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COMPARATIVE STUDY ON PHOSPHATE REMOVAL IN BHAVANI RIVER WATER BY SELECTED BACTERIAL ISOLATES

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Abstract—The water available for daily consumption may be contaminated by natural sources or industrial effluents. Nowadays, eutrophication is having more influence over drinking water sources like lake, river, streams etc., it is mainly due to the discharge of domestic sewage directly into the water bodies without any treatment and awareness. Domestic sewage is rich in nutrients like nitrate and phosphate. The permissible limit value of phosphate is 0.02mg/l. If this value is exceeded beyond permissible limit, it may lead to diseases like blue baby syndrome and has adverse effect on surrounding environment. For treating water, biological method is more advantageous than conventional methods. In this study, the characterization of river water and bacteria species is to be studied. The present study showed that pseudomona, aeruginosa, staphylococcusaureus, kleibsellaspcies bacterium to remove the phosphate effectively. The removal efficiency of phosphate is 70% and the contact time is 10 days.

Key words:bacterialspecies,water, Phosphate removal efficiency.

1. INTRODUCTION

Phosphorus in water contributes to health and environmental threats as they are linked tllnesse as well as ecosystem disruption via algal blooms in contaminated water bodies. Based on above perspectives a comparative study was conducted on local freshwater bacteria pseudomonas aeruginosa, staphylococcus sp., klebsiellasp.,.Bacterial performance in removing phosphorus was evaluated by measuring phosphorus content of MWS incubated with the strains for 7 days. Instantaneous readings were taken every 48 hours to determine periodic levels of the nutrients phosphate. As such, phosphorus in water have been recognized as priority targets for removal, with maximum contaminant levels (MCL) set in place to regulate water quality.

Phosphorus levels do not fluctuate as much, with this nutrient persisting from early on alongside other forms of phosphorous compounds in the water production line. The significant phosphorus content in water is largely blamed on detergent use, where predetergent days saw only 3 to 4 mg/L of phosphorus in treated water compared to the 10 to 20 mg/L concentrations reported more recently. In addition to detergents, phosphorous compounds are also used for corrosion control in water supply as well as industrial cooling systems.The aim of the present study is to determine the optimum water nutrient concentration for growth of bacterial sp. and to assess the removal efficiencies of phosphorus from surface water.

MATERIALS AND METHODS:

STUDY AREA

First of all water is collected from bhavani river near erode region which is tested for its basic parameters. That area is highly polluted with domestic sewage which is rich in nutrients, especially nitrate and phosphate. If the

nutrients exceed their permissible limits may cause ill effects for humans and also a source for algal blooms which will result in eutrophication. Because of this the oxygen will be depleted gradually, conversely ecosystem get disturbed.

LOCATION OF STUDY AREA

ALGAL BLOOMS IN BHAVANI

RIVER WATER

ANALYTICAL PROCEDURE

DETERMINATION OF P^H:

(1) p^H:

p^H can be viewed as an abbreviation for power of hydrogen or more completely, power of the concentration of hydrogen ion. It says that the p^H is equal to the negative log of the hydrogen ion concentration, or $p^H = -\log [H^+]$.

$p^H = -\log [H_3O^+]$.

After calibration with buffer solution, rinse the electrode with DW and wipe gently. Take the sample in a beaker. Bring the temperature of the sample to room temperature. Dip the electrode in the beaker in such a way that bulb of the electrode deep in to sample. Bring the temperature to homogeneity by stirring. Record the reading from display, which will give the p^H value of the sample.

TURBIDITY

In this process we are used the Jackson candle turbidity method to find the turbidity of the water sample. Turbidity, a measure of the light – transmitting properties of water, is another test used to indicate the quality of waste discharges and natural waters with respect to colloidal and residual suspended matter

The measurement of turbidity is based on comparison of the intensity of light scattered by a sample to the light scattered by a reference suspension under the same conditions

The results of turbidity measurements are reported as nephelometric turbidity units (NTU) Colloidal matter will scatter or absorb light and thus prevent its transmission.

It should be noted that the presence of air bubbles in the fluid will cause erroneous turbidity readings. In general, there is no relationship between turbidity and the concentration of total suspended solids in untreated waste water. There is, however, a reasonable relationship between turbidity and total suspended solids for the settled and filtered secondary effluent from the activated sludge process

TOTAL SUSPENDED SOLIDS

A filtered sample containing the dissolved solids is evaporated to dryness at 180°C. The residue is known as TDS.

The estimation is carried out with the filtrate collected after the filtration of the sample containing suspended solids. Take an evaporating dish cleaned with chromic acid and rinsed well with tap water, then with DDW. Dry it overnight in an oven set at 180°C.Cool the dish in a desiccating cabinet to room temperature. Weigh until a constant weight is achieved. In a pre-weighed evaporating dish, place 100ml filtrate collected in the estimation of TSS. Place it in an oven set at 180°C until all the filtrate evaporates, leaving behind the dissolved solids. Cool in a desiccating cabinet and weigh the dish. Repeat the steps of cooling and weighing until a constant weight is achieved. Record the final weight.

NITRATE

The reaction with the nitrate and brucine produces yellow colour that can be used for the colorimetric estimation of nitrate. The intensity of colour is measured at 410 nm. The method is recommended only for concentration of 0.1– 2.0 mg/L NO_3^- N.

All strong oxidising and reducing agent interfere. Sodium arsenite is used to eliminate interference by residual chlorine; sulphanilic acid eliminates the interferences byNO_2^- N and chloride interference is masked by addition of excess NaCl. High concentration of organic matter also may interfere in the determination. Nitrate standards are prepared in the range 0.1–1.0 mg/L diluting 1.00, 2.00, 4.00, 7.00 and 10.0 mL standard nitrate solution to 10 mL with distilled water.

If residual chlorine is present 1 drop of sodium arsenite solution is added for each 0.1 mg Cl_2 and mixed. Set up a series of reaction tubes in test tube stand. Add 10 mL sample or a portion diluted to 10 mL to the reaction tubes. Place the stand in a cool water bath and add 2 mL NaCl solution and mix well. Add 10 mL H_2SO_4 solution and again mix well and allow cooling. The stand is then placed in a cool water bath and add 0.5 ml brucine-sulphanilic acid reagent.

Swirl the tubes and mix well and place the tubes in boiling water bath at temperature 95°C. After 20 minutes, remove the samples and immerse in cool water bath. The sample are then poured into the dry tubes of spectrophotometer and read the standards and sample against the reagent blank at 410 nm. Prepare a standard curve for absorbance value of standards (minus the blank) against the concentration of NO_3^- N. Read the concentration of NO_3^-N in the sample from the known value of absorbance.

PHOSPHATE

A phosphate in acidic condition reacts with ammonium molybdate to form molybdophosphoric acid which is then reduced to molybdenum blue by adding stannous chloride. The intensity of the blue colored complex is measured spectrophotometrically, which is directly proportional to the concentration of phosphate present in the sample

Take 50 ml of filtered and clear sample. Add 2 ml of ammonium molybdate solution and 5 drops of stannous chloride. Measure the blue color developed at 690 nm on a spectrophotometer using a distilled water blank with the same chemicals. Note down the readings of spectrophotometer after 5 minutes but before 12 minutes of the addition of the last reagent

.

Find out the concentration of the phosphate with the help of the standard curve. Use standard phosphate solution and prepare the standard curve in

the range of 0.0 to 1.0 mg/L of PO_4^-P at the interval of 0.1 mg/L by treating in the same way as the sample.

DISSOLVED OXYGEN

Dissolved oxygen is the amount of oxygen dissolved in a given quantity of water at a particular temperature and pressure. DO level is one of the indicator that a water body is polluted by organic matter. If it is less than 6 to 7 mg/L the water is said to be polluted water. It is one of the important parameters to find the purity of the water body. It is determined by wrinkler's method.

Bacteria can reproduce by binary fission, by asexual mode, or by budding. Generally, they reproduce by binary fission, in which the original cell becomes two new organisms. The time required for each division, which is termed the generation time, can vary from days to less than 20 min. For example, if the generation time is 30 min, one bacterium would yield 16,777,216 bacteria after a period of 12h.

Assuming spherical –shaped bacteria with a l um diameter and specific gravity of 1.0, the weight of 1 cell is approximately 5.0 x 1013 g. In 12 h the bacteria mass would be about 8.4 x 10-6 g or 8.4 g; thus the number of cells is quite large compared to the mass.

Collect the sample in 300ml BOD bottle. Add 2ml of Winkler's A solution and 2ml of Winkler's B solution well below the surface through the walls. Stopper

immediately to remove air bubbles and mix carefully by inverting bottle up and down.

Allow the brown precipitate to settle down leaving clear supernatant. Add conc. Sulphuric acid drop by drop till precipitate digested. Restopper the bottle and mix by inverting several times for complete dissolution.

A yellow colored solution appears. Take 50ml samples in conical flask. Add few drops of starch indicator and titrate against 0.025N $Na_2S_2O_3$ solution. Note down the reading until the color changes from blue to colorless.

BACTERIA'S CULTURE MEDIUM

Preparation of media

A common liquid medium is used for growing bacteria is nutrient broth medium. It contains beef extract, peptone and sodium chloride. This medium can be supplemented with other substances like sugar and organic salts to meet the requirements of any particular organisms. This media kept always sterile until they are used.

Liquid growth media contains nutrient are usually solidified by the addition of agar agar is the complex polysaccharide consist of 3-6 anhydrogalactose and D-pyranose free of nitrogen. It produced from red algae belongs to gellatiumgracilaria and other genera. It has no nutritional value. It liquifies on heating at 100ºC and hardens into jelly by cooling at 40ºC-45ºC.

The composition of nutrient broth is beef extract 3grams,peptone 5grams,sodium chloride 5 grams, distilled water one litre with a p^H in the range between 7.2 ± 0.2.

The composition of agar media is beef extract 3grams,peptone 5grams,sodium chloride 5 grams, distilled water one litre with a p^H in the range between 7.2 ± 0.2.

Preparation of nutrient broth

Distilled water is taken in a conical flask and above ingredients is weighed and dissolved in distilled water. p^H of solution is adjusted to 7.2 ± 0.2. The nutrient broth is distributed in test tubes and plug tubes with cotton. The medium is sterilised at 121ºC for 15 minutes in anautoclave. After 15 minutes the test tubes are removed and kept in a sterile condition for culture of microorganism.

Preparation of nutrient agar medium

Distilled water is taken in a conical flask and above ingredients is weighed and dissolved in distilled water. p^H of solution is adjusted to 7.2 ± 0.2. The nutrient broth is distributed in test tubes and plug tubes with cotton. The medium is sterilised at 121ºC for 15 minutes in an autoclave. After 15 minutes the test tubes are removed and kept in a sterile condition for culture of micro organisms. Now the prepared agar is poured into the petri dish containing nutrient broth. It will get densified within 10 minutes. For culturing we can adopt streak method. It is nothing but using inoculation loop we can streak it in a zig zag manner. We can see the culture of micro organisms which is formed as colonies.

Culturing of bacteria

The bacteria used for removal of nutrients are pseudomonas aeruginosa,staphylococcusaureus, kleibsella sp.,. It is having capability of removing nutrients by taking those as source of food.. They were obtained from the Microbiology Department .The test isolates were stored in nutrient agar slants and incubated at 4 ºC until needed.

Prior use, each isolate was first streaked in nutrient agar slants and incubated at 37 ºC for 24 h to ascertain their purity, after which the isolates were sub-cultured into nutrient broth. For each experiment, only 18-24 h old broth cultures of the isolates were used for nutrientremoval study.

RESULTS AND DISCUSSIONS:

GENERAL:

From this study results shows that water sample is rich in phosphate beyond permissible limit, it is found to be 0.3mg/l. So they are highly affecting human's life as well as surrounding water sources. The reduction of

Before usage, the water was first dispensed in 200 mL quantity in 250 mL Erlenmeyer's flasks. All flasks containing the water were cotton-plugged and sterilized at 121ºC for 15min at 15 psi in an autoclave. After sterilization, flasks were first incubated for 24 h to ascertain that there was no growth from any contaminant.

Nutrient removal study

To the sterile water in Erlenmeyer's flasks, a known population of the respective isolates was inoculated. In this study, four different initial biomasses 6.31 x 10^8cfu/mL, 1.26 x 10^9cfu/mL, 1.89 x 10^9cfu/mL and 2.52 x 10^9cfu/mL (*Pseudomonas* sp.), [2.91 x 10^8cfu/mL, 5.82 x 10^8cfu/mL, 8.73 x 10^8cfu/mL and 1.16 x 10^9cfu/mL (*Klebsiella*sp.), 7.1 x 10^8cfu/mL,1.42 x 10^9cfu/mL, 2.13 x 10^9cfu/mL and 2.84 x 10^9cfu/mL (*Staphylococcus* sp.) is used for investigation. The estimation of the initial biomasses was carried out using standard plating techniques

After inoculation, the inoculated flasks were incubated in a rotary shake at 120 revolutions per minute (rpm) at 25ºC. Immediately after inoculation (referred to as 0 h in this study) and every 24 h for 96h, aliquot samples were aseptically taken from each flask for the estimation of the pH, growth rate, phosphate concentration in the water, using standard procedures.

In all cases, phosphate concentrations in the water were analysed using the ascorbic acid method and the salicylate methods, respectively. All experiment analyses were carried out in triplicate. Also, all reagents used were of analytical grades. In all cases, an uninoculated control was also set up for each batch of experiment

CHARACTERISTICS OF WATER:

S. No	Parameters	Range (mg/l)
1.	P^H	9.2*
2.	Turbidity	2.7
3.	Total dissolved solids	280
4.	Dissolved oxygen	0.74
5.	Nitrate	3.0
6.	Phosphate	0.3

* Value on p^H scale

TABLE 1: EFFECT OF CONTACT TIME WITH BACTERIA PSEUDOMONAS AERUGINOSA

Time (days)	phosphate removal (mg/L)		Phosphate removal (%)
	initial	final	
1	0.3	0.285	5
2	0.3	0.279	7
3	0.3	0.275	7
4	0.3	0.260	13.33
5	0.3	0.250	16.67
6	0.3	0.238	20.66
7	0.3	0.225	25
8	0.3	0.190	36.67
9	0.3	0.100	66.67
10	0.3	0.090	70

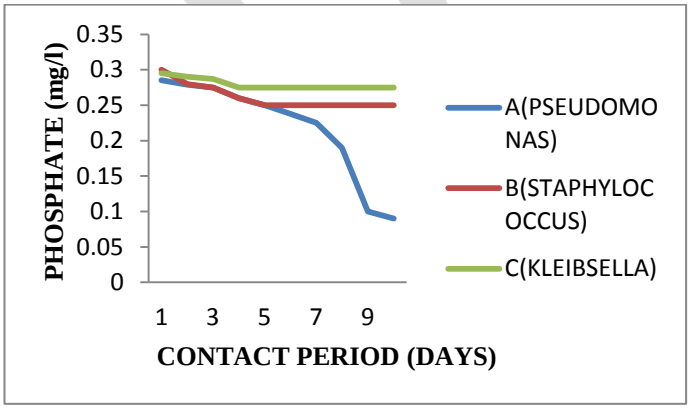
TABLE2: EFFECT OF CONTACT TIME WITH BACTERIA STAPHYLOCOCCUS AUREUS

Time (days)	phosphate removal (mg/L)		Phosphate removal (%)
	initial	final	
1	0.3	0.3	0
2	0.3	0.28	6.66
3	0.3	0.275	7
4	0.3	0.260	13.33
5	0.3	0.250	16.67
6	0.3	0.250	16.67
7	0.3	0.250	16.67
8	0.3	0.250	16.67
9	0.3	0.250	16.67
10	0.3	0.250	16.67

TABLE 3: EFFECT OF CONTACT TIME WITH BACTERIA KLEIBSELLA SP.,

Time (days)	phosphate removal (mg/L)		Phosphate removal (%)
	initial	final	
1	0.3	0.295	1.67
2	0.3	0.29	3.33
3	0.3	0.287	4.33
4	0.3	0.275	8.33
5	0.3	0.275	8.33
6	0.3	0.275	8.33
7	0.3	0.275	8.33
8	0.3	0.275	8.33
9	0.3	0.275	8.33
10	0.3	0.275	8.33

REMOVAL EFFICIENCY OF PHOSPHATE USING BACTERIUM



SUMMARY AND CONCLUSION

GENERAL

Results from this study shows that river water is highly enriched with nutrient like phosphate which is harmful for both the humans and environment. The characterization of river water and bacterial species are studied. The effective reduction of phosphate is up to 70% which is evident from the above fig. Here the bacterium is cultured and the detention period is found for effective removal of phosphate. The comparison is made between three types of bacterium. Among those pseudomonas species showed the effective removal of nutrients from the surface water.