Phosphorus reduction in batch culture using seed sludge of wastewater

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Abstract. Phosphorus is a chemical substance and is a kind of nutrient that is essential for the growth of the organism. This key element tends to be growth-limiting-nutrient in lake ecosystems. The high concentration of phosphorus is toxic and can cause a major effect on the water body. Excessive nutrients may lead to eutrophication which will cause the depletion of oxygen. The anoxic waters would reduce the water quality of the surface water and disrupt the stability of the ecosystem. Thus, it is important to measure the phosphorus in a surface water system and to limit the amount of phosphorus going into the water body. Thus, this research aims to evaluate the phosphorus reduction using seed sludge taken from wastewater treatment in Kolej Mawar, UiTM, Shah Alam, Selangor. In the wastewater, biological phosphorus removal (BPR or Bio-P) or the enhanced biological phosphorus removal (EBPR) is the biological process where this substance is incorporated into cell biomass and subsequently removed from the process as a result of sludge wasting. Here, seed sludge from wastewater was used in a batch culture experiment using two different solutions of disodium hydrogen phosphate (Na₂HPO₄) and dipotassium hydrogen phosphate (K₂HPO₄) in a synthetic medium. The phosphorus reduction and the percentage removal were observed in these two solutions in one-week incubation time. It can be observed that the percentage removal of phosphorus in Na₂HPO₄ solution was higher (88% and 96%) compared to K₂HPO₄ solutions (70% and 29%). From the results obtained, the phosphorus can significantly be reduced using the seed sludge from the wastewater in short incubation time within one week. Thus, a sustainable approach in green technology can be further investigated and enhanced to overcome the environmental problems in reducing the phosphorus effluent discharge into the surface water system.

1. Introduction
Water is vital in our daily life as this component has been considered as top priority and necessities. Nowadays, water needs to be purified and treated before being used. Currently, most of the freshwater resources such as lakes, rivers and reservoirs have been polluted. This has resulted from the vast
development and high growth of population in the urban area. Mismanagement of solid waste and improper treatment of wastewater led to the reduction of water quality in the surface water system [1]. Due to that, the best management practice is vital to preserving the water quality for sustainable water resources.

Communities had already faced the water shortage that frequently happens due to the inconsistent climate change. In the Selangor state of Malaysia, water scarcity would create a huge problem and significantly affected the economic activities and reduced the quality of life for the inhabitants [2]. The water shortage in Selangor that happened due to climate change as well as due to the polluted water resources would cause the increased in operating cost and efficient treatment for treated water. Thus, a sustainable approach to life cycle assessment of the surface water system needs to be further investigated [3]. Several pollutants such as nitrogen and phosphorus have become a major problem for the deterioration of water quality.

Effluent discharge from wastewater treatment plants needs to be properly treated before being discharged into the river. Furthermore, the sources of the nutrients coming from agricultural activity, manufacturing products, food, and beverages (F&B) industries have contributed to the excessive loading of nutrients in the surface water system [4]. The high concentration of phosphorus stimulates the growth of plankton and aquatic plants such as the increase of algal bloom which will lead to eutrophication in the surface water ecosystem. Thus, it is essential to have an efficient technology to enhance the water quality of the effluent.

Accordingly, this study is to evaluate the effectiveness of seed sludge coming from the wastewater treatment plant in the removal of phosphorus using a batch culture experiment. The reduction of phosphorus was observed in the synthetic medium for one-week duration.

2. Material and methods

2.1 Preparation of synthetic medium and seed sludge

The seed sludge was taken from Kolej Mawar Treatment Plant in UiTM Shah Alam. Previously, the wastewater characteristics for influent and effluents for this wastewater treatment plant has been determined. The synthetic medium shown in table 1 was prepared for the batch culture experiment. The medium solution was prepared by diluting 0.005 g/L of iron (III) chloride (FeCl₃), 0.002 g/L of manganese (II) sulfate hydrate (H₂MnO₅S), 0.011 g/L of calcium chloride (CaCl₂), 0.16 g/L of magnesium sulphate heptahydrate (MgSO₄·7H₂O), 1.0 g/L of ammonium persulfate (NH₄)₂S₂O₈ and 10 g/L of D-Glucose, Anhydrous (C₆H₁₂O₆) with 1 L of deionized (DI) water. The amount of glucose used is dominating the other substance since glucose is used as the carbon source to increase the phosphorus solubilization. Then, 10.9 g/L of di-potassium hydrogen phosphate (K₂HPO₄) and disodium hydrogen phosphate (Na₂HPO₄) was diluted in two different beakers containing 1 L of dilution water, respectively [5]. All solutions prepared and autoclaved for 15 minutes to neutralize the potentially infectious agents or bacteria that might cause problems and errors in the results.

| Chemical Composition                      | Weight (g/L) |
|------------------------------------------|--------------|
| Iron (III) chloride (FeCl₃)              | 0.005        |
| Manganese (II) sulfate hydrate (H₂MnO₅S)| 0.002        |
| Calcium chloride (CaCl₂)                | 0.011        |
| Magnesium sulphate heptahydrate (MgSO₄·7H₂O) | 0.16      |
| Ammonium persulfate (NH₄)₂S₂O₈          | 1.0          |
| D-Glucose, Anhydrous (C₆H₁₂O₆)         | 10           |

Table 1. Synthetic medium in one (1) litre bottle [5].
2.2 Batch culture experiment
Batch culture experiment was performed using the 250 mL conical flask. The conical flask was filled with 150 mL of the combination of seed sludge from wastewater sample, phosphate solution and medium synthetic solution. Two types of phosphate solutions were used with four different initial concentrations (high and low concentration). These two solutions were di-sodium hydrogen phosphate (Na$_2$HPO$_4$), and di-potassium hydrogen phosphate (K$_2$HPO$_4$) solutions that were mixed separately with the medium solution and the seed sludge of wastewater sample in four different flasks labelled A, B, C and D. Both solutions were tested to compare the performance of phosphate removal in the batch experiment. Initially, the phosphorus concentrations in the flasks were determined in day 0 and incubated in the mechanical shaker for one-week at room temperature of 25°C ± 2°C under aerobic conditions.

2.3 Parameter detection
The phosphorus concentrations were measured daily for one-week incubation time. The measurement of phosphorus was obtained by spectrophotometer [6].

3. Results and discussion

3.1 Batch culture
In this study, the batch culture experiment has been conducted to remove the phosphorus biologically using the seed sludge coming from the wastewater of Kolej Mawar Treatment Plant. This experiment was conducted in aerobic conditions with synthetic medium prepared for the phosphate solution. The microorganism in the seed sludge of wastewater will consume the nutrients and therefore, will reduce the phosphorus concentration.

3.2 High phosphorus concentration
Table 2 shows the concentration of two solutions, di-potassium hydrogen phosphate (K$_2$HPO$_4$) and disodium hydrogen phosphate (Na$_2$HPO$_4$) in Flasks A and B, respectively. At the end of the experiment, the phosphorus concentration was reduced depending on the incubation time. Both concentrations measured at day 5 for the Flask A and Flask B were decreased to 1.68 mg/L and 0.39 mg/L, correspondingly.

Figure 1 shows that there is a sudden decrease between day 2 to day 4. The phosphorus concentration begins to reduce after the second day due to the limitation of the nutrient supplied in this batch culture experiment. The decreasing of the phosphorus concentration is because of the exponential growth of the microorganisms in the acceleration phase [7]. During this exponential growth phase, the microorganism starts to grow rapidly by consuming all the nutrients available. Due to the limitation of nutrients supplied for this experimental process, the microorganisms in the wastewater have limited nutrient sources to survive for a longer period. As all nutrients consumed, the concentration of the phosphorus measured will decrease due to the amount of phosphate uptake by the microorganism [8].

The pH of the culture medium was changed during the batch culture experiment. The mixed culture medium in Flask A has pH values ranging from 8.38-8.84 compared to the mixed culture medium in Flask B having pH values between 8.49-8.77. Most of the microorganisms in the wastewater like bacteria, fungi, yeast, protozoa, and microalgae do utilize the dissolved orthophosphate, polyphosphate, and organic phosphate for their growth [7]. Specifically, the polyphosphate accumulating organisms (PAOs) was the type of bacteria that can consume a large amount of phosphate, thus reducing the phosphorus concentration in the wastewater. Candidatus Accumulibacter phosphatis (A. phosphatis) is the examples of the polyphosphate accumulating organisms (PAOs) [9,10].
Table 2. Results and the pH value for Flasks A and B with high phosphorus concentration.

| Days   | Concentration in Flask A (effluent sample + K$_2$HPO$_4$ + medium solution) mg/L | pH  | Concentration in Flask B (effluent sample + Na$_2$HPO$_4$ + medium solution) mg/L | pH  |
|--------|---------------------------------------------------------------------------------|-----|---------------------------------------------------------------------------------|-----|
| Day 0  | 5.6                                                                              | 8.34| 3.15                                                                             | 8.49|
| Day 1  | 5.59                                                                             | 8.55| 3.15                                                                             | 8.49|
| Day 2  | 3.93                                                                             | 8.56| 1.18                                                                             | 8.72|
| Day 3  | 2.63                                                                             | 8.73| 0.68                                                                             | 8.77|
| Day 4  | 2.54                                                                             | 8.84| 0.6                                                                              | 8.77|
| Day 5  | 1.68                                                                             | 8.84| 0.39                                                                             | 8.77|
| Removal Percentage (%) | 70%                                                                              |     | 88%                                                                              |     |

Figure 1. The phosphorus concentration of Flask A and Flask B during the batch culture experiment.

The percentages of phosphorus removal in Flask A and Flask B were 70% and 88%, respectively. This shows that phosphorus is used as the nutrient by the microorganism in the batch flasks to grow and multiply. It can be observed that the biological treatment using the seed sludge showed that the phosphorus could be decreased within one-week duration. It is important to investigate the performance using a sustainable approach to control the phosphorus concentration in the effluent discharge [7].

3.3 Low phosphorus concentration

Table 3 and figure 2 shows the phosphorus concentration at the end of 5 days for Flask C and Flask D (1.84 and 0.08 mg/L). From day 0 to day 3, the data recorded for both solutions have slightly decreased. However, from day three onwards, the phosphorus concentration has gradually declined until day 5.

The microorganisms in the wastewater sample are always competing for nutrients for their survival. For the PAOs, the nearest competitor is the non-PAOs called the glycogen-accumulating organisms (GAOs). GAOs compete with PAOs for the volatile fatty acids (VFAs) as the energy sources for the microbial production. The increase of the GAOs will deteriorate the phosphate removal since the GAOs cannot synthesize the polyphosphate. Apart from that, the increase of the GAOs also possibly due to the high amount of medium solution added into the culture medium, which is rich in glucose [7].
The pH values recorded for this batch culture experiment are within the range of 7.52 - 8.72 for both flasks. The percentage of removal for Flask C was 29%, very low compared to Flask B with 96% removal. The significant decreased of the phosphorus concentration has been observed in the solution of disodium hydrogen phosphate (Na$_2$HPO$_4$) compared to the di-potassium hydrogen phosphate (K$_2$HPO$_4$) solution.

The effluent sample that has been treated using the batch culture experiment can reduce the amount of the phosphorus content in the receiving water body. When there is a limiting amount of phosphorus discharged into the receiving water, the productivity of aquatic plants and algae can be controlled since the nutrient sources are in short supply. Water quality was impaired due to the excessive nutrients loading. The oversupplied nutrients may cause eutrophication, which is the excessive plants and algae growth, leading to high biochemical oxygen demand (BOD) as the algae decomposed and used up available oxygen supplied. A further effect of eutrophication is the threatening on fish survival and other aquatic organisms in the aquatic ecosystem [11].

**Table 3.** Results and the pH value for the Flask C and D with low phosphorus concentration.

| Days  | Concentration in Flask C (effluent sample + K$_2$HPO$_4$ + medium solution) mg/L | pH  | Concentration in Flask D (effluent sample + Na$_2$HPO$_4$ + medium solution) mg/L | pH  |
|-------|---------------------------------------------------------------------------------|-----|---------------------------------------------------------------------------------|-----|
| Day 0 | 2.58                                                                             | 8.41| 1.86                                                                             | 8.66|
| Day 1 | 2.46                                                                             | 8.32| 1.96                                                                             | 8.72|
| Day 2 | 2.44                                                                             | 7.52| 1.92                                                                             | 8.48|
| Day 3 | 2.58                                                                             | 7.56| 1.35                                                                             | 8.47|
| Day 4 | 1.86                                                                             | 7.72| 0.12                                                                             | 8.46|
| Day 5 | 1.84                                                                             | 7.75| 0.08                                                                             | 8.43|
|       | **Removal Percentage (%)**                                                       |     | **Percentage (%)**                                                              |     |
|       | 29%                                                                              |     | 96%                                                                              |     |

**Figure 2.** The phosphorus concentration of Flask C and Flask D during the batch culture experiment.
4. Conclusion
The batch culture experiment using seed sludge from wastewater of Kolej Mawar Treatment Plant has significantly reduced the phosphorus concentration in the synthetic medium. The di-sodium hydrogen phosphate (Na$_2$HPO$_4$) with high concentration had greatly reduced phosphorus in the effluent sample. The small amount of phosphorus in the effluent discharged can control the nutrients level in the receiving water, and a healthy ecosystem can be ensured without the excess of algae growth which leads to the eutrophication. Enhanced biological phosphorus removal (EBPR) in the secondary treatment process can help to remove the phosphorus in advance. More research on the polyphosphate accumulating organisms (PAOs) needs to be explored to investigate the potential to remove phosphorus in the water.

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