REMOVAL OF PHOSPHATE IN POND BY USING MICROORGANISMS

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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 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 pseudomonas aeruginosa species bacterium to remove the phosphate effectively.

Key words: *Bacterial species, water, Phosphate removal efficiency*.

Introduction

Phosphorus in water contributes to health and environmental threats as they are linked to illnesses as well as ecosystem disruption via algal blooms in contaminated water bodies. Based on above perspectives a comparative study was conducted on local fresh water bacteria pseudomonas aeruginosa 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.

Literature Review

Removal of Phosphate Using Pseudomonas

Olaolu T.D. et. al studied the presence of excessive amounts of phosphate in waste water which have detrimental effect on humans and living organisms. They investigated the effect of initial biomass concentration on the nutrient removal efficiency in waste water under shaking flasks condition. Four different species were used like Klebsiellasp., pseudomonas sp., staphylococcus sp.,

lysinibcillus sp., Aliquot samples were taken at the beginning of the study and every 24 h for 96h for the estimation of growth rate, pH, phosphate and nitrate concentrations in the wastewater, using standard methods. The results revealed only slight phosphate decreases in the wastewater after the expiration of incubation. After 96 h incubation 68.36 to 90.67 %, 91.80 to 95.29 %, 3.20 to 11.48 % and 86.77 to 94.33 % of nitrate was removed in the presence of the Klebsiellasp., Pseudomonas sp., Lysinibacillussp. and Staphylococcus sp., respectively.

Analytical Procedure

Determination of PH

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

$PH = -\log [H_3O^+]$.

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.

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Determination of 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 1total 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

Determination of 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.

Determination of 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 spectrophoto metrically, which is directly proportional to the concentration of phosphate present in the sample. 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.

Determination of 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. 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 an hydro galactose 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, and 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 an autoclave. After 15 minutes the test tubes are removed and kept in a sterile condition for culture of micro organisms.

Preparation of Nutrient Agar Medium

Distilled water is taken in a conical flask and above ingredients is weighed and dissolved in distilled water. pH 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. 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 4ºC 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 nutrient removal study.

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 synthetic wastewater 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, staphylococcus aureus and kleibsella) are 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 96 h, aliquot samples were aseptically taken from each flask for the estimation of the pH, growth rate, phosphate and nitrate concentrations in the wastewater, 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.

Preparation of Sample Curve

It was prepared by dilution method. First of all, sample was prepared as per standards. 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. Find out the concentration of the phosphate with the help of the standard curve. The graph was plotted by using concentration in the X axis and absorbance in Y axis. Before that blank reading was noted by using distilled water and the absorbance was calculated from the blank reading.

Conclusion

From this study it concluded that the water used for drinking are mainly contaminated by domestic and industrial sewage. The permissible limit of phosphate is 0.5mg/l but water sample is having beyond limit as 0.8mg/l. For this it needs to be treated by using bacteria. The contact time for pseudomonas was about 10 days. The effective reduction of phosphate is up to 70% by the bacteria pseudomonas aeruginosa. Here the bacterium was cultured and the detention period is found for effective removal of phosphate.

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