Journal of Scientific Research & Reports
3(14): 1875-1885, 2014; Article no. JSRR.2014.14.004

SCIENCEDOMAIN *international*
www.sciencedomain.org

Investigation of the Efficiency of Selected Bacterial and Fungal Species in the Removal of Phosphate and Sulphate from Wastewater

S. O. Dahunsi[1], O. B. Akpor[1*] and R. Aransiola[2]

[1]Microbiology Unit, Department of Biological Sciences, Landmark University, PMB 1001, Omu Aran, Kwara State, Nigeria.
[2]Department of Biochemistry, University of Ilorin, Kwara State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OBA conceptualized the study, designed the methodology, assisted in the laboratory analysis and wrote the first draft of the manuscript. Author SOD assisted in the design of the methodology, laboratory analysis and interpretation of the results. Author RA assisted in laboratory analysis, interpretation of results and proofread the first draft of the manuscript. All authors read and approved the final manuscript.

Review Article

Received 15th April 2014
Accepted 28th May 2014
Published 13th June 2014

ABSTRACT

The aim of this study was to ascertain the efficiency of selected bacteria (*Pseudomonas*, *Staphylococcus*, *Klebsiella* and *Lysinibacillus* species) and fungi (*Aspergillus niger*, *Aspergillus flavus*, *Fusarium* and *Absidia* species) in the removal of phosphate and sulphate compounds from wastewater. The study was carried out under shaking flasks conditions. The test wastewater was filtered, sterilised and inoculated with the respective test bacterial and fungal isolates. Just after inoculation with a test isolate and every 24 h for 96 h, aliquot wastewater sample was taken from each flask aseptically for the estimation of phosphate and sulphate concentration in the wastewater. The results revealed remarkable phosphate and sulphate removal within the first 24 h and 48 h, respectively of incubation in the presence of three of the bacterial (*Pseudomonas*, *Staphylococcus* and *Klebsiella*) isolates, after which increases were observed. In the presence of the fungal isolates, remarkable decreases in phosphate and sulphate levels in the wastewater were observed after the first 48 h of incubation in the presence of the *Aspergillus niger* and *Aspergillus flavus*. In the presence of the *Fusarium* spp., there were

**Corresponding author: E-mail: akpor.oghenerobor@lmu.edu.ng;*

consistent decreases in phosphate and sulphate levels with time. No remarkable decreases in phosphate and sulphate levels were observed with time in the presence of the *Lysinibacillus* and *Absidia* species. The study was able to give an insight into the phosphate and sulphate removal ability of the test isolates under the experimental conditions.

Keywords: Sulphate; phosphate; wastewater; removal.

1. INTRODUCTION

The continuous increase in the human population coupled with infrastructural developments has led to greater quantities of wastes being disposed into the environment every day [1]. Also, improper disposal of wastewater has led to outbreaks and widespread of disease in different parts of the world and thus calls for wastewater management and proper treatment. Wastewater may contain high levels of nutrients, which when excessively released to the environment can lead to the undesirable growth of microorganisms and hence eutrophication [2].

Although phosphorus is useful in the production of phospholipids, ATP and nucleic acid, which are all important components of living organisms, when present in excess amount in receiving water bodies is the leading cause of eutrophication [3].The eutrophication of water bodies is mainly due to the presence of excess phosphorus and nitrogen [4,5,6]. The most recognizable manifestation of eutrophication is the presence of algal blooms, which results in low dissolved oxygen in water bodies, death of aquatic life and the depletion of desirable flora and fauna [7,8,9].

In recent years, attention has shifted from chemical treatment methods to biological methods for the removal of nutrients from wastewater. This is stimulated partly by the enforcement of more stringent legislation to protect the environment stimulates. It is reported that although investment costs of chemical treatment plants are lower than for biological treatment plants, the annual operating costs are significantly higher for chemical than biological treatment processes [10].

The presence of sulphate in wastewaters is due to a spectrum of industrial processes, such as paper and pulp production, sea food processing, potato starch production, tanneries, fermentation, fertilizers, ore processing mining. The presence of excess amount of sulphate in drinking water high in sulphate is reported to cause to adverse health effects to animals, leading. Animals that consume drinking water with excessive sulphate concentrations experience numerous health problems, such as poor conception, decreased immune response and growth rate and poor absorption of trace minerals [11]. Apart from the health impacts on humans and animals, a sulphate level, which is above 500 mg/L (acceptable levels set by many environmental legislation), could cause other problems, such as corrosion of water transport systems and of concrete [12]. A number of treatment techniques for sulphate rich waters have been developed in recent years, with selection of a criteria for each of the processes based on a variety of technical, economic, environmental and legislative factors [13]. Biological sulphate removal processes are indicated to be beneficial to mining industries experiencing acid mine drainage problems, since they result in removal of sulphate, in a pH increase, which neutralizes the acidity of the treated water and also in metal removal [14].

The aim of this study therefore was to investigate the efficiency of selected eight microbial isolates, consisting of four bacterial and four fungal species in the removal of phosphate and sulphate from wastewater under shaking flaks conditions.

2. MATERIALS AND METHODS

A total of eight microorganisms, consisting of four bacteria (*Pseudomonas* spp., *Staphylococcus* spp., *Klebsiella* spp. and *Lysinibacillus* spp.) and four fungi (*Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp. and *Absidia* spp.) were used for this investigation. The isolates were part of the laboratory stock of the Department of Biological Sciences, Landmark University, Omu-Aran, Nigeria and have previously been used for phosphate and nitrate removal studies in synthetic wastewater [15,16] hence the need to test them on natural wastewater.

The test wastewater used for this study was obtained from the Landmark University Commercial Farms, located in Omu-Aran, Nigeria. Before usage, the wastewater was filtered using Whatman No1 filter paper in 200 mL quantity into 250 mL capacity conical flasks. After filtration, the wastewater was supplemented with sodium acetate (5 g/L), potassium nitrate (0.15 g/L) and magnesium sulphate (0.5 g/L) and then sterilised in an autoclave for 15 min at 121ºC at 15 psi. To ascertain the efficiency of the sterilisation process, the sterilised wastewater was plated in nutrient agar and sabouraud dextrose agar plates for bacterial and fungal growths, respectively. Only sterilised wastewater that showed no growth after plating in the media were used for the study.

To each of the sterilised flasks containing wastewater, 0.5 mL of the respectively test isolates suspended in sterile normal saline (0.85% NaCl) was added aseptically and incubated at 30ºC in a rotary shaker at a shaking speed of 150 rpm. Immediately after inoculation and every 24 h for the 96 h, aliquot wastewater samples were aseptically removed from each flask for the analysis of total phosphate and sulphate, using standard procedures [17]. All experiment setups were done in triplicate. The reagents that were used for the study were all of analytical grades

3. RESULTS AND DISCUSSION

As shown in Fig. 1, in the presence of the *Pseuodomonas* spp., remarkable decrease in phosphate concentration from 22.83 mg/L to 14.57 mg/L in the wastewater was observed within the first 24 h of incubation, after which there was an increase with time. After the 96 h incubation period, phosphate concentration was found to be 18.31 mg/L. For sulphate in the presence of the *Pseudomonas* spp., concentrations were not observed to follow any pattern

When inoculated with the *Staphylococcus* spp., phosphate levels also showed maximum decreases after 24 h incubation, decreasing from 20.47 mg/L to 13.18 mg/L, after which there were consistent increases with time until after 72 h, before a slight decrease was observed. At the end of incubation, the final concentration was observed to be 17.77 mg/L. In the presence of the isolate, sulphate levels in the wastewater showed consistent decreases with time, until after 48 h incubation, before a drastic increase was observed at 72 h. A maximum sulphate decrease from 2643.04 mg/L to 1638.92 mg/L was observed after 48 h incubation (Fig. 2).

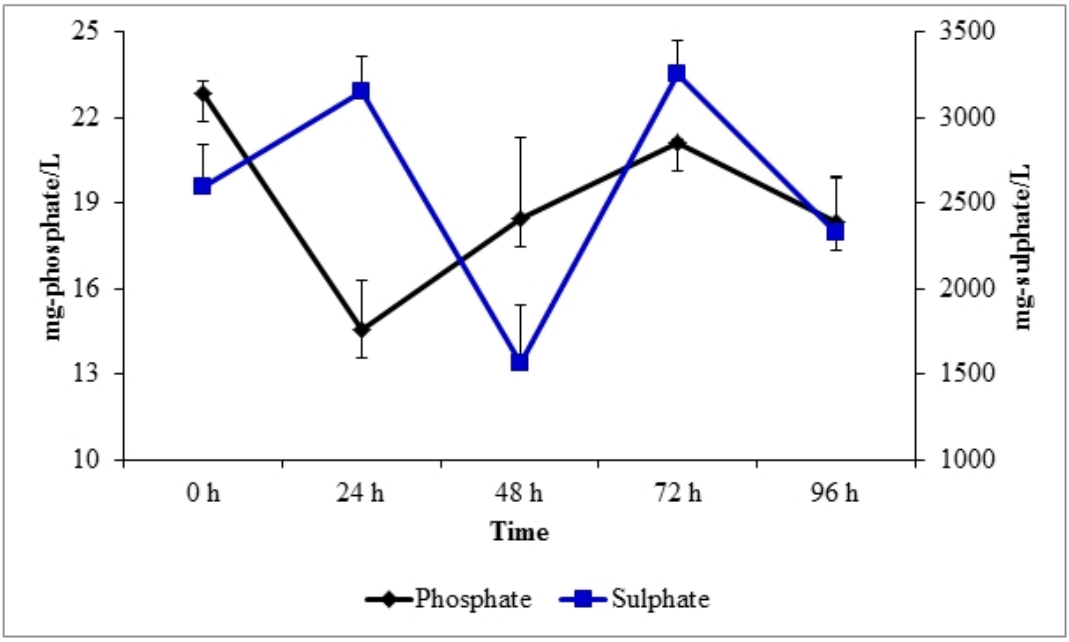

Fig. 1. Variation in phosphate and sulphate concentrations in the presence of the *Pseudomonas* spp

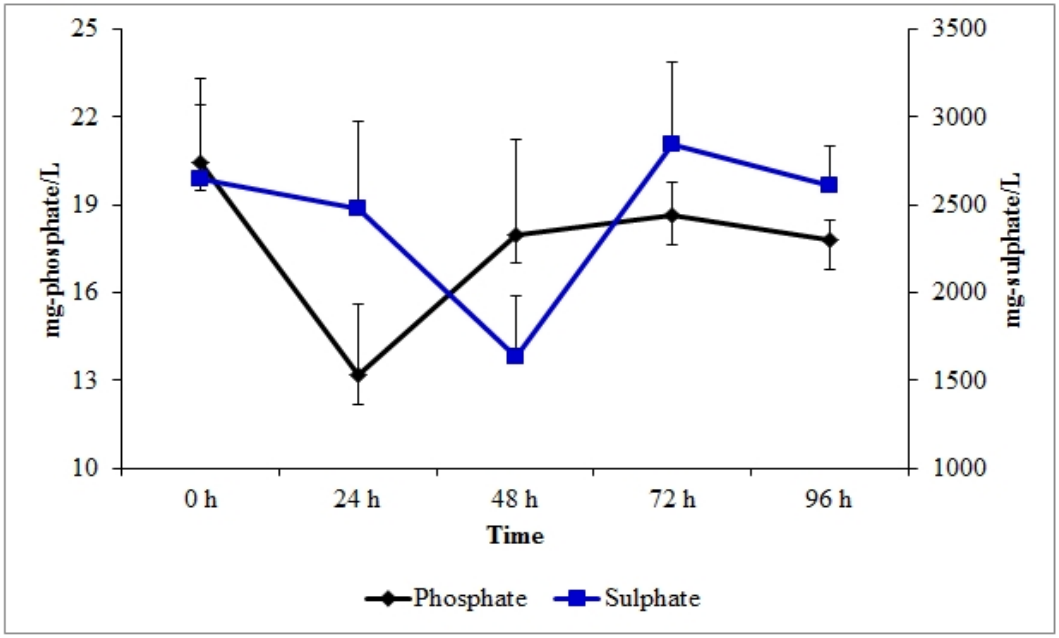

Fig. 2. Variation in phosphate and sulphate concentrations in the presence of the *Staphylococcus* spp

In the presence of the *Klebsiella* spp., phosphate concentration in the wastewater was observed to decrease from 21.05 mg/L to 14.54 mg/L within 24 h of incubation, after which there was an increase. At the end of the 96 h incubation, phosphate level was observed as 17.84 mg/L. In the case of sulphate concentration in the wastewater, the lowest and highest concentrations were observed at 48 h and 72 h incubation, respectively. At the expiration of the period of incubation, concentration was observed to increase from 2181.38 mg/L at 0 h to 2371.81 mg/L at 96 h (Fig. 3).

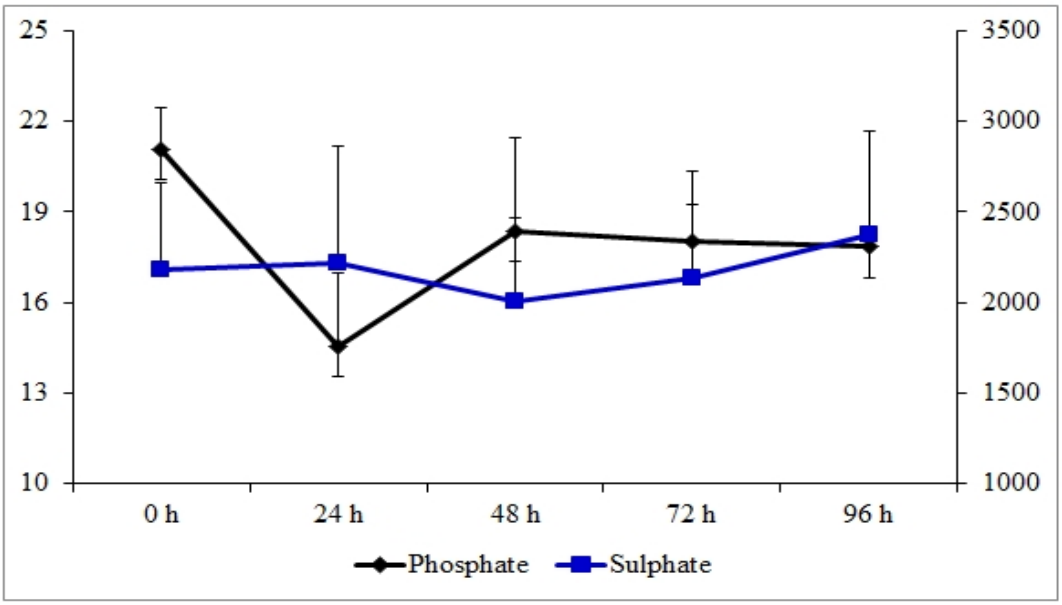

Fig. 3. Variation in phosphate and sulphate concentrations in the presence of the *Klebsiella* spp

As shown in Fig. 4, no remarkable decreases in phosphate and sulphate concentration in the wastewater were observed throughout the period of sampling in the presence of the *Lysinibacillus* spp. At the end of the 96 h incubation period, phosphate and sulphate levels in the wastewater were observed to decrease from 20.83 mg/L to 17.17 mg/L and from 2319.88 mg/L to 2383.35 mg/L, for phosphate and sulphate, respectively (Fig. 4).

With respect to the *Aspergillus niger*, phosphate concentration in the wastewater was observed to show maximum decrease within 24 h incubation, after which there was consistent increase with time until the after 96 h when there was a slight decrease. At the end of incubation, phosphate level showed a decrease from 23.83 mg/L at 0 h to 17.49 mg/L after 96 h (Fig. 5). In the case of sulphate levels in the wastewater in the presence of the *Aspergillus niger*, no remarkable decrease in concentration was observed within 24 h of incubation. At the end of the 96 h incubation period, sulphate levels showed a decrease from 3479.81 mg/L at 0 h to 2602.65 mg/L (Fig. 5).

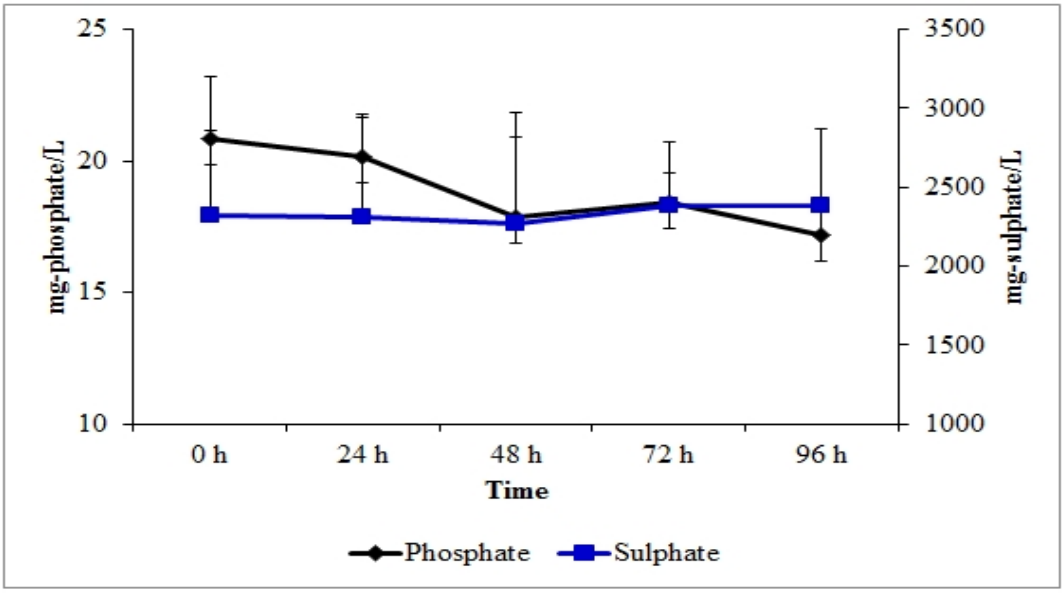

Fig. 4. Variation in phosphate and sulphate concentrations in the presence of the *Lysinibacillus* spp

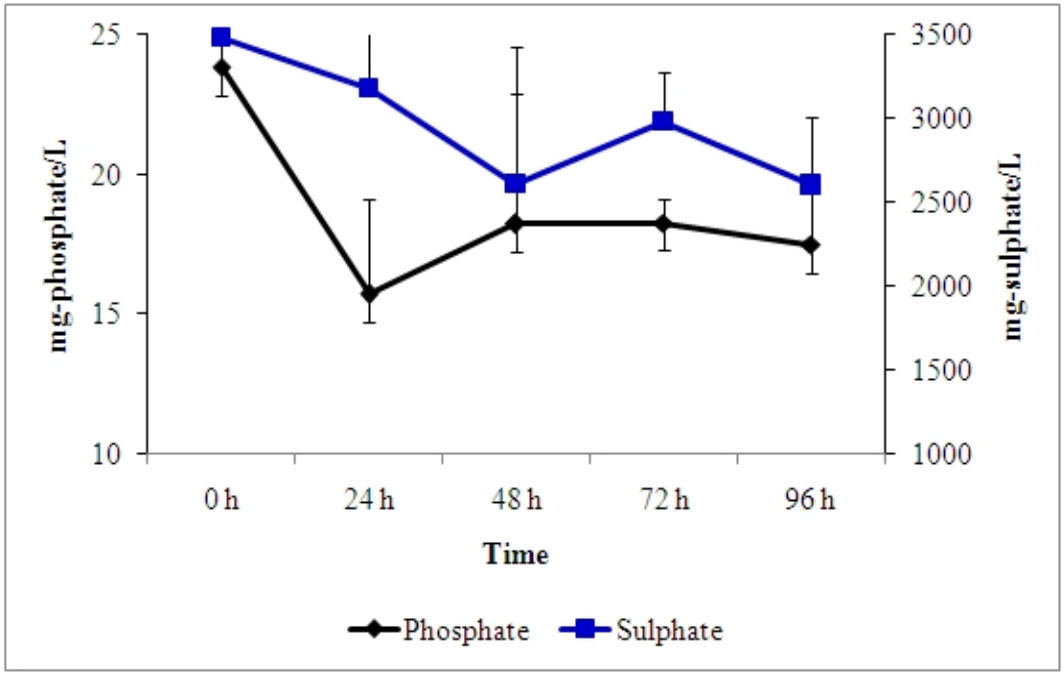

Fig. 5. Variation in phosphate and sulphate concentrations in the presence of the *Aspergillus niger*

In the presence of the *Aspergillus flavus*, no remarkable decrease in phosphate concentration in the wastewater was observed throughout the period of incubation. At the expiration of the period of incubation, phosphate levels in the wastewater showed a decrease from 22.67 mg/L at 0 h to 18.14 mg/L at 96 h. For sulphate concentration in the wastewater in presence of the *Aspergillus flavus*, remarkable decrease in concentration was observed after 24 h incubation, after which there was an increase with time till 72 h before a further decrease was observed. After the 96 incubation time, sulphate levels decreased from an initial concentration of 3000.83 mg/L to a final level of 2764.23mg/L (Fig. 6).

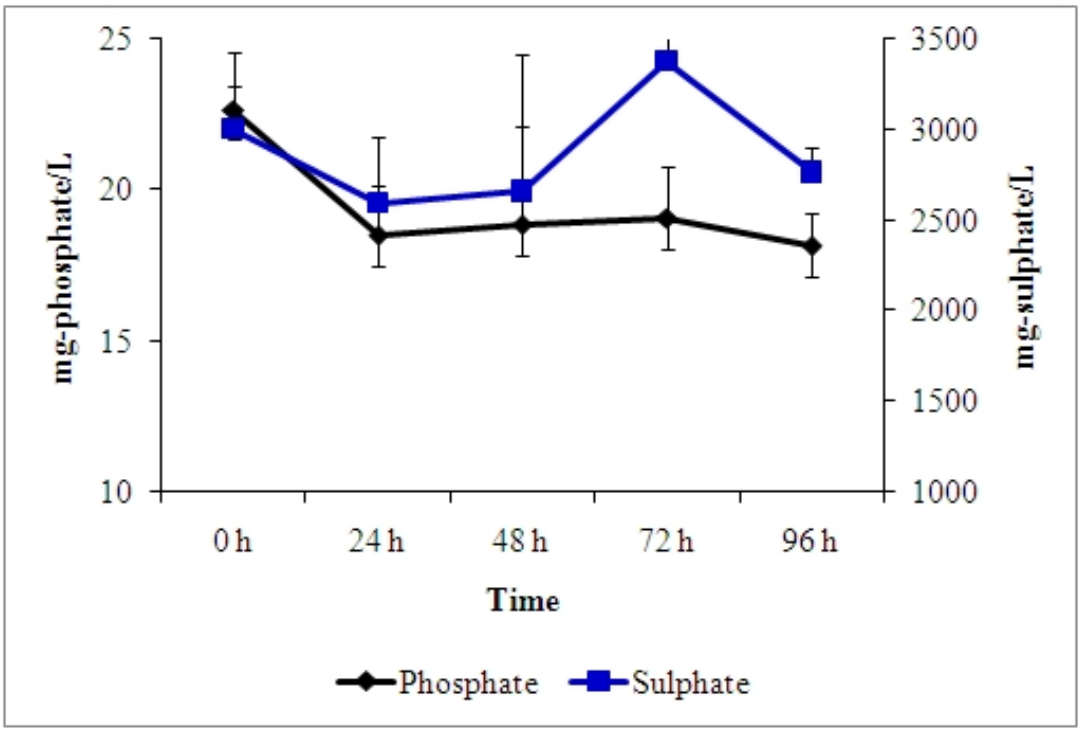

Fig. 6. Variation in phosphate and sulphate concentrations in the presence of the *Aspergillus flavus*

When inoculated with the *Fusarium* spp., phosphate levels showed slight decreases with time till after 72 h incubation, before a slight increase was observed. From an initial level of 24.25 mg/L, phosphate level was observed to decrease to 18.43 mg/L. In the case of sulphate concentration in the wastewater, there were consistent remarkable decreases in concentration with time. At the expiration of incubation, sulphate levels showed a decrease from an initial concentration of 3364.40 mg/L to a final concentration of 1869.75 mg/L (Fig.7). As shown in Fig. 8, in the presence of the *Absidia* spp., no remarkable decreases in phosphate and sulphate concentrations in the wastewater were observed throughout the period of incubation, although slight decreases were observed at the expiration of incubation time. Phosphate and sulphate levels in the presence of the *Absidia* spp. showed a decrease from initial concentrations of 22.36 mg/L and 2798.03 mg/L to final levels of 17.61 mg/L and 2787.31 mg/L, respectively (Fig. 8).

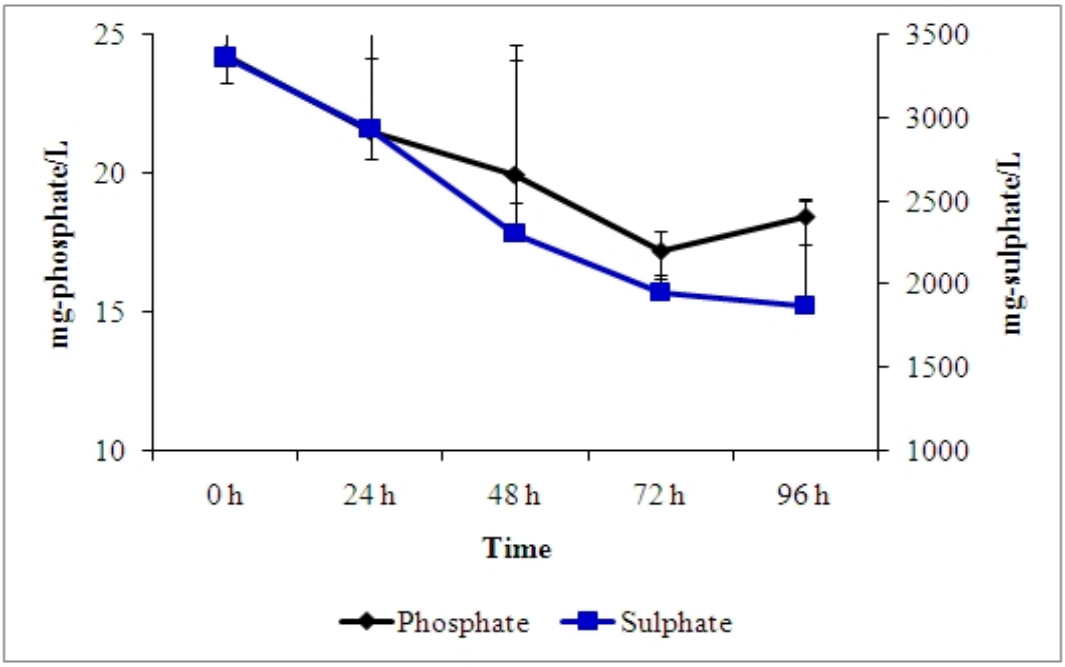

Fig. 7. Variation in phosphate and sulphate concentrations in the presence of the *Fusarium* spp

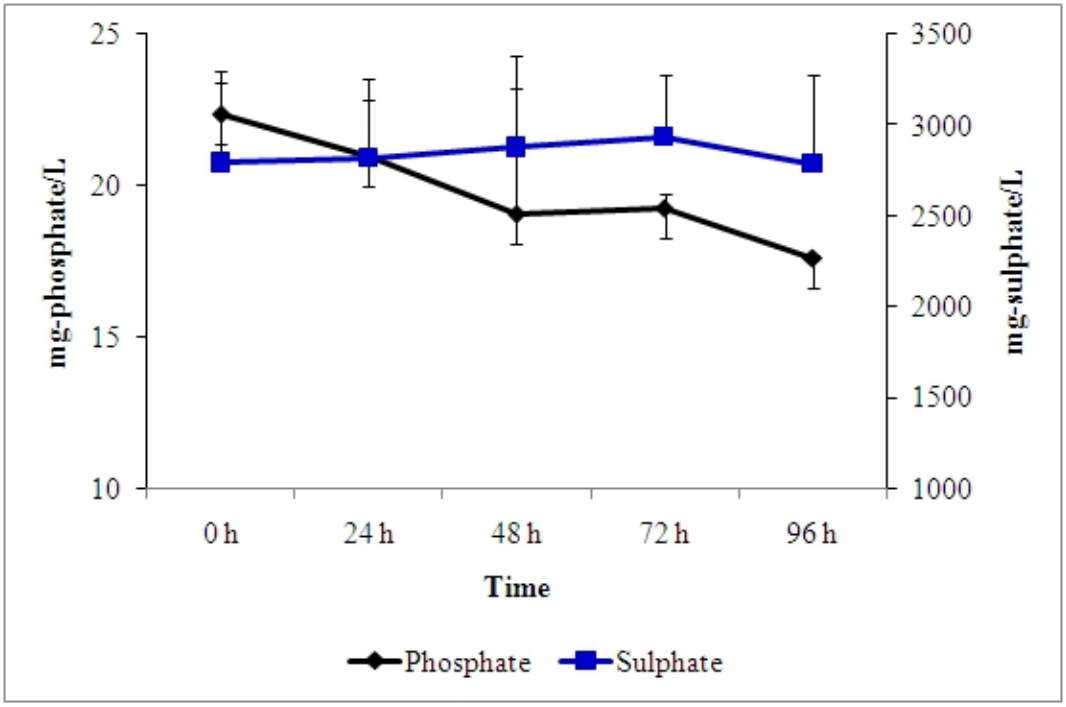

Fig. 8. Variation in phosphate and sulphate concentrations in the presence of the *Absidia* spp

This study made use of sodium acetate as the external carbon source in the wastewater. The use of acetate as external carbon source in nutrient removal studies have been reported by earlier investigators [18,19]. It is indicated also that efficient application of sulphate reducing bacteria in bioremediation processes is dependent on the selection of an energy source. Although some sulphate reducing bacteria have the ability to utilize different contaminants in the environment, including halogenated compounds and constituents of petroleum hydrocarbons as source of energy, the general preference is said to be for low molecular weight organic compounds [20].

In the present study, three of the test bacterial (*Pseuodmonas* spp., *Staphylococcus* spp. and *Klebsiella* spp.) isolates and one of the fungi (*Aspergillus niger)* showed remarkable phosphate removal after 24 h incubation. Although the mechanism of removal of phosphate by the isolates was not investigated in the present study, it is indicated that the mechanism of biological phosphate removal from wastewater is achieved in two ways; stoichiometric coupling to microbial growth and enhanced storage in the biomass as polyphosphate, which is also referred to as ``luxury uptake'', which is said to be the key mechanism in the enhanced biological phosphate removal process [21].

The further increase of phosphate and sulphate after initial reduction especially after 24 h incubation period as seen with some isolates in this study could be that the microbial biomass became saturated with absorbed nutrients and could no longer take up more and as the incubation progressed, they tend to release some of the already absorbed nutrients back into the medium.

In the case of sulphate removal, this study revealed significant decreases in concentrations after 48 h incubation in the presence of the *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger.* In the presence of the *Fusarium* spp., a steady decrease in concentration was observed with time throughout the period of incubation. In a study by Maree et al. [22], on integrated process for biological sulphate removal and sulphur recovery, in the presence of sufficient carbon and energy source, concentration was removed consistently down to 200 mg/L from an initial concentration of 2203 mg/L. It is also reported that in other studies, optimal sulphate reduction rates is achieved when sufficient carbon and energy source are present and when pH is higher than 7.5 and lower than 8.5 [23].

This investigation was carried out at incubation temperature of 30ºC. The biological sulphate removal is indicated to depend on the performance of the sulphate reducing bacteria. In the presence of organic matter and sulphate, bacteria have the ability to reduce sulphate to sulphide. The majority of sulphate reducing bacteria are said to be mesophilic although they can perform within temperature range of 10-50ºC. With a rise in temperature, growth, chemical and enzymatic reactions in the cell is indicated to proceed at faster rates. A study by Greben and co-workers [23] on the effect of different parameters on the biological volumetric and specific sulphate removal rates, carried out at temperatures of 15ºC and 25ºC, maximum sulphate was revealed that although that sulphate was reduced at 15ºC, the reduction rate was lower than at 25ºC [23].

4. CONCLUSION

The present study was aimed at ascertaining the efficiency of test bacterial and fungal species in the removal of phosphate and sulphate from wastewater was able to reveal the following:

- In the presence of three of the test bacterial species, phosphate removal under the experimental condition was remarkably removed from the wastewater within the first 24 h of incubation in, after which there was an increase in concentration
- For sulphate concentration in the presence of the bacterial species, remarkable removal was only observed after the first 48 h of incubation, after which there was observed increases in concentration.
- In the presence of the *Aspergillus niger* and *Aspergillus flavus*, remarkable decreases in phosphate and sulphate levels were only observed after the first 48 h of incubation, after which increases were observed.
- There were consistent decreases in phosphate and sulphate levels with time in the presence of the *Fusarium* species.
- Both the *Lysinibacillus* and *Absidia* species did not show any remarkable phosphate and sulphate removal ability throughout the period of incubation

Although we cannot conclude that this study was exhaustive, it has still been able to provide an insight to the phosphate and sulphate removal ability of the test isolates under the experimental conditions. The effects of several factors, such as temperature, pH, carbon sources and carbon-source concentrations on the phosphate and sulphate removal ability of the isolates are the subject of further investigation.

Despite the fact that the study cannot be considered to be exhaustive, the findings were able to reveal the role of temperature in the sulphate removal from wastewater by the test bacterial and fungal isolates under the experimental conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dahunsi SO, Oranusi US. Haematological response of *Clarias gariepinus* to rubber processing effluent. Annual Review and Research in Biology. 2013;3(4):624-635.
2. Akpor OB, Momba MNB, Okonkwo JO. The effects of pH and temperature on phosphate and nitrate uptake by wastewater protozoa. African Journal of Biotechnology. 2008;7(13):2221-2226.
3. Becker EW. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge; 1994.
4. Bektas N, Akbulut H, Inan H, Dimoglo A. Removal of phosphate from aqueous solutions by electrocoagulation. Journal of Hazardous Materials. 2004;106:101–105.
5. Chiban M, Soudani A, Sinan F, Persin M. Characterization and application of dried plants to remove heavy metals, nitrate and phosphate ions from industrial wastewaters in a batch system. CLEAN - Soil, Air, Water. 2011a;39:376-283.
6. Chiban M, Soudani A, Sinan F, Persin M. Single, binary and multi-component adsorption of some anions and heavy metals on environmentally friendly Carpobrotus edulis plant, Colloids and Surfaces B: Biointerfaces. 2011b;82:267-276.
7. De-Bashana LE,Yoav BB. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). Water Research. 2004;38(19):4222–4246.
8. Ramothokang TR, Simelane SC, Bux F. Biological nitrogen and phosphorus removal by filamentous bacteria in pure culture. Water Institute of South Africa (WISA) Biennial Conference, Durban, South Africa; 2006.

9. Sathasivan A. Biological phosphorus removal processes for wastewater treatment. Encyclopeadia of Life Support Systems; 2011. Available at: http://eolss.net/Eolss-sampleAllChapter.aspx. Accessed 20/03/2014.
10. Baetens D. Biological phosphorus removal. Modelling and Experimental Design; 2001. Available at: www.biomath.ugent.be/biomath/publications/.../baetensdanielle_sum.pdf. Accessed 20/03/2014.
11. Zoski E. Biological Sulphate Removal for Livestock Production; 2009. Available at: www.wbdc.sk.ca. Accessed 20/03/2014.
12. Al-Zuhair S, El-Naas, MH, Al-Hassani, H. Sulfate inhibition effect on sulfate reducing bacteria. Journal of Biochemistry and Technology. 2008;1(2):39-44.
13. Macingova E, Luptakova A. Removal of sulphates from mining waters using the sulphate-reducing bacteria. Mineralia Slovaca. 2010;42:333-336.
14. Greben HA, Matshusa MP, Maree JP. The biological Sulphate removal technology. 9th International Mine Water Congress; 2006.
Available at: www.imwa.info/docs/imwa_2005/IMWA2005_048_Greben.pdf. Accessed 20/03/2014.
15. Adelani-Akande TA, Akpor OB, Aderiye BI. Investigation of the role of selected fungal strains in the removal of phosphate and nitrate in synthetic wastewater. Annual Research & Review in Biology. 2014;4(7):1045-1058.
16. Akpor OB, Olaolu TD, Okolie EC. The effect of temperature on nitrate and phosphate uptake from synthetic wastewater by selected bacteria species. British Microbiology Research Journal. 2014;4(3):328-342.
17. APHA. Standard Methods for the Examination of Water and Wastewater, 22nd edition. APHA, Washington D.C; 2012.
18. Liu Y, Chen Y, Zhou Q. Effect of initial pH control on enhanced biological phosphorus removal from wastewater containing acetic and propionic acids. Chemosphere. 2007;66:123-129.
19. Bharati B, Kumar GP. A study on efficiency of five different carbon sources on sulfate reduction. Journal of Environmental Research and Development. 2012;7(1):416-420.
20. Hussain A, Qazi JI. Biological sulphate reduction using watermelon rind as a carbon source. Biologia (Pakistan). 2012;58(1-2):85-92.
21. Van Loosdrecht MCM, Heijnen JJ. Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Research. 1998;32(11):3193-3207.
22. Maree JP, Netshidaulu I, Strobos G, Nengovhela R, Geldenhuys AJ. 2004. Integrated process for biological sulphate removal and sulphur recovery. Proceedings of the Water Institute of Southern Africa (WISA) Biennial Conference, Cape Town, South Africa; 2004.
23. Greben HA, Bologo H, Maree JP. Effect of different parameters on the biological volumetric and specific sulphate removal rates. Paper presented at the Biennial Conference of the Water Institute of Southern Africa, Durban, South Africa; 2002.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=560&id=22&aid=4907