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# Phosphorus uptake of bacterial isolates from eutrophic river system

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**Abstract**
The general objective of this study was to isolate and screen bacteria from eutrophic river system that luxuriously uptake phosphorus. Six bacterial colonies were differentiated based on cultural characters. Except isolate 1, the remaining five isolates could reduce the phosphorus concentration in flasks by more than 50% after 72 hours. Isolate 4 had the lowest final phosphorus concentration (2.85±0.23 g/L), thus, had the highest percent phosphorus reduction (59.95%). The per cent phosphorus reduction of the six isolates after 72 hours was not statistically significant when compared, therefore, any of the six isolates once identified could be used in future bioremediation studies.

**Keywords:** Phosphorus, phosphorus uptake, bioremediation, eutrophic river

## 1. Introduction
Phosphorus is one of the major nutrients needed by plants, animals and humans. It is considered as the 11th most abundant element in the earth's crust. The concentration of phosphorus is typically low under natural conditions, thus, it limits the growth of aquatic organisms [1, 2]. Fish can absorb phosphorus from the water, but due to the low waterborne phosphorus concentration, dietary supplementation is necessary. The excess of this mineral in fish diets provides higher levels of excreted phosphorus, with this being the main cause of eutrophication in the aquatic environment, impairing water quality [3]. Phosphorus exists in three forms namely organic phosphorus, orthophosphate and polyphosphate [4].
Phosphorus is also considered as pollutant in high concentration. The addition of phosphate ion is a serious environmental problem because it contributes to an increased eutrophication process in inland bodies of water [2]. The possible sources of this ion into aquatic environments include household sewage water, industrial effluents, fertilizers, cleaning preparations and boiler waters [2].
Over-enrichment of nutrients leads to harmful algal blooms and eventually depletion of oxygen. In a global scale, harmful algae blooms are considerably more widespread and frequent than they were a decade ago, a situation that is expected to further deteriorate by 2020. Since 1960, the number of documented hypoxic areas has doubled every decade. For example, in 2007, a total of 415 eutrophic and hypoxic coastal systems were identified [5].
Bioremediation is a process wherein organic wastes are biologically degraded under controlled conditions, or to levels below concentration limits as established by regulatory authorities[6]. Living organisms, particularly microbes are being used to facilitate the degradation of environmental contaminants into less toxic forms. The microorganisms to be used may be naturally living to a contaminated area or may be introduced to the contaminated sites. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes [7]. However, the use of microorganisms for bioremediation requires an understanding of all physiological, microbiological, ecological, biochemical and molecular aspects involved in pollutant transformation [8].
Phosphorus is taken up by microbes for their cellular maintenance, synthesis of nucleic acids, construction of cell membranes and chemical energy transfer reactions within cells [4]. Study of Krishnaswamy [3] revealed that *Bacillus* sp., *Pseudomonas* sp. and *Enterobacter* sp. were efficient in removing phosphate in synthetic medium. The general objective of this study was to isolate and screen bacteria from eutrophic river system that luxuriously uptake phosphorus.

## 2. Materials and Methods
### 2.1. Isolation of bacterial colonies
Two series of 10-fold dilutions ($10^{-3}$ and $10^{-4}$) of eutrophic river sediment was be made in sterile distilled water. The diluted sample of 0.1 mL was streaked onto Trypticase Soy Agar (TSA) and incubated at 37 ˚C for 18 to 24 hours.

### 2.2. Cultural characterization of bacterial colonies
Cultural characters (*e.g.* size, amount of growth, opacity, form, surface texture, margin or edge, consistency, elevation and growth characteristics in nutrient broth) were considered in differentiating the bacterial colonies that grown in TSA plates. Colonies that exhibit different cultural characters were used in the phosphorus-uptake experiment.

### 2.3. Phosphorus-uptake experiment
Flasks with 250 mL capacity were filled up with 50 mL of sterile distilled water supplemented with 0.5% glucose. Five milliliters of bacterial suspension (turbidity of 0.5 MacFarland standards) was added in each flask. Using a synthetic source, the phosphorus concentration in flasks was adjusted to 7 g/L. The flasks were sealed and incubated at room temperature for 72 hours with constant mixing. The final concentration of phosphorus after 72 hours was determined using the prescribed procedures in the laboratory manual of Aquatic Ecology of the College of Fisheries in Central Luzon State University. The percent reduction in phosphorus concentration was computed using the below formula:

$$\text{Percent reduction (\%)} = \frac{\text{Initial phosphorus-final phosphorus}}{\text{Initial phosphorus}} \times 100$$

## 3. Results and Discussion
Six bacterial colonies were differentiated based on cultural characters such as size, amount of growth, opacity, form, surface texture, margin or edge, consistency, elevation and growth characteristics in nutrient broth. The colonies were purified on TSA plates before subjected to phosphorus-uptake experiment for period of 72 hours. The data on final phosphorus concentration and percent reduction after 72 are presented in Table 1. Except isolate 1, the remaining five isolates could reduce the phosphorus concentration by more than 50%. Isolate 4 had the lowest final phosphorus concentration (2.85±0.23), thus, highest phosphorus reduction (59.95%). Statistical comparison showed that the final phosphorus concentration and percent phosphorus reduction of the six isolates were not significant ($p$>0.05), therefore, any of the isolate once identified could be used in future bioremediation studies.

**Table 1:** The ability of the six bacterial isolates to uptake phosphorus in their cells as supported by per cent phosphorus reduction.

| Isolates | Initial Phosphorus (g/L) | Final Phosphorus (g/L)* | Reduction (%)* |
|---|---|---|---|
| 1 | 7.05 | 3.66±0.23 | 48.04 |
| 2 | 7.05 | 3.31±0.25 | 53.12 |
| 3 | 7.05 | 3.26±0.25 | 53.70 |
| 4 | 7.05 | 2.85±0.23 | 59.95 |
| 5 | 7.05 | 3.17±0.35 | 55.04 |
| 6 | 7.05 | 3.33±0.59 | 52.74 |

*Not statistically significant when means (±SD) are compared between rows ($p$>0.05)

Phosphorus is recognized as one of the major nutrients required by living organisms and has been involved in major physiological processes. It can be further classified into two, namely; the soluble reactive phosphorus (SRP) which is readily used by aquatic plants and algae and the other one is the total phosphorus which includes the dissolved and particulate forms of phosphorus [9]. The concentration of phosphate in water bodies vary from 0.005 to 10 ppm depending on the source of phosphate near the water body. Phosphate levels greater than 1.0 ppm may interfere with coagulation in water treatment plants. The Environmental Protection Agency (EPA) has fixed the standard phosphate level of 0.015 ppm for water supply, 0.025 ppm for aquatic life, 0.05 ppm for lakes and 0.02 ppm for mountain lakes [10].

The addition of phosphorus as phosphate ion is one of the serious environmental problems that results to the eutrophication of lakes and other natural waters [4]. Phosphorus is the key element of concern because the natural occurrence of phosphorus in surface water bodies is minimal. Therefore, even a minute amount of phosphorus entering or becoming soluble in a water body can trigger a single algal bloom. Algal bloom can lower light penetration and dissolved oxygen levels, and it also causes aesthetics degradation of surface water bodies [11]. The addition of phosphorus as phosphate fertilizers in soil in excessive amount causes serious environmental problems in the form of eutrophication. The main sources of phosphate in aquatic environment is through household sewage water containing detergents and cleaning preparations, agricultural effluents containing fertilizers as well as industrial effluent from fertilizer and soap industries [2].

Bioremediation is the used of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms [7]. Microorganisms for bioremediation purposes is achieved by the addition of growth substances, nutrients, terminal electron acceptor/donors or some combination thereby resulting in an increase in organic pollutant degradation. The energy and carbon are obtained through the metabolism of organic compounds by the microbes involved in bioremediation processes[12]. Bioremediation strategies are more effective because it can be implemented *in situ* [13].

Microbial strategies for the removal of environmental pollutants from contaminated areas can provide attractive alternative to traditional methods such as incineration. Currently, phosphates are biologically removed by living microorganisms such as bacteria, microalgae, yeasts, protozoa, fungi and macrophytes. The quantity of eliminated phosphate depends on the net production of living biomass[4].

Enhanced Biological Phosphate Removal (EBPR) uses the metabolism of specific bacteria, which under certain conditions accumulate large amounts of phosphate as intercellular polyphosphate. These bacteria use the degradation of polyphosphate to produce energy under aerobic conditions with the release of phosphate into the wastewater. The wastewater is then treated in an aerobic basin. Here the polyphosphate bacteria used stored carbon reserves to produce energy for growth and to replenish their stores of phosphorus. The result is a net removal of phosphate from the wastewater [4].

In the study of Krishnaswamy [4], among 38 isolates, bacteria that belong to *Bacillus* sp., *Pseudomonas* sp. and *Enterobacter* sp. were screened and identified as predominant phosphate utilizers based on the minimum inhibitory concentration (MIC) test after 72 hours of incubation. The removal efficiency of soluble phosphates varied with strains. Various microorganisms are capable of utilizing phosphate as a sole source of phosphorus and these microbial transformations have been proposed as key steps in the phosphorus cycle in nature [14]. The *Pseudomonas* sp., *Aerobacter* sp., *Moraxella* sp., *Escherichia coli*, *Mycobacteria* sp., *Beggiatoa* sp. and *Klebsiella* sp. have the ability to accumulate phosphorus at approximately 1 to 3% of the cell dry mass [15].

## 4. Conclusion

The per cent phosphorus reduction of the six isolates ranged from 48.04 to 59.95% after 72 hours' phosphorus-uptake experiment with no statistical significance when compared, therefore, any of the six isolates once identified could be used in future bioremediation studies.

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