS (Total dissolved solid), chloride, DO (Dissolved oxygen).

Due to increase in population, advanced agricultural practices, industrialization, man-made activity, water was being highly polluted with different contaminants as reported by Sagar *et al*. (2012) where the water was a vital resource for human survival and the availability of good quality water was an indispensable feature for preventing diseases and improving quality of life. It was necessary to know details about different physico-chemical parameters such as color, temperature, total hardness, pH, sulphate, chloride, DO, BOD, COD, alkalinity used for testing of water quality.

Total ammonium nitrogen concentration was identified as a key limiting factor in intensive aquaculture system as per the study conducted by Grommen *et al*. (2002). They have used a suspension of nitrifying cells (ammonia binding inoculums liquid, ABIL) to shorten the startup period of a biofilter. Results showed removal of total ammonia from 10 mg $l^{-1}$ to below the detection limit within a period of 4 days provided adequate aeration and the dissolved oxygen levels were maintained. Likewise, Raja *et al.* (2015) mentioned that aquaculture was one of the fastest on rising sectors in the world today. An aquaculture system was diversified as fresh, brackish and marine water culture systems. However, the occurrence of the disease was a major constraint for its sustainability. Probiotics are often employed to control bacterial pathogens in the aquaculture systems.

Bajaj (2017) in his study have collected data on environmental factors such as temperature, pH, dissolved oxygen levels and nutrient levels from local ponds and have tried to correlate this with fish productivity.

Kroupovai *et al.* (2005) reported nitrite was an intermediate in the oxidation of ammonium to nitrate. An elevated ambient nitrite concentration a potential problem for freshwater fish since nitrite was actively taken up across the gills in competition with chloride. He concluded that the most important ones are water quality parameters are (e.g. pH, temperature, cation, anion and oxygen concentration), length of exposure, fish species, fish size and age, and individual fish susceptibility. Chloride concentration in water was considered one of the most important factors influencing nitrite toxicity to fish. The importance of individual factors was assessed and re-evaluated continuously.

Different parameters were seen in the aquaculture system like the pH, EC, total hardness, TDC etc. which usually leads to the show the pollution level in any water bodies and thus by using the different bacteria (single / in combination) we could find the capacity of the bacteria to lower the pollution concentration in the aquarium condition, which could be beneficial for the fish to grow well under the aquatic condition as well. Along with this they are also capable of changing the various physico-chemical factors like pH, BOD, COD, DO. Thus, the co-inoculation of both heterotrophic and autotrophic bacteria helps for improvement in various physico-chemical parameters as described earlier and in the above context various scientists have given their experimental results which have been below: Mahobe and Mishra (2013) reported that the various physico-chemical parameters with their respective ranges such as temperature varied between 27.4 °C and 28.3 °C during summer and 18.4 °C -19 °C in winter season. pH of pond water was taken and it ranges between the 7.1-7.9 and 6.8-7.3 in the summer and winter season respectively indicating the permissible limits. Electrical conductivity concentration in the ionized substances present in the E.C. shows the range of 601-1220 and 590-980 micro mhos./cm in the summer and winter respectively. Dissolved oxygen which is important for the process of decomposition of organic matter was shown in the range of 2.5 - 4.5 to 3.4 - 5.4 mg $l^{-1}$. Similarly, the Biological Oxygen Demand (B.O.D.) level in sample -6 is highest in both the season (summer -78 and winter- 69) mg $l^{-1}$. Total dissolved solid (TDS) value of the water samples were between the 390.7 to 793 mg $l^{-1}$ and 351 to 657 mg $l^{-1}$ in summer and winter respectively. Likewise, the Nitrate concentration was ranged from the 0.0 to 47.84 mg $l^{-1}$.

Bhatnagar and Devi (2013) reported all the parameters like -Temperature, Turbidity, Dissolved oxygen (DO), Biochemical oxygen demand (BOD), pH were categorized in the 3 different ranges viz, Acceptable range, Desirable range and Stress condition range and they have given differed ranges for ideal physicochemical properties like; Temperature (15-35, 20-30 and <12, >35) °C, Turbidity (30-80 and <12, >80) cm, Dissolved oxygen (3-5, 5 and <5, >8) mg $l^{-1}$, Biochemical Oxygen Demand (3-6, 1-2 and >10) mg $l^{-1}$, pH (7-9.5, 6.5-9 and <4, >11).

Sapkale *et al*. (2013) found that the maximum weight gain conversion of feeding efficiency and survival was found at the pH of 7.5 and is best suitable for its overall growth.

Shrivastava *et al.* (2007) reported that the various physico-chemical characteristics ranges of the Bilaspur pond such as pH, Electrical Conductivity, Dissolve oxygen, Total Dissolved Solids are 7.31 to 7.92, 124 to 680 µS/cm, 4.6 to 6.4 mg $l^{-1}$, 104 to 398 mg $l^{-1}$ respectively.

Roberts and Palmeiro (2008) reported sub optimal pH and the fluctuations in the pH may resulting into the lethargy, stress, skin irradiation/ lesions, behavioral changes (attempt to jump outside the aquarium), change in skin color, gill irritation, increased muscle production, mortality and respiratory sign.

Swarnakar and Choubey (2016) analysed different pond water of Raipur city and found physico-chemical parameters such as pH, Turbidity, Dissolve Oxygen (DO), Nitrate concentration are in the range of 7.6 to 9.36, 3.1 to 30.2 cm, 3.1 to 3.2 mg $l^{-1}$ and 0.4 to 5.1 mg $l^{-1}$, respectively.

Physico-chemical parameters of Raipur ponds were recorded for pH, Electrical Conductivity, Dissolve Oxygen, Total Dissolved Solids. The values of these parameters were 6.5 to 7.6, 453 to 1225 µS/cm, 6.1 to 8.3 mg $l^{-1}$, 1288 to 2475 mg $l^{-1}$, respectively as reported by Yadav *et al.* (2016)

### 2.4. Inoculation effect of heterotrophic chemoautotrophic bacteria alone & in combination on fish growth in aquaculture system.

The performance of different bacteria is having the different results as per their capacity to convert the toxic material into harmless one. Thus, we need to examine upon which bacterial combination or sole bacteria is having the maximum benefit to the aquatic organism (fish) as there is a synergistic effect upon mixing of two different bacteria in water bodies.

Sharma and Bhukhar (2000) studied the effect of a probiotic product (Aquazyme-TM-1000) on the pond water quality and growth of common carp (*Cyprinus carpio var. communes* L.). No significant influence was noticed on water quality. Zooplankton production was however, higher in probiotic treated waters (598 and 599 No. $l^{-1}$ respectively). The fish growth was significantly greater in the highest dose of probiotic as compared to other treatments and control.

Dewan *et al.* (1991) concluded that the water quality of the ponds treated with probiotics was observed to be suitable for survival and growth of fish culture.

Lakshya and Viraj (2014) studies the usefulness of probiotics in water quality and bacterial flora in fish pond where the culture used of *Nitrosomonas* and *Nitrobacter* were having the dose of 1.62 kg/ha and 0.82 kg/ha respectively in two ponds where one is being kept as the control pond. Chemoautotrophic bacteria (*Nitrosomonas*) relative loads in Pond-A, Pond-B and Pond-C are ranged from 2.01 x $10^3$ to 4.80 x $10^3$ cfu/ml, 2.25 x $10^3$ to 4.90 x $10^3$ cfu/ml and 1.24 x $10^3$ to 2.00 x $10^3$ cfu/ml respectively. Whereas, chemoautotrophic bacteria (*Nitrobacter*) relative loads in Pond-A, Pond-B and Pond-C were 2.20 x $10^3$ to 4.95 x $10^3$ cfu/ml, 2.10 x $10^3$ to 4.15 x $10^3$ cfu/ml and 1.10 x $10^3$ to 2.15 x $10^3$ cfu/ml respectively. In which he found that the probiotics has a tremendous effect upon the significant growth and maintaining the optimum water quality parameters which finally results in the fish growth. Which further also helps in improving the water quality along with the prevention of any bacterial disease in the fish pond.

Sahu and Datta (2018) studied the effect of pH on the growth and survival of fish in which the 5 treatments were used pH – 7 (control), 5 ± 0.25, 6 ± 0.25, 8 ±

0.25 and 9 ± 0.25 and among these he found that the weight gain (%) and survival % was higher in the control group, pH 7. $LC_{50}$ was 5.71 and 1%, 50 %, 99 % survivals were 7.53, 5.71 and 3.89 in the acidic range respectively. At last, the conclusion was that the optimum pH range for captative growth, culture and survival for juveniles was found between 6.5 -7.5 and in addition to the pH above 8.2 and 5.7 may cause serious stress to fish in captive condition and the rate of mortality rate may even cross 50 %.

Putra *et al.* (2019) studied the effect of cultivation of fishes in the biofloc system (with heterotrophic and autotrophic bacteria) to figure out the survival rate and growth of red tilapia (*Oreochromis* sp.). They added different organic sources of carbon into culture medium namely -molasses, tapioca flour, white sugar and control. He finds that the best growth of the tilapia fish was seen when the molasses was used as a carbon source (Absolute weight growth was 8.22 ± 0.329 g, daily growth rate was 7.86 ± 0.158 %, Survival Ratio was 92.22 ± 1.93 %).

Putra *et al.* (2020) according to him the biofloc refers to use of the heterotrophic and autotrophic bacteria. Molasses, which is a liquid by product of the sugar industry, may provide adequate carbon for heterotrophic bacteria that use it as a source of development. Thus, later the best results in the fish growth were seen when the molasses starter was used in the pond resulting into better fish growth.

In the pond water, if it is less polluted then the weight and length of fish was seen to be increased as per the study conducted by Bajaj, (2017). As when the ammonia level reaches > 0.1 mg $l^{-1}$ which has as several ill effects upon the fish like – gill damage, destroy mucous producing membranes, poor feeding conversion and reduced disease resistance at concentration less than the lethal concentration, kidney failure, Osmoregulatory imbalance, sub-lethal effects like reduced growth. In such case when the chemoautotrophic bacteria were introduced to such polluted pond then it helps in lowering the ammonia concentration which ultimately helps in overall fish growth (increase in fish length and fish weight). Here the desirable range of ammonia is 0 - <0.025 mg $l^{-1}$ and the acceptable range is 0 - 0.05 mg $l^{-1}$ was seen when the study conducted by Bhatnagar and Devi (2013).

As per various scientists there were some acceptable range and desirable range which has a direct effect upon the significant growth of fish, where as per the Thomforde (2004) the desirable range for nitrite is 0.-1 mg $l^{-1}$ and acceptable range is < 4 mg $l^{-1}$. Bhatnagar *et al.* (2004) seen that the concentration of nitrite 0.02 – 1.0 ppm range is lethal to many fish species, and the lethal concentration for many warm water fish is > 1.0 ppm whereas 0.02 ppm is an acceptable range. Similarly, OATA (2008) reported that it should not exceed 0.2 mg $l^{-1}$ in freshwater and 0.125 mg $l^{-1}$ in seawater.

The ammonia being very ill effect upon the fish health thus it is necessary to study the lethal and toxic effect level concentration in the water and to retain within the safe range for better growth and development. Sub-lethal effects occur to fish at concentration 0.01 to 0.3 mg $l^{-1}$ and the toxic level for unionized ammonia is 0.6 and 2.0 mg $l^{-1}$ (EIFAC, 1973 and Robinette, 1976). 0.1 mg $l^{-1}$ is a maximum limit for ammonia concentration as seen in aquatic organism was reported by Meade,1985; Santhosh and Singh, 2007. Swann, 1997 and OATA, 2008 reported the safest concentration level for ammonia is 0.02 ppm.

Barik *et al.* (2018) reported that the decrease in the ammonia nitrogen concentration from 10 mg $l^{-1}$ to below the minimum limit 0.3 mg $l^{-1}$ was being achieved within the 3 Days after inoculation (DAI) of microbial inoculums with aeration in water. Aeration and microbial application plays an important role in increasing the nitrification. Therefore, it may be concluded that the application of bioremediators decreased ammonia and nitrite nitrogen. 2-3 mg $l^{-1}$ is the toxic level concentration for the aquatic life thus is very important for its remediations in polluted water bodies. As the ammonia at high concentration results into poor growth and survival of fish and ionized and un-ionized both form of ammonia has a toxic effect on fish.

Bohn *et al.* (2001) concluded that when the oxygen is under lower concentration then nitrate-nitrogen ($NO_3$-N) function as a alternative source of electron acceptor by the heterotrophy. In his investigation initial nitrate level were very low in polluted water because the nitrification process was very poor (because of poor nitrifiers, poor dissolved oxygen and high COD) which shows improvement later due to the nitrifiers inoculation and aeration. In the comparison between the aerated and unaerated aquaria the higher nitrate-nitrogen recovery shows higher nitrification rate under the aerated condition with the sufficient dissolved oxygen.

In the aquaculture system the excess ammonia nitrogen used to remove by the nitrifiers which has been used as a bioremediators/ probiotics due to which the growth of fish has been significantly increased in fish length and fish weight as per reported by Prabhu *et al*., 1999, Shariff *et al*., 2001 and Sambasivam *et al.,* 2002.

Acidic pH(5.5) the reduction in fish growth rate has been reported as seen by Menendez, 1976 ; Craig and Baski, 1977. Kilmmel, (1993) in the study it was observed that in the pH range 8.0 and 6.0 fish weight loss has been seen due to the imbalance in haemostasis. Acidic pH(5.0) induce reduction in feeding and the growth of fish was reported by Beamish (1972).

In the fish *B. bendelensis* the range of fish length and weight was observes as 10.8-15 cm and 12.9-38.8 g respectively was seen when the culture of the heterotrophic and chemoautotrophic bacteria was being used and where the relative condition factor for fish growth was highest when these bacteria were used thus leads to a significant growth in the fish length and weight as studied by Singh and Nautiyal, (2017).

Sunitha and Padmavati (2013) studied that when the ponds were treated with the Total Heterotrophic Bacteria (THB) with the relative loads on Pond-A is 5.25 x $10^5$ to 7.80 x $10^5$ cfu/ml, Pond-B is 5.42 x $10^5$ to 7.80 x $10^5$ cfu/ml and Pond-C is 20.5 x $10^5$ to 4.72 x $10^5$ cfu/ml respectively and similarly the Chemoautotrophic bacteria (*Nitrosomonas*) relative loads in Pond-A, Pond-B and Pond-C are ranged from 2.01 x $10^3$ to 4.80 x $10^3$ cfu/ml, 2.25 x $10^3$ to 4.90 x $10^3$ cfu/ml and 1.24 x $10^3$ to 2.00 x $10^3$ cfu/ml respectively. Whereas, chemoautotrophic bacteria (*Nitrobacter*) relative loads in Pond-A, Pond-B and Pond-C were 2.20 x $10^3$ to 4.95 x $10^3$ cfu/ml, 2.10 x $10^3$ to 4.15 x $10^3$ cfu/ml and 1.10 x $10^3$ to 2.15 x $10^3$ cfu/ml respectively. Where the (Gross and Net yields) obtained respectively are 33.97 & 32.29 t/ha/332 days, 33.66 & 32.04 t/ha/332 days and 29.53 and 28.09 t/ha/332 days in the Pond-A, Pond-B and Pond-C respectively. At last, the fish weight gained during the harvesting time in the 3 ponds indicates that the fish grow well in the Pond-A and Pond-B than the control Pond-C. Hence, higher yield can be achieved with the better survival, disease resistance and growth by using the probiotics (Total Heterotrophic Bacteria and Chemoautotrophic bacteria) in aquaculture ponds.

Two nitrifying bacterial cultures *Nitrosomonas* species at the rate of 2 kg/ha and *Nitrobacter* species at the rate of 1 kg/ha respectively were used in treated pond. *Nitrosomonas* loads ranged from $2.10 \times 10^3$ to $3.90 \times 10^3$ with a mean of $3.00 \times 10^3$ cfu/ml in treated pond, and $1.10 \times 10^3$ to $2.00 \times 10^3$ with a mean of $1.46 \times 10^3$ cfu/ml in control pond, whereas, *Nitrobacter* loads in treated and untreated pond ranged from $2.60 \times 10^3$ to $4.30 \times 10^3$ and $1.30 \times 10^3$ to $2.40 \times 10^3$ with mean values of $3.29 \times 10^3$ and $1.75 \times 10^3$ cfu/ml. The gross yields obtained in control and treated pond is 27.20 and 31.00 t/ha. However, net yields in the ponds are 25.41 and 29.22 t/ha respectively. The fish yield indicates that the fish grow well in nitrifying bacteria treated pond than in control pond as reported by Sharma and Thakur (2018).

Sunitha and Padmavati (2013) reported in the nitrogen cycle involves the oxidation of the ammonia to nitrite by the bacteria *Nitrobacter*. Thus, we know that the ammonia is unable to remove from the water body but it can be however it can be converted to non-toxic nitrate by nitrifying bacteria and with the means of probiotic treatment and after the treatment it was seen that the probiotics (*Nitrosomonas* and *Nitrobacter*) used in the pond under such conditions resulting into (Gross yield and Net yield) in Pond-A, Pond-B and Pond-C are 33.97 & 32.29 t/ha/332days and 33.66 & 32.04 t/ha/332 days and 29.53 & 28.09 t/ha/332days respectively. This result shows that the fish growth was very good in the probiotics treated pond (Pond-A and Pond-B) whereas the growth was lower in case of controlled Pond-C.

Bhatnagar and Devi (2013) seen that chemoautotrophic bacteria have a direct effect upon the conversion of ammonia into nitrite which was further converted into nitrate thus removing the chance of getting adversally affected by the nitrite as per being the silent killer for fish.

# CHAPTER- III

# MATERIALS AND METHODS

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"**. The materials used and methodologies followed are present as below:

## 3.1 General

### 3.1.1 Glassware

In this experiment the glassware used for the present investigation was cleaned with tap water and then treated with chromic acid solution (60 g potassium dichromate + 1000 ml distilled water + 60 ml concentrate sulphuric acid) then washed with tap water. The glassware thus cleaned was rinsed with double distilled water and autoclaved before use.

### 3.1.2 Chemicals

Standard chemicals, reagents, culture media etc. required for the experimentation were obtained from Department of Agricultural Microbiology, College of Agriculture Raipur, Indira Gandhi Krishi Vishwavidyalaya Raipur, Chhattisgarh.

## 3.2 Location of experimental site

The present investigation was carried out at the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya Raipur (Chhattisgarh). Selected autotrophic (nitrifying) and heterotrophic bacterial isolates were isolated and applied in to sets of growth room experiment and fish aquariums in the green house of the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya Raipur. Water biochemical-chemical analysis and demonstration of promising treatment combination effect were also done.

### 3.3 Sampling of Soil and Water for Bacterial Isolation

Twenty-five soil samples (0-15 cm) were collected from corner bottom of 25 fish ponds belonging to Raipur district (Chhattisgarh). The composit sample was 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 urea polluted water in order to observe their efficiency especially their effect on rate of fish growth.

### 3.4 Isolation of bacteria

#### (a)Isolation of autotrophic (nitrifying) bacteria

Nitrifying bacteria were isolated from moist fish pond soil by using serial dilution and plating method (Schmidt and Belser, 1982) by using specific agar media (Ford, 1988). The agar plates were incubated at 30$^{o}$C for 4 weeks. Desirable colonies were picked up for their multiplication and testing in the broth media. Similarly these bacterial isolates also isolated from the samples of polluted pond water (Table 4.1, Plate 1a-c and Plate 2).

### 3.5 Testing of bacteria

#### (a) Testing of autotrophic (nitrifying) bacteria

The ammonia broth medium and nitrite broth medium were used for multiplication and testing of *Nitrosomonas s*and *Nitrobacter* isolates, respectively (James and Natalie, 1992).

#### *Nitrosomonas* culture medium

The chemical composition of ammonia broth medium for *Nitrosomonas* was as follows:

1) Ammonium sulfate $(NH_4)_2$ $SO_4$ - 2.00g

2) Magnesium sulfate ($MgSO_4$ $7H_2O$) - 0.50g

3) Ferrous sulfate ($FeSO_4$ $7H_2O$) - 0.03g

4) Sodium chloride (NaCl) - 0.30g

5) Magnesium carbonate ($MgCO_3$) - 10.00g

6) Di-potassium hydrogen phosphate ($K_2HPO_4$) - 1.00g

7) Double distilled water - 1000ml

***Nitrobacter* culture media**

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

1) Sodium nitrite ($NaNO_2$) - 1.00g

2) Magnesium sulfate ($MgSO_4.7H_2O$) - 0.50g

3) Ferrous sulfate ($FeSO_4.\ 7H_2O$) - 0.03g

4) Sodium chloride (NaCl) - 0.30g

5) Sodium carbonate ($Na_2CO_3$) - 1.00g

6) Di-potassium hydrogen phosphate ($K_2HPO_4$) - 1.00g

7) Double distilled water - 1000ml

Fifty ml of the ammonia broth medium and the nitrite broth medium were taken in Erlenmeyer flasks (250 ml) and sterilized by autoclaving for 30 minutes at 15 lb/sq inch pressure.

One ml of autotrophic (nitrifying) bacterial suspension was inoculated into appropriately labeled flasks, A and B. Flask A contained the *Nitrosomones* in-oculated ammonium broth medium and flask B contained the *Nitrobacter* inoculated nitrite broth medium while, flask C contained un-inoculated broth medium for control. All the flasks were incubated up to four weeks at 30° C temperature.

Inoculated and incubated media were periodically checked for activities of autotrophic (nitrifying) bacteria by measuring the decrease of ammonia and/or nitrite concentrations in the flasks. Nitrogen spot test method (James and Natalie, 1992) was also used for testing the presence of ammonia, nitrite and nitrate in the flasks at various intervals *i.e.* at 15 days after inoculation (DAI). Simultaneously, other tests including Gram's stain slides were also prepared for the characterization purpose of the isolates. These isolates were further confirmed through microscopic observations by using phase contrast Leica DMRBE microscope. Selection of effective nitrifying bacterial isolates was made on the basis of their growth performance and qualitative testing.

**(b) Isolation of heterotrophic bacteria**

Heterotrophic bacteria were isolated from the muddy moist fish pond soil just after collection of samples by using serial dilution and plating method (Schmidt and Belser, 1982). In this connection, specific medium was used for isolation of heterotrophic bacterium and their multiplication-testing as bio-remediators for polluted water in order to increase fish growth rate (Table 4.2, Plate 1a-c, 2).

About 1 gram of the muddy soil was aseptically transferred, using a sterilized dropper, into a sterile test tube containing 9 ml of the diluents. This gave $10^{-1}$ dilution. Subsequently, up to six fold ($10^{-6}$) serial dilutions were prepared from the $10^{-1}$ dilution (Joel and Amajuoyi; 2010). Further, the aliquot of appropriate dilution was used for inoculation on its agar medium. The inoculated agar plates were incubated at $28 \pm 2^{o}C$ up to 72 hrs. The identified selected colonies were counted at 24 hrs intervals as described by Joel and Amajuoyi, (2010).

The chemical composition of the heterotrophic medium for heterotrophic bacteria was as follows: (composition for 1 liter)

| | |
|---|---|
| Agar | -20.0g |
| $Na_2HPO_4$ | -7.9g |
| $KH_2PO_4$ | -1.5g |
| $NH_4Cl$ | -0.3g |
| $MgSO_4.7H_2O$ | -0.1g |
| Yeast extract solution | -10.0ml |
| Trace elements solution SL10 | -1.0ml |
| pH | - 7.5 |

**Testing of heterotrophic bacteria**

Fifty ml of the broth medium were taken in Erlenmeyer flasks (250 ml) and sterilized by autoclaving for 30 minutes at 15 lb/sq inch pressure. Then after 1ml of heterotrophic bacterial suspension was inoculated into appropriately labeled flasks. Flask contained the broth medium. All the flasks were incubated up to 1 weeks at 28 ± 2$^{o}$C. Broth of inoculated and incubated flasks was periodically checked for activities of ammonification and denitrification by measuring the concentration of ammonia and nitrate in the flasks.

## 3.6 Multiplication of bacteria

### (a) Autotrophic (nitrifying) bacteria

A new batch of flasks containing appropriate medium were inoculated by using 4 weeks old, 1ml inoculum and incubated in shaking incubator (200 rpm) for another 4 weeks at 30$^{o}$C temperature. Appropriate pH (8.0) was maintained during incubation period. After 4 weeks of inoculation, inoculum was stored in refrigerator for their further use especially in fish aquarium system.

### (b) Heterotrophic bacteria

A new batch of flasks containing appropriate medium were inoculated by using 1 weeks old, 1ml inoculum and incubated in shaking incubator (200 rpm) for another 1weeks at 30$^{o}$C temperature. Appropriate pH (7.5) was maintained during incubation period. After 1 week of inoculation, inoculum was stored in refrigerator for their further use in the present investigation.

## 3.7 Characterization and identification of autotrophic and heterotrophic bacteria

Selected bacterial isolates (autotrophic NS8, NB8 and heterotrophic PS5, PS16) were characterized morphologically and biochemically by using standard methodology (Table 4.2b, 4.4, Plate 3-5) as described in Bergeys Manual of Determinative Bacteriology.

### 3.8 Experimental detail

A series of experiments was (Preliminary experiment, fish aquarium green house experiment and demonstration trial of potential bioremediators) conducted in the present investigation in order to test and identify eco-friendly promising bacterial bioremediators for polluted fish pond water especially of urban area in order to increase fish growth rate.

#### 3.8.1 Preliminary experiment

**(a) Preliminary in vitro experiment without fish was conducted with a set of 500ml conical flasks containing polluted pond water with following treatments**

| Abbre. | Treatments |
|---|---|
| **$T_1$** | No inoculums and no chemical |
| **$T_2$** | Organic source of N (Urea 2 ppm) + No inoculums |
| **$T_3$** | Organic source of N (Urea 2 ppm) + $I_1$ |
| **$T_4$** | Organic source of N (Urea 2 ppm) + $I_2$ |
| **$T_5$** | Organic source of N (Urea 8 ppm) + No inoculums |
| **$T_6$** | Organic source of N (Urea 8 ppm) + $I_1$ |
| **$T_7$** | Organic source of N (Urea 8 ppm) + $I_2$ |
| **$T_8$** | Organic source of N (Urea 12 ppm) + $I_1$ |

$I_1$ = Inoculum @ 100µl $l^{-1}$ (Heterotrophic bacteria broth)
$I_2$ = Inoculum @ 100µl $l^{-1}$ each ( NS8 : *Nitrosomonas* sp. NB8: *Nitrobacter* sp. and PS5=*Micrococcus luteus* CP001628 bacterial isolate, PS16=*Ochrobactrum pituitosum* AM490609)

**(b) Preliminary in vitro experiment without fish was conducted with 5% molasses amended water of polluted pond in 500ml conical flasks with same set of treatments (Plate 6-7).**

**(c) Preliminary in vitro experiment without fish was conducted with 1% skimmed milk amended water of polluted pond in 500ml conical flasks with same set of treatments (Plate 6-7).**

**Application of urea**

Application of urea as nitrogenous organic compound to create different levels of water pollution artificially to test bacterial bioremediators in vitro conditions as mentioned in the treatments details. The in-vitro preliminary experiment was planned without fish culture. The treatments were replicated thrice in CRD. At different intervals, analysis for important parameters (N transformations) was done.

**Application of bioremediators**

The mixed inoculum of *Nitrosomonas NS* 8 and *Nitrobacter NB*8 isolates and PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609 heterotrophic bacterial isolates were used @ 100µl $l^{-1}$ as inoculum.

**Determination of ammonium and nitrate**

The concentration of ammonia and nitrate was determined by Kjeldhal method by using Devarda's alloy. The Devarda's alloy which 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) of nitrate to ammonium. The solution still has a high pH so that the $NO_3$ converted to $NH_4$ easily which can be again distilled off for analysis.

**3.8.2 (a) Fish aquarium green house experiment**

The fish aquarium green house experiment was very important experiment of the present investigation. It was especially planned to observe influence of bacterial bioremediators on fish growth rate under artificially urea polluted aquarium conditions containing polluted pond water and ordinary water in 1:1 ratio. Out of the 25 ponds, the water was collected from near by polluted pond.

**Treatment details**

| Abbre. | Treatments |
|---|---|
| $T_1$ | No inoculums and no chemical |
| $T_2$ | Organic source of N (Urea 2 ppm) + No inoculums |
| $T_3$ | Organic source of N (Urea 2 ppm) + $I_1$ |
| $T_4$ | Organic source of N (Urea 2 ppm) + $I_2$ |
| $T_5$ | Organic source of N (Urea 8 ppm) + No inoculums |
| $T_6$ | Organic source of N (Urea 8 ppm) + $I_1$ |
| $T_7$ | Organic source of N (Urea 8 ppm) + $I_2$ |
| $T_8$ | Organic source of N (Urea 12 ppm) + $I_1$ |

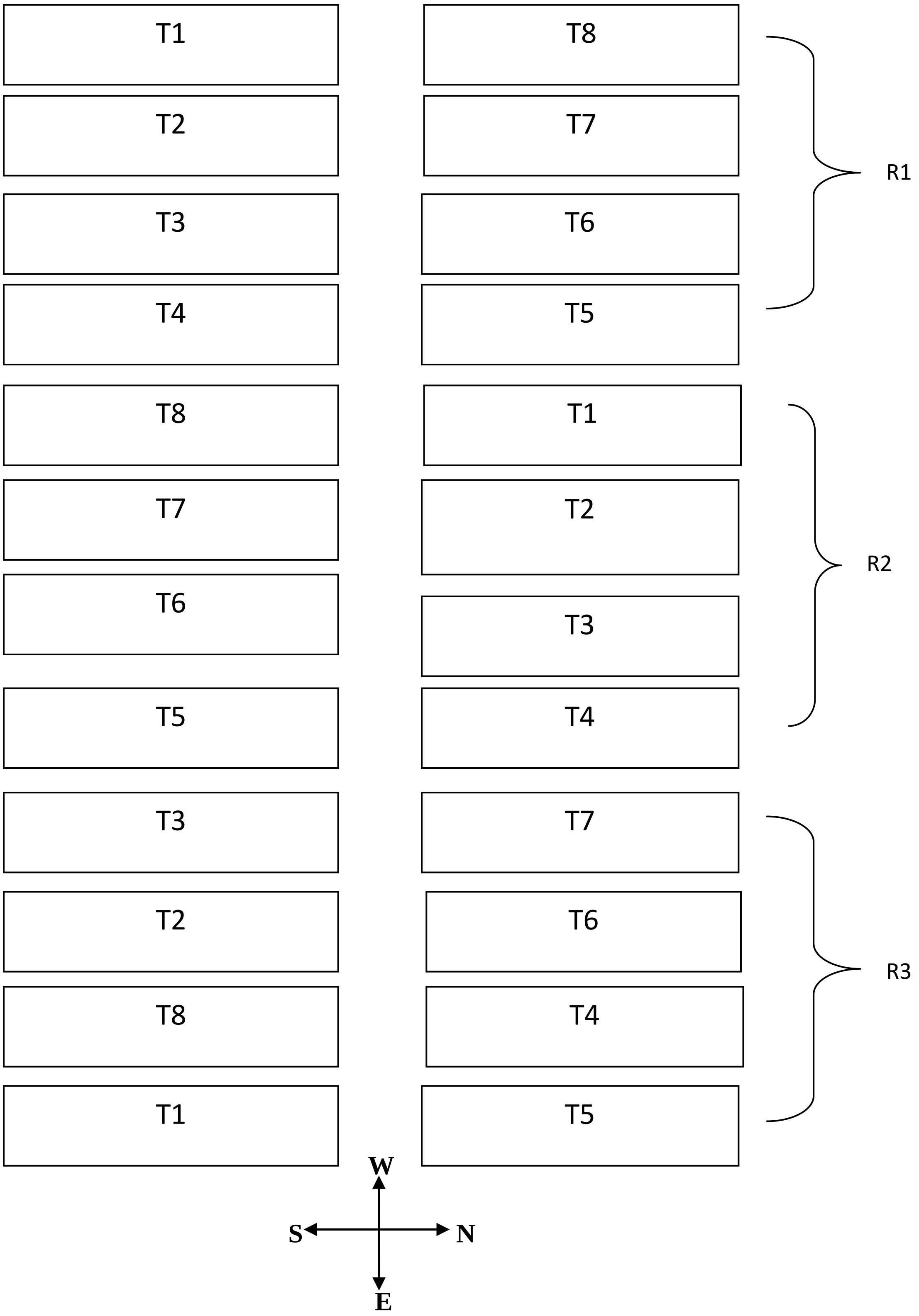
$I_1$ = Inoculum @ 15µl $l^{-1}$ (Heterotrophic bacteria broth)

$I_2$ = Inoculum @15µl $l^{-1}$ each ( NS8 : *Nitrosomonas* sp. NB8: *Nitrobacter* sp. and PS5= *Micrococcus luteus* CP001628 bacterial isolate, PS16 = *Ochrobactrum pituitosum* AM490609)

**(b) The fish aquarium experiment was also conducted with 15 ppm molasses amended water of polluted pond with same set of treatments**

This study was carried out in twenty-four aquarium (volume 75x45x30 $cm^3$) containing 70.0 litre water under green house conditions.

## Experimental layout plan



**Application of urea**

Application of urea as nitrogenous organic compound to create different levels of water pollution artificially to test bacterial bioremediators in green house conditions as mentioned in the treatments details. The experiment was planned with fish culture. The treatments were replicated thrice in CRD. Similar to that of preliminary experiment, at different intervals, analysis for important parameters (N transformations) of this experiment was also done.

**Application of bioremediators in aquarium**

The mixed inoculum of *Nitrosomonas NS8* and *Nitrobacter NB8* isolates and PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609 heterotrophic bacterium were used @ 15µl each inoculum $l^{-1}$ in fish aquaculture water, while they were used @100 µl each inoculum $l^{-1}$ water in the study without fish. The inoculum dose was adjusted and applied in such a way so that commercially viable dose can be fixed to large scale fish farmers.

**Physico-Chemical Analysis**

**pH, Temperature, Turbidity, EC, TDS and Dissolved Oxygen**

These chemical properties were determined electrometrically with a multi-parameter data logger (Hanna model HI991300).

**pH**

The pH was measured by pH meter. For soil pH by using 1:2 soil : water suspension.

**Temperature**

The temperature of water was one important parameter which directly influences some chemical reactions in aquatic ecosystem, the temperatures was recorded by thermometer (Ganpati 1943).

**Electrical Conductivity**

Electrical conductivity was measured by conductivity meter. Elicomicro processor based conductivity meter, model 1601. Electrical conductivity indicates the capacity of electrical current that passed through the water.

**Total Dissolved Solid**

TDS was measured with the help of digital TDS meter, E.I., model 651. Electrical conductivity of water samples correlates with the concentration of TDS of water. All samples are non saline as per the salinity classification. (Table-2) as suggested by Robinove *et al.,* (1958).

**Classification of water on the basis of salinity value**

TDS (ppm) Description

<1000 non saline

1000-3000 slightly saline

3000-10,000 moderately saline

>10,000 Very Saline

**Turbidity**

Turbidity was measured by digital turbidity meter model 33I EI.The suspension of particles in water interfering with passage of light was called turbidity (Mahobe and Mishra, 2013).

**3.9 Fish growth study**

**3.9. 1 Release of fish**

Ten common carp (*Cyprinus carpio var. communis*) fry were stocked in each aquarium.

**3.9.2 Fish food**

Proper fish feed was used. Feed composition was- crude protein about 28%, crude fat about 3%, crude fiber about 4% and moisture maximum 10%.

**3.9.3 Measurement of fish growth**

Growth of fish was studied in terms of weight in gram and length in cm (Bajaj, 2017).

## 3.10 Statistical analysis

The data recorded on each parameter under study were statistically analysed following CRD design to test the statistical significance of variance among different treatment means as influenced with the application of different treatments of microorganisms and organic source of N on fish growth rate and N transformations.

# CHAPTER- IV
# RESULTS AND DISCUSSION

The present investigation was conducted at the Department of Agricultural Microbiology, Indira Gandhi Agricultural University, Raipur during 2017-2019 to isolate and select effective autotrophic (nitrifying) and heterotrophic bacterial isolates from soil-water of polluted ponds of Raipur District of Chhattisgarh State in order to enhance eco-friendly fish production especially in polluted urban water bodies.The investigation included i) Preliminary experiment ii) Fish aquarium green house experiment iii) Experiment to demonstrate promising combination of bio-inoculants.

**Background of the study**

Present investigation was planned as an important part of study for exploiting natural microbial resources in order to formulate bacterial inoculants to be used as bio-remediators for chemical pollutants including urea and ammonia of water bodies especially urban water bodies for eco-friendly sustainable fish cultivation.

## 4.1. Location of soil and water samples collected for isolation of nitrifying autotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh)

Data presented in (Table 4.1, Plate 1a, 1b, & 1c) related to information about *Nitrosomonas* and *Nitrobacter* isolates which were isolated from 25 soil and water samples of polluted ponds belonging to area of Raipur, Chhattisgarh. Further these isolates were tested for their ability to convert ammonia into nitrate. Results of qualitative analysis (Table 4.2b, 4.5- 4.8) clearly indicate that ammonia started to transform after two weeks of inoculation of nitrifying bacterial isolates under controlled conditions. Selection of effective nitrifying bacterial isolates was made on the basis of rate of conversion of ammonia into nitrate (James and Natalie, 1992).

Isolation of nitrifying bacteria was done by using serial dilution-plating method as also practiced by several researchers (Ford, 1988; Grunditz and Dalhammar, 2001; Scmidit and Belser 1982). They clearly indicate that isolates of nitrifying bacteria can be obtained by making serial diluation of soil samples. Li *et al.*,(2001) reported that nitrifying bacteria can be isolated by making appropriate serial diluation of drained fish pond soil. In this connection, Grunditz and Dalhammar (2001) reported that presence of nitrifying bacteria can be observed up to $10^{-7}$diluation. However, in the present studies, isolation was made from $10^{-3}$ dilution. Which bears low population density of nitrifying bacteria in soils of Chhattisgarh (Gupta *et al.*,2002).

During isolation of nitrifying bacteria *i.e. Nitrosomonas* and *Nitrobacter*, both media become turbid and this further confirms the presence of nitrifying bacteria. Gram's staining and microscopic observation also gave confirmation of presence of nitrifying bacteria.

Further selected isolates of *Nitrosomonas* and *Nitrobacter* were multiplied for their exploitation as bioremediator in simulated aquaculture system. *Nitrosomonas* and *Nitrobacter* isolates were also isolated from drained fish ponds by Barik *et al*., (2005).They expressed similar views and reported that soil of Chhattisgarh plains was exposed to extreme dry and hot climate during summer resulting in to drastic loss in population of mesophilic microbes including nitrifying bacteria. Further, similar view was also given by Scmidit and Belser, (1982). They suggested that $10^{-3}$ dilution can be consider as appropriate diluation for isolation of nitrifying bacteria from drained fish pond soil.

Nitrification has been reported to get inhibited in polluted waters (Gruditz and Dalhammar, 2001). *Nitrosomonas* and *Nitrobacter* genera were also isolated from polluted pond water by Barik *et al.,* (2018) and Watson *et al*., (1989) and from activated sludge by Grunditz and Dalhammar, (2001) and Du *et al*., (2003), biofilm in aquarium by Bower and Turner (1981), garden soil by James and Natalie (1992) and from pond mud by Li *et al*., (2001). Selection of effective nitrifying bacterial isolates was made on the basis of rate of colour change due to nitrifying process James and Natalie, (1992). Selected isolates of *Nitrosomonas* and *Nitrobacter* were multiplied for their exploitation as bioremediator in aquaculture system. For fast multiplication purpose, temperature and pH were maintained at 30$^{o}$C and 8, respectively throughout the 4 weeks of incubation period as described by Grunditz and Dalhammar, (2001).

**Table 4.1. Location of soil and water samples collected for isolation of nitrifying autotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh**

| S.No. | Village /N.Nigam Ward Name | Location of pond | | | Isolate No. | | | |
|---|---|---|---|---|---|---|---|---|
| | | Latitude | Longitude | Pond Name | *Nitrosomonas* | | *Nitrobacter* | |
| | | | | | Soil | Water | Soil | Water |
| 1. | Purena | 21°14'1.070"N | 81°40'17.73"E | 1st Purena pond, | NS1S | NS1W | NB1S | NB1W |
| 2. | Purena | 21°14'0.120"N | 81°40'23.00"E | 2nd Purena pond, | NS2S | NS2W | NB2S | NB2W |
| 3. | Amlidih | 21°13'21.08"N | 81°40'11.28"E | 1st Amlidih pond | NS3S | NS3W | NB3S | NB3W |
| 4. | Amlidih | 21°13'27.45"N | 81°40'31.07"E | 2nd Amlidih pond | NS4S | NS4W | NB4S | NB4W |
| 5. | Katora | 21° 14' 5.44"N | 81° 39' 18.0"E | Katora Talab pond | NS5S | NS5W | NB5S | NB5W |
| 6. | Telibandha | 21°14'23.45"N | 81°39'39.48"E | Marine drive pond | NS6S | NS6W | NB6S | NB6W |
| 7. | Tikrapara | 21°13'39.57"N | 81°38'26.22"E | NariyaTikraparapond | NS7S | NS7W | NB7S | NB7W |
| 8. | Math para | 21°13'16.55"N | 81°37'31.98"E | 1st Dudhadhari pond | NS8S | NS8W | NB8S | NB8W |
| 9. | Math para | 21°13'42.04"N | 81°37'44.89"E | 2nd Dudhadhari pond | NS9S | NS9W | NB9S | NB9W |
| 10. | Kankali para | 21° 14' 10.75"N | 81° 37' 42.00"E | Kankaliparapond | NS10S | NS10W | NB10S | NB10W |
| 11. | Kalibadi | 21°13'48.58"N | 81°37'59.83"E | Budha Talab pond | NS11S | NS11W | NB11S | NB11W |
| 12. | Mova | 21°16'6.500"N | 81°40'18.77"E | Mova pond | NS12S | NS12W | NB12S | NB12W |
| 13. | Pandri | 21°13'39.36"N | 81°38'26.64"E | NariyaPandripond | NS13S | NS13W | NB13S | NB13W |
| 14. | Raja para | 21° 14' 52.69"N | 81° 39' 16.0"E | Raja Talab pond | NS14S | NS14W | NB14S | NB14W |
| 15. | KailashpuriKushalpur | 21°12'53.00"N | 81°42'37.66"E | Dabri pond | NS15S | NS15W | NB15S | NB15W |
| 16. | KailashpuriKushalpur | 21° 13' 49.10"N | 81° 37' 15.24"E | Kho-Kho pond | NS16S | NS16W | NB16S | NB16W |
| 17. | KailashpuriKushalpur | 21° 13' 39.85"N | 81° 36' 48.58"E | Pahadi pond | NS17S | NS17W | NB17S | NB17W |
| 18. | Kushalpur | 21°13'58.44"N | 81°37'10.62"E | Gittikhadan pond | NS18S | NS18W | NB18S | NB18W |
| 19. | Kushalpur | 21°14'4.12"N | 81°37'9.68"E | Malsat pond | NS19S | NS19W | NB19S | NB19W |
| 20. | Professor colony Kushalpur | 21° 13' 17.3"N | 81° 37' 29.99"E | BhaiyaTalabpond | NS20S | NS20W | NB20S | NB20W |
| 21. | Purana basti | 21° 13' 44.3"N | 81° 37' 37.31"E | Maharaja pond | NS21S | NS21W | NB21S | NB21W |
| 22. | Math purena | 21° 13' 19.9"N | 81° 37' 56.17"E | 1st Chiranjiv pond | NS22S | NS22W | NB22S | NB22W |
| 23. | Math purena | 21° 12' 39.0"N | 81° 38' 31.00"E | 2nd Chiranjiv pond | NS23S | NS23W | NB23S | NB23W |
| 24. | Kharun | 21° 12' 59.36"N | 81° 35' 16.00"E | Kharun | NS24S | NS24W | NB24S | NB24W |
| 25. | Sarona | 21° 17' 15.43"N | 81° 36' 42.00"E | Sarona pond | NS25S | NS25W | NB25S | NB25W |

NS =*Nitrosomonas* bacterial isolate, NB = *Nitrobacter* bacterial isolate

**Amlidih pond** (Latitude/Longitude 21°13'21.08"N/81°40'11.28"E)

**Bhaiya talab** (Latitude/Longitude21° 13' 17.3"N /81° 37' 29.99"E)

**Budha talab**(Latitude/Longitude21°13'48.58"N/81°37'59.83"E)

**Plate 1a : General view of ponds situated South side of Raipur city (landmark Jaistambh considered for soil-water sampling).**

**Kho-Kho pond** (Latitude/Longitude21° 13' 49.10"N/81° 37' 15.24"E)

**Math purena pond** (Latitude/Longitude21° 13' 19.9"N/81° 37' 56.17"E)

**Dudhadhari pond** (Latitude/Longitude 21°13'16.55"N/81°37'31.98"E)

**Plate 1b : General view of ponds situated South side of Raipur city (landmark Jaistambh considered for soil-water sampling).**

**Katora Talab** (Latitude/Longitude21° 14' 5.44"N/81° 39' 18.0"E)

**Raja Talab** (Latitude/Longitude21° 14' 52.69"N/81° 39' 16.0"E)

**Telibandha** (Latitude/Longitude 21°14'23.45"N/81°39'39.48"E)

**Plate 1c : General view of ponds situated East side of Raipur city (landmark Jaistambh considered for soil-water sampling).**

**Kho-Kho pond** (Latitude/Longitude21° 13' 49.10"N /81° 37' 15.24"E)

**Pahadi pond** (Latitude/Longitude  21° 13' 39.85"N/81° 36' 48.58"E)

**Plate 2 : General view of ponds situated West South side of Raipur city, (landmark Jaistambh  considered for soil-water sampling).**

### 4.2(a). Location of soil samples collected for isolation of heterotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh)

Heterotrophic bacteria were isolated from 25 soil samples of polluted ponds belonging to near by area of Raipur, Chhattisgarh (Table 4.2a, Plate 2). Further these isolates were tested for their ability to convert ammonia into nitrate by using proper medium (Table 4.2a, Fig.1, Plate 1-5)

During isolation of denitrifying bacteria *Ochrobactrum pituitosum* AM 490609 (PS16) and *Micrococcus luteus* CP001628 (PS5), the broth medium become more turbid with in a week this further confirms that they belong to heterotrophic group of bacteria. Gram's staining and microscopic observation also gave confirmation of presence of these bacteria.

Further selected heterotrophic bacterial isolates were multiplied for their testing as bioremediators in simulated aquaculture system. *Ochrobactrum pituitosum* and *Micrococcus luteus* strains were also isolated from polluted fish ponds water by Huber *et al.,* 2010. Similarly, *Ochrobactrum pituitosum* genera were also isolated from industrial environment by Scholz *et al.*, (2010). Selection of effective denitrifying bacterial isolates was made on the basis of rate of colour change due to denitrifying process (Lin, 2004, Sunitha and Padmavathi, 2013).

**Table 4.2(a) Location of soil samples collected for isolation of heterotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh)**

| S.No. | Village /N.Nigam Ward Name | Location of pond | | | Isolate No. |
|---|---|---|---|---|---|
| | | Latitude | Longitude | Pond Name | Heterotrophic bacteria of pond soil |
| 1. | Purena | 21°14'1.070"N | 81°40'17.73"E | 1st Purena pond, | **PS1** |
| 2. | Purena | 21°14'0.120"N | 81°40'23.00"E | 2nd Purena pond, | PS2 |
| 3. | Amlidih | 21°13'21.08"N | 81°40'11.28"E | 1st Amlidih pond | PS3 |
| 4. | Amlidih | 21°13'27.45"N | 81°40'31.07"E | 2nd Amlidih pond | PS4 |
| 5. | Katora | 21° 14' 5.44"N | 81° 39' 18.0"E | Katora Talab pond | **PS5** |
| 6. | Telibandha | 21°14'23.45"N | 81°39'39.48"E | Marine drive pond | **PS6** |
| 7. | Tikrapara | 21°13'39.57"N | 81°38'26.22"E | NariyaTikraparapond | PS7 |
| 8. | Math para | 21°13'16.55"N | 81°37'31.98"E | 1st Dudhadhari pond | PS8 |
| 9. | Math para | 21°13'42.04"N | 81°37'44.89"E | 2nd Dudhadhari pond | PS9 |
| 10. | Kankali para | 21° 14' 10.75"N | 81° 37' 42.00"E | Kankaliparapond | **PS10** |
| 11. | Kalibadi | 21°13'48.58"N | 81°37'59.83"E | Budha Talab pond | **PS16** |
| 12. | Mova | 21°16'6.500"N | 81°40'18.77"E | Mova pond | PS12 |
| 13. | Pandri | 21°13'39.36"N | 81°38'26.64"E | NariyaPandripond | PS13 |
| 14. | Raja para | 21° 14' 52.69"N | 81° 39' 16.0"E | Raja Talab pond | PS14 |
| 15. | Kailashpuri Kushalpur | 21°12'53.00"N | 81°42'37.66"E | Dabri pond | PS15 |
| 16. | Kailashpuri Kushalpur | 21° 13' 49.10"N | 81° 37' 15.24"E | Kho-Kho pond | PS16 |
| 17. | Kailashpuri Kushalpur | 21° 13' 39.85"N | 81° 36' 48.58"E | Pahadi pond | PS17 |
| 18. | Kushalpur | 21°13'58.44"N | 81°37'10.62"E | Gittikhadan pond | PS18 |
| 19. | Kushalpur | 21°14'4.12"N | 81°37'9.68"E | Malsat pond | PS19 |
| 20. | Professor colony Kushalpur | 21° 13' 17.3"N | 81° 37' 29.99"E | BhaiyaTalabpond | PS20 |
| 21. | Purana basti | 21° 13' 44.3"N | 81° 37' 37.31"E | Maharaja pond | PS21 |
| 22. | Math purena | 21° 13' 19.9"N | 81° 37' 56.17"E | 1st Chiranjiv pond | PS22 |
| 23. | Math purena | 21° 12' 39.0"N | 81° 38' 31.00"E | 2nd Chiranjiv pond | PS23 |
| 24. | Kharun | 21° 12' 59.36"N | 81° 35' 16.00"E | Kharun | PS24 |
| 25. | Sarona | 21° 17' 15.43"N | 81° 36' 42.00"E | Sarona pond | **PS25** |

PS= Heterotrophic bacteria isolate, PS5=*Micrococcus luteus*CP001628 bacterial isolate, PS16 =*Ochrobactrum pituitosum* AM490609 bacterial isolate

### 4.2(b). Ammonia and nitrate dynamics in polluted pond water as influenced by selected pond soil heterotrophic bacterial isolates under controlled conditions.

Heterotrophic bacteria isolates were isolated from 25 pond soils belonging to area of Raipur district, Chhattisgarh. Among these, 7 isolates (PS1, PS3, PS5, PS6, PS10, PS16 and PS25) were selected on the basis of their growth performance during isolation from pond soils. These selected isolates were further tested for their ability for ammonia transformations as presented in Table-4.2b (Fig 1, Plate 3).

Data presented in Table 4.2b revealed that out of 7 isolates, PS-5 and PS-16 isolates found more effective with reference to conversion of ammonia.The ammonia concentration due to PS-5 was decreased from 0.20 mg $l^{-1}$ (15 DAI) to 0.09 mg $l^{-1}$ (30 DAI). Similarly, the PS16 was also showed the decreasing result from 0.15 mg $l^{-1}$ (15 DAI) to 0.05 mg $l^{-1}$ (30 DAI). These findings are close to findings of Barik *et al.,* 2018.

In the table 4.2(b), the nitrate concentration due to PS-5 was increased from 135 mg $l^{-1}$ (15 DAI) to 170 mg $l^{-1}$ (30 DAI) because of effective transformation of ammonia. Similarly, the PS16 also gave similar type of promising result in order to reduce more harmful concentration of ammonia by nitrification process. The nitrate concentration increased from 139 mg $l^{-1}$ (15 DAI) to 171 mg $l^{-1}$ (30 DAI). These findings are also close to findings of Mahobe and Mishra, 2013 and Barik *et al*., 2018.

**Table 4.2(b). Ammonia and nitrate dynamics in polluted pond water as influenced by selected pond soil heterotrophic bacterial isolates under controlled condition.**

| S.No. | Isolate No. | Ammoniaconcentration (mg $l^{-1}$) | | Nitrate concentration (mg $l^{-1}$) | | Turbidity (NTU) |
|---|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 15 DAI | At 30 DAI | At 30 DAI |
| **1.** | **PS1** | 0.31 | 0.21 | 116 | 160 | 239 |
| **2.** | **PS3** | 0.29 | 0.20 | 120 | 157 | 250 |
| **3.** | **PS5** | **0.20** | **0.09** | **135** | **170** | **274** |
| **4.** | **PS6** | 0.25 | 0.19 | 121 | 162 | 194 |
| **5.** | **PS10** | 0.22 | 0.12 | 121 | 165 | 206 |
| **6.** | **PS16** | **0.15** | **0.05** | **139** | **171** | **270** |
| **7.** | **PS25** | 0.25 | 0.20 | 123 | 168 | 220 |
| | **CD(0.05)** | 0.02 | 0.01 | 5.09 | 9.59 | 9.89 |

PS 5 = *Micrococcus luteus* CP001628, PS16 = *Ochrobactrum pituitosum*AM490609

Note =Water were collected from polluted pond which was contaminated by open drain water

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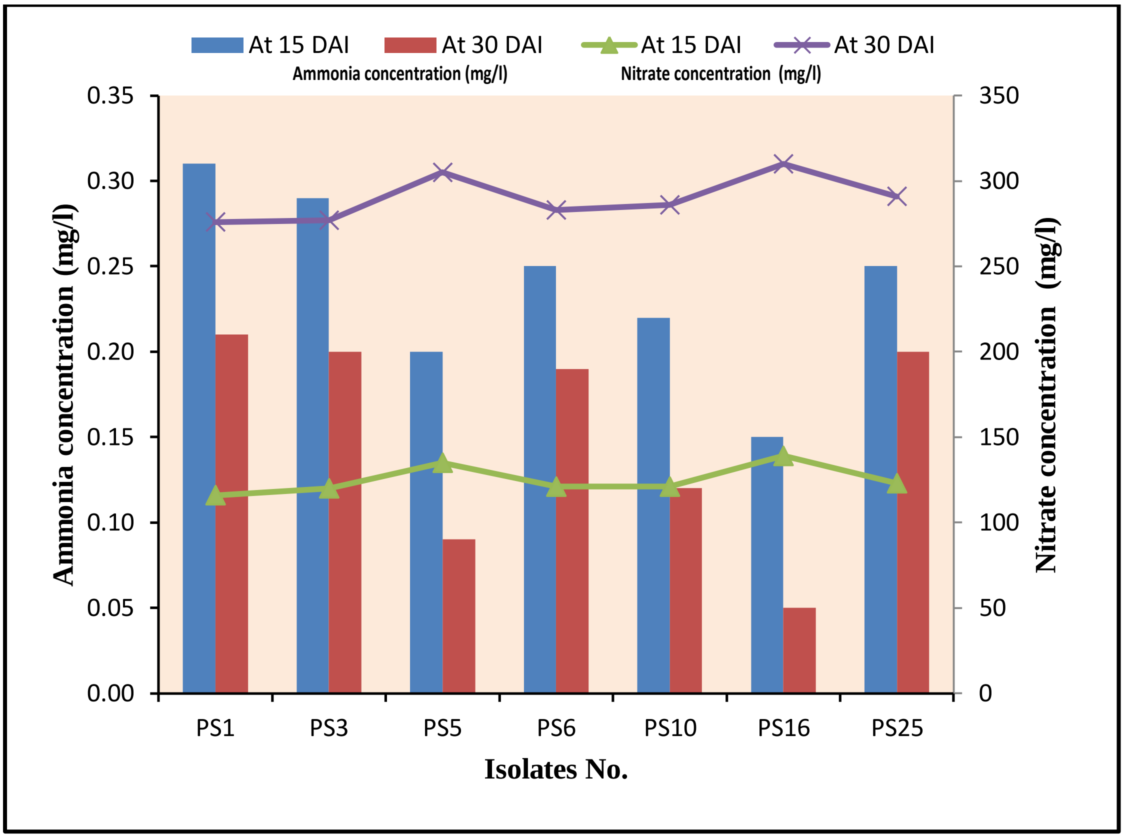



At 15 DAI At 30 DAI At 15 DAI At 30 DAI

Ammonia concentration (mg/l) Nitrate concentration (mg/l)

Heterotrophic bacterial isolates, isolated from:

| | | | |
|---|---|---|---|
| PS1 | - First Purena pond, Purena, | PS3 | - FirstAmlidih pond, Amlidih |
| PS5 | - Katora Talab pond, Katora Talab, | PS6 | - Marine drive pond, Telibandha |
| PS10 | - Kankalipara pond, Kankali Para, | PS16 | - Kho-Kho pond, Kushalpur |
| PS25 | - Gittikhadan pond, Kushalpur | | |

**Fig. 1 : Ammonia and nitrate dynamics in polluted pond water as influenced by selected pond soil heterotrophic bacterial isolates under controlled conditions.**

**Plate 3 : Isolation of heterotrophic bacteria from pond water and soil samples**

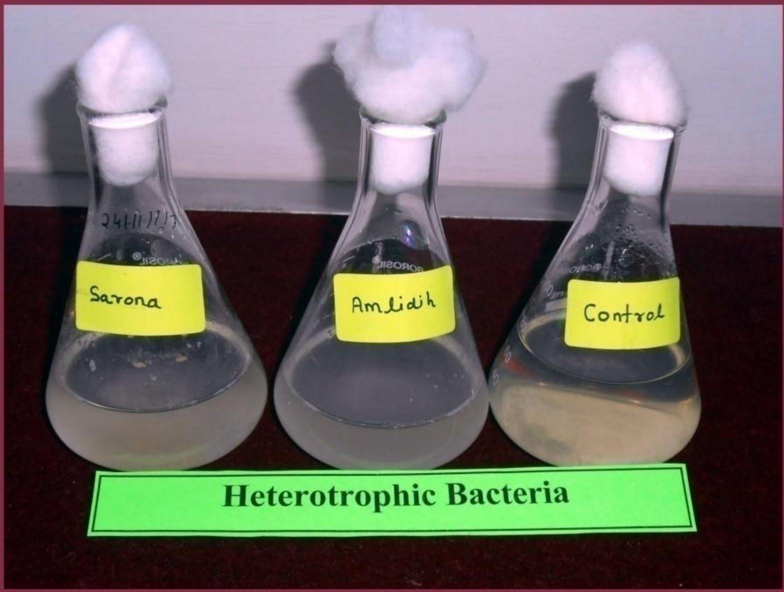




**Plate 4 : General views of growth of heterotrophic bacterial isolates.**

### 4.3. 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, Twenti 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 4.3 ( Plate 1-5) .

Pond water lowest EC (17x10 micro mhos/cm) was associated with Kankali para pond.While, highest value of water EC (38x10micro mhos/cm) recorded in Kailashpuri Kusalpur dabri pond. Similarly, lowest TDS was also associated with Kailashpuri Kusalpur pahadi pond (235 mg $l^{-1}$) followed by Amlidih first pond (250 mg $l^{-1}$). Highest TDS value was observed with water of Kailashpuri Kusalpur dabri pond (508 mg $l^{-1}$). Pond water pH was recorded towards alkaline range, the minimum value was 7.4 while, highest pH was recorded 8.2

Pond soil lowest EC (20x10 micro mhos/cm) was associated with Kailashpuri Kusalpur Pahadi pond and Math purena first chranjeevi pond. While, highest value of soil EC (40x10 micro 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 chranjeevi pond (256 mg $l^{-1}$) While, 545 mg $l^{-1}$ value was considered as highest which was associated with Kailashpuri Kusalpur dabri pond. The highest pH of pond soil was recorded 8.4 while, 7.6 remained lowest.

Measurement of and EC, pH were conducted in the water and soil samples by the help of hand-hold portable digital pH, TDS and EC meter, respectively. In this study the pond soil pH range was observed from 7.6 to 8.4 which was almost similar to the findings of Bajaj, (2017). The acceptable range of the pH was 7-9.5, desirable range was 6.5-9 and in stress condition it reached to $<4$ and $>11$ as reported by Bhatnagar and Devi, (2013). The pH of the water collected from different ponds ranged between 8.20 to 7.4. These observations are close to observations of Shrivastava *et al.,* 2007; Ogbonna and Chinomso, 2010.

**Table 4.3. 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 µhmos/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. | KailashpuriKushalpur | Dabri pond | 7.5 | 38×10 | 508 | 8.4 | 40×10 | 545 |
| 16. | KailashpuriKushalpur | Kho-Kho pond | 8.0 | 22×10 | 356 | 8.2 | 25×10 | 394 |
| 17. | KailashpuriKushalpur | 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 |

**Plate 5: Quantitative and qualitative testing of different hetrotrophic and autotrophic bacterial isolates for N transformations.**

**4.4. Morphological and bio-chemical characteristics of autotrophic and heterotrophic bacterial isolates.**

Data presented in Table 4.4 (Plate 3-5) related to information about autotrophic (nitrifying) and heterotrophic bacterial isolates which were isolated from 25 soil and water samples of polluted fish ponds belonging to area of Raipur and nearby Raipur, Chhattisgarh. Selected autotrophic (nitrifying) and heterotrophic bacteria were characterized with respect to colony characteristics and biochemical properties as per Bergey's Manual of Determinative Bacteriology, (Williams *et al*., 1994). These autotrophic and heterotrophic isolates were motile (polar flagella) and non-motile in properties, rod/ round in shape and showed gram-negative/positive behaviour during gram staining. They also showed negative/positive reaction for nitrate reduction test and positive for oxidase and catalase test. Mixed reactions were observed for ammonia utilization test, nitrate reduction, urease and motility tests as mentioned in Table 4.4.

In a study conducted by Phirke (2014) autotrophic (nitrifying) bacteria was found gram negative, motile rod, oxidase positive, liquefied gelatin were produced which is in close agreement with observations of present investigation.

**Table 4.4. 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 characterized 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) | (+ve) |
| | Cell shape | Long rod | Short rod | Short rod | Round |
| | Cell arrangement | Motile (polar flagella) | Non motile | Non motile | Non motile |
| **Biochemical characteristics** | Oxidase test | (+) | (+) | (+) | (+) |
| | Catalase test | (+) | (+) | (+) | (+) |
| | Ammonia utilization test | (+) | (-) | (-) | (+) |
| | Nitrate reduction test | (-) | (+) | (+) | (-) |
| | Urease test | (+) | (+) | (-) | (+) |
| | Motility test | (+) | (+) | (+) | (-) |

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

## 4.5. Ammonia and nitrate dynamics in 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 see reducton in the ammonium concentration in polluted pond water under controlled conditions. Data presented in Table 4.5 (Fig 2, Plate 6-7,9) about ammonium and nitrate concentration which 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 (T2-T8), lowest ammonia concentration detected in T4 (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, were as the ammonia concentration in the polluted pond water after 15 days showed maximum concentration but after 30 DAI showed decrease in ammonia concentration in polluted pond water mostly bcause 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.

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 done 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 about (3.01 ppm) as shown at 15 DAI (35.42 ppm) and at 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 at 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) and at 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 was having the least effect upon lowering the ammonia concentration from 7.17 (15 DAI) to 7.00 ppm (30 DAI).

Similar type of observation was made by Bhatnagar and Devi, 2013 who reported that acceptable range of ammonia was up to 0.05 ppm and desirable range was 0 - < 0.025 ppm and under stress condition the concentration ranges > 0.3ppm.The ammonia nitrogen was decreased from maximum level 10 mg $l^{-1}$ to minimum level 0.34 mg $l^{-1}$ when inoculated with 2.5 µl $l^{-1}$ as reported by Barik, *et al.* 2018.

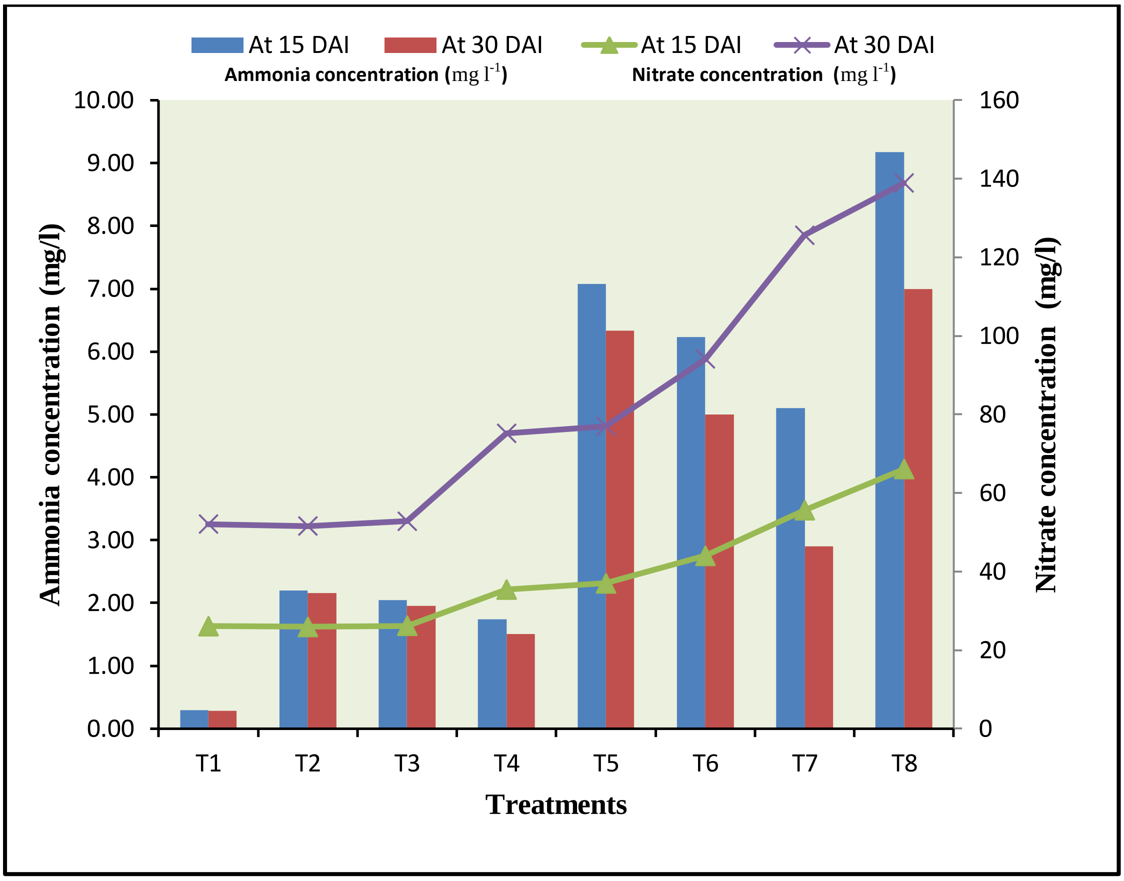
Among 3 levels of the urea concentration as 2 ppm, 8 ppm, 12 ppm in which under the 2-ppm urea concentration when comparing the treatments $T_3$ & $T_4$ with the treatment $T_2$ for the analysis of the nitrate concentration, the best result similar to that of ammonium level, the better result associated 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 $T_6$ & $T_7$ treatment, 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 was having the least effect upon increasing the nitrate concentration from 15 DAI (66.11 ppm) to 30 DAI (70.00 ppm). Similar type of observation was made by Bhatnagar and Devi, 2013. Findings of present investigation were similar to the findings of Ogbonna and Chinomsa, 2010.

The quantitative analysis clearly showed that initial concentration of ammonia and nitrate of water of polluted pond remained statistically at par as concentration associated at different intervals with treatment $T_1$ i.e. absolute control.

**Table 4.5. Ammonia and nitrate dynamics in 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 $l^{-1}$) | | Nitrate concentration ppm(mg $l^{-1}$) | |
|---|---|---|---|---|---|
| | | **At 15 DAI** | **At 30 DAI** | **At 15 DAI** | **At 30 DAI** |
| **T1** | No inoculums and no chemical | 0.289 | 0.281 | 26.1 | 26.0 |
| **T2** | Organic source of N (Urea 2 ppm) + No inoculums | 2.20 | 2.16 | 25.9 | 25.7 |
| **T3** | Organic source of N (Urea 2 ppm) + $I_1$ | 2.05 | 1.95 | 26.2 | 26.6 |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 1.74 | 1.51 | 35.42 | 39.77 |
| **T5** | Organic source of N (Urea 8 ppm) + No inoculums | 7.08 | 6.33 | 36.99 | 40.0 |
| **T6** | Organic source of N (Urea 8 ppm) + $I_1$ | 6.23 | 5.00 | 44.00 | 50.15 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 5.10 | 2.90 | 55.62 | 70.00 |
| **T8** | 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 culture of NS8, NB8 and PS5, PS16




At 15 DAI At 30 DAI Ammonia concentration (mg/l) At 15 DAI At 30 DAI Nitrate concentration (mg/l)

| | |
|---|---|
| T1 | No inoculums and no chemical |
| T2 | Organic source of N (Urea 2 ppm) + No inoculums |
| T3 | Organic source of N (Urea 2 ppm) + $I_1$ |
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T5 | Organic source of N (Urea 8 ppm) + No inoculums |
| T6 | Organic source of N (Urea 8 ppm) + $I_1$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |
| T8 | Organic source of N (Urea 12 ppm) + $I_1$ |

**Fig. 2: Ammonia and nitrate dynamics in water of polluted pond as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled conditions.**

**Plate 6: View of testing of bacterial isolates for an transformations in polluted pond water with and without molasses and skimed milk amendment polluted pond water.**

**Plate 7 : Study of microbial transformations of organic N**

### 4.6. Ammonia and nitrate dynamics in molasses amended water of polluted pond as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled conditions.

The data presented in Table 4.6 (Fig. 3 and Plate 6-7,9 ) clearly revealed that at 2-ppm organic N containing urea concentration, treatment T4 gave best results with reference to decease in the ammonium concentration from 15 DAI (1.41 ppm) to 30 DAI (1.27 ppm) and increase in nitrate concentration from 30.0 to 44.2 ppm when comparing the treatments of $T_3$ & $T_4$ with the treatment $T_2$ for the analysis of the ammonia concentration. Similarly at 8-ppm concentration of organic N-urea when comparing between the $T_5$ with $T_6$ & $T_7$ treatment, we got the best result with treatment $T_7$ with reference to reduce ammonia concentration.The decrease in the ammonia concentration from 15 DAI(4.77ppm) to 30 DAI(2.73ppm). While the next organic N level of 12 ppm, it was having the least effect upon lowering the ammonia concentration from 8.33 ppm at 15 DAI to 6.52 ppm at 30 DAI.

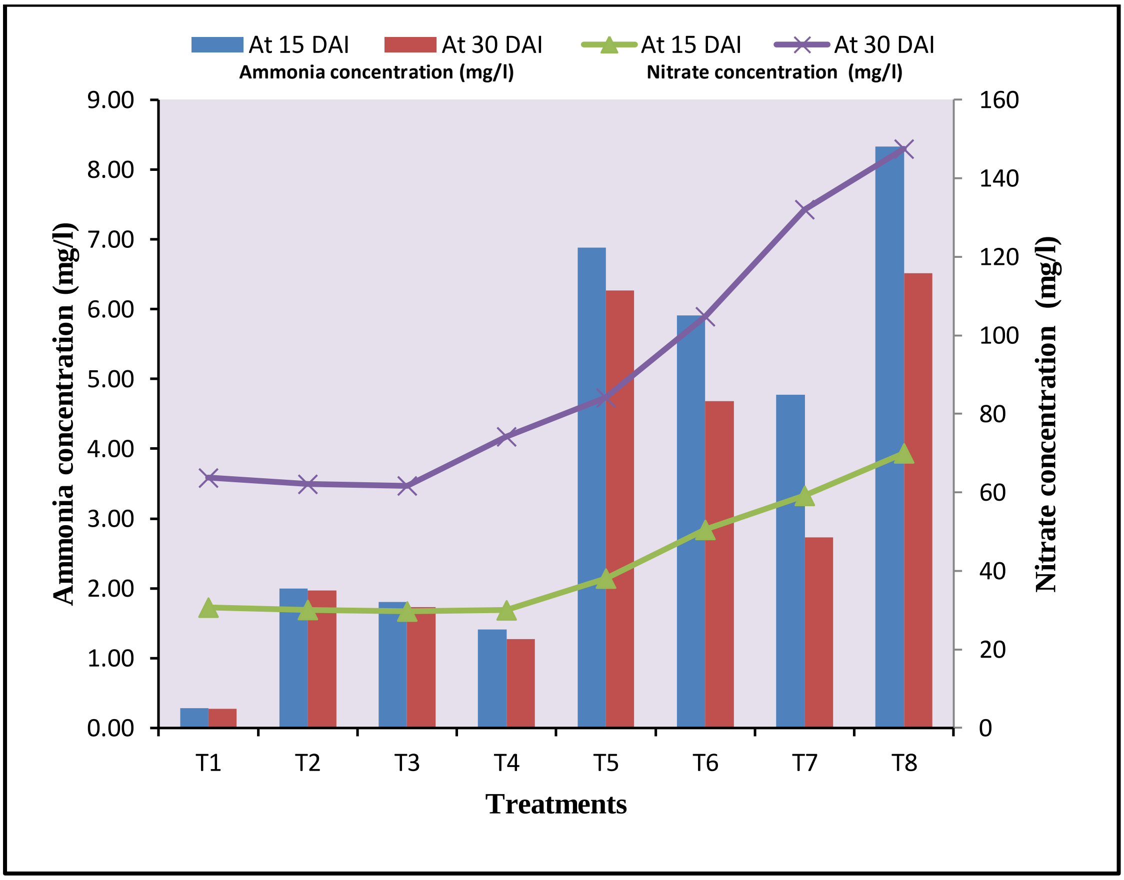
Similar finding has been obtained that molasses pretreatment can significantly stimulate the process of bacterial degradation of ammonia in the submerged condition. These type of finding of present investigation are close to findings of Muter *et al.*, 2013. The ammonia-nitrogen range was 0.02 – 0.07 mg $l^{-1}$, nitrite-nitrogen range was between 0.20 – 0.43 mg $l^{-1}$, whereas in the nitrate-nitrogen 0.90 – 3.20 mg $l^{-1}$ (Putra *et al.*, 2020) and the carbon source from the molasses was effective in reducing the concentration of ammonia, when cultured with the biofloc technique (Putra *et al.*, 2019).The ammonia concentration was decreasing as per the results given by Yadav *et al.*, 2016.

The best result associated with the $T_4$ treatment as it showed higher rate of nitrification resulting in significant dereasing ammonia concentration and increase the concentration of $NO_3$ . The increase in nitrate from 15 DAI (30.00ppm) to 30 DAI (44.2 ppm). Similarly at 8-ppm concentration of organic N (in the form of urea), when comparing between the $T_5$ with $T_6$ and $T_7$ treatment, treatment $T_7$ gave the best result among them, as it was showing the increase in the nitrate concentration from 15 DAI (59.17 ppm) to 30 DAI (72.90 ppm) mainly because of nitrification. In the next urea concentration level of 12-ppm, it was having the least effect upon increasing the nitrate concentration from 15 DAI (70.00 ppm) to 30 DAI (77.50 ppm). Similar finding was obtained by Muter *et al.,* (2013) who emphasized molasses pretreatment which can significantly stimulated the process of bacterial degradation of ammonia in the submerged condition. The ammonia concentration range was 0.02 – 0.07 mg $l^{-1}$, Nitrite concentration range was between 0.20 – 0.43 mg $l^{-1}$ . Carbon source from the molasses was effective in reducing the concentration of ammonia when cultured with the biofloc technique (Putra *et al.*,2019) and also another finding was that the nitrate concentration was increasing under the similar in-vitro condition as reported by Yadav *et al.,* 2016.

**Table 4.6: Ammonia and nitrate dynamics in molasses amended* 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 $l^{-1}$) | | Nitrate concentration ppm(mg $l^{-1}$) | |
|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 15 DAI | At 30 DAI |
| **T1** | No inoculums and no chemical | 0.280 | 0.278 | 30.7 | 33.0 |
| **T2** | Organic source of N (Urea 2 ppm) + No inoculums | 2.00 | 1.97 | 30.1 | 32.0 |
| **T3** | Organic source of N (Urea 2 ppm) + $I_1$ | 1.80 | 1.73 | 29.7 | 32.0 |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 1.41 | 1.27 | 30.0 | 44.2 |
| **T5** | Organic source of N (Urea 8 ppm) + No inoculums | 6.88 | 6.27 | 38.10 | 46.0 |
| **T6** | Organic source of N (Urea 8 ppm) + $I_1$ | 5.91 | 4.68 | 50.5 | 54.3 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 4.77 | 2.73 | 59.17 | 72.9 |
| **T8** | Organic source of N (Urea 12 ppm) + $I_1$ | 8.33 | 6.52 | 70.00 | 77.5 |
| | CD (0.05) | 0.23 | 0.09 | 6.05 | 6.75 |

* = 5% Molasses water solution,
$I_1$ = Mixed culture of heterotrophic bacteria (PS5, PS16), $I_2$ = It was a mixture of culture of NS8,NB8 and PS5, PS16




At 15 DAI At 30 DAI (Ammonia concentration (mg/l)) At 15 DAI At 30 DAI (Nitrate concentration (mg/l))

| | |
|---|---|
| T1 | No inoculums and no chemical |
| T2 | Organic source of N (Urea 2 ppm) + No inoculums |
| T3 | Organic source of N (Urea 2 ppm) + $I_1$ |
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T5 | Organic source of N (Urea 8 ppm) + No inoculums |
| T6 | Organic source of N (Urea 8 ppm) + $I_1$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |
| T8 | Organic source of N (Urea 12 ppm) + $I_1$ |

**Fig. 3 : Ammonia and nitrate dynamics in molasses amended water of polluted pond as influenced by autotrophic and heterotrophic bacteria under controlled conditions.**

**4.7. Ammonia and nitrate dynamics in skimmed milk amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled conditions.**

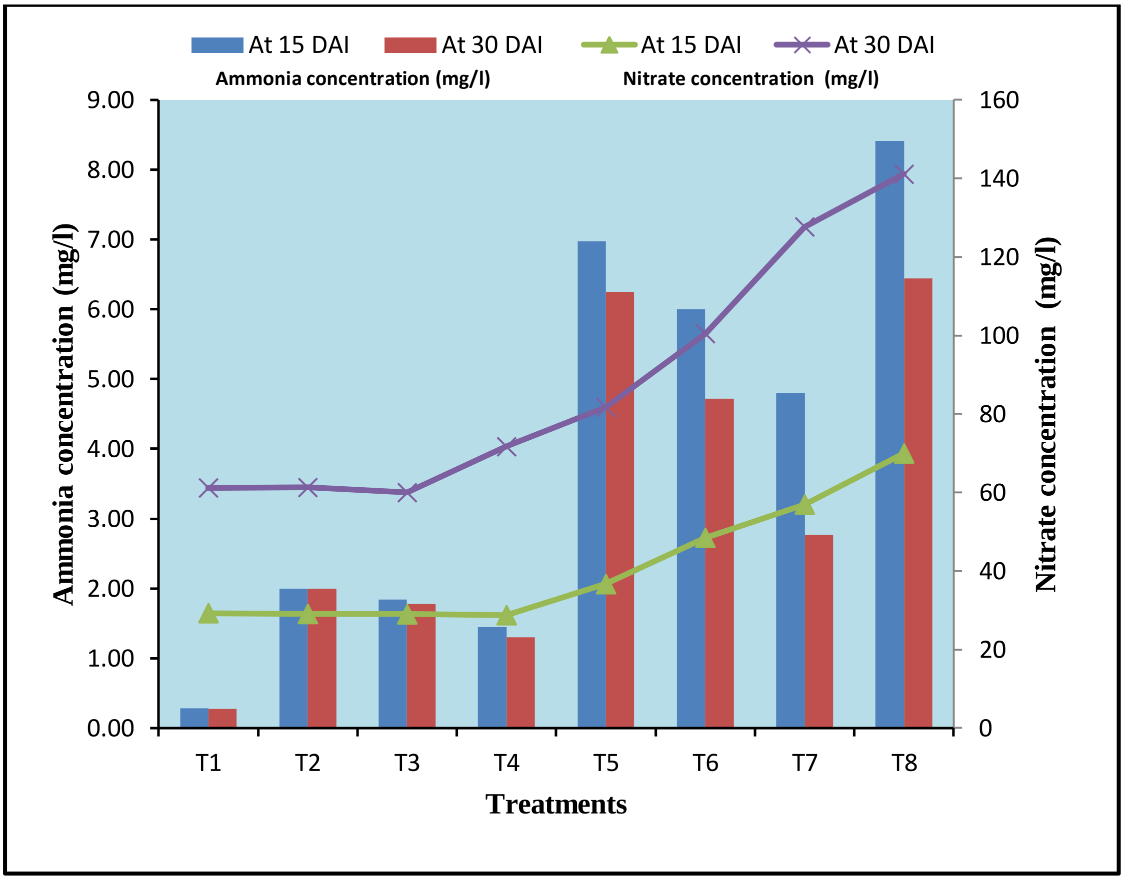
Data presented in Table 4.7 (Fig. 4 and Plate 6-7, 9) related to ammonia and nitrate dynamics in skimmed milk amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled conditions at 15 and 30 DAI. The ammonia concentration results recorded at 15 DAI (0.28 to 8.41 ppm) and 30 DAI (0.275 to 6.44 ppm). The ammonia concentration clearly showed decreasing trend from 15 DAI to 30 DAI. The minimum ammonia concentration obtained in the $T_4$ treatment (1.46 to 1.00 ppm) at 2 ppm organic N source and $T_7$ also gave similar type of minimum ammonia concentration at 8 ppm organic N source (4.80 to 2.77 ppm) *i.e*. reduced by 2.03 ppm. Thus, in both the treatments significant reduction in the ammonia concentration was observed mainly due to nitrification process. However at the level of 12 ppm urea, the maximum concentration of ammonia was associated with$T_8$ (8.41 to 6.44 ppm). Similar finding has also been obtained by Phirke ;2014.

In the present investigation the nitrate concentration was observed at 15 DAI and 30 DAI, We have used the 3 levels of the urea N concentration (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 $T_2$ with respect to nitrate concentration, best result was associated with $T_4$ treatment, as it helps in decreasing ammonia concentration and resulting in significant increasing the level of nitrate concentration in the treatments from 15 DAI (28.8 ) to 30 DAI (42.9 ppm). Similarly at 8-ppm of urea concentration when comparing $T_5$ with $T_6$ & $T_7$ treatment, we observed the superiority of treatment $T_7$ over other treatments of comparison. Increase in the nitrate concentration from 15 DAI (57 ppm) to 30 DAI (70.07 ppm) was recorded. In the next urea level of 12-ppm, it was having the least effect upon decreasing ammonia and increasing the nitrate concentration from 15 DAI (70.0 ppm) to 30 DAI (71.1 ppm). Similar finding has been reported as the acceptable range of nitrate was (0 - 100 ppm) and desirable range was (0.1 - 4.5 ppm) as reported by Shrivastava *et al.*,(2007). The result was also similar to the finding of Mahobe and Mishra (2013).

**Table 4.7: Ammonia and nitrate dynamics in skimmed milk* amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled condition.**

| Abbre. | Treatments | Ammonia concentration ppm(mg $l^{-1}$) | | Nitrate concentration ppm(mg $l^{-1}$) | |
|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 15 DAI | At 30 DAI |
| **T1** | No inoculums and no chemical | 0.280 | 0.275 | 29.3 | 31.9 |
| **T2** | Organic source of N (Urea 2 ppm) + No inoculums | 2.00 | 2.00 | 29.1 | 32.2 |
| **T3** | Organic source of N (Urea 2 ppm) + $I_1$ | 1.84 | 1.78 | 29.0 | 31.0 |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 1.45 | 1.30 | 28.8 | 42.9 |
| **T5** | Organic source of N (Urea 8 ppm) + No inoculums | 6.97 | 6.25 | 36.7 | 45.0 |
| **T6** | Organic source of N (Urea 8 ppm) + $I_1$ | 6.00 | 4.72 | 48.5 | 52.0 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 4.80 | 2.77 | 57.0 | 70.7 |
| **T8** | Organic source of N (Urea 12 ppm) + $I_1$ | 8.41 | 6.44 | 70.0 | 71.1 |
| | CD (0.05) | 0.23 | 0.09 | 5.95 | 6.70 |

* = 1% Skimmed milk amended polluted pond water solution,
$I_1$ = Mixed culture of heterotrophic bacteria (PS5, PS16), $I_2$ = It was a mixture of culture of NS8,NB8 and PS5, PS16




At 15 DAI   At 30 DAI   Ammonia concentration (mg/l)

At 15 DAI   At 30 DAI   Nitrate concentration (mg/l)

| | |
|---|---|
| T1 | No inoculums and no chemical |
| T2 | Organic source of N (Urea 2 ppm) + No inoculums |
| T3 | Organic source of N (Urea 2 ppm) + $I_1$ |
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T5 | Organic source of N (Urea 8 ppm) + No inoculums |
| T6 | Organic source of N (Urea 8 ppm) + $I_1$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |
| T8 | Organic source of N (Urea 12 ppm) + $I_1$ |

**Fig. 4 : Ammonia and nitrate dynamics in skimmed milk amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different levels of organic under controlled conditions.**

**4.8. Ammonia and nitrate dynamics in molasses amended polluted pond water and ordinary water (1:1) in fish aquarium as influenced by autotrophic and heterotrophic bacteria under controlled conditions.**

The data presented in Table 4.8 (Fig.5 and Plate 8-10) clearly revealed that out of 3 levels of the organic N containing urea concentration ( 2 ppm, 8 ppm and 12 ppm), 2-ppm organic N concentration when comparing the treatments $T_3$ and $T_4$ with treatment $T_2$ for the ammonia transformations, the best result was associated with $T_4$ treatment as it helped in decreasing the level of ammonia concentration from 15 DAI (1.07 ppm) to 30 DAI (0.95 ppm). Similarly at 8-ppm concentration when comparing between $T_5$ with $T_6$ and $T_7$ treatments, we got the best result with $T_7$ as the harmful ammonia concentration for fish growth reduced from 4.41 ppm at 15 DAI to 1.35 ppm at 30 DAI. In the next concentration level of organic N i.e. 12-ppm, it was having the least effect for lowering the ammonia concentration from 7.73 ppm at 15 DAI to 5.10 ppm at 30 DAI. It clearly indicated the effectiveness of bioremediators remained successful up to level of 8 ppm organic N concentration but can not work properly at 12 ppm urea concentration wrt to reduction of harmful level of ammonia for fish cultivation.

The treatment T4 when comparing the treatments $T_3$ & $T_4$ with the treatment $T_2$ for the nitrate concentration, we obtained the best result with the $T_4$ treatment as it showed higher rate of nitrification resulting in increased the level of nitrate concentration from 15 DAI (33.00 ppm) to 30 DAI (39.50 ppm). While, at 8-ppm concentration of organic N , when comparing between the $T_5$ with $T_6$ & $T_7$ treatment, we got that the treatment $T_7$ was showing the best result among them, as it helped to decrease ammonia concentration and resulting increase in the nitrate concentration from 15 DAI (55.00 ppm) to 30 DAI (68.90 ppm).

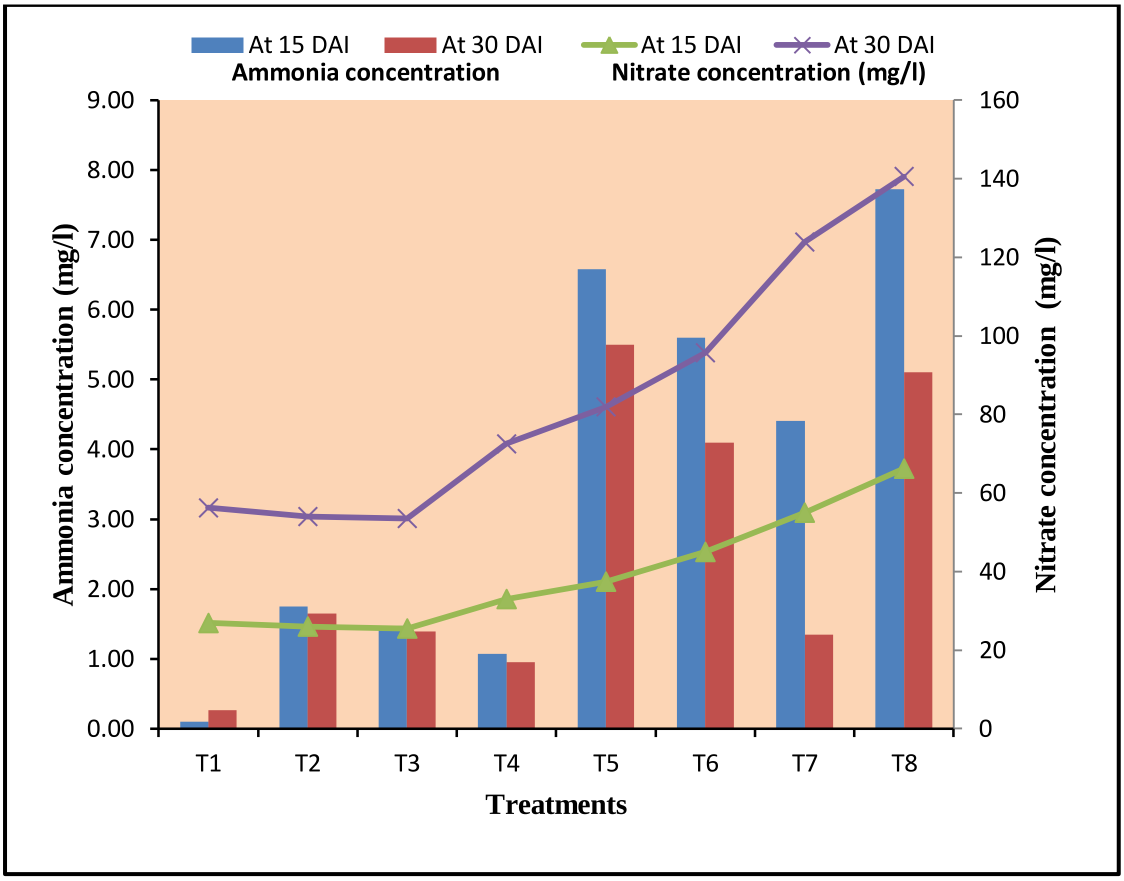
Similar type of findings reported by Muter *et al.,* (2013) that molasses pre treatment can significantly stimulate the process of bacterial attachment on the ceramic carrier and the degradation of ammonia in the submerged condition. As per using the molasses, they noticed that it reduced the ammonia concentration and increased the nitrate concentration in the polluted pond water. Ammonia-nitrogen range was 0.02 – 0.07 mg $L^{-1}$, whereas nitrate-nitrogen was 0.90 – 3.20 mg $L^{-1}$

Carbon source from the molasses was effective in reducing the concentration of ammonia, nitrite and nitrate when cultured with the biofloc technique (Putra, *et al.*, 2019, Yadav *et al.*, 2016).

**Table 4. 8: Ammonia and nitrate dynamics in molasses amended* polluted pond water and ordinary water (1:1) fish aquarium as influenced by autotrophic and heterotrophic bacteria under controlled condition.**

| Abbre. | Treatments | Ammonia concentration ppm(mg $l^{-1}$) | | Nitrate concentration ppm(mg $l^{-1}$) | |
|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 15 DAI | At 30 DAI |
| **T1** | No inoculums and no chemical | 0.25 | 0.26 | 26.9 | 29.3 |
| **T2** | Organic source of N (Urea 2 ppm) + No inoculums | 1.75 | 1.65 | 26.0 | 28.0 |
| **T3** | Organic source of N (Urea 2 ppm) + $I_1$ | 1.47 | 1.39 | 25.5 | 28.0 |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 1.07 | 0.95 | 33.0 | 39.5 |
| **T5** | Organic source of N (Urea 8 ppm) + No inoculums | 6.58 | 5.50 | 37.43 | 44.5 |
| **T6** | Organic source of N (Urea 8 ppm) + $I_1$ | 5.60 | 4.10 | 45.0 | 50.7 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 4.41 | 1.35 | 55.00 | 68.9 |
| **T8** | Organic source of N (Urea 12 ppm) + $I_1$ | 7.73 | 5.10 | 66.2 | 74.4 |
| | CD(0.05) | 0.14 | 0.17 | 5.61 | 6.22 |

*15ppm molasses water solution, $I_1$= Mixed culture of heterotrophic bacteria (PS5, PS16), $I_2$ =It was a mixture of culture of NS8, NB8 and PS5, PS16




At 15 DAI At 30 DAI At 15 DAI At 30 DAI
Ammonia concentration Nitrate concentration (mg/l)

| | |
|---|---|
| T1 | No inoculums and no chemical |
| T2 | Organic source of N (Urea 2 ppm) + No inoculums |
| T3 | Organic source of N (Urea 2 ppm) + $I_1$ |
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T5 | Organic source of N (Urea 8 ppm) + No inoculums |
| T6 | Organic source of N (Urea 8 ppm) + $I_1$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |
| T8 | Organic source of N (Urea 12 ppm) + $I_1$ |

**Fig.5 : Ammonia and nitrate dynamics in molasses amended polluted pond water and ordinary water (1:1) of fish aquarium as influenced by autotrophic and heterotrophic bacteria under controlled conditions.**


**Plate 8a:General view of fish aquarium green house experiment at time of promising bacterial inoculation**

**Plate 8 b: General view of fish aquarium green house experiment after 15 days of promising bacterial inoculation.**


**Plate 8c : Cleaning of aquarium at 15 days intervals.**


**Plate 9 : Quantitative study of microbial transformations of N.**

**4.9. Fish weight and fish length dynamics in water of polluted pond water and ordinary water (1:1) of fish aquarium as influenced by autotrophic and heterotrophic bacteria.**

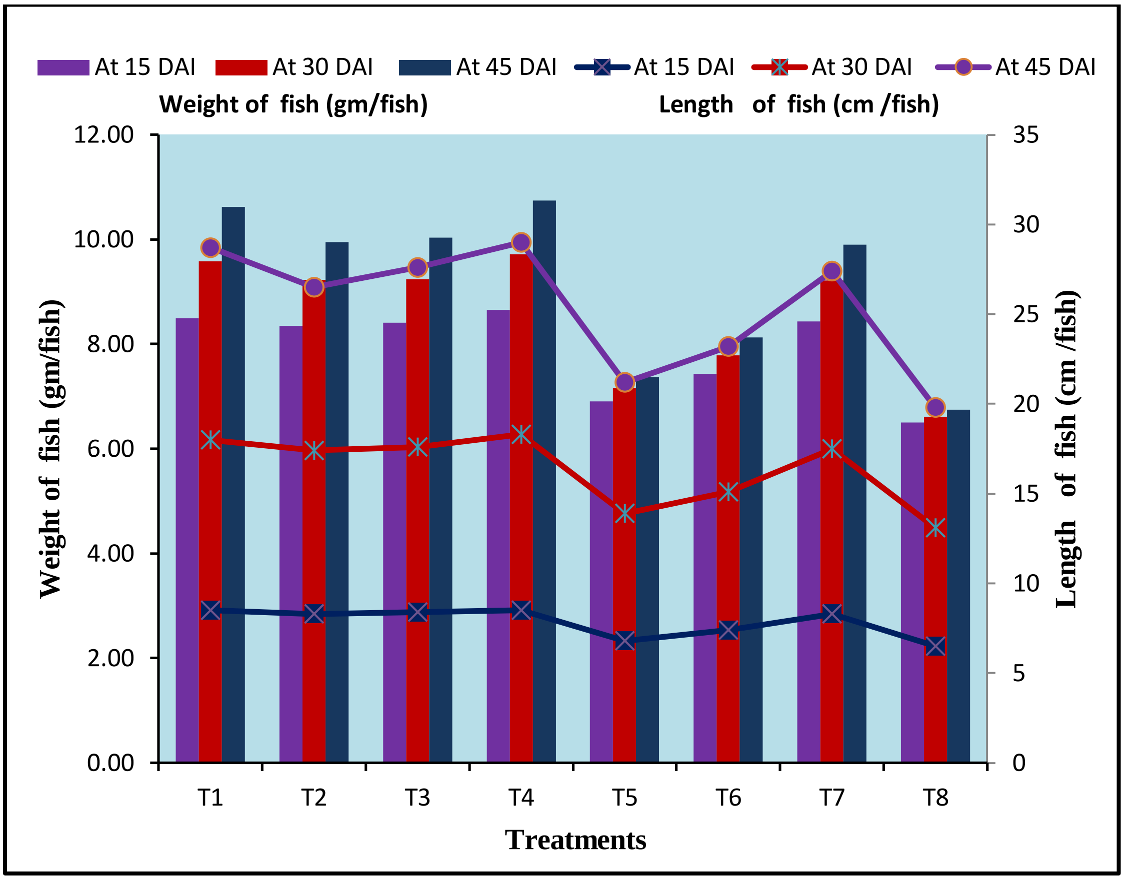
Data presented in Table- 4.9 (Fig. 6 and Plate 8-10) indicated that variation in fish weight (g) and fish length (cm) in mixture of polluted pond water and ordinary water (1:1) has been observed due to different treatments c of bioremediators. It was observed that at 2 ppm urea level, $T_2$ gave average fish weight 8.35, 9.23 and 9.95 g at 15, 30 and 45 DAI respectively and $T_3$ produced 8.41, 9.2 and 10.03 g/fish weight at 15, 30 and 45 DAI respectively. While, the best result was associated with $T_4$ treatment. $T_4$ gave 8.65, 9.72 and 10.74 g/fish weight at 15, 30 and 45 DAI respectively. Likewise, at 8ppm urea level comparing $T_5$ with $T_6$. Got 6.91, 7.16 and 7,37 g/fish weight due to $T_5$ at 15, 30, 45 DAI and .43, 7.78, 8.13 g/fish weight due to $T_6$ at 15, 30, 45 DAI. Between $T_6$ and $T_7$ treatments the $T_7$ treatment showed the best result. The data of this table revealed that the treatments $T_4$ and $T_7$ are the most effective treatments over other treatments under the present investigation.

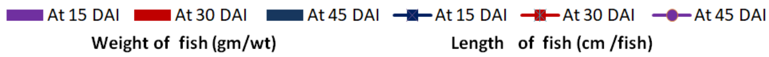
Likewise, the fish length gradually increased and recorded average fish length was 8.5, 9.8 and 10.7 cm due to $T_4$ at 15, 30, 45 DAI, respectively. The best results were associated with $T_4$ followed $T_7$.

**Table 4.9: Fish weight and fish length dynamics in polluted pond water and ordinary water (1:1) of fish aquarium as influenced by autotrophic and heterotrophic bacteria.**

| Abbre. | Treatments | Weight of fish (gm/fish) | | | Length of fish (cm/fish) | | |
|---|---|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 45 DAI | At 15 DAI | At 30 DAI | At 45 DAI |
| **T1** | No inoculums and no chemical | 8.5 | 9.58 | 10.62 | 8.5 | 9.5 | 10.7 |
| **T2** | Organic source of N (Urea 2 ppm) + No inoculums | 8.35 | 9.23 | 9.95 | 8.3 | 9.1 | 9.1 |
| **T3** | Organic source of N (Urea 2 ppm) + $I_1$ | 8.41 | 9.24 | 10.03 | 8.4 | 9.2 | 10.0 |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 8.65 | 9.72 | 10.74 | 8.5 | 9.8 | 10.7 |
| **T5** | Organic source of N (Urea 8 ppm) + No inoculums | 6.91 | 7.16 | 7.37 | 6.8 | 7.1 | 7.3 |
| **T6** | Organic source of N (Urea 8 ppm) + $I_1$ | 7.43 | 7.78 | 8.13 | 7.4 | 7.7 | 8.1 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 8.43 | 9.21 | 9.90 | 8.3 | 9.2 | 9.9 |
| **T8** | Organic source of N (Urea 12 ppm) + $I_1$ | 6.50 | 6.61 | 6.75 | 6.5 | 6.6 | 6.7 |
| | CD | 1.50 | 1.64 | 1.33 | 1.33 | 1.29 | 1.67 |

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




| | |
|---|---|
| T1 | No inoculums and no chemical |
| T2 | Organic source of N (Urea 2 ppm) + No inoculums |
| T3 | Organic source of N (Urea 2 ppm) + $I_1$ |
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T5 | Organic source of N (Urea 8 ppm) + No inoculums |
| T6 | Organic source of N (Urea 8 ppm) + $I_1$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |
| T8 | Organic source of N (Urea 12 ppm) + $I_1$ |

**Fig. 6 : Fish weight and length dynamics in water of fish aquarium as influenced by autotrophic and heterotrophic bacteria.**

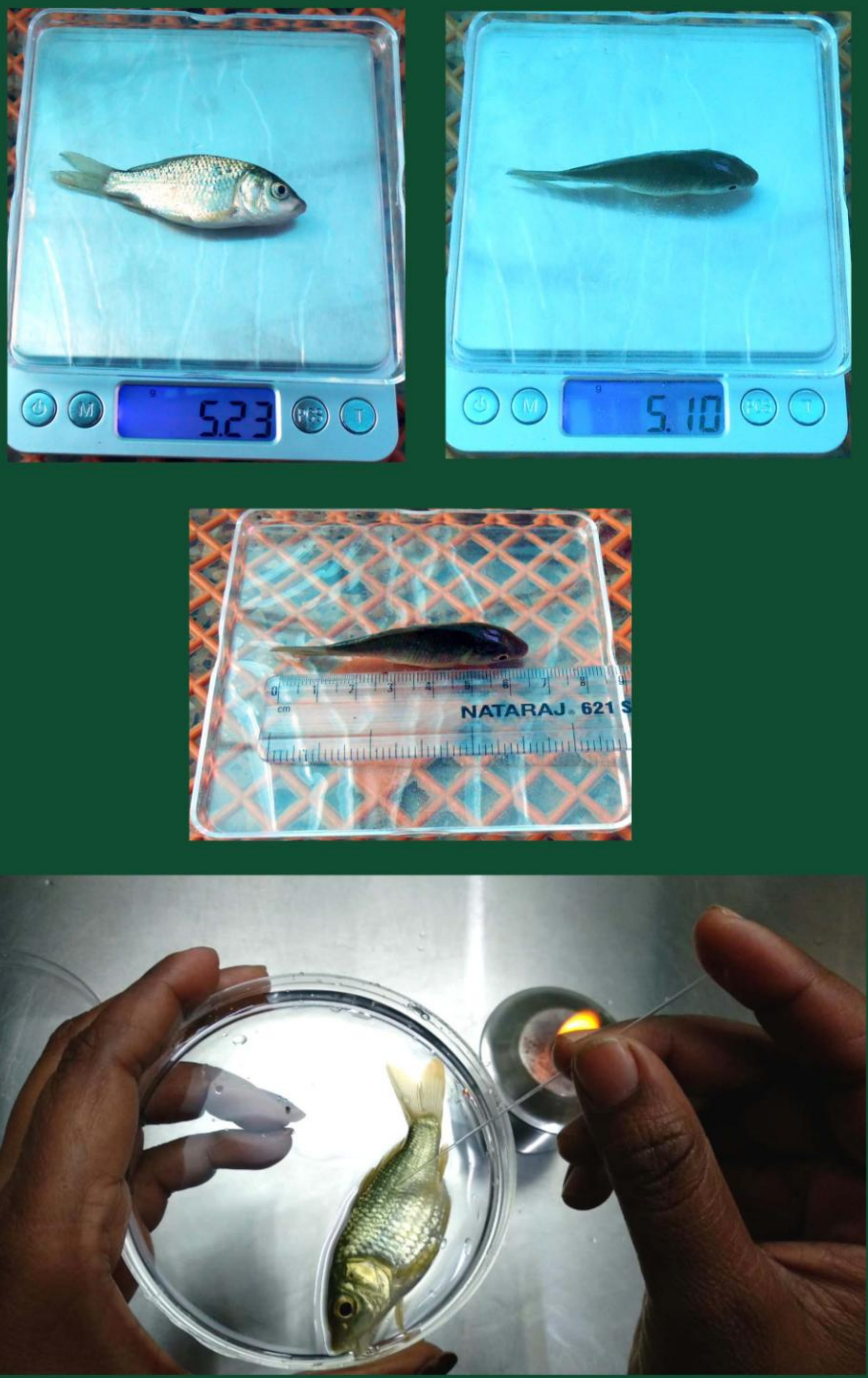



**Plate 10 : Influence of promising bacterial inoculation on fish growth parameters at different level of organic nitrogen.**

### 4.10. Fish weight and fish length dynamics in molasses amended polluted pond water and ordinary water (1:1) of fish aquarium as influenced by autotrophic and heterotrophic bacteria under controlled conditions.

The data presented in the Table 4.10 (Fig. 7 and Plate 8-10), wrt variations in fish weight (g) and fish length (cm) clearly showed additive positive effect of effect of bioremediators with molasses. It can be concluded from the study that in green house aquarium conditions, highest fish growth rate was associated with Treatment $T_4$ and T7 at 2 and 8 ppm urea levels, respectively. $T_8$ showed lowest fish growth rate. The growth rate was observed statistically at par due to treatment $T_7$, $T_4$, $T_2$ and $T_1$ but differ significantly due to $T_1$-$T_4$, $T_6$-$T_7$ over $T_5$ and T8. Similarly more fish growth rate was associated with $T_3$ over $T_6$. It can be concluded from the data of present investigation that better fish growth rate can be obtained in deep cemented tank (WxLxH: 42.5x67x95 $cm^3$ ) over green house aquarium environment. Further, it can also be concluded that bacterial bio remediators can help to obtained desirable fish growth rate up to 8 ppm organic - urea level by faster rate of nitrification. It was significant finding of this investigation that almost same fish growth rate at up to 8 ppm level of organic N urea can be obtained statistically similar to that of fish growth rate at 2 ppm level of organic N urea because of inoculation of effective location specific mixed culture of desirable autotrophic and heterotrophic bacterial inocula (N*itrosomonas NS8* and *Nitrobacter NB8* isolates and PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609 bacteria).

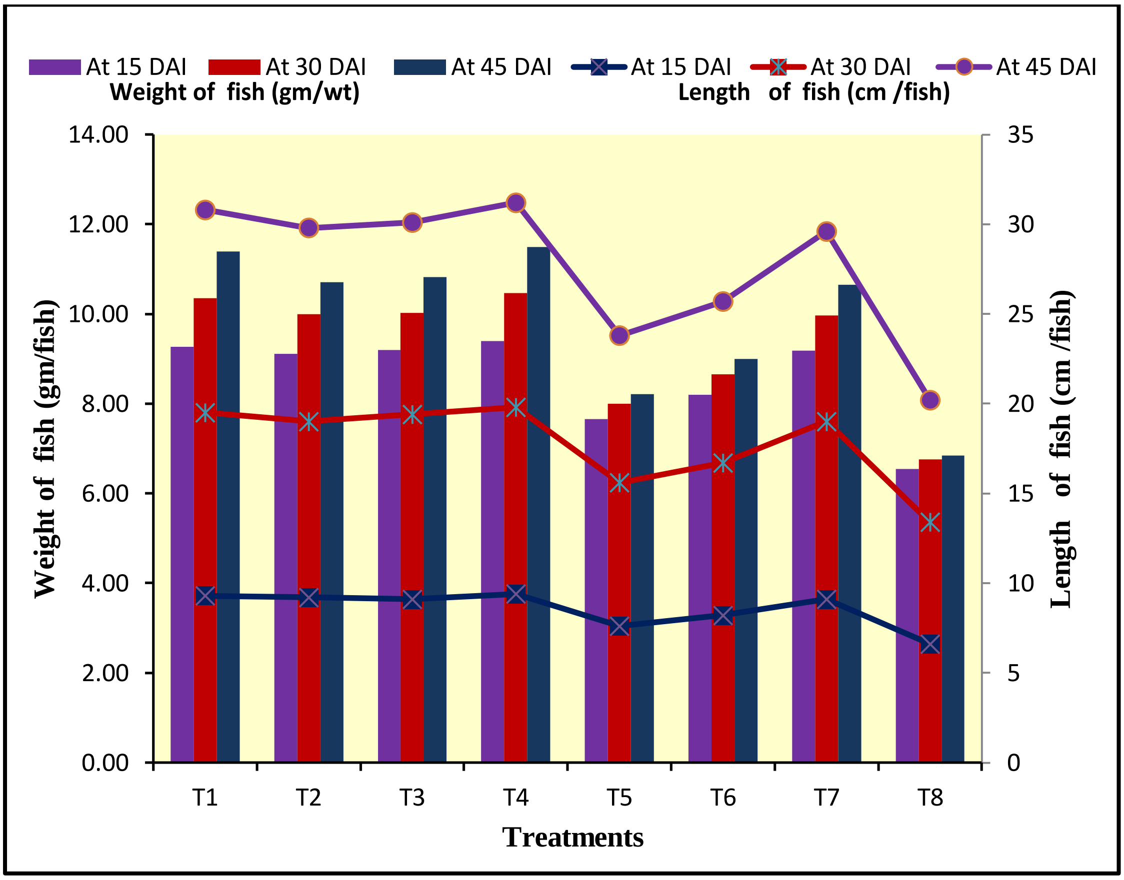
The maximum fish weight due to $T_4$ was recorded at 15 DAI 9.40 g/fish, at 30DAI 10.47g/fish and at 45DAI 11.49 g/fish. Treatments $T_4$ and $T_7$ are the most effective treatments over others.

Likewise, the fish length was almost similar to that of trends of fish weight associated with $T_4$ and $T_7$. These findings are similar to that of Bajaj, 2017.

**Table 4.10. Fish weight and fish length dynamics in molasses amended*polluted pond water and ordinary water (1:1) of fish aquarium as influenced by autotrophic and heterotrophic bacteria under controlled conditions.**

| Abbre. | Treatments | Weight of fish (gm/fish) | | | Length of fish (cm /fish) | | |
|---|---|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 45 DAI | At 15 DAI | At 30 DAI | At 45 DAI |
| **T1** | No inoculums and no chemical | 9.27 | 10.35 | 11.39 | 9.3 | 10.2 | 11.3 |
| **T2** | Organic source of N (Urea 2 ppm) + No inoculums | 9.11 | 9.99 | 10.71 | 9.2 | 9.8 | 10.8 |
| **T3** | Organic source of N (Urea 2 ppm) + $I_1$ | 9.20 | 10.03 | 10.82 | 9.1 | 10.3 | 10.7 |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 9.40 | 10.47 | 11.49 | 9.4 | 10.4 | 11.4 |
| **T5** | Organic source of N (Urea 8 ppm) + No inoculums | 7.65 | 8.00 | 8.21 | 7.6 | 8.0 | 8.2 |
| **T6** | Organic source of N (Urea 8 ppm) + $I_1$ | 8.20 | 8.65 | 9.00 | 8.2 | 8.5 | 9.0 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 9.18 | 9.96 | 10.65 | 9.1 | 9.9 | 10.6 |
| **T8** | Organic source of N (Urea 12 ppm) + $I_1$ | 6.54 | 6.76 | 6.85 | 6.6 | 6.8 | 6.8 |
| | CD | 1.61 | 1.01 | 1.56 | 0.95 | 0.73 | 0.80 |

* = 15ppm molasses amended water, $I_1$= Mixed culture of heterotrophic bacteria (PS5, PS16), $I_2$ =It was a mixture of culture of NS8,NB8 and PS5, PS16




At 15 DAI At 30 DAI At 45 DAI — Weight of fish (gm/wt)

At 15 DAI At 30 DAI At 45 DAI — Length of fish (cm /fish)

| | |
|---|---|
| T1 | No inoculums and no chemical |
| T2 | Organic source of N (Urea 2 ppm) + No inoculums |
| T3 | Organic source of N (Urea 2 ppm) + $I_1$ |
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T5 | Organic source of N (Urea 8 ppm) + No inoculums |
| T6 | Organic source of N (Urea 8 ppm) + $I_1$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |
| T8 | Organic source of N (Urea 12 ppm) + $I_1$ |

**Fig. 7 : Fish weight and fish length dynamics in molasses amended polluted pond water and ordinary water (1:1) of fish aquarium as influenced by autotrophic and heterotrophic bacteria.**

### 4.11. Demonstration of performance of promising mixed culture of autotrophic and heterotrophic bacteria on rate of fish growth.

In aquarium fish culture experiment, among all the treatments tested, the performance of $T_4$ & $T_7$ found better over other teatments at 2 ppm and 8 ppm urea levels. Hence, it was planned for furthers testing in deep cemented tank environment to provide more suitable environment almost similar to that of natural fish farming conditions. In this connection, deep cemented tank (WxLxH: 42.5x67x95 $cm^3$ ) were prepared for testing of most effective treatments (Table 4.11, Fig 8 and plate 10-11).

During the demonstration trial of promising treatment, treatment $T_4$ gave average fish weight 10.4, 11.6 and 12.7g at 15, 30 and 45 DAI while, $T_7$ produced average fish weight 9.9, 10.8 and 11.9 g at the 15, 30 and 45 DAI, respectively. Similarly $T_4$ gave average fish length 10.3, 11.6 and 12.3 cm at the 15, 30 and 45 DAI, while $T_7$ gave 9.8, 10.9 and 11.9 cm at the 15, 30 and 45 DAI, respectively.

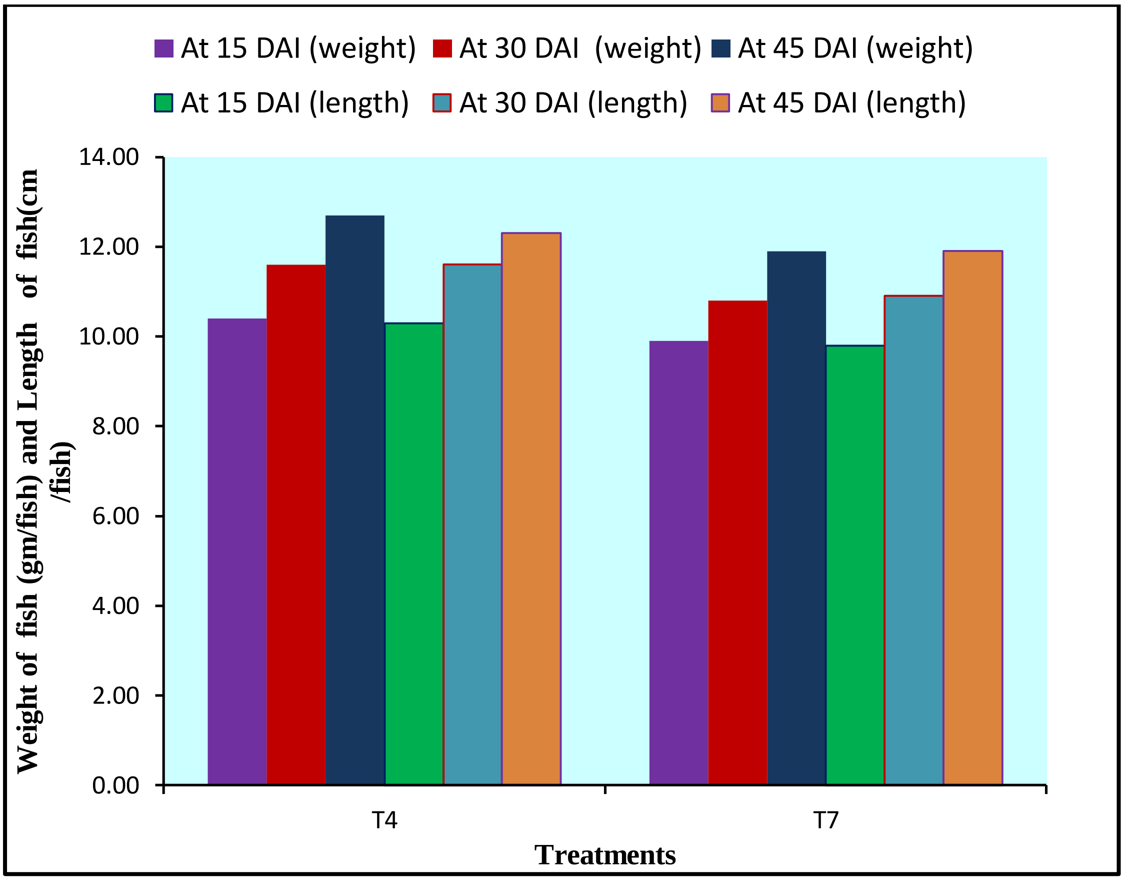
It was clearly concluded from the data of present investigation that better fish growth rate can be obtained in deep cemented tank (WxLxH: 42.5x67x95 $cm^3$ ) over green house aquarium environment. It can also be concluded that bacterial bio remediators can help to obtained desirable fish growth rate up to 8 ppm organic N-urea level by faster rate of nitrification. It was significant finding of this investigation that almost same fish growth rate at up to 8 ppm level of organic N urea can be obtained statistically similar to that of fish growth rate at 2 ppm level of organic N urea because of inoculation of effective location specific mixed culture of desirable autotrophic and heterotrophic bacterial inocula (N*itrosomonas NS8* and *Nitrobacter NB8* isolates and PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609 bacteria).

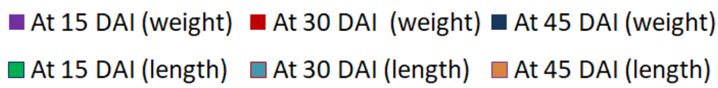
**Table 4.11. Demonstration of performance of promising mixed culture of autotrophic and heterotrophic bacteria in molasses amended* polluted pond water and ordinary water (1:1 ratio)**

| Abbre. | Treatments | Weight of fish (gm/fish) | | | Length of fish (cm/fish) | | |
|---|---|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 45 DAI | At 15 DAI | At 30 DAI | At 45 DAI |
| **T4** | Organic source of N (Urea 2 ppm) + $I_2$ | 10.4 | 11.6 | 12.7 | 10.3 | 11.6 | 12.3 |
| **T7** | Organic source of N (Urea 8 ppm) + $I_2$ | 9.9 | 10.8 | 11.9 | 9.8 | 10.9 | 11.9 |

* = 15ppm molasses amended water

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




| | |
|---|---|
| T4 | Organic source of N (Urea 2 ppm) + $I_2$ |
| T7 | Organic source of N (Urea 8 ppm) + $I_2$ |

**Fig. 8: Demonstration of performance of promising mixed culture of autotrophic and heterotrophic bacterial isolates on fish growth rate under deep tank environment.**

**Plate 11 : Conformation of performance of promising bacterial isolates for increasing fish growth rate in molasses amended water of polluated pond and ordinary water (1:1) in deep tank environment.**

## CHAPTER-V

# SUMMARY AND CONCLUSIONS

Keeping in views that more concentration of ammonia is toxic to fish growth and survival and must be reduced/ removed or converted into harmless substances before it builds up to lethal levels in the urban polluted fish pond water, the present study entitled **"Bioremediation of polluted pond water for increasing fish production"** was conducted at the Department of Agricultural Microbiology, College of Agriculture Raipur, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the year 2017-2019. In this connection, 25 soil and water samples were collected from polluted ponds of capital city Raipur area and nearby area for isolation of autotrophic (nitrifying) and heterotrophic local bacterial isolates especially in order to exploit them as bio remediators for ammonia polluted urban water bodies for enhancing fish production. Further, the collected samples were analyzed for the different quality parameters like pH, EC and TDS and isolation of autotrohic and heterotrophic bacterial isolates. The isolated bacterial isolates were selected to examine their effect on N transformations especially to decrease ammonia concentration with and without molasses and skimmed milk amendment and fish culture. The observations of organic N transformations were recorded properly in microbial growth room and green house aquarium environment at 15 days time intervals. The observations related to growth rate in terms of fish weight and length were also recorded properly in aquarium green house and deep cemented tank environment at 15 days time intervals.

Both combinations of inoculants $I_1$ (mixed culture of bacterial isolates N*itrosomonas* NS8 and *Nitrobacter* NB8) and $I_2$ (The mixed culture of local bacterial isolates N*itrosomonas* NS8 and *Nitrobacter* NB8 and PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609) were used and the got better result of the $I_2$ in the aquatic ecosystem.

It can be concluded from the study that in green house aquarium conditions, highest fish growth rate was associated with Treatment $T_4$ while $T_8$ gave lowest fish growth rate. The growth rate was observed statistically at par due to treatment $T_7$, $T_4$, $T_2$ and $T_1$ but differ significantly due to $T_1$-$T_4$, $T_6$-$T_7$ over $T_5$ and $T_8$. Similarly, more fish growth rate was associated with T3 over T6. It can be concluded from the data of present investigation that better fish growth rate can be obtained in deep cemented tank (W×L×H: 42.5×67×95 $cm^3$) over green house aquarium environment. It can also be concluded that bacterial bio remediators can help to obtained desirable fish growth rate up to 8 ppm organic N level by faster rate of microbial nitrification. It was significant finding of this investigation that almost same fish growth rate at up to 8 ppm level of organic N can be obtained statistically similar to that of fish growth rate at 2 ppm level of organic N because of inoculation of effective location specific mixed culture of desirable autotrophic and heterotrophic local bacterial isolates.

The nitrifying autotrophic and denitrifying heterotrophic bacteria were isolated, tested and proliferated under the controlled conditions. Where the application of the nitrifying and heterotrophic bacterial isolates was applied and tested with and without fish viz., In conical flask (with polluted pond water with and without molasses and skimmed milk), In aquarium green house conditions (with polluted pond water: ordinary water (1:1) and molasses amended water), cement tank (to confirm the performance of promising treatment of mixed culture of autotrophic and heterotrophic bacteria) from where we concluded as follows :

1. Upon colleting the water and soil samples from different 25 different ponds near by Raipur for the bacterial isolations, best performing local bacterial isolates of *Nitrosomonas* and *Nitrobacter* (soil-NS8 and NB8) were selected for further study. The isolates originally isolated from soil of 1st Dudhadhari pond

2. The local heterotrophic bacterial isolates PS5 *Micrococcus luteus* CP001628 and PS16 *Ochrobactrum pituitosum* AM490609 showed the best result among other local heterotrophic bacterial isolates (Table 4.2b) with reference to Organic N transformations.

3. The pH range for all the samples collected from the soil (7.9) and water (7.8) has observed between the acceptable range (pH: 7- 9.5).

4. The dual inoculation of heterotrophic and autotrophic bacterial isolates found better than single inoculation of autotrophic bacterial isolates wrt Organic N transformations to mitigate ammonium pollution in urban water bodies.

5. Use of molasses 15 ppm along with mixed culture of heterotrophic and autotrophic bacterial isolates found better for enhancing fish growth rate.

**Suggestion for future work**

- The results of present investigation should be tested under natural field condition.
- Isolates of nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* need to be inoculated in eutrophic culture ponds under different climatic conditions.
- Bacterial isolates of other nitrifiers such as *Psudomonas* can also be studied to see the effect on bioremediation of ammonical and nitrite nitrogen.
- In addition to autotrophic nitrifiers, heterotrophic beneficial bacteria can also be used as bioremediator as the anaerobic condition prevails in eutrophic pond.
- Several doses of microbial inoculation can be studied for a long time, as paucity of time did not allow such work in this study.
- Aeration by the use of different aerators and consequent remediation can be studied in culture ponds.
- Removal of water quality parameters like, physio-chemical parameters, BOD and COD load through rapid mineralization by the group of bacteria which are equipped with all hydrolytic enzymes such as protease, lipase, amylase, etc. There are several species of *Bacillus, Actinomycetes* and *Coryneform* bacteria also, which should be applied in such culture system along with nitrifying bacteria

# REFERENCES


Abe, T., Ushiki, N., Fujitani, H. and Tsuneda, S. 2017. A rapid collection of yet unknown ammonia oxidizers in pure culture from activated sludge. Water Research, 108: 169-178.

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

Barik, P., Ram, R., Haldar, C. and Vardia, H.K. 2018. Study on nitrifying bacteria as bioremediator of ammonia in simulated aquaculture system. Journal of Entomology and Zoology Studies, 6(3): 1200-1206.

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. Agriculture, 10(2): 49-52.

Beamish, R.J. 1972. Lethal pH for the white suker Catostomus commersoni (Lacepede). Transactions of the American Fisheries Society, 101(2): 355-358.

Bhatnagar, A. and Devi, P. 2013. Water quality guidelines for the management of pond fish culture. International Journal of Environmental Sciences, 3(6): 1980-2009.

Bhatnagar, A., Jana, S.N., Garg, S.K. Patra, B.C., Singh, G. and Barman, U.K., 2004, Water quality management in aquaculture, In: Course Manual of summer school on development of sustainable aquaculture technology in fresh and saline waters, CCS Haryana Agricultural, Hisar (India),: 203-210.

Blain, K.S., Tucker, Jr. J.W., Neidig, C.L., Vermeer, G.K., Cooper, V.R. and Jarrell, J.L. 1998. Bacterial management strategies for stock enhancement of warm water marine fish: a case study with common snook (*Centropomus undecimalis*). Bulletin of Marine Science, 62(2): 573-588.

Bohn, H. L., McNeal, B. L., and O'Connor, G.A. 2001. Acid soils. Soil Chemistry. Third Edition. John Wiley and Sons, New York, NY 303,: 48-66.

Bower, C.E. and Turner, D.T. 1981. Nitrification in closed sea water aquariums: Effects of simulated power failures. Aquaculture, 2(1): 1-3.

Boyd, C.E. and Ahmad, T. 1987. Evolution of aerations for channel cat fish farming. Bulletin 584, agricultural experimental station, Auburn, AL. USA, 125.

Craig, G.R. and Baski, W.F. 1977. The effects of depressed pH on flagfish reproduction, growth and survival. Water Research, 11(8): 621-626.

Dewan, D., Wahab, M. A., Beveridge, M.C.M., Rahman, M.H. and Sarkar, B.K. 1991. Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carp fry and fingerlings grown in extensively managed, rain-fed ponds in Bangladesh. Aquaculture Research, 22(3): 277-294.

Diestra, E., Esteve, I., Burnat, M., Maldonado, J. and Solé, A. 2007. Isolation and characterization of a heterotrophic bacterium able to grow in different environmental stress conditions, including crude oil and heavy metals. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 1: 90-99.

Douillet, P.A. and Langdon, C.J. 1994. Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture, 119(1): 25-40.

Du, G., Geng, J., Chen, J. and Lun, S. 2003. Mixed culture of nitrifying bacteria and denitrifying bacteria for simultaneous nitrification and denitrification. World Journal of Microbiology and Biochemistry, 19: 433-437.

European Inland Fisheries Advisory Commission (EIFAC) 1973. Water quality criteria for European freshwater fish: Report on ammonia and inland fisheries. Water Research, 7(7): 1011-1022.

Feng - Jing Gao, Luo, X., Wu, G. X., Li, T., & Peng, Y. Z. 2013. Quantitative analyses of the composition and abundance of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in eight full-scale biological wastewater treatment plants. Bioresource technology, 138, 285-296.

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.

Ganapathi, S.V., 1943. An ecological study of a garden pond containing abundant zooplankton. Proc. Indian Academic of Sciences, 17: 41-58.

Gibson, L.F., Woodworth, J. and George, A.M. 1998. Probiotic activity of *Aeromonas* media on the Pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. Aquaculture, 169(1-2): 111-120.

Grommen, R., Hautenghum, I.V., Wambeke, M.V. and Verstracte, W. 2002. An improved nitrifying enrichment to remove ammonia and nitrite from fresh water aquaculture systems. Aquaculture, 211(1-4): 111-124.

Grunditz, C. and Dalhammar, G. 2001. Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. Water Research, 35(2): 433-440.

Gupta, B.P., Joseph, K.O., Muralidhar, M. and Krishnani, K.K. 2002. Effect of commercially available bioaugmentors on the removal of ammonia and sulphide from brackish water. Indian Journal of Fisheries, 49(3): 229-233.

Hoang, P.H., Nguyen, H.T., Trung, T.T., Tran, T.T., Do, L.P. and Le, T.N.C. 2016. Isolation and selection of nitrifying bacteria with high biofilm formation for treatment of ammonium polluted aquaculture water. Journal of Vietnamese Environment, 9(1): 33-40.

Hu, A., Yao, T., Jiao, N., Liu, Y., Yang, Z. A. O. and Liu, X. 2010. Community structures of ammonia oxidising archaea and bacteria in high altitude lakes on the Tibetan Plateau. Freshwater Biology, 55(11): 2375-2390.

Hu, L.X. and Liu, Y.L. 2002. Treatment of wastewater with modified sequencing batch biofilm reactor technology. Journal of Shanghai University (English Edition), 6(3): 248-254.

Huber, B., Scholz, H.C., Kämpfer, P., Falsen, E., Langer, S. and Busse, H.J. 2010. Ochrobactrum pituitosum sp. nov., isolated from an industrial environment. International journal of systematic and evolutionary microbiology, 60(2): 321-326.

James, G.C. and Natalie, S. 1992. A laboratory manual of general microbiology. Benjamin/Cumming Publishing Company, Inc.California,492p.

Joel, O. and Amajuoyi, C.S. (2010): Determination of the concentration of ammonia that could have lethal effect on fish pond. ARPN J. Engineer. Appl. Sci. pp 1-4.

Joseph, K.O., Muralidhar, M., Gupta, B.P. and Krishnani, K.K. 2002. Effect Of Commercially Available Bioaugmentors on the removal of Ammonia And Sulphide From Brackish Water. Indian Journal of Fisheries, 49(3): 229-233.

Kartal, B., Wessels, H.J., Van Der Biezen, E., Francoijs, K.J., Jetten, M.S., Klotz, M. G. and Stein, L.Y. 2012. Effects of nitrogen dioxide and anoxia on global gene and protein expression in long-term continuous cultures of *Nitrosomonas eutropha* C91. Applied and Environmental Microbiology, 78(14): 4788-4794.

Kilmmel, W.G. 1993. The impact of acid mine drainage on the stream. The Pennsylvania Academic Science Publication. Pennsylvania, 24: 434.

Kroupova, H., Machova, J. and Svobodova, Z. 2005. Nitrite influence on fish: a review. Veterinarni Medicina, 50(11): 461.

Lakshmipriya, V.P. and Sivakumar, P.K. 2012. Isolation and characterization of total heterotrophic bacteria and exopolysaccharide produced from mangrove ecosystem. International Journal of Pharmaceutical and Biological Archives, 3(3): 679-684.

Lakshya, T. and Viraj, R. 2014. Usefulness of probiotics in civilizing water quality and bacterial flora in fish ponds. Advances in Aquaculture and Fisheries Management 2(1): 102-108.

Li, L., Weinberg, C.R., Darden, T.A., and Pedersen, L.G. 2001. Gene selection for sample classification based on gene expression data: study of sensitivity to choice of parameters of the GA/KNN method. Bioinformatics, 17(12): 1131-1142.

Lin, Y., Kong, H., He, Y., Kual, L. and Inamori, Y. 2004. Simultaneous nitrification and denitrification in a membrane bioreactor and isolation of heterotrophic nitrifying bacteria. Japanese Journal of Water Treatment Biology, 40(3), 105-114.

Liu, H.M., Cao, L., Lu, P., Ni, H., Li, Y.X., Yan, X. and Li, S.P. 2012. Biodegradation of butachlor by *Rhodococcus* sp. strain B1 and purification of its hydrolase (ChlH) responsible for N-dealkylation of chloroacetamide herbicides. Journal of Agricultural and Food Chemistry, 60(50): 12238-12244.

Lloyd, R. and Jordan, D.H. 1964. Some factors affecting the resistance of rainbow trout (Salmo gairdneri Richardson) to acid waters. International Journal of Air and Water Pollution, 8: 393-403.

Longeri, L. and Moroni, M. 1986. Ammonium-oxidizing nitrifying bacteria from Chilean soils. Turrialba (IICA), AGRIS, 259-262.

Mahobe, H. and Mishra, P. 2013. Study of Physico-Chemical Characteristics of Water Ponds of Rajnandgaon Town, Chhattisgarh. International Journal of Scientific and Engineering Research, 4(8): 738-748.

Meade, J.W. 1985. Allowable ammonia for fish culture. The Progressive Fish-Culturist, 47(3): 135-145.

Meincke, M., Ahlers, B., Krause-Kupsch, T., Krieg, E., Meyer, C., Sameluck, F. and Bock, E. 1988. Isolation and characterization of endolithic nitrifiers. In VIth International Congress on deterioration and conservation of stone. *Proceedings Vie Congrès International sur l'altération et la conservation* *de la pierre. Actes. Torun,* 12-14. 09.1988 (pp. 15-23). Nicholas Copernicus University.

Menendez, R. 1976. Chronic effects of reduced pH on book trout (*Salvelinus fontinalis*). Journal of the Fisheries Boad of Canada, 33(1): 118-123.

Mevel, G. and Chambroux, S. 1981. A study on nitrification in the presence of prawn (*Penacus japonicus*) in marine closed systems. *Aquaculture,* 23(1-4): 29-43.

Moriarty, D.J. 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture, 164(1): 351-358.

Muter, O. Mihailova, A., Berzins, A., Shvirksts, K., Patmalnieks, A., Strikauska, S., and Grube, M. 2013. Optimization of nitrification process by a bacterial consortium in the submerged biofiltration system with ceramic bead carrier. J Microb Biochem Technol, 6: 148-153.

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.

Ojeda, J.Y.K., Collazos-Lasso, L.F. and Arias-Castellanos, J.A. 2018. Dynamics and use of nitrogen in Biofloc Technology-BFT. Aquaculture, Aquarium, Conservation and Legislation, 11(4): 1107-1129.

Ornamental Aquatic Trade Association (OATA), 2008. Water Quality Criteria ornamental fish. Company Limited by Guarantee and Registered in England No2738119 Registered Office Wessex House, 40 Station Road, Westbury, Wiltshire, BA13JN, UK,

Park, M.R., Park, H. and Chandran, K. 2017. Molecular and KineticCharacterization of Planktonic Nitrospira spp. Selectively Enriched from Activated Sludge. Environmental Science and Technology, 51(5): 2720-2728.

Patricia. A., Robertson, L. A. and Gijs Kuenen, J. 1995. Nitrification and denitrification by *Thiosphaera pantotropha* in aerobic chemostat cultures. FEMS Microbiology Ecology, 18(4): 305-315.

Phirke, N.V. 2014. Characterization of enriched cultures of nitrifying bacteria from black cotton soil of Purna basin. International Journal of Advances in Pharmacy, Biology and Chemistry, 3(4): 869-866.

Prabhu, N.M., Nazar, A.R., Rajagopal, S. and Khan, S.A. 1999. Use of probiotics in water quality management during shrimp culture. Journal of Aquaculture in the Tropics, 14(3): 227-233.

Prosser, J.I. and Embley, T.M. 2002. Cultivation-based and molecular approaches to characterisation of terrestrial and aquatic nitrifiers. Antonie van Leeuwenhoek, 81(1-4): 165-179.

Putra, I., Effendi, I., Lukistyowati, I. and Tang, U.M. 2019. Growth and survival rate of red tilapia (*Oreochromis* sp.) cultivated in the brackish water tank under biofloc system. In International Conference of CELSciTech 2019-Science and Technology track (ICCELST-ST 2019) (pp. 96-99). Atlantis Press.

Putra, I., Effendi, I., Lukistyowati, I., Tang, U.M., Fauzi, M., Suharman, I. and Muchlisin, Z.A. 2020. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia Oreochromis niloticus system. F1000 Research, 9.

Raja, S., Nandhini, E., Sahana, K. and Dhanakkodi, B. 2015. Beneficial and destructive effects of probiotics in aquaculture systems International Journal of Fisheries and Aquatic Studies, 2(3): 153-159.

Reddy, A.D., Subrahmanyam, G., Kumar, N.S., Karunasagar, I and Karunasagar, I. 2015. Isolation of ammonia oxidizing bacteria (AOB) from Fish processing effluents. National Academy Science Letters, 38(5): 393-397.

Roberts, H. and Palmeiro, B.S. 2008. Toxicology of aquarium fish. *Veterinary clinics of North America: exotic animal practice*, 11(2): 359-374.

Robinette, H.R. 1976. Effect of Selected Sublethal Levels of Ammonia on the Growth of Channel Catfish (*Ictalurus punctatus*), Progressive Fish Culturist, 38: 26- 29.

Robinove, C.J., Langford, R.H. and Brookhart, J.W. 1958. *Saline-water resources of North Dakota* (No. 1428). US Government Printing Office, 245.

Ruiz-Ponte C, Samain, J.F. and Nicolas, J.L. 1998. Antibacterial activity exhibited by the marine strain Roseobacter sp. Actes de colloques-IFREMER, 166-8.

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

Sagar, T.P., Singh, S., Singh, V., Baghel, S.R. and Dwivedi, A.K. 2012. Effect of water pH on growth and production of fish in the Coka Dam Papara Distt. Satna (MP). International Journal of Pharmacy and Life Sciences, 3(9): 483.

Sahu, S. and Datta, S. 2018. Effect of Water pH on Growth and Survival of *Trichogaster lalius* (Hamilton, 1822) Under Captivity. International Journal of Current Microbiology and Applied Sciences, SP7: 3655-3666.

Sambasivam, S., Chandran, R. and Khan, S.A. 2002. role of probiotics on the environment of shrimp pond. Journal of Environmental Biology, 24(1): 103-106.

Santhosh, B. and Singh, N.P. 2007. Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Center, Publication no.29.

Sapkale, P.H., Singh, R.K. and Desai, A.S. 2013. Effect of different water temperatures and pH on the growth, specific growth rate and feed conversion efficiency of spawn to fry of common carp, *Cyprinus carpio*. International Journal of Environment and Waste Management, 12(1): 112-120.

Satoh, K., Takizawa, R., Sarai, M., Sato, N., Takahashi, R., and Tokuyama, T. 2004. Two kinds of ammonia-oxidizing bacteria isolated from biologically deodorizing plants in cold district. Journal of bioscience and bioengineering, 98(3): 207-210.

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

Scholz, H.C., Huber, B., Kämpfer, P., Falsen, E., Langer, S. and Busse, H.J. 2010. *Ochrobactrum pituitosum* sp. nov., isolated from an industrial environment. International journal of systematic and evolutionary microbiology, 60(2): 321-326.

Shariff, M., Yusoff, F.M., Devaraja, T.N. and Rao, P.S.S. 2001. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp. *Penaeus monodon* (Fabricius) Ponds. Aquaculture Research, 32(3): 181-187.

Sharma, G.K. and Thakur, A. 2018. Bioremediation of Farm Ponds for Improving Water Quality and Fish Productivity in Bastar Plateau. advances in Bioresearch, 9(3): 81-85.

Sharma, O.P. Bhukhar, S.K.S. 2000. Effect Of Aquazyn-Tm-1000, A probiotic on the water quality and growth O*cyprinus Carpio* Var. *Communis* (L). Indian Journal of Fisheries, 47(3): 209-213.

Shrivastava, S.K., Vajpai, K., Shrivastava, D.K., and Vajpai, S.K. 2007. Study of physicochemical quality of pond water in Bilaspur, Chhattisgarh. Current World Environment, 3(1): 97.

Singh, U., and Nautiyal, P. 2017. Length-weight relationship and relative condition factor of some hill stream fishes, India. International Journal of Fisheries and Aquatic Studies 3: 525-529.

Sohier, L. and Bianchi, M. 1983. The regulation of ammonia and nitrite rates by nitrifying bacteria in mariculture ponds. Biophysical-and-biochemical mechanism of transfers in the trophic chaines in the marine environment. Groupment– pour-1. Advancement-deta biochime. Marine, France. 9(3): 265-273.

Sorokin, D.Y., Muyzer, G., Brinkhoff, T., Kuenen, J.G. and Jetten, M.S. 1998. Isolation and characterization of a novel facultatively alkaliphilic *Nitrobacter* species, N. alkalicus sp. nov. *Archives of microbiology*, 170(5): 345-352.

Spieck, E. and Lipski, A. 2011.Cultivation, Growth Physiology, and Chemotaxonomy of Nitrite-Oxidizing Bacteria. Methods in enzymology, 486(Part A), 109-130.

Sunitha, K. and Padmavathi, P. 2013. Influence of Probiotics on Water Quality and Fish Yield in Fish Ponds. International Journal of Pure & Applied Sciences & Technology, 19(2): 48-60.

Suwa, Y., Imamura, Y., Suzuki, T., Tashrio, T. and Urushigawa, Y. 1994. Ammonia-oxidizing bacteria with different sensitivities to ammonium sulphate in activated sludges. Water Research (Oxford)(United Kingdom).

Swann, L.D. 1997. A Fish Farmer's Guide to Understanding Water Quality, Aquaculture Extension Illinois, Purdue University, Indiana Sea Grant Program Fact Sheet AS-503.

Swarnakar, A.K. and Choubey, S. 2016. Testing and analysis of pond water in Raipur City. International Journal of Science and Research, 5(4): 1962-1965.

Thandar, S.M., Ushiki, N., Fujitani, H., Sekiguchi, Y., and T Suneda, S. 2016. Ecophysiology and Comparative Genomics of *Nitrosomonas* mobilis Ms1 Isolated from Autotrophic Nitrifying Granules of Wastewater Treatment Bioreactor. Frontiers in Microbiology, 7: 1869.

Thomforde, H. K. 2004. Understanding your fish pond water analysis report (pp. 1-4). Cooperative Extension Program, University of Arkansas at Pine Bluff, US Department of Agriculture and county governments cooperating.

Van Rijin, J., Diab, S., Shiloh, M. 1984. Mechanism of ammonia transformation in fish ponds. European Mari Culture. Society Bredene Belgium, 8: 17-40.

Verma, S. and Khan, J.B. 2015. Analysis of Water Quality by Physico-Chemical Parameters in Fateh Sagar Talab in Bagar, Dist. of Jhunjhunu (Raj.), India. Department of Botany. Journal of Pharmacy and Biological Sciences, 10(5): 41-45.

Watson, S.W., Bock, E., Harms H., Koops, H.P. and Hooper, A.B. 1989. Family Nitrobacteraceae, In Bergey's Manual of Systematic Microbiology, (3). pp. 1808-1834.

Williams, S.T., Krieg, N.R., Sneth, P.H.J. and Half, J.G.B. 1994. Manual of determinative bacteriology, ninth edition. pp. 447-455.

Wu, J.T., Erickson, A.J., Tsao, K.C., Wu, T.L. and Sun, C.F. 2000. Elevated serum chromogranin A was detectable in patients with carcinomas at advanced disease stages. Annals of Clinical and Laboratory Science, 30(2): 175-178.

Yadav, A., Sahu, P.K., Chakradhari, S., Rajhans, K.P., Ramteke, S., Dahariya, N.S., and Patel, K.S. 2016. Urban pond water contamination in India. Journal of Environmental Protection, *7*(1): 52.

Yang HaoFeng; Xie Liu; Zhou JunLi; Zhang Ting; Yang Yue Ping 2013. Guangxi University, Guangxi, China Journal Genomics and Applied Biology*,* 32(4): 453-458.

Zavarzin, G. and Legunkova, R. 1959. The morphology of Nitrobacter winogradskyi. Microbiology, 21(1): 186-190.

# APPENDIX-I

**_Nitrosomonas_ culture medium**

The chemical composition of ammonia broth medium for *Nitrosomonas* was as follows:

| | |
|---|---|
| Ammonium sulfate $(NH_4)2\ SO_4$ | 2.00gm |
| Magnesium sulfate (MgSO4 $7H_2O$) | 0.50gm |
| Ferrous sulfate ($FeSO_4$ $7H_2O$) | 0.03gm |
| Sodium chloride (NaCl) | 0.30gm |
| Magnesium carbonate ($MgCO_3$) | 10.00gm |
| Dipotassium 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 ($MgSO_4.7H_2O$) | - 0.50g |
| Ferrous sulfate ($FeSO_4$. $7H_2O$) | - 0.03g |
| Sodium chloride (NaCl) | - 0.30g |
| Sodium carbonate ($Na_2CO_3$) | - 1.00g |
| Di-potassium hydrogen phosphate ($K_2HPO_4$) | - 1.00g |
| Double distilled water | - 1000ml |

Fifty ml of the ammonia broth medium and the nitrite broth medium were taken in Erlenmeyer flasks (250 ml) and sterilized autoclaving for 30 minutes at 15 lb/inch 2 pressure.

**_Ochrobactrum pituitosum_ culture media**

Composition of MacConkey Agar Composition

| Ingredients | Amount |
|---|---|
| Peptone | 17 gm |
| Proteose peptone (meat and casein) | 3 gm |
| Lactose monohydrate | 10 gm |
| Bile salts | 1.5 gm |
| Sodium chloride | 5 gm |
| Neutral red | 0.03 gm |
| Crystal Violet | 0.001 gm |
| Agar | 13.5 gm |
| Distilled water | 1000 ml |

**Preparation of media:**

Suspend 49.53 grams of dehydrated medium in 1000 ml of distilled water and heat to boiling to dissolve the medium completely then sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes thereafter cool it to 45°C -50°C and then mix well before pouring into sterile Petri plates.

**Nutrient Agar medium (gm/litre)**

| | |
|---|---|
| Yeast extract | 2.0 |
| Peptone | 5.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |
| Distilled water | 1000 ml |
| pH | 7.4 |

***Micrococcus luteus* culture media (gm/litre)**

| | |
|---|---|
| Yeast extract | 3.0 |
| Peptone | 5.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |
| Distilled water | 1000 ml |
| Beef extract | 1.5 |
| Glucose | 1.0 |

pH 7.4 ± 0.2 @ 25°C

**Preparation of media:**Add components to distilled water and bring volume to 1.0 L and mix thoroughly. Gently heat and bring to boiling. Distributed into tubes or flask. Autoclaving at 15 lbs pressure (121°C) for 15 minutes thereafter cool it to 45°C -50°C and then mix well before pouring into sterile Petri plates.

**Gelatin liquefaction test**

| | |
|---|---|
| Peptone | 5.0g |
| Beef | 3.0g |
| Gelatin | 120g |
| Agar | 10.0g |
| Distilled Water | 1000ml |

All the components, except agar, were added to distilled water and pH was adjusted. Agar was mixed by contrast heating and stirring. The medium was than autoclaved at 15 lbs pressure for 20 min.

## Reagent used

### 2.1. Gram'sstains:

I. Crystal violet

a) Solution A

| | |
|---|---|
| Crystal violet (90% dye content) | 2.0 g |
| Ethylalcohol (95%) | 20.0 ml |

b) Solution B

| | |
|---|---|
| Ammonium oxalate | 0.8 g |
| Distilled water | 80.0 ml |

Crystal violet was dissolved in ethyl alcohol and the ammonium oxalate in distilled water. Then solution A and B was mixed.

ll. Gram's iodine

| | |
|---|---|
| Iodine | 1.0 g |
| Potassium iodide | 2.9 g |
| Distilled water | 300.0 ml |

Make a homogeneous préparation of the iodine and iodide using mortar and pestle. Transfer the contents to a reagent bottle and add water to make a total of 300 ml. Store the solution in a glass stoppered brown bottle.

III. Ethyl alcohol (95 %)

| | |
|---|---|
| Ethyl alcohol (100 %) | 95.0 ml |
| Distilled water | 5.0 ml |

IV. Safranin

| | |
|---|---|
| Safranin (2.5% sol. + 95% ethylalcohol) | 10.0 ml |
| Distille water | 100 ml |

**2.4. $H_2O_2$ solution (3 %)**

| | |
|---|---|
| Hydrogen peroxide | 3.0 ml |
| Distilled water | 97.0 ml |

**2.5. Crystal violet Reagent (1%)**

| | |
|---|---|
| Crystal violet | 1gm |
| Distilled water | 99ml |

**Nitrate Test Solution:**

a) Solution A

| | |
|---|---|
| Sulfanilic acid | 8.0 g |
| Acetic acid (5N) | 1000 ml |

b) Solution B

| | |
|---|---|
| α – naphthylamine | 5.0 g |
| Acetic acid (5N) | 1000 ml |

# Appendix-II

## Weekly meteorological data during fish growth period of aquarium in 2018-19

| Met. Week | Max.temp(°C) | Min temp.(°C) | Rainfall (mm) | RH (%)(I) | RH (%)(II) | Evaporation (mm) | Sun Shine (hr) |
|---|---|---|---|---|---|---|---|
| Jun 18-24 | 38 | 26.6 | 26.4 | 76 | 44 | 55.4 | 8.6 |
| Jun 25-01 | 33.5 | 26 | 16.9 | 84 | 60 | 39.2 | 4.4 |
| Jul 02 - 08 | 33.8 | 25.4 | 1.6 | 87 | 60 | 33.7 | 3.6 |
| Jul 09 -15 | 31.1 | 25 | 199.2 | 94 | 86 | 18.8 | 0.9 |
| Jul 16 - 22 | 30.4 | 25.4 | 75.8 | 93 | 82 | 18.8 | 0.4 |
| Jul 23 -29 | 28.64 | 25 | 51.4 | 88 | 76 | 15.5 | 0.1 |
| Jul 30 - 05 | 31.9 | 25.4 | 31 | 88 | 67 | 26.9 | 2.3 |
| Aug 06 - 12 | 30 | 24.8 | 103.4 | 92 | 88 | 20.2 | 1.2 |
| Aug 13 -19 | 30.3 | 25.3 | 101.2 | 94 | 79 | 24.7 | 2.9 |
| Aug 20-26 | 29 | 24.6 | 60.4 | 93 | 79 | 18 | 0.6 |
| Aug 27 - 02 | 28.3 | 24.1 | 275 | 96 | 86 | 14.7 | 0.2 |
| Sep 03 - 09 | 29.2 | 23.9 | 30.2 | 93 | 57 | 22.7 | 1.1 |
| Sep 10 -16 | 32.6 | 25.1 | 0 | 90 | 55 | 27.9 | 6.4 |
| Sep 17 -23 | 31 | 24.1 | 32.8 | 92 | 68 | 19.2 | 3.6 |
| Sep 24 -30 | 32.9 | 25 | 11 | 93 | 59 | 25.6 | 7.8 |
| Oct 01 - 07 | 34 | 23.8 | 0 | 91 | 44 | 28.1 | 8 |
| Nov 12-18 | 31.5 | 14.4 | 0 | 86 | 29 | 23.3 | 9.1 |
| Nov. 19-25 | 31.4 | 15.3 | 0 | 88 | 28 | 23.2 | 7.9 |
| Nov. 26- 02 | 29.3 | 13.6 | 0 | 89 | 33 | 19.7 | 7.5 |

| Met. Week | Max.temp(°C) | Min temp.(°C) | Rainfall (mm) | RH (%)(I) | RH (%)(II) | Evaporation (mm) | Sun Shine (hr) |
|---|---|---|---|---|---|---|---|
| Dec. 03-09 | 28.2 | 14.3 | 0 | 87 | 38 | 17.4 | 4.4 |
| Dec. 10 -16 | 27.4 | 15.7 | 0 | 86 | 51 | 13.9 | 1.2 |
| Dec. 17-23 | 22.1 | 11.0 | 47.2 | 90 | 57 | 15.3 | 4.5 |
| Dec. 24-31 | 25.1 | 8.6 | 0 | 86 | 28 | 16.6 | 7.5 |
| Jan. 01-07 | 27.4 | 8.5 | 0 | 88 | 28 | 16.8 | 6.6 |
| Jan. 08-14 | 27.1 | 10.2 | 0 | 87 | 34 | 16.5 | 6.1 |
| Jan. 15-21 | 28.1 | 9.2 | 0 | 85 | 21 | 18.7 | 6.8 |
| Jan. 22-28 | 26.3 | 14.3 | 23.6 | 85 | 53 | 18.5 | 4 |
| Jan. 29-04 | 26.4 | 9.5 | 0 | 87 | 24 | 20.6 | 8.2 |
| Feb.05-11 | 28.8 | 12.5 | 3.4 | 81 | 36 | 21.3 | 7.6 |
| Feb. 12-18 | 30.2 | 13.6 | 9 | 84 | 34 | 20.4 | 8.3 |
| Feb. 19-25 | 33.1 | 17.0 | 0 | 81 | 30 | 30.3 | 9.1 |
| Feb. 26-04 | 31 | 17.3 | 0.2 | 72 | 36 | 30.7 | 7.8 |
| Mar.05-11 | 33.3 | 17.6 | 0 | 70 | 32 | 40.3 | 8.9 |
| Mar. 12-18 | 35.6 | 21.6 | 0 | 72 | 33 | 36.7 | 6.8 |
| Mar. 19-25 | 34.5 | 19.8 | 9.2 | 80 | 28 | 37.1 | 8.4 |
| Mar. 26-01 | 38.2 | 20.6 | 10.8 | 64 | 19 | 44.5 | 8.7 |
| Apr . 02-08 | 39.7 | 23.4 | 0 | 50 | 18 | 53.2 | 8.3 |
| Apr. 09-15 | 40.8 | 24.5 | 0 | 47 | 20 | 60.4 | 8.3 |

# Appendix-III

## Weekly meteorological data during fish growth period of aquarium in 2019-20

| Met. Week | Max. temp(°C) | Min temp.(°C) | Rainfall (mm) | RH (%)(I) | RH (%)(II) | Evaporation (mm) | Sun Shine (hr) |
|---|---|---|---|---|---|---|---|
| Jun 18-24 | 37.4 | 26.3 | 45.4 | 82 | 54 | 41.3 | 4.6 |
| Jun 25-01 | 36.8 | 27.5 | 45.4 | 78 | 53 | 39 | 5 |
| Jul 02-08 | 29.5 | 24.7 | 45.4 | 91 | 84 | 16.6 | 0.4 |
| Jul 09-15 | 33.5 | 26.1 | 13.2 | 82 | 59 | 39.1 | 3.6 |
| Jul 16-22 | 35.5 | 26.4 | 26.1 | 84 | 55 | 36.3 | 7.5 |
| jul 23-29 | 32.3 | 25.5 | 8.4 | 88 | 77 | 21.3 | 2.7 |
| jul 30-05 | 28 | 24.4 | 99 | 90 | 82 | 14.5 | 0.5 |
| Aug 06-12 | 30.2 | 25.3 | 185.6 | 89 | 83 | 24.3 | 2.9 |
| Aug 13-19 | 30.8 | 25.4 | 49.2 | 90 | 74 | 18.1 | 3.8 |
| Aug 20-26 | 31.7 | 25.2 | 27.8 | 92 | 75 | 21.9 | 3.2 |
| Aug 27-02 | 30.9 | 25.1 | 53.8 | 92 | 73 | 18.2 | 1.3 |
| Sep 03-09 | 29.5 | 24.9 | 239 | 94 | 87 | 11.6 | 0.5 |
| Sep 10-16 | 30.7 | 25 | 9.2 | 92 | 74 | 21.6 | 4.3 |
| Sep 17-23 | 33 | 25.5 | 2.4 | 88 | 63 | 29.6 | 8.2 |
| Sep 24-30 | 30.2 | 24.2 | 178.1 | 91 | 74 | 17.7 | 3.8 |
| Oct 01-07 | 32 | 24.3 | 1.8 | 90 | 64 | 25.3 | 7.5 |
| Nov 12-18 | 30 | 16 | 0 | 90 | 38 | 20 | 9 |
| Nov 19-25 | 30 | 15 | 0 | 89 | 38 | 20 | 8 |
| Nov 26- 02 | 30 | 16 | 0 | 90 | 43 | 20 | 6 |
| Dec. 03-09 | 28 | 13 | 0 | 84 | 34 | 21 | 8 |
| Dec. 10 -16 | 30 | 15 | 0 | 91 | 48 | 17 | 5 |
| Dec. 17-23 | 27 | 14 | 1 | 88 | 42 | 17 | 5 |
| Dec. 24-31 | 26 | 12 | 0 | 81 | 35 | 22 | 6 |
| Jan. 01-07 | 23 | 13 | 19 | 84 | 55 | 15 | 4 |
| Jan. 08-14 | 25 | 11 | 3 | 90 | 46 | 16 | 6 |
| Jan. 15-21 | 29 | 14 | 0 | 88 | 48 | 17 | 6 |
| Jan. 22-28 | 29 | 13 | 0 | 87 | 39 | 20 | 7 |
| Jan. 29-04 | 26 | 14 | 0 | 76 | 46 | 22 | 4 |
| Feb. 05-11 | 21 | 14 | 49 | 94 | 66 | 12 | 2 |

# RESUME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RESEARCH ARTICLE

# Study on Heterotrophic and Chemo-autotrophic Bacteria (nitrifying) as Bioremediator of Ammonia and Nitrate in the Simulated Aquaculture System

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## ABSTRACT

*The present investigation was carried out at the Department of Agricultural Microbiology, College of Agriculture Raipur, Indira Gandhi Krishi Vishwavidyalaya, and 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 70.00 liter 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 the contaminated pond water, inoculation of autotrophic and heterotrophic bacteria positively affected ammonia concentration and heterotrophic bacteria (Micrococcus luteus and Ochrobactrum pituitosum) PS5 and PS16isolateare performing best results in decreasing the ammonia concentration and increasing the nitrate concentration, respectively. Use of molasses 5 ppm along with the mixed culture of heterotrophic and autotrophic bacterial isolates found better for enhancing fish growth rate. Use of 1ppm skimmed milk found positively affected ammonia concentration at 15 DAI (0.28 to 8.41 ppm) and 30 DAI (0.275 to 6.44 ppm), respectively.*

**Keywords:** *Autotrophic Bacteria, Heterotrophic Bacteria, Isolation, Molasses, Skimmed milk*

## 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 manufactured ponds constitute a significant source to 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.

Heterotrophic bacteria use ammonia from consumed food, fecal waste, etc. as nitrogen sources that are decomposed anaerobically. However, for bacterial growth and energy generation, supplemental carbon sources are needed. Their decomposition depends on environmental factors such as temperature and oxygen. Thus, the use of labile sources such as molasses and dextrose, as well as the use of sources with lower dissolution, as in the case of plant meals, are necessary as carbon sources to heterotrophic bacteria [2-3].

Commercially available nutrient amendments with complex compositions that are known to stimulate microbial growth include molasses. Molasses contains about 50% sugar in the form of sucrose, glucose, and fructose, and is rich in mineral elements [4]. In recent years, manipulation of C:N ratio infeed has shown promising results in aquaculture [2]. The C:N ratio can be manipulated by the application of various carbohydra te sources such as molasses, rice flour, tapioca powder, etc. Plant meals are alternative carbon sources that contrast with molasses in their cost and dissolution in culture water. BFT farming system is viable with low-cost carbon-rich sources [3]. Recommend using low-cost carbon sources because it is an alternative that enables economics sustainability, provides an additional source of protein, and improves the nutritional efficiency of the culture system [5].

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 in order to increase sustainable, eco-friendly fish production. Bacteriological nitrification was the most 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 a 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. Therefore, the study aims to develop microbial biofloc for culture using carbohydrate materials (sugarcane molasses) as a carbon source to boost production by improving the conversion of nutrients into harvestable products while maintaining good water quality.

## 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 the 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.

### Isolation of autotrophic (nitrifying) bacteria

Autotrophic (nitrifying) bacteria were isolated from moist fish pond soil utilizing serial dilution and plating [6] using nitrifying agar media containing red phenol [7]. 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

After collecting samples, heterotrophic bacteria were isolated from the muddy moist fish pond soil using the serial dilution and plating process [6]. In this relation, for the isolation of the heterotrophic 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.



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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 [8]. 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. They 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, Yeast 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 $NH_4$ easily, which can be again distilled off for analysis [9].

## RESULTS

**Location of soil and water samples collection for isolation of nitrifying autotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh)**

Data presented in (Table 1) related to information about *Nitrosomonas* and *Nitrobacter* isolates which were isolated from 25 soil and water samples of polluted 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 indicate that ammonia started to transform after two weeks of inoculation of nitrifying bacterial isolates under controlled conditions. The selection of effective nitrifying bacterial isolates was made based on the rate of conversion of ammonia into nitrate [10].

Isolation of nitrifying bacteria was done by using the serial dilution-plating method as also practiced by several researches [6, 7 and 11]. They indicate that isolates of nitrifying bacteria can be obtained by making serial dilution of soil samples. [12] reported that nitrifying bacteria could be isolated by making appropriate serial dilution of drained fish pond soil. In this connection, [11] said that the presence of nitrifying bacteria could be observed up to $10^{-7}$diluation. However, in the present studies, isolation was made from $10^{-3}$ dilution, which bears a low population density of nitrifying bacteria in soils of Chhattisgarh.

**Location of soil and water samples collection for isolation of heterotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh**

Heterotrophic bacteria were isolated from 25 soil and water samples of polluted ponds belonging to the area of Raipur, Chhattisgarh (Table 2a). Further, these isolates were tested for their ability to convert ammonia into nitrate by using the proper medium.

During isolation of denitrifying bacteria *Ochrobactrum*

**Table 1.** Location of soil and water samples collection for isolation of nitrifying autotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh

| S.No. | Location of pond | | | | Isolate No. | | | |
|---|---|---|---|---|---|---|---|---|
| | Village /N. Nigam Ward Name | Latitude | Longitude | Pond Name | *Nitrosomonas* | | *Nitrobacter* | |
| | | | | | Soil | Water | Soil | Water |
| 1. | Purena | 21°14'1.070"N | 81°40'17.73"E | 1st Purena pond, | NS1S | NS1W | NB1S | NB1W |
| 2. | Purena | 21°14'0.120"N | 81°40'23.00"E | 2nd Purena pond, | NS2S | NS2W | NB2S | NB2W |
| 3. | Amlidih | 21°13'21.08"N | 81°40'11.28"E | 1st Amlidih pond | NS3S | NS3W | NB3S | NB3W |
| 4. | Amlidih | 21°13'27.45"N | 81°40'31.07"E | 2nd Amlidih pond | NS4S | NS4W | NB4S | NB4W |
| 5. | Katora | 21° 14' 5.44"N | 81° 39' 18.0"E | Katora Talab pond | NS5S | NS5W | NB5S | NB5W |
| 6. | Telibandha | 21°14'23.45"N | 81°39'39.48"E | Marine drive pond | NS6S | NS6W | NB6S | NB6W |


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|---|---|---|---|---|---|---|---|---|
| 7. | Tikrapara | 21°13'39.57"N | 81°38'26.22"E | NariyaT-ikraparapond | NS7S | NS7W | NB7S | NB7W |
| 8. | Math para | 21°13'16.55"N | 81°37'31.98"E | 1stDudhadhari pond | NS8S | NS8W | NB8S | NB8W |
| 9. | Math para | 21°13'42.04"N | 81°37'44.89"E | 2ndDudhadhari pond | NS9S | NS9W | NB9S | NB9W |
| 10. | Kankali para | 21° 14' 10.75"N | 81° 37' 42.00"E | Kankalipara-pond | NS10S | NS10W | NB10S | NB10W |
| 11. | Kalibadi | 21°13'48.58"N | 81°37'59.83"E | Budha Talab pond | NS11S | NS11W | NB11S | NB11W |
| 12. | Mova | 21°16'6.500"N | 81°40'18.77"E | Mova pond | NS12S | NS12W | NB12S | NB12W |
| 13. | Pandri | 21°13'39.36"N | 81°38'26.64"E | NariyaPandri-pond | NS13S | NS13W | NB13S | NB13W |
| 14. | Raja para | 21° 14' 52.69"N | 81° 39' 16.0"E | Raja Talab pond | NS14S | NS14W | NB14S | NB14W |
| 15. | Kailashpuri-Kushalpur | 21°12'53.00"N | 81°42'37.66"E | Dabri pond | NS15S | NS15W | NB15S | NB15W |
| 16. | Kailashpuri-Kushalpur | 21° 13' 49.10"N | 81° 37' 15.24"E | Kho-Kho pond | NS16S | NS16W | NB16S | NB16W |
| 17. | Kailashpuri-Kushalpur | 21° 13' 39.85"N | 81° 36' 48.58"E | Pahadi pond | NS17S | NS17W | NB17S | NB17W |
| 18. | Kushalpur | 21°13'58.44"N | 81°37'10.62"E | Gittikhadan pond | NS18S | NS18W | NB18S | NB18W |
| 19. | Kushalpur | 21°14'4.12"N | 81°37'9.68"E | Malsat pond | NS19S | NS19W | NB19S | NB19W |
| 20. | Professor colony Kushalpur | 21° 13' 17.3"N | 81° 37' 29.99"E | BhaiyaTalab-pond | NS20S | NS20W | NB20S | NB20W |
| 21. | Purana basti | 21° 13' 44.3"N | 81° 37' 37.31"E | Maharaja pond | NS21S | NS21W | NB21S | NB21W |
| 22. | Math purena | 21° 13' 19.9"N | 81° 37' 56.17"E | 1stChiranjiv pond | NS22S | NS22W | NB22S | NB22W |
| 23. | Math purena | 21° 12' 39.0"N | 81° 38' 31.00"E | 2ndChiranjiv pond | NS23S | NS23W | NB23S | NB23W |
| 24. | Kharun | 21° 12' 59.36"N | 81° 35' 16.00"E | Kharun | NS24S | NS24W | NB24S | NB24W |
| 25. | Sarona | 21° 17' 15.43"N | 81° 36' 42.00"E | Sarona pond | NS25S | NS25W | NB25S | NB25W |

NS =*Nitrosomonas* bacterial isolate, NB = *Nitrobacter* bacterial isolate

**Table 2a.** Location of soil and water samples collection for isolation of heterotrophic bacteria (Tehsil and District: Raipur; Chhattisgarh)

| S.No. | Location of pond | | | | Isolate No. |
|---|---|---|---|---|---|
| | Village /N.Nigam Ward Name | Latitude | Longitude | Pond Name | Heterotrophic bacteria of pond soil |
| 1. | Purena | 21°14'1.070"N | 81°40'17.73"E | 1stPurena pond, | **PS1** |
| 2. | Purena | 21°14'0.120"N | 81°40'23.00"E | 2ndPurena pond, | PS2 |
| 3. | Amlidih | 21°13'21.08"N | 81°40'11.28"E | 1stAmlidih pond | PS3 |
| 4. | Amlidih | 21°13'27.45"N | 81°40'31.07"E | 2ndAmlidih pond | PS4 |
| 5. | Katora | 21° 14' 5.44"N | 81° 39' 18.0"E | Katora Talab pond | **PS5** |
| 6. | Telibandha | 21°14'23.45"N | 81°39'39.48"E | Marine drive pond | **PS6** |
| 7. | Tikrapara | 21°13'39.57"N | 81°38'26.22"E | NariyaTikraparapond | PS7 |
| 8. | Math para | 21°13'16.55"N | 81°37'31.98"E | 1stDudhadhari pond | PS8 |
| 9. | Math para | 21°13'42.04"N | 81°37'44.89"E | 2ndDudhadhari pond | PS9 |
| 10. | Kankali para | 21° 14' 10.75"N | 81° 37' 42.00"E | Kankaliparapond | **PS10** |


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|---|---|---|---|---|---|
| 11. | Kalibadi | 21°13'48.58"N | 81°37'59.83"E | Budha Talab pond | **PS16** |
| 12. | Mova | 21°16'6.500"N | 81°40'18.77"E | Mova pond | PS12 |
| 13. | Pandri | 21°13'39.36"N | 81°38'26.64"E | NariyaPandripond | PS13 |
| 14. | Raja para | 21° 14' 52.69"N | 81° 39' 16.0"E | Raja Talab pond | PS14 |
| 15. | Kailashpuri Kushalpur | 21°12'53.00"N | 81°42'37.66"E | Dabri pond | PS15 |
| 16. | Kailashpuri Kushalpur | 21° 13' 49.10"N | 81° 37' 15.24"E | Kho-Kho pond | PS16 |
| 17. | Kailashpuri Kushalpur | 21° 13' 39.85"N | 81° 36' 48.58"E | Pahadi pond | PS17 |
| 18. | Kushalpur | 21°13'58.44"N | 81°37'10.62"E | Gittikhadan pond | PS18 |
| 19. | Kushalpur | 21°14'4.12"N | 81°37'9.68"E | Malsat pond | PS19 |
| 20. | Professor colony Kushalpur | 21° 13' 17.3"N | 81° 37' 29.99"E | BhaiyaTalabpond | PS20 |
| 21. | Purana basti | 21° 13' 44.3"N | 81° 37' 37.31"E | Maharaja pond | PS21 |
| 22. | Math purena | 21° 13' 19.9"N | 81° 37' 56.17"E | 1stChiranjiv pond | PS22 |
| 23. | Math purena | 21° 12' 39.0"N | 81° 38' 31.00"E | 2ndChiranjiv pond | PS23 |
| 24. | Kharun | 21° 12' 59.36"N | 81° 35' 16.00"E | Kharun | PS24 |
| 25. | Sarona | 21° 17' 15.43"N | 81° 36' 42.00"E | Sarona pond | **PS25** |

PS= Heterotrophic bacteria isolate, PS5=*Micrococcus luteus*CP001628 bacterial isolate, PS16 =*Ochrobactrum pituitosum* AM490609 bacterial isolate

*pituitosum*AM 490609(PS16) and *Micrococcus luteus* CP001628 (PS5), media become more turbid within a week. This further confirms that they belong to the heterotrophic group of bacteria. Gram's staining and microscopic observation also confirmed the presence of these bacteria.

**Ammonia and nitrate dynamics in polluted pond water as influenced by selected pond soil heterotrophic bacterial isolates under controlled conditions.**

Heterotrophic bacteria isolates were isolated from 25 pond soils belonging to the area of Raipur district, Chhattisgarh. Among these, 7 isolates (PS1, PS3, PS5, PS6, PS10, PS16, and PS25) were selected based on their growth performance during isolations from pond soils. Data presented in Table 2b revealed that out of 7 isolates, PS-5 and PS-16 isolates were found more effective concerning conversion of ammonia. The ammonia concentration due to PS-5 was decreased from 0.20 mg/l (15 DAI) to 0.09 mg/l (30 DAI). Similarly, the PS16 was also showed a decreasing result from 0.15 mg/l (15 DAI) to 0.05 mg/l (30 DAI). These findings are close to the findings of [13].

In table 4.2(b), the nitrate concentration due to PS-5 was increased from 135 mg/l (15 DAI) to 170 mg/l (30 DAI) because of the effective transformation of ammonia. Similarly, the PS16also gave the similar type of promising result to a reduced harmful concentration of ammonia by nitrification process. The nitrate concentration increased from139 mg/l (15 DAI) to 171 mg/l (30 DAI). These findings are also close to results of [13 and 14].

**Table 2b.** Ammonia and nitrate dynamics in polluted pond water as influenced by selected pond soil heterotrophic bacterial isolates under controlled conditions.

| Sr. No. | Isolate No. | Ammonia concentration (mg/l) | | Nitrate concentration (mg/l) | |
|---|---|---|---|---|---|
| | | At 15 DAI | At 30 DAI | At 15 DAI | At 30 DAI |
| 1. | PS1 | 0.31 | 0.21 | 116 | 160 |
| 2. | PS3 | 0.29 | 0.20 | 120 | 157 |
| 3. | PS5 | **0.20** | **0.09** | **135** | **170** |
| 4. | PS6 | 0.25 | 0.19 | 121 | 162 |
| 5. | PS10 | 0.22 | 0.12 | 121 | 165 |
| 6. | PS16 | **0.15** | **0.05** | **139** | **171** |
| 7. | PS25 | 0.25 | 0.20 | 123 | 168 |
| | CD(0.05) | 0.02 | 0.01 | 5.09 | 9.59 |

PS 5 = *Micrococcus luteus* CP001628, PS16 = *Ochrobactrum pituitosum*AM490609

Note =Water were collected from the polluted pond which was contaminated by open drain wate.

**Ammonia and nitrate dynamics in molasses amended water of polluted pond as influenced by autotrophic and heterotrophic bacteria under controlled conditions.**

The data presented in Table 3 revealed that at 2-ppm organic N level, $T_4$ gave best results with reference to decrease in the ammonium concentration from 15 DAI (1.41 ppm) to 30 DAI (1.27 ppm) and increase in nitrate concentration from 30.0 to 44.2 ppm when comparing the treatments of $T_3$ and $T_4$ with the treatment $T_2$ for

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the analysis of the ammonia concentration. Similarly, at 8-ppm concentration of organic N when comparing the $T_5$ with $T_6$ and $T_7$ treatment, we got the best result with treatment $T_7$. The decrease in the ammonia concentration from 15 DAI(4.77) to 30 DAI(2.73). While the next organic N level of 12ppm, it had the most negligible effect upon lowering the ammonia concentration from 8.33 ppm at 15 DAI to 6.52 ppm at 30 DAI.

A similar finding has been obtained that molasses pretreatment can significantly stimulate the process of bacterial attachment on the ceramic bottom pieces for faster degradation of ammonia in the submerged condition. These types of the finding of the present investigation are close to results of [15]. The ammonia-nitrogen range was 0.02 – 0.07 mg $L^{-1}$, the nitrite-nitrogen field was between 0.20 – 0.43 mg $L^{-1}$, whereas in the nitrate-nitrogen 0.90 – 3.20 mg $L^{-1}$ and the carbon source from the molasses was effective in reducing the concentration of ammonia, when cultured with the biofloc technique [16]. The ammonia concentration was decreasing as per the results given by [17].

**Table 3.** Ammonia and nitrate dynamics in molasses amended* water of polluted pond as influenced by autotrophic and heterotrophic bacteria under controlled conditions.

| Abbre. | Treatments | Ammonia concentration ppm(mg/l) | | Nitrate concentration ppm(mg/l) | |
|---|---|---|---|---|---|
| | | At 15 DAI | At-30DAI | At 15DAI | At-30DAI |
| $T_1$ | No inoculums and no chemical | 0.280 | 0.278 | 30.7 | 33.0 |
| $T_2$ | Organic source of N (Urea 2 ppm) + No inoculums | 2.00 | 1.97 | 30.1 | 32.0 |
| $T_3$ | Organic source of N (Urea 2 ppm) + $I_1$ | 1.80 | 1.73 | 29.7 | 32.0 |
| $T_4$ | Organic source of N (Urea 2 ppm) + $I_2$ | 1.41 | 1.27 | 30.0 | 44.2 |
| $T_5$ | Organic source of N (Urea 8 ppm) + No inoculums | 6.88 | 6.27 | 38.10 | 46.0 |
| $T_6$ | Organic source of N (Urea 8 ppm) + $I_1$ | 5.91 | 4.68 | 50.5 | 54.3 |
| $T_7$ | Organic source of N (Urea 8 ppm) + $I_2$ | 4.77 | 2.73 | 59.17 | 72.9 |
| $T_8$ | Organic source of N (Urea 12 ppm) + $I_1$ | 8.33 | 6.52 | 70.00 | 77.5 |
| | CD | 0.23 | 0.09 | 6.05 | 6.75 |

* = 5% Molasses water solution,

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

**Ammonia and nitrate dynamics in skimmed milk amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different levels of organic under controlled conditions.**

Data presented in Table 4related to ammonia and nitrate dynamics in skimmed milk amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different organic N levels under controlled conditions at 15 and 30 DAI. The results were recorded at 15 DAI (0.28 to 8.41 ppm) and 30 DAI (0.275 to 6.44 ppm), respectively. However, the ammonia concentration showed a decreasing trend from 15 DAI to 30 DAI, in both intervals (15 DAI & 30 DAI).The minimum ammonia concentration obtained in the $T_4$ treatment (1.46 to 1.00 ppm) at 2 ppm organic N source and $T_7$ also gave minimum ammonia concentration at 8 ppm organic N source application at 30DAI (4.80 to 2.77 ppm) *i.e.*, reduced by 2.03 ppm, thus in both the treatments significant reduction in the ammonia concentration was observed. When we used the area at 12 ppm, the maximum concentration of ammonia was recorded in $T_8$ (8.41to 6.44 ppm). A similar finding has been obtained by [18].

**Table 4.** Ammonia and nitrate dynamics in skimmed milk* amended polluted pond water as influenced by autotrophic and heterotrophic bacteria with different levels of organic N under controlled condition.

| Abbre. | Treatments | Ammonia concentration ppm(mg/l) | | Nitrate concentration ppm(mg/l) | |
|---|---|---|---|---|---|
| | | At 15 DAI | At-30DAI | At 15DAI | At-30DAI |
| $T_1$ | No inoculums and no chemical | 0.280 | 0.275 | 29.3 | 31.9 |
| $T_2$ | Organic source of N (Urea 2 ppm) + No inoculums | 2.00 | 2.00 | 29.1 | 32.2 |
| $T_3$ | Organic source of N (Urea 2 ppm) + $I_1$ | 1.84 | 1.78 | 29.0 | 31.0 |
| $T_4$ | Organic source of N (Urea 2 ppm) + $I_2$ | 1.45 | 1.30 | 28.8 | 42.9 |



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| | | | | | |
|---|---|---|---|---|---|
| $T_5$ | Organic source of N (Urea 8 ppm) + No inoculums | 6.97 | 6.25 | 36.7 | 45.0 |
| $T_6$ | Organic source of N (Urea 8 ppm) + $I_1$ | 6.00 | 4.72 | 48.5 | 52.0 |
| $T_7$ | Organic source of N (Urea 8 ppm) + $I_2$ | 4.80 | 2.77 | 57.0 | 70.7 |
| $T_8$ | Organic source of N (Urea 12 ppm) + $I_1$ | 8.41 | 6.44 | 70.0 | 71.1 |
| | CD (0.05) | 0.23 | 0.09 | 5.95 | 6.70 |
| | | | | | |

* = 1% Skimmed milk amended polluted pond water solution,

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

## DISCUSSION

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 presence of nitrifying bacteria. Additionally selected isolates of *Nitrosomonas* and *Nitrobacter* were multiplied for their exploitation as bio-remediator in simulated aquaculture systems. *Nitrosomonas* and *Nitrobacter* strains were also isolated from drained fish ponds by [13]. They expressed similar views and reported that the soil of Chhattisgarh plains was exposed to an arid and hot climate during summer resulting in drastic loss in the population of mesophilic microbes, including nitrifying bacteria:

Further, a similar view was also given by [6]. They suggested that $10^{-3}$ dilution can be considered as appropriate diluation for isolation of nitrifying bacteria from drained fish pond soil. Nitrification has been reported to get inhibited in polluted waters [11]. *Nitrosomonas* and *Nitrobacter* genera were also isolated from polluted pond water by [13 and 19] and from activated sludge by [11], (2001) and [20], biofilm in the aquarium by [21], garden soil by [10], and from pond mud by [12]. Selection of effective nitrifying bacterial isolates was made on the basis of the rate of colour change due to thenitrifying process [10]. Selected isolates of *Nitrosomonas* and *Nitrobacter* were multiplied for their exploitation as bioremediator in aquaculture systems. For fast multiplication purposes, temperature and pH were maintained at 30°C and 8, respectively, throughout the 4 weeks of incubation period as described by [11].

Further selected heterotrophic bacterial isolates were multiplied for their testing as bioremediators in a simulated aquaculture system. *Ochrobactrum pituitosum* and *Micrococcus luteus* strains were also isolated polluted from fish ponds water by [22]. *Ochrobactrum pituitosum* genera were also isolated from the industrial environment by [23]. The selection of effective denitrifying bacterial isolates was made on the basis of the rate of colour change due to denitrifying process [24].

The best result associated with the $T_4$ treatment as it showed a higher rate of nitrification, the increase in nitrate from 15 DAI (30.00ppm) to 30 DAI (44.2 ppm). Similarly, at 8-ppm concentration of organic N, when comparing between the $T_5$ with $T_6$ and $T_7$ treatment, got the treatment $T_7$ was also having the best result among them as it was showing the increase in the nitrate concentration from 15 DAI (59.17 ppm) to 30 DAI (72.90 ppm). In the next urea concentration level of 12-ppm, it had the most negligible effect upon increasing the nitrate concentration from 15 DAI (70.00 ppm) to 30 DAI (77.50 ppm). A similar finding was obtained by [15], who emphasized molasses pretreatment, which can significantly stimulate the process of bacterial attachment on the ceramic carrier and the degradation of ammonia in the submerged condition. The ammonia-nitrogen range was 0.02 – 0.07 mg $L^{-1}$, Nitrite-nitrogen range was between 0.20 – 0.43 mg $L^{-1}$ whereas in the Nitrate-Nitrogen 0.90 – 3.20 mg $L^{-1}$ and carbon source from the molasses was effective in reducing the concentration of ammonia when cultured with the biofloc technique [16] and also another finding was that the nitrate concentration was increasing under the similar in-vitro condition as reported by [17].

In the present investigation, the nitrate concentration was observed at 15 DAI and 30 DAI, we have used the 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 $T_3$ and $T_4$ with the treatment $T_2$ with respect to nitrate concentration we obtained the best result in $T_4$ treatment as it helps in increasing the level of nitrate concentration in the treatments from 15 DAI (28.8) to 30 DAI (42.9 ppm) and under the 8-ppm concentration when comparing $T_5$ with $T_6$ and $T_7$ treatment we get that the treatment $T_7$ was also having the best result among them as it was shown an increase in the nitrate concentration from 15 DAI (57 ppm) to 30 DAI (70.07 ppm). In the next urea level of 12-ppm, it had the most negligible effect upon increasing the nitrate concentration from 15 DAI (70.0 ppm) to 30 DAI (71.1 ppm). A similar finding has been reported as the acceptable range of nitrate was



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(0 - 100 ppm), and the desirable range was (0.1 - 4.5 ppm) and under stress conditions, the concentration ranges >100, <0.01 as reported by [25]. The result was also similar to the finding of [14], were observed maximum nitrate concentration in the sample (Moti talab, Rajnandgaon).

## CONCLUSION

Upon collecting the water and soil samples from different 25 different ponds of different village/n. nigam/ward the best performance of *Nitrosomonas* (soil-NS8S and water-NS8W) and *Nitrobacter* (soil-NB8S and water-NB8W) were 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 have 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] Avnimelech, Y. 2009. Biofloc Technology: *A Practical Guide Book*. World Aquaculture Society, Baton Rouge, Louisiana, ISBN: 9781888807165, Pages: 182.

[3] Crab, R., Defoirdt, T., Bossier, P. and Verstraete, W. 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture*. 351: 356–357.

[4] Hemmerly, T.E. 1983. Traditional method of making sorghum molasses. *Econ Bot*. 37: 406–409.

[5] Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Adhikary, R.K., Rahman, S.M.S., Azim, M.E., Verreth, J.A.J. 2010. Effects of carbohydrate source for maintaining a high C:N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems. *Aquaculture*. 37: 46–301.

[6] 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.

[7] 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.

[8] Joel, O.F., 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.

[9] Ogbonna, J., 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.

[10] James, G.C. and Natalie, S. 1992. A laboratory manual of general microbiology. Benjamin/Cumming Publishing Company, Inc.California, 492p.

[11] Grunditz, C. and Dalhammar, G. 2001. Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. *Water Research*. 35 (2): 433-440.

[12] Li, L., Weinberg, C.R., Darden, T.A., and Pedersen, L.G. 2001. Gene selection for sample classification based on gene expression data: study of sensitivity to choice of parameters of the GA/KNN method. *Bioinformatics*. 17(12): 1131-1142.

[13] 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.

[14] Mahobe, H., and Mishra, P. 2013. Study of Physico-Chemical Characteristics of Water Ponds of Rajnandgaon Town, Chhattisgarh. *International Journal of Scientific & Engineering Research*. 4(8), 738-748.

[15] Muter, O. Mihailova, A., Berzins, A., Shvirksts, K., Patmalnieks, A., Strikauska, S., and Grube, M. 2014. Optimization of nitrification process by a bacterial consortium in the submerged biofiltration system with ceramic bead carrier. *J MicrobBiochem Technol*, 6: 148-53.

[16] Putra, I., Effendi, I., Lukistyowati, I., Tang, U. M., Fauzi, M., Suharman, I., and Muchlisin, Z. A. 2020. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia Oreochromis niloticus system. *F1000 Research*, 9.

[17] Yadav, A., Sahu, P. K., Chakradhari, S., Rajhans, K. P., Ramteke, S., Dahariya, N. S., and Patel, K. S. 2016. Urban pond water contamination in India. *Journal of Environmental Protection*. 7(01), 52.


http://dx.doi.org/10.53709/CHE.2021.v02i01.003

[18] Phirke, N. V. 2014. Characterization of enriched cultures of nitrifying bacteria from black cotton soil of Purna basin. *International Journal of Advances in Pharmacy, Biology* and *Chemistry*. 3(4): 869-866.

[19] Watson, S.W., Bock, E., Harms H., Koops, H.P. and Hooper, A.B. 1989. *Family Nitrobacteraceae, In Bergey's Manual of Systematic Microbiology*, (3). *pp*. 1808-1834.

[20] Du, G., Geng, J., Chen, J. and Lun, S. 2003. Mixed culture of nitrifying bacteria and denitrifying bacteria for simultaneous nitrification and denitrification. *World journal of microbiology* and *biochemistry*. 19:433-437.

[21] Bower, C.E. and Turner, D.T. 1981. Nitrification in closed sea water aquariums: Effects of simulated power failures. *J. Aquaricult*. 2(1): 1-3.

[22] Huber, B., Scholz, H. C., Kämpfer, P., Falsen, E., Langer, S., and Busse, H. J. 2010. Ochrobactrum pituitosum sp. nov., isolated from an industrial environment. *International journal of systematic and evolutionary microbiology*. 60(2): 321-326.

[23] Scholz, H.C., Huber, B., Kämpfer, P., Falsen, E., Langer, S., and Busse, H. J. 2010. *Ochrobactrum pituitosum* sp. nov., isolated from an industrial environment. *International journal of systematic and evolutionary microbiology*. 60(2), 321-326.

[24] 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.

[25] Shrivastava, S.K., Vajpai, K., Shrivastava, D.K. and Vajpai, S.K. 2007. Study of physicochemical quality of pond water in Bilaspur, Chhattisgarh. *Current World Environment*. 3(1): 97.

RESEARCH ARTICLE

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

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## 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