International Journal of Biotech Trends and Technology
ISSN: 2249 – 0183 /doi:10.14445/22490183/IJBTT-V10I4P603

Volume 10 Issue 4, 20-29, Oct-Dec 2020
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Bioremediation: Assessment of Microbial Strain and Cost Estimations for the Rejuvenation of Tilyar Lake, India

Neeraj Gahlawat[1], Sanjay Kumar Koli[2], Uma Shanker[*3]

[1]*Project Officer, Technical & Spokesman, National Mission for Clean Ganga, Ministry of Water Resources, River Development & Ganga Rejuvenation, New Delhi-110001, India*
[2]*Trainee Engineer, Air Lab, Delhi Pollution Control Committee, New Delhi-110006, India*
[*3]*Corresponding author – Trainee Engineer, Delhi Pollution Control Committee, Government of NCT of Delhi, New Delhi-110006, India*

Abstract *- The quality/portability of water that is consumed defines the baseline of protection against many diseases and infections. The present study aimed to calculate the monthly variation of physicochemical parameters of Tilyar Lake and the most suitable microbial strain for the survival of aquatic life. Water samples were collected from each sampling station and analyzed for estimation of pH, Electrical Conductivity (EC), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Dissolved Oxygen (DO), Phosphate (PO_4^{3-}), Nitrate (NO_3^-). During the study 12 different microbial strains have been selected from which Pseudomonas aeruginosa strain was found to be the most effective and consumes less dissolved oxygen as compared to 11 different microbial strains. Pseudomonas aeruginosa can work at a wide range of temperatures and consumption of DO is least as compared to the other 11 microbes during the experiments, the total of 138 liters of Pseudomonas aeruginosa sample needs to be added at a rate of 35.71µl/l (V/V).*

Keywords - *Microbial Strains, Bioremediation, Biotechnology, Water Treatment, Lake Rejuvenation.*

I. INTRODUCTION

About 97% of the earth's surface in the oceans and seas and about 2% of water is locked in the polar ice caps and glaciers, while only 1% is available as fresh water in the rivers, lakes, streams, and groundwaters for human consumption. The quality of this 1% of water is deteriorating due to heavy pollution stresses from suppressing anthropogenic activities rapidly. Several degraded water bodies pose serious water scarcity problems (Sharma 2015).

Bioremediation is relatively a new technology that uses microbes to degrade organic matter (Sumit Pal and Vimala Y, 2012) and has undergone more intense investigation in recent decades due to contamination and release of toxic compounds into the water bodies from rapid industrialization and urbanization (Adnan et al. 2013). Biological treatment methods using bacterial and fungal decolorization, adsorption by bacterial and fungal biomass (Ramachandran et al.,2013). One important property of bioremediation is that it is carried out in a non-sterile environment that comprises a lot of microorganisms (Ahmed et al., 2015). Microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy (Ijah and Antai, 1988; Ezeji et al., 2005; Antai and Mgbomo) Bioremediation is a pollution treatment expertise that uses biological systems to catalyze the destruction such as pesticides (Naphade et. al,2013) or transformation of various chemicals, to less harmful forms thus organic wastes are organically degraded under controlled conditions to levels (NITHYA SREENIVASAN, PRAVEEN KUMAR G, SUNEETHA V, 2015) below concentration limits established by regulatory authorities (McCutcheon, Medina, and Larson 2004). Bioremediation is a technology that utilizes the metabolic potential of living organisms (yeast, fungi, or bacteria) to clean up contaminated environments by degrading the hazardous substances into less toxic, yeast and fungi release high-affinity iron-binding compounds that chelate iron. The Fe^{3+} chelates are formed outside the cell walls and are taken up by the cells. In Saccharomyces cerevisiae, elimination of metals is done by their precipitation as sulfides e.g. Cu^{2+} is precipitated as CuS. The best-known example of microbial metal metabolism is the mining microbe Thiobacillus ferrooxidans. These bacteria and other allied species derive energy by using metallic sulphides.

Staphylococcus, Bacillus, Pseudomonas, Citrobacteia, Klebsilla, and Rhodococcus are organisms that are commonly used in bioremediation mechanisms (Connors and Barnsley 1982). These mechanisms include bioaugmentation, in which microbes and nutrients are added to the contaminated site, and biostimulation, in which nutrients and enzymes are added to supplement the intrinsic microbes of the site. It was concluded that it is obligate aerobe (Pseudomonas species) and ability to grow on different substrates; the minimum nutrient concentration required for their growth is also very low, and also found that Pseudomonas aeruginosa can adapt well in different nutrient conditions (Fulekar and Geetha 2008). It is possible to extract the enzymes in the lab and

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they can be used on the contaminated sites to speed up the biodegradation process. Similarly, Aspergillus niger secretes enzymes which can absorb Zinc and Copper ions (Price, Classen, and Payne 2001). Most of the biological methods are used in treating wastewater and oil spills. It was found that bioremediation can be applied to eat up the oil up to 70% in just 3 weeks (Hozumi, Tsutsumi, and Kono 2000). Using scale-up techniques, Bacillus special, Pseudomonas special, and Streptomyces sp. can be used in bioremediation of pesticide cypermethrin up to a concentration of 100 mg/L (Boricha and Fulekar 2009). Bacillus sp. can decolorize the distillery effluent up to 22% where Pseudomonas aeruginosa can remove the color up to 67%. 1% glucose as carbon and energy source is to be added in case of Bacillus sp. (Mohana, Desai, and Madamwar 2007)(Shah Maulin P,2017).

II. OBJECTIVE AND NOVELTY

The objective of this study is to determine the physicochemical parameters of Tilyar Lake and the microbial strain which is paramount suitable for degradation of organic matter at a wide range of temperature and consumes less dissolved oxygen for the further natural treatment of the lake water.

III. MATERIAL AND METHODS

A. Study Area

The Tilyar Lake has been selected for the study located at geographical coordinates (28°52′53 N, 76°38′12″ E) in Rohtak district of Haryana (India) as shown in fig 1. and situated 5 km from Rohtak city (Singh and Laura 2012). Lake Complex is spread in 132 acres of area (Tyor and Tanwar, 2013). Tilyar Lake has stagnant and forms a flawless green belt in the surrounding area and is used for entertaining activities (Juneja and Choudhary, 2018). There is a tourist resort called the Tilyar Resort near the lake that has a restaurant, a zoo, shops, a bar, and a children's park. Tilyar Lake has been described in table 1.

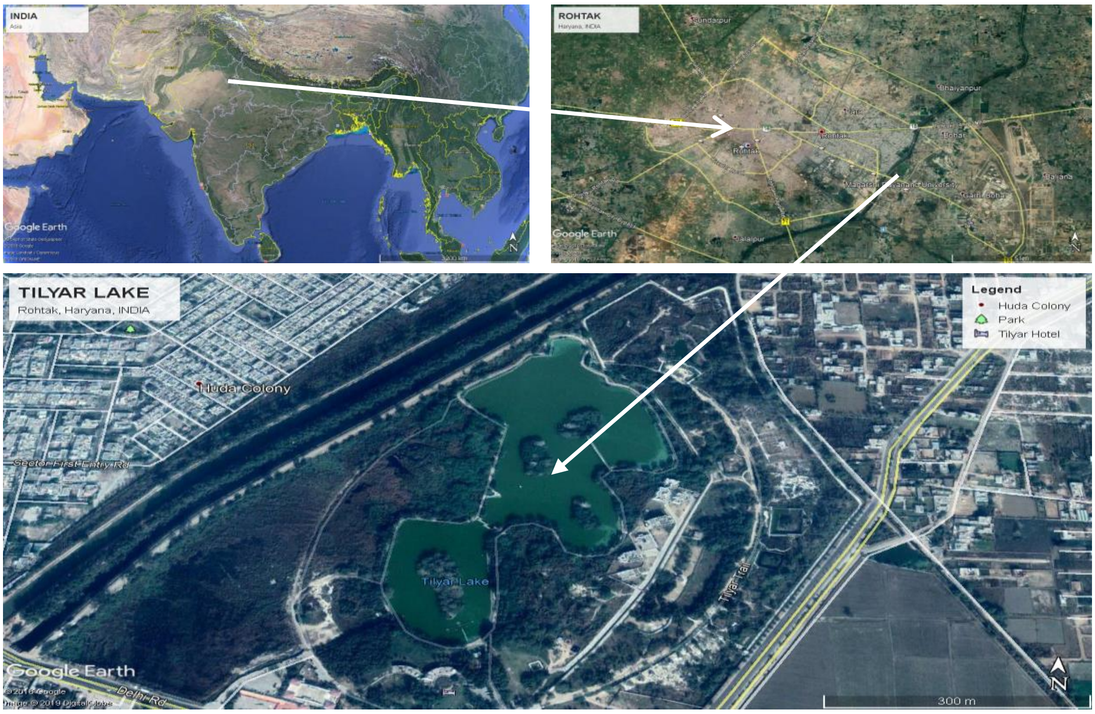

Fig 1: Geographical Mapping of Tilyar Lake.

TABLE I
Information about Tilyar Lake (Format for details is as per NLCP Guidelines, 2008)

General		
1	Name of the water body	Tilyar Lake
2	Location (Describe; Name, nearest Town, District & State)	Tilyar Lake, Rohtak, Haryana
3	Geographical co-ordinates (latitude/longitude)	Latitude 28°52′53 N Longitude 76°38′12″ E
4	Lake type	Artificial
5	Area, acres (Full water level)	22.1 acres
6	Mean depth, m (Full water level)	3.01 m
Hydrology		
1	Source of water (inflow)	Rainfall Canal (Bhalot minor)
2	Outflow, if any (describe)	Two pumps were installed in September 2012 of 1 cusec

		capacity each.
3	Water level changes (annual), meters	Not Available
4	Does the lake dry out completely?	Rarely
5	Has sewer been provided	No sewer is discharged to the lake.
6	Sewage treatment	Not Available
7	Solid waste disposal in the lake (if any)	Visitors throw solid waste in the lake.
8	Describe any prominent/special features	The lake serves tourism purposes besides conserving the aquatic rich biodiversity.
Water quality and pollution		
1	Sources	Silt in inlet water Other (solid waste).
2	Level	Inlet water is very turbid especially during the monsoon period.
3	Trophic status	Appears to be oligotrophic, but more data is required.
Bio-Diversity		
1	Aquatic plants	Submerged. Emergent. Free-floating.
2	Aquatic Animals	Zooplankton. Algae. Fish.

		Reptiles.
3	Name important/ rare/ endemic/ exotic species	34 species of birds out of which 5 are migratory birds.
Functions and values		
1	Water used for	Conservation of biodiversity. Recreation (tourism).
2	Use of biological resources	Fish
3	Functions of lake	Groundwater recharge. Tourism: Local & National. Supports Biodiversity.
Major problems		
1	Observed Problems	Reduction in-depth (siltation) Pollution of lake water by tourists Aquatic Weeds Excessive load on agricultural water.

B. Sampling Collection and Analysis of Water

Five sampling sites within the lake were selected for water sampling. All the samples were brought in duplicate to minimize the analysis errors. The adopted five sampling sites within the lake are shown in fig 2 and the water samples were analyzed for BOD, COD, DO, pH, Conductivity, TDS, Phosphate, Nitrate, and Turbidity has been discussed in table 2.

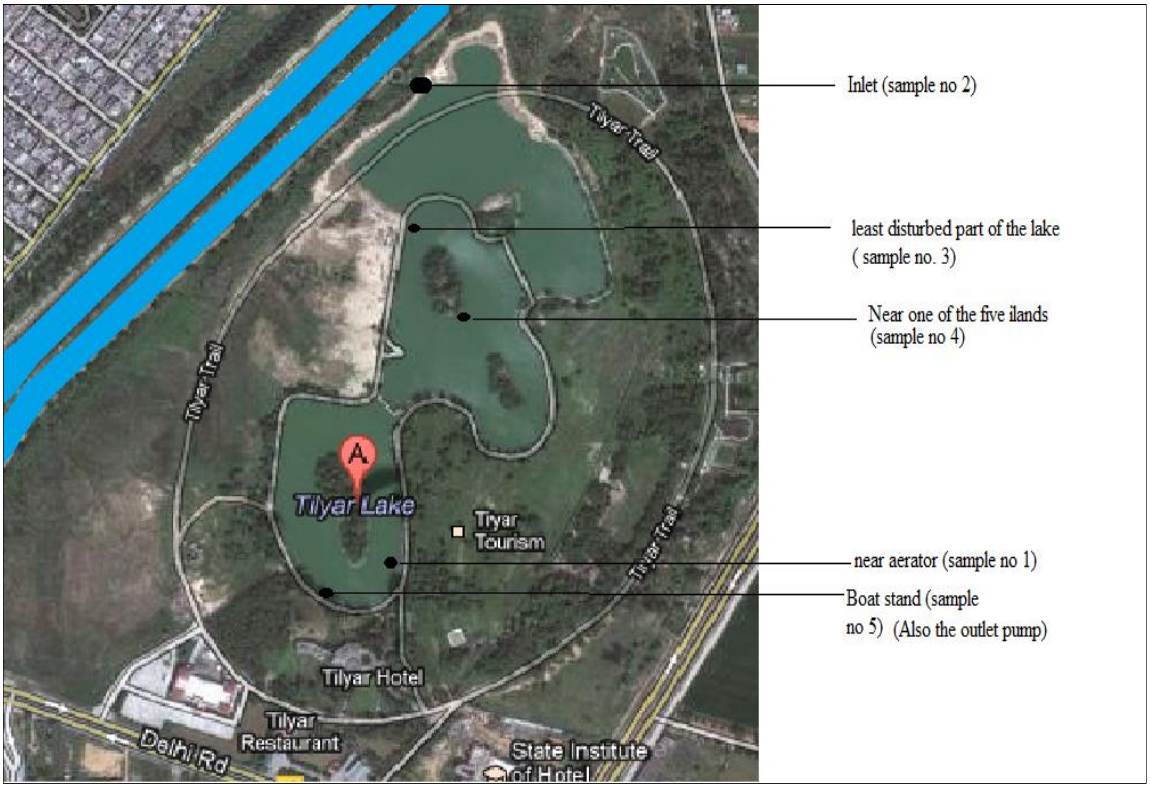

Fig 2: Sampling locations

IV. RESULT AND DISCUSSION

A. Physicochemical Parameters

a) pH

It is one of the most important water quality parameters due to the effect on the performance of treatment units and supply lines. It plays an important role in clarification and disinfection. BIS has a prescribed permissible limit of 6.5-8.5. The pH value of river samples in the study area was in the range of 8.1 to 8.8.

b) Electrical Conductivity (µS/cm)

Conductivity is a measurement of the ability of an aqueous solution to carry an electrical current. Conductivity in water is affected by the presence of dissolved ions such as sodium, potassium, calcium, magnesium, iron, chloride, nitrate, sulfate, phosphate, etc. Organic compounds do not conduct electric current very well and hence their contribution to conductivity is very low. Conductivity is a useful parameter to establish water quality. Significant changes in conductivity could then be an indicator that a discharge or some other source of pollution has entered the water resource. The conductivity of collected samples varies between 198.3µS/cm to 204.4µS/cm.

c) Total Dissolved Solids (mg/L)

Total dissolved solids (TDS) are the term used to describe the inorganic salts and small amounts of organic matter present in dissolved form. The presence of dissolved solids in water may affect its taste. BIS has prescribed 500 mg/L as the acceptable limit and 2000 mg/L as the permissible limit for TDS in absence of an alternate source of drinking water. The guideline is not health-based but based on palatability. TDS in the river varies from 94.3 mg/L to 126.9 mg/L. The monthly variation of pH, EC, TDS, and turbidity are shown in figure 3.

d) Turbidity

Turbidity may be caused by inorganic or organic constituents. Turbidity in the analyzed river samples of the study area varies from 25.7 NTU (Nephelometric Turbidity Unit) to 32.7 NTU.

e) Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Dissolved Oxygen (DO)

COD is considered an important water quality parameter for representing the degrees of organic pollution and is strictly controlled by environmental regulatory agencies (Li et al. 2018). The COD can be a maximum of 2.5 times the BOD value but during the study, high COD values have been observed. This suggests that industrial effluent is being added to the Bhalot minor's water in the upstream stretch. The higher BOD values also suggest the invasion of domestic sewage. As the solubility of gases increases in the cold season and as the temperature increases the solubility of gases decreases in the lake. The DO concentration increased in November and decreases as temperature increases. The DO values must not be less than 4 mg/L for the survival of aquatic organisms including fishes but during the study period, the DO value reaches 2.6 mg/L.

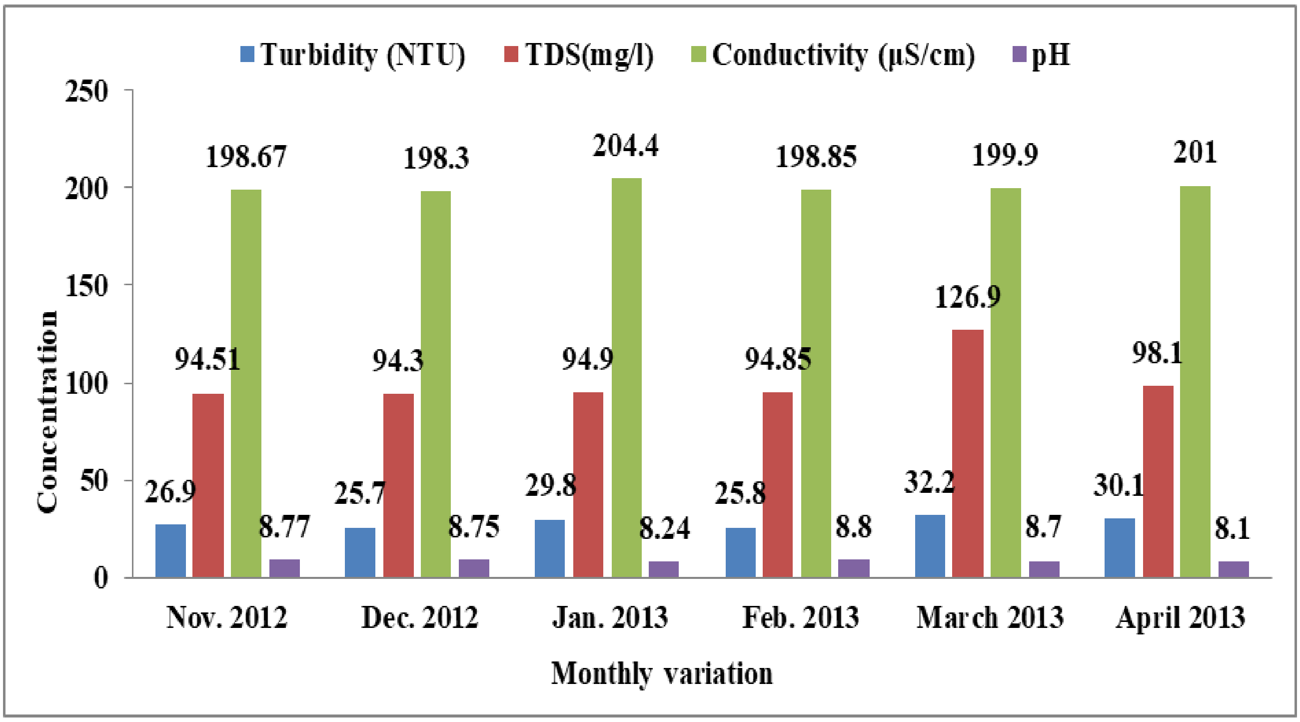

Figure 3 Monthly variation of Turbidity (NTU), Total Dissolved Solids (TDS), Conductivity (µS/cm) and pH.

TABLE II
Observed variations in physicochemical parameters of Tilyar Lake

S.No.	Parameters	Nov 2012	Dec 2012	Jan 2013
1.	DO(mg/L)	8.8	2.6	3.2
2.	BOD (mg/L)	15.2	20.8	18.1
3.	COD (mg/L)	172.3	180.5	184
4.	pH	8.77	8.75	8.24
5.	Conductivity (µS/cm)	198.7	198.3	204.4
6.	TDS(mg/L)	94.51	94.3	94.9
7.	Nitrate(mg/L)	1.58	1.75	1.95
8.	Phosphate(mg/L)	1.94	1.65	2.06
9.	Turbidity (NTU)	26.9	25.7	29.8
S.No.	**Parameters**	**Feb 2013**	**March 2013**	**April 2013**
1.	DO(mg/L)	2.6	2.9	3.1
2.	BOD (mg/L)	22.4	24.5	19.2
3.	COD (mg/L)	179	191.3	171
4.	pH	8.8	8.7	8.1
5.	Conductivity (µS/cm)	198.85	199.9	201
6.	TDS(mg/L)	94.85	126.9	98.1
7.	Nitrate(mg/L)	1.35	2.0	1.62
8.	Phosphate(mg/L)	1.89	1.71	1.93
9.	Turbidity (NTU)	25.8	32.2	30.1

f) Nitrate (mg/L) and Phosphate (mg/L)

Nitrate (NO_3) is found naturally in the environment and is an important plant nutrient. Nitrate can reach both surface water and groundwater as a consequence of agricultural activity, from wastewater disposal. The presence of nitrate in drinking water is a potential health hazard when present in large quantities. Nitrites are formed by the reduction of nitrate in the human body, which combines with hemoglobin in the blood to forms methemoglobin that leads to methemoglobinemia (blue baby syndrome) in infants.

The combination of nitrates with amines, amides, or other nitrogenous compounds through the action of bacteria in the digestive tract results in the formation of potentially carcinogenic nitrosamines. According to the Indian Standard for drinking water, the maximum allowable nitrate concentration in drinking water is 45 mg/L as NO_3. The minimum and maximum values of the nitrate and phosphate in the lake samples vary from 1.35 to 2 mg/L and 1.65 mg/L to 2.06 mg/L as described in figure 4.

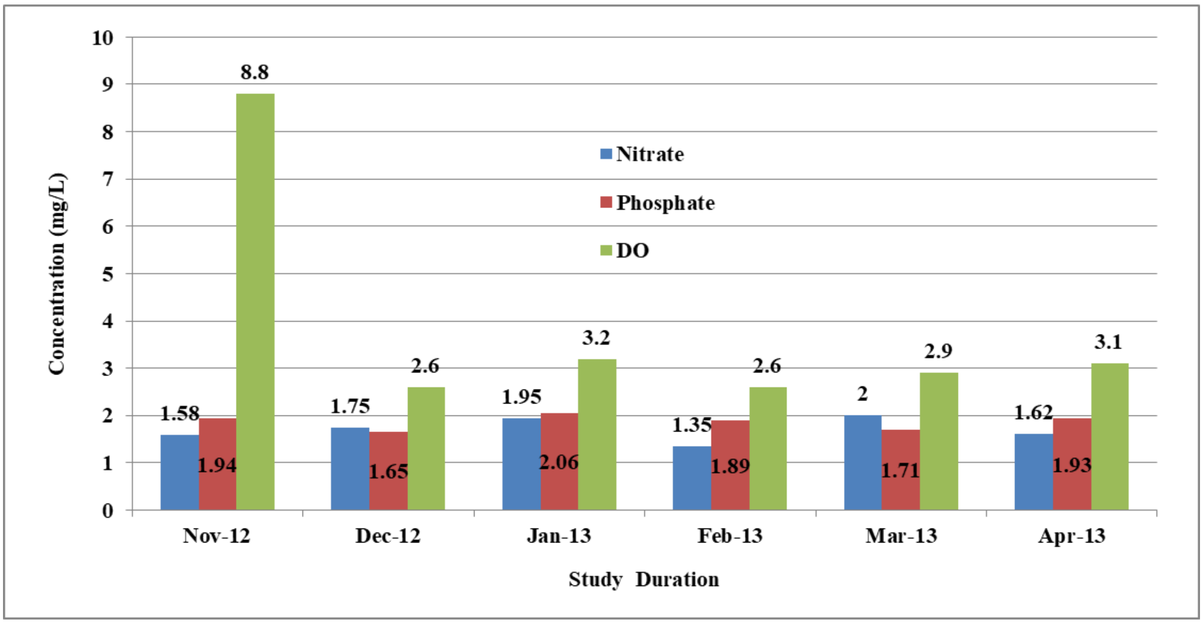

Fig 4: Monthly variation of Dissolved oxygen (DO), nitrate (mg/L) and phosphate (mg/L).

B. Effect of various microbial strains on DO and BOD

The DO (dissolved oxygen) of water decreases because of the degradation of biological and chemical matter. In our experiments, the organic matter was targeted. If we add such microbes to water that are capable of consuming the organic matter at a faster rate, the decrease in DO will be less. Twelve (12) Microbial species are first selected based on a literature survey and availability. These microbial strains were Lactococcus sp., Protius valgarus, Staphylococcus sp., Nocardia sp, Faecalis alcoligenes, Pseudomonas aeruginosa, Cunninghanella elegance, Candida tropitalis, Aspergillus niger, Aspergillus brasillinesis, Streptomyces kanamyecticus, and Bacillus subtilis.

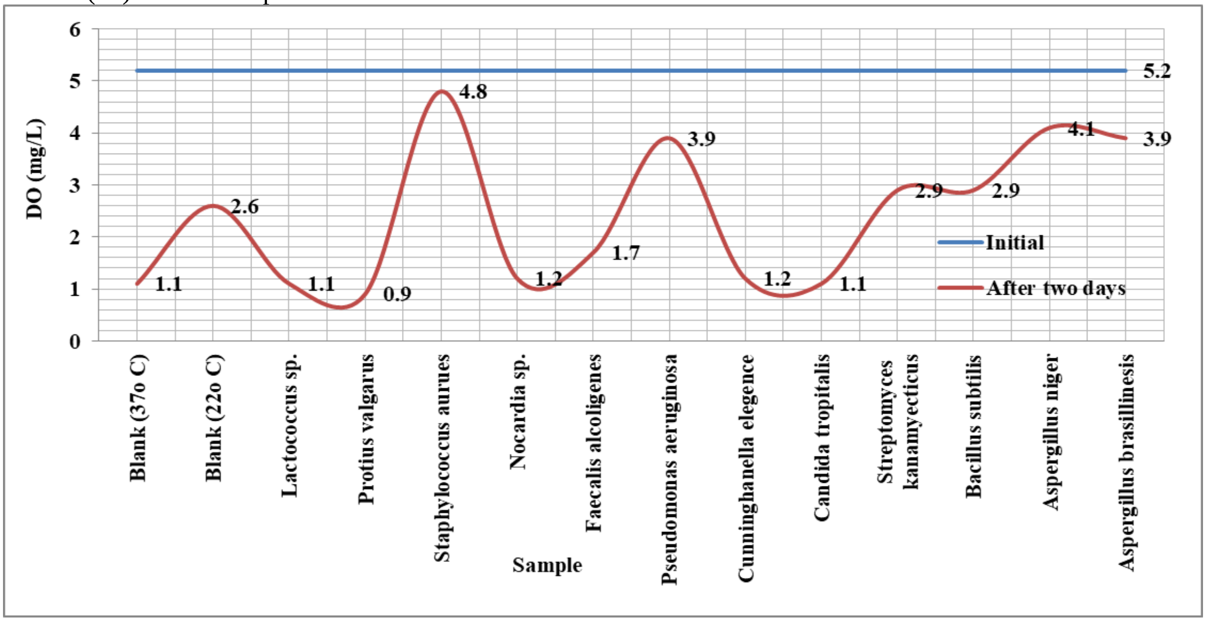

Fig 5: DO consume by the Microbial Strains after two days

From fig.5, the four bacterial and two fungal strains were found to be less DO demand. Staphylococcus aurues, Pseudomonas aeruginosa, Streptomyces kanamyecticus, Bacillus subtilis, Aspergillus niger, and Aspergillus brasillinesis. A fall of oxygen below 4.8 mg/L, the chronic criterion can cause hypoxia/anoxia conditions and other adverse effects on the health of the aquatic system, resulting in declination in the inhabitants of important aquatic animals. These have some significant detrimental effects on ecological health, economic health, and stability of aquatic systems of the water body (Prasad et al. 2014). The temperature at the located site varies from 5º – 45º C, in a different season of the year and this may affect the activities of the microbes in the lake. The persistence of the microbes and the composition of the hydrocarbons also depends on the temperature of the environment which can inhibit the growth and activity of the microbes (Das and Chandran 2010).

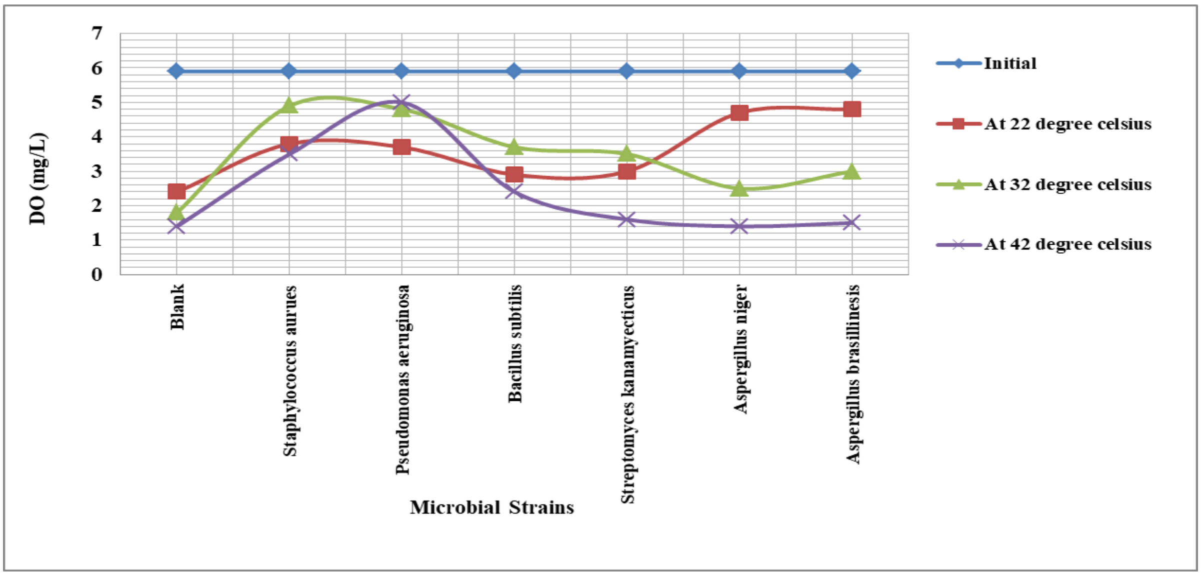

Fig 6: Effect of microbial strains on DO at different temperatures.

Results have been shown in fig. 6 and DO consume by the microbes after two days of incubation, where, Pseudomonas aeruginosa is found to be highly efficient at the higher temperature (32 – 42º C), Aspergillus niger and Aspergillus brasillinesis became inactive at that temperature but most efficient at low temperature (22º C). The activity of the psychrotrophic Pseudomonas putida, supposed to be appreciable for the treatment of wastewater at the temperature of (10-25°C) (Onysko, Budman, and Robinson 2000)(Margesin 2000). At the region of sub-zero temperature, the transport channel of the microbial cell may closer the entire cytoplasm and cause, most oleophilic microbes to metabolically inactive (Macaulay 2015)(YANG et al. 2009). The variations in the degradation rate due to the effect of temperature on the enzymatic activity also known as the Q10 effect (Margesin 2000).

The reduction in the BOD and COD majorly depends on the concentration of organic matter contained in the waste or wastewater (Tchobanoglous, Burton, and Stensel 2003). Some microorganism depends on the type of nutrients and others are live on the ability to compete for the nutrients (Oljira, Muleta, and Jida 2018). An experimental study has been carried out to chequer the efficiency of the determined microbial strains at an optimum temperature of each microbial strain has been summarized in fig. 6 and the DO drop after the two days of incubation period along with temperature variation has been described in fig. 7.

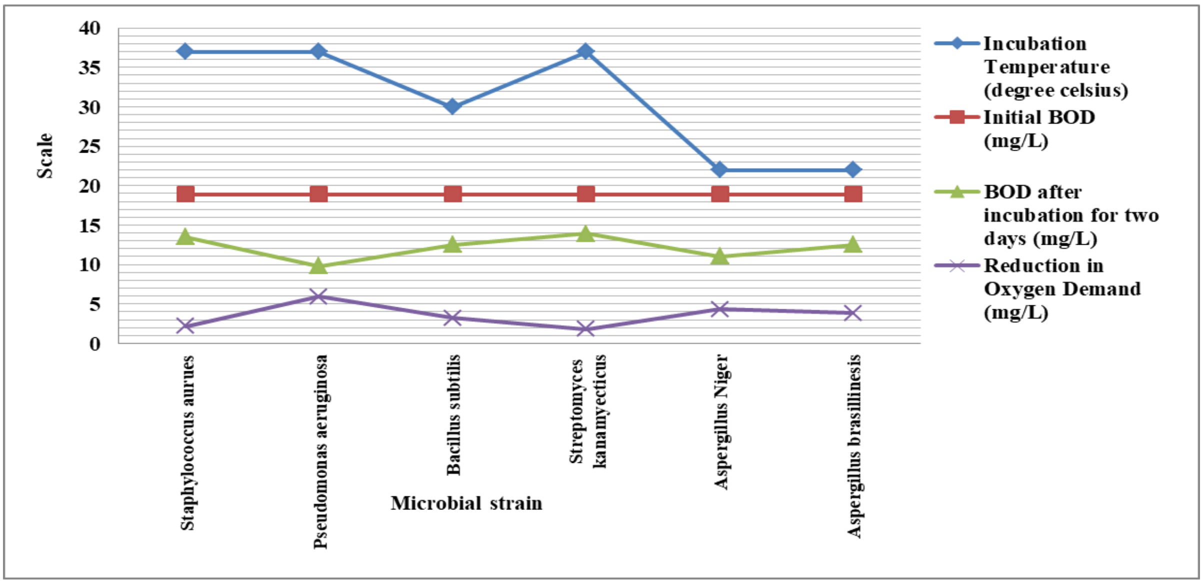

Fig 7: Efficiency of the microbial strains at their optimum temperature

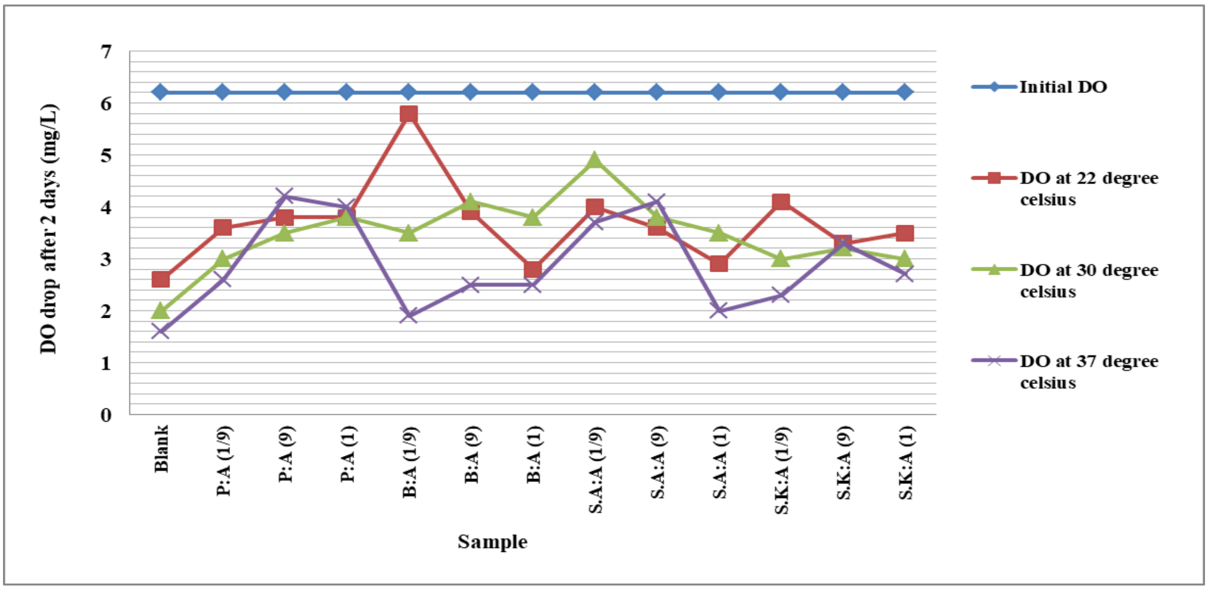

Fig 8: Efficiency of the microbial strains (Pseudomonas aeruginosa (P), Aspergillus brasillinesis (A), Bacillus subtilis (B), Staphylococcus aurues (S) in the mixture at different ratio

An attempt, to check the compatibility of two or more strains has been made and founded that the selected strains do not have any special effect on DO when the different amounts of two strains were mixed and then added to water samples. As the fungal species have a much lower optimum growth temperature as compared to the bacterial strains, the Aspergillus niger strain mixed with the other bacterial strains in different concentrations and founded that all the strains performed in a better, individually, and Aspergillus brasillinesis strain when mixed with the bacterial strains, the results were battered in case of individual strains.

a) Optimization of the Concentration of Inoculum

To optimize the quantity of the bacterial strain best suited for the bioremediation, water samples were incubated with different amounts of bacterial strains, the BOD of water samples observed to be reducing with time in figure 8.

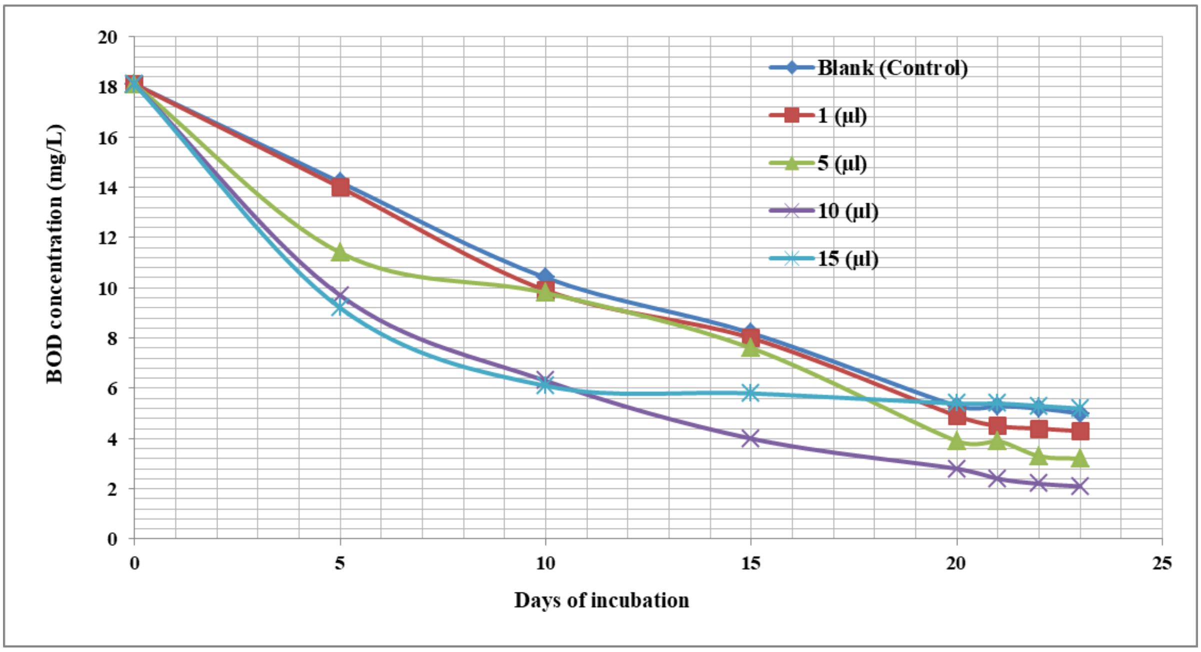

Fig 9: Shows the BOD variation using Pseudomonas Aeruginosa in different volumes of inoculum.

From fig. 9, It has been founded that, 10 µl of inoculum gives the best results in BOD reduction. The BOD value reduced from 18.1 to 2.1 mg/Lin only 23 days, the reduction in the blank sample was 72.38%, whereas, in the case of 10 µl inoculum, it reduces to 88.89%.

b) Dosage Required

An experiment has been done for bioremediation of the selected site and founded that the visible colony having 10^6 cells. For the concentration of 10^5 cells/ml, 10^6 cells are mixed well using the vortex mixer, in 10 ml of sterile media and incubated the samples with 10 µl sample, for having the concentration of 100 colony forming units (cfu's) per 281 ml of water samples. Considering the calculated concentration as 10 µl sample/281 ml, the sample required for the bioremediation is, 35.71µl/L. The founded volume of the lake was 291MCM. Spread plate technique used to analyze the Most Probable Number (MPN). Five dilutions of water samples were checked after incubation of 20 days and 23 days. Initially, the bacterial samples were cultivated on Nutrient Agar (N.A.) medium, where the selective media was used for the other microbial strains present in the lake water. Cetrimide Agar is used to isolating the Pseudomonas aeruginosa strain. It was found that 112 cfu's after 20 days, 108 cfu's after 24 days and 96 cfu's on the 30^{th} day of incubation per 281 ml were present in the water samples. From the adopted study, it has been found that the lake water has the BOD of 18.1mg/L, can be treated by adding 138 liters of microbial sample to the lake at a concentration of 35µl/L.

V. COST ESTIMATES

The cost of a conservation plan for Bioremediation has been estimated and is given along with O&M cost for three years. The cost estimates are divided into two categories as one-time cost and Reoccurring cost. One time cost includes the cost to be incurred in Biological components, hiring the experts for one time, and the beautification of the lake. Lake Beautification includes shoreline development, boundaries, gate, parking facilities, etc. Also, the rates of media are taken care of as per the quotation received from the supplier. The following table 3 shows the one-time cost required for the conservation plan:

TABLE III
One-time, required cost estimation

S.No.	Component	Rates
1.	Nutrient agar	Rs. 3704 per kg
2.	Cetrimide agar	Rs. 4358 per kg
3.	Revival and maintenance of	Lump-sum

	microbes	
4.	Hiring experts	30,000 per person
5.	Lake beautification	Lump-sum
6.	Public participation	5% of the project cost
S.No.	**Component**	**Quantity**
1.	Nutrient agar	138 kg
2.	Cetrimide agar	15 kg
3.	Revival and maintenance of microbes	-------
4.	Hiring experts	2 experts
5.	Lake beautification	-------
6.	Public participation	--------
S.No.	**Component**	**Total cost (in lakh Rs.)**
1.	Nutrient agar	5.11
2.	Cetrimide agar	0.65
3.	Revival and maintenance of microbes	5.00
4.	Hiring experts	0.60
5.	Lake beautification	20.00
6.	Public participation	1.56
7.	**Total cost**	**32.92**

The re-occurring cost includes the cost of water quality monitoring and the wages of the hired experts permanently. The cost of analyzing three samples per month is calculated and standard rates given by CPCB are taken as reference. The parameters selected for analysis are BOD, COD, DO, Phosphates, Nitrates, and Biological quantitative analysis of *Pseudomonas aeruginosa*. The salaries of the experts are decided by taking the water quality staff on a part-time basis. The re-occurring cost is summarized below in table 4:

TABLE IV
Estimation of re-occurring cost

S.No.	**Component**	**Rates**
1.	Water samples analysis	2050
2.	Salary of the nodal officer	35,000
3.	Salary of clerk	15,000
4.	Salary of water quality monitoring staff	15,000
S.No.	**Component**	**Quantity**
1.	Water samples analysis	3
2.	Salary of the nodal officer	1
3.	Salary of clerk	1
4.	Salary of water quality monitoring staff	1
S.No.	**Component**	**Total monthly cost (in Rs.)**
1.	Water samples analysis	6150
2.	Salary of the nodal officer	35,000
3.	Salary of clerk	15,000
4.	Salary of water quality monitoring staff	15,000
5.	**Total**	**71,150**

The total capital cost of the project is estimated at 32.92 lakhs and a reoccurring cost of Rs. 71 thousand will incur. For the sustainability of the project, the revenue generation possibilities need to be explored.

VI. CONCLUSION

Bioremediation was found to be an effective method for the conservation of lakes related to their self-cleansing property. In the proposed study out of the 12 strains, Pseudomonas aeruginosa strain found to be most effective as it can speed up the natural biodegradation process of organic matter at a faster, and can work efficiently at a wide range of temperature and consumption of DO is least as compared to the other 11 microbes during the experiments. The self-cleaning property of the lake can be maintained by adding the number of microbes related to the volume of the lake, by keeping the concentration as calculated. As per standards of best-designated usage of water, values of BOD, COD, and DO are much higher in the lake and are not suitable for recreational activities. The addition of sewage and industrial effluent is suspected in the upstream stretch of the Lake from Bhalot Minor's water.

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