International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

---

# Effect of Initial pH on Sulphate and Phosphate Uptake from Wastewater by Selected Bacterial and Fungal Species

O. B. Akpor [a]*, S. O. Dahunsi [a], R. Aransiola [b]

[a] *Microbiology Unit, Department of Biological Sciences, Landmark University, PMB 1001, OmuAran, Kwara State, Nigeria*

[b] *Department of Biochemistry, University of Ilorin, Kwara State, Nigeria*

**Abstract**

This study was aimed at investigating the effect of pH on sulphate and phosphate uptake from wastewater by selected bacterial and fungal species. A total of four each of bacterial and fungal isolates were used under shaking flasks conditions. The wastewater was supplemented with sodium acetate to serve as external carbon source at a concentration of 5 g/L. Immediately after inoculation with the respective isolates and at 24 h intervals, for the next 96 h, aliquot wastewater samples were taken from each flask for the estimation of total phosphate and sulphate in the wastewater, using standard procedures. The results revealed remarkable sulphate removal at pH 12. Apart from the *Klebsiella* sp., which showed high phosphate removal at pH 6, none of the isolates showed high phosphate removal at the different pH ranges. From an initial concentration of 480.22 mg/L, sulphate levels in the wastewater inoculated with the respective isolates were observed to decrease after 24 h incubation to 256.23 mg/L, 271.23 mg/L, 234.30 mg/L and 254.77 mg/L, in the presence of the *Aspergillus niger, Aspergillus flavus, Fusarium and Absidia* species, respectively. For bacterial isolates, concentrations of sulphate after 24 h incubation were 290.85 mg/L, 218.14 mg/L, 278.16 mg/L and 298.93 mg/L, in the presence of the *Pseudomonas*, *Klebsiella, Lysinibacillu*s and *Staphylococcus* species, respectively. The study was able to reveal the optimum pH for sulphate and phosphate uptake by the test microbial species.

***Keywords:*** Sulphate; Bacteria; Fungi; pH

---

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

## 1. Introduction

A high rate of industrialisation has led to the contamination of wastewater influents with high sulphate and phosphate concentrations. The increase in sulphate concentrations in receiving water bodies is mainly due to wastewater from metallurgical, mining, chemical and textile industries among others. In addition with hydrogen carbonates and chlorides, sulphates are the main anions in natural water ecosystems, with concentrations ranging from ten to hundreds of mg/L in typical ground and surface waters. In some wastewater effluents, sulphate levels can be as high as 4000 mg/L, which is drastically above the acceptable limits of 500 mg/L, as set by various environmental legislations. The presence of sulphate in water, which can be reduced to hydrogen sulphide, can lead to the corrosion of copper, iron and carbon steel. Apart from the corrosion effects, hydrogen sulphide has a unique odour, which said to be very harmful to the environment [1, 13]. Similarly, a high phosphate concentration above the recommended limit of 1 mg/L in receiving water bodies leads to eutrophication [16].

It is reported that the accepted sulphate level is determined mainly from its taste threshold, which is estimated to range from 200 to 500 mg/L, depending on the associated cation. A high sulphate concentration is indicated to act as laxative, especially when in combination with magnesium ion. Under normal conditions, adults are affected at levels of 1000 mg/L, although children could be affected at lesser concentrations. Hydrogen sulphide, which is a product of sulphate reduction, is very toxic. It is known to inhibit a number of enzyme systems and an irritant to the epithelium of the mucous membrane. On inhalation, hydrogen sulphide can paralyse the respiratory centre, with instant death likely to occur at concentrations of 2800 mg/L [9]. The eutrophication of water bodies leads to algal blooms, which impair water quality. Algal blooms results in oxygen depletion for other aquatic life in the ecosystem [11].

Due to the negative impacts of high sulphate and phosphate concentrations in water, a lot of efforts have been made in the treatment of sulphate and phosphate-rich waters. A number of techniques, which include precipitation, membrane separation, desalination and biological methods, are currently in use in the removal of sulphate from water. Because of the relatively low cost and energy consumption, coupled with the environmental friendliness, when compared with physicochemical methods, biological methods are the preferred choice [3, 5, 7].

In microbial sulphate removal, the sulphate-reducing microorganisms compete with methane-producing micro-organisms for the available organic carbon, resulting in the formation of hydrogen sulphide. It is indicated that during the treatment of high-sulphate wastewater, a high concentrations of sulphur compounds hinder the treatment of wastewater and leads to the production of methane gas. This phenomenon is therefore said to result from the microbiological reduction of sulphates into sulphides. For an efficient biological sulphate removal process, there is the need for a suitable and stable pH [15]. An enhanced biological phosphate removal, which is an economical and sustainable method of phosphate removal, is currently adopted in many wastewater treatment systems. As with sulphate removal, in enhanced biological phosphate removal systems, it is indicated that pH plays as important role [16]. This study was therefore aimed at investigating the role of pH in the sulphate and phosphate removal from wastewater by selected fungal and bacterial strains.

## 2. Materials and Methods

Wastewater source and composition: The wastewater used for the study was collected from the Landmark University Commercial Farms in OmuAran, Kwara State, Nigeria. It was filtered using Whatman No 1 filter paper in 250 mL capacity flasks. After filtration, each of the flasks containing the filtered wastewater was supplemented with sodium acetate (5 g/L), peptone (2.5 g/L) and magnesium sulphate (0.5 g/L) and then autoclave at 121 $^{o}$C for 15 min at 15 psi. Before sterilization, the pH of each of the medium in each flask was adjusted to the desired one, using an electronic pH meter.

Test microorganisms: A total of eight organisms, consisting of four each of bacteria and fungi were used for the study. The bacteria isolates were *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Lysinibacillus* species while the fungal isolates were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* and *Absidia* species. The isolates were obtained from the laboratory stock of the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. Prior to use, each of the isolates were plated in petri dishes (nutrient agar for the bacteria and potato dextrose agar for fungi) to ascertain their purity before storing at 4 $^{o}$C in the refrigerator until when needed.

Nutrient uptake studies: This study was carried out at four (3, 6, 9 and 12) different pH range. To each experimental flask, one mL of the test isolates prior suspended in normal saline (0.85 % NaCl in distilled water w/v) was inoculated. The inoculated flasks were then incubated in an incubating shaker at a speed of 150 rpm at 30 $^{o}$C. Immediately after inoculation and at 24 h interval for 96 h, aliquot wastewater sample was removed from each flask for the estimation of sulphate concentration, using standard methods (APHA, 2012). In each batch of experiment, an uninoculated control was also set up. All experimental setups and analyses were carried out in triplicate.

## 3. Results

As shown in Fig. 1, the variation in sulphate concentration in the wastewater at the end of the 96 h incubation period in the presence of the *Aspergillus niger* showed a change from 491.07 to 659.82 mg/L, from 488.86 to 470.32 mg/L, from 493.38 to 468.99 mg/L and from 480.14 to 452.44 mg/L for pH 3, 6, 9 and 12, respectively. Remarkable decreases in sulphate concentration were observed after 24 h for pH 9 (266.62 mg/L) and pH 12 (256.23 mg/L).In the case of phosphate concentration in the wastewater inoculated with the *Aspergillus niger* at the different pH, at the expiration of incubation, concentrations were change from initial value of 75.60 mg/L, 80.39 mg/L, 76.83 mg/L and 75.36 mg/L to 68.48 mg/L, 72.75 mg/L, 97.65 mg/L and 78.06 mg/L, for pH 3, 6, 9 and 12, respectively. Remarkable decreases in concentration to 34.81 mg/L (24 h) and 52.83 mg/L (72 h) were observed only at pH 6 (Fig. 1).

In the presence of *Aspergillus flavus*, after the 96 h incubation period, sulphate concentration was observed to change from 491.07 to 712.22 mg/L, from 491.86 mg/L to 586.72 mg/L, from 493.38 to 422.51 mg/L and from 494.14 to 282.70 mg/L, at pH 3, 6, 9 and 12, respectively. He most remarkable decreases in sulphate concentration were observed after 48 h, for pH 9 and after 96 h, for pH 12, decreasing from 493.38 mg/L to 192.14 mg/L and from 494.14 to 282.70 mg/L, respectively (Fig. 2).

In the case of phosphate concentration in the wastewater, concentration was observed to change from 75.60 to 62.32 mg/L for pH 3, from 80.39 to 75.31 mg/L for pH 6, from 76.83 to 75.46 mg/L, from 76.83 to 85.46 mg/L for pH 9 and from 75.36 to 88.02 mg/L, for pH 12 (Fig. 2)

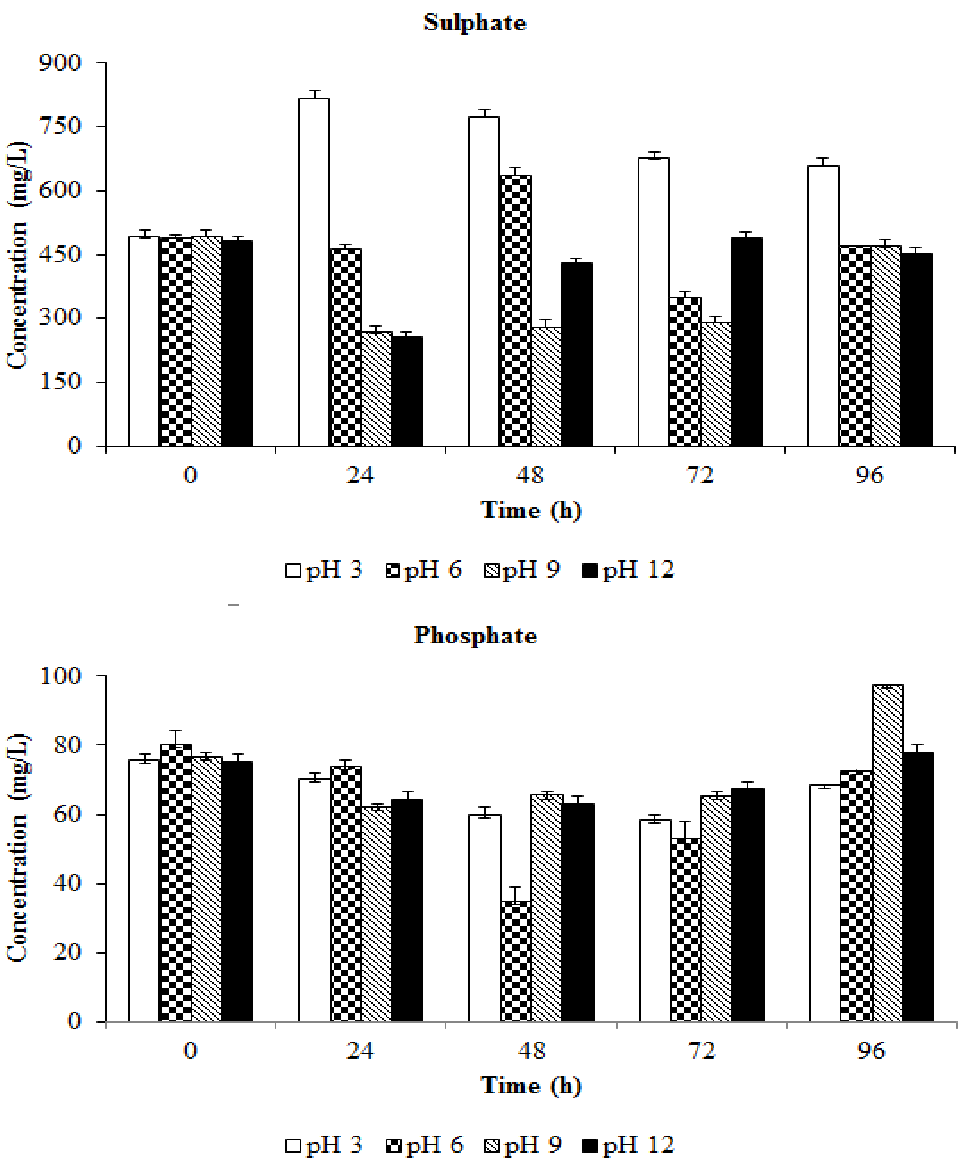


**Fig. 1: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Aspergillus niger***

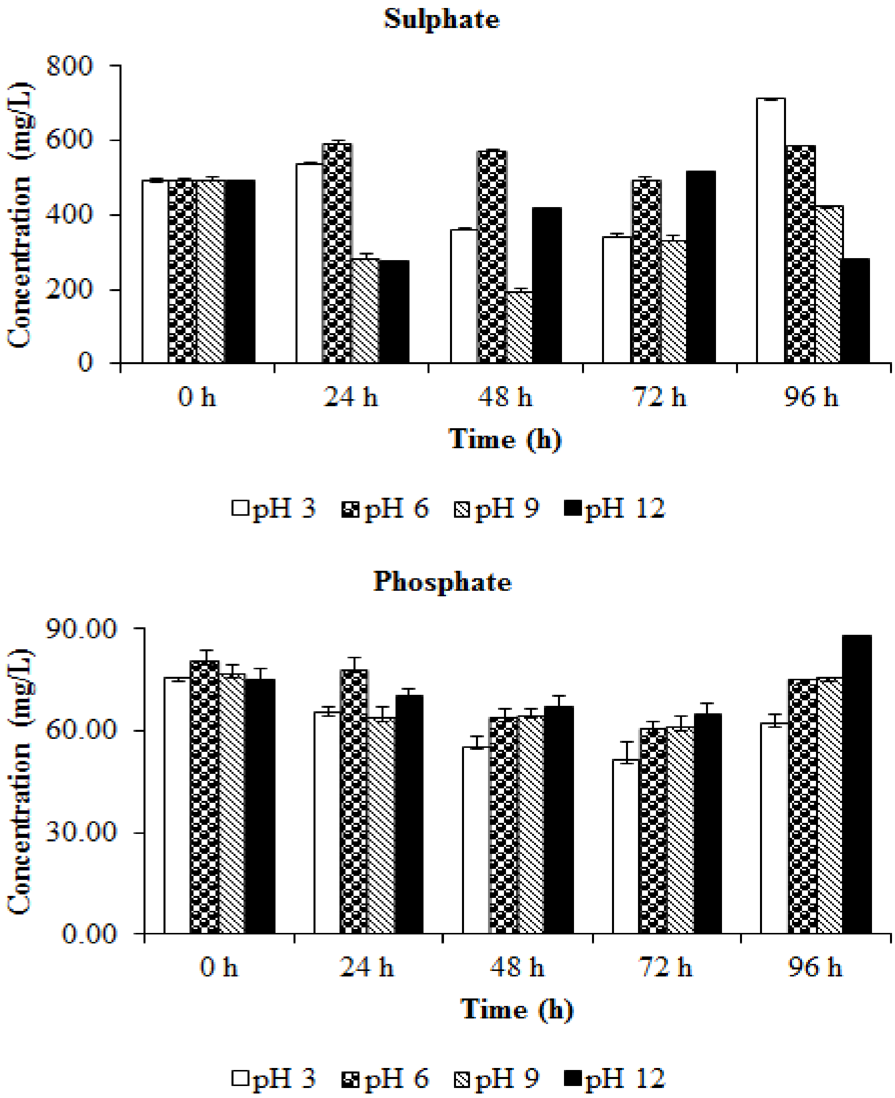


**Fig. 2: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Aspergillus flavus***

The variation in sulphate and phosphate concentrations in the wastewater in the presence of the *Fusarium* sp. is shown in Fig. 3. Sulphate concentration was observed to vary from initial values of 517.07 mg/L, 518.86 mg/L, 519.38 mg/L and 513.14 mg/L to final concentrations of 639.42 mg/L, 538.40 mg/L, 500.92 mg/L and 240.07 for pH 3, 6, 9 and 12, respectively. At pH 9, lowest reduction in concentration (249.30 mg/L) was observed after 24 h incubation while for pH 12, the lowest concentration was observed after 96 h incubation (Fig. 3).

In the case of phosphate in presence of the *Fusarium* sp., concentration was observed to reduce from 75.60 to 66.78 mg/L and from 80.39 to 77.14 mg/L for pH 3 and 6 respectively while increase in values was observed from 76.83 to 86.46 mg/L and 75.36 to 86.08 mg/L for pH 9 and 12 respectively.

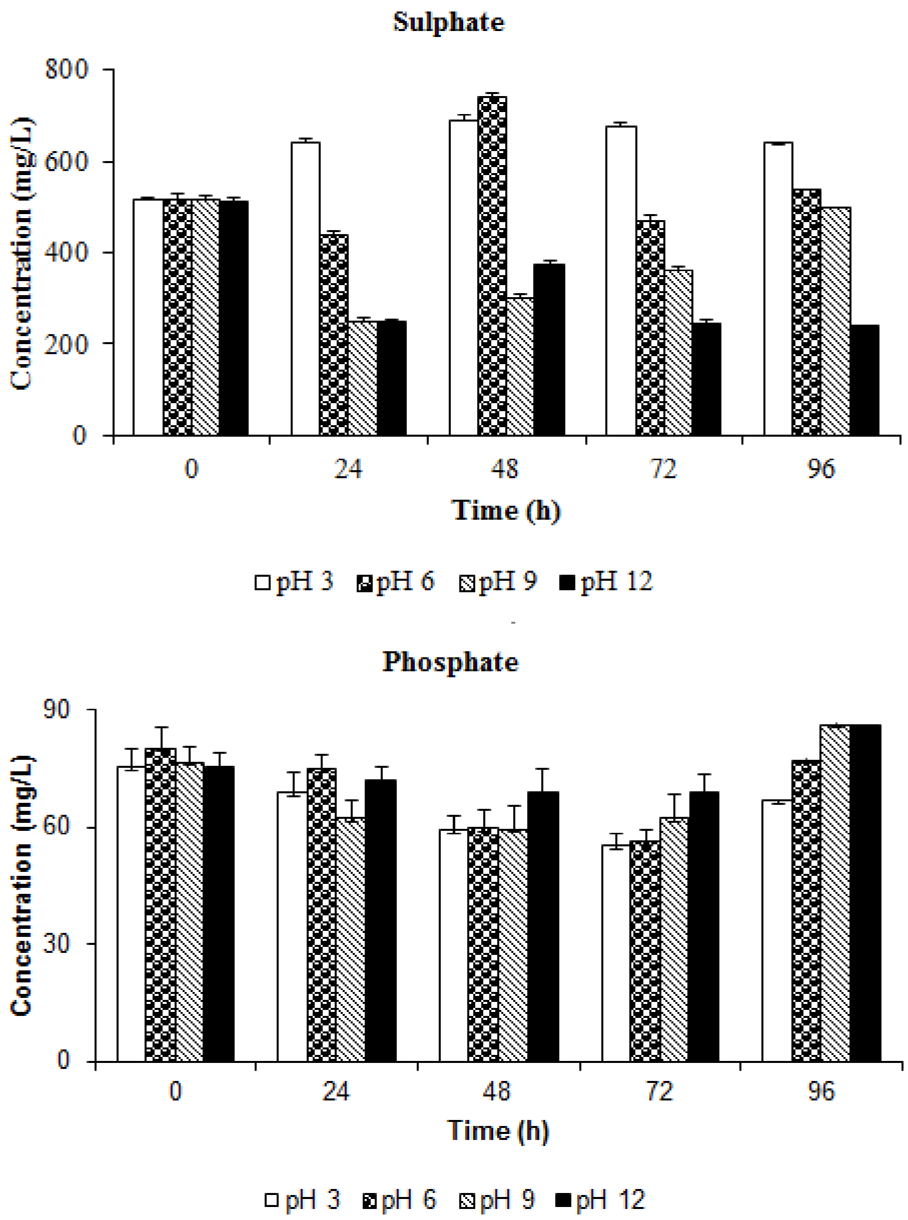


**Fig. 3: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Fusarium* sp.**

With the exception of pH 3, in the presence of the *Absidia* sp., sulphate concentration in the wastewater was observed to decrease after the 96 h incubation. At the expiration of the 96 h, the concentration of phosphate was observed to increase from 517.07 to 717.56 mg/L for pH 3 and decreased from 515.86 mg/L, 519.38 mg/L and 512.14mg/L to 363.57 mg/L, 468.60 mg/L and 230.84 mg/L, for pH 6, 9 and 12, respectively (Fig. 4). The lowest reduction in concentration (170.82) was observed after 48 h incubation for pH 9.

For phosphate concentration in the wastewater in the presence of the *Fusarium* sp., there was a reduction from 75.60 to 59.24 mg/L, for pH 3, while increased values (from 80.39 to 82.10 mg/L, 76.83 to 84.13 mg/L and 75.36 to 79.44 mg/L) were obtained for pH 6, 9 and 12 respectively after the 96 h incubation period (Fig. 4).

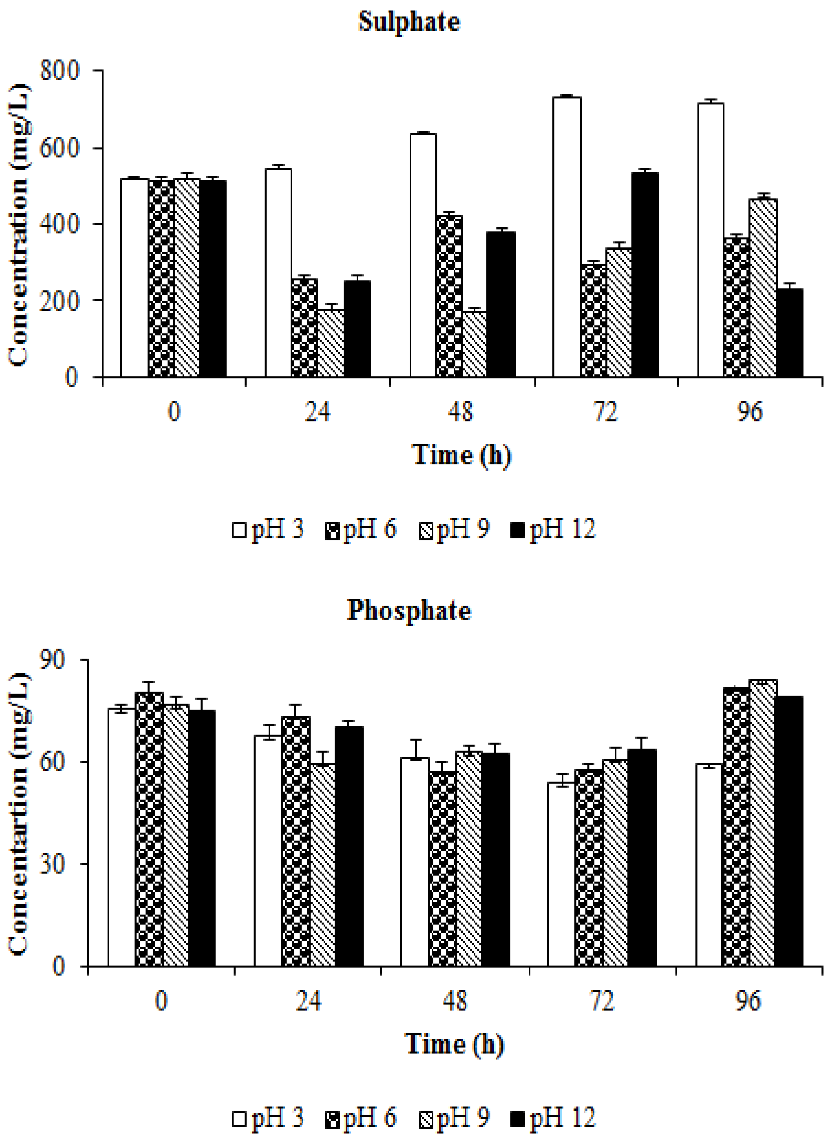


**Fig. 4: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Absidia* sp.**

As shown in Fig. 5, in the presence of the *Klebsiella* sp., sulphate concentration in the wastewater was observed to show minimum decrease after 24 h incubation for pH 6, 9 and 12. At the expiration of the 96 h incubation period, sulphate levels were observed to vary from initial concentrations of 517.07 mg/L, 515.86 mg/L, 519.38 mg/L and 520.14 mg/L to final concentrations of 581.71 mg/L, 408.58 mg/L, 424.74 mg/L and 353.18 mg/L, for pH 3, 6, 9 and 12, respectively (Fig. 5).

In the case of phosphate levels in the wastewater in the presence of the *Klebsiella* sp., remarkable decrease in concentration was only observed after 96 h for pH 6. At other pH levels, phosphate concentrations were observed to increase after 96 h. At the end of the 96 h incubation, phosphate levels were observed to vary from 75.60 to 77.35 mg/L, from 80.39 to 24.85 mg/L, from 76.83 to 77.07 mg/L and from 75.36 to 84.04 mg/L, for pH 3, 6, 9 and 12, respectively (Fig. 5).

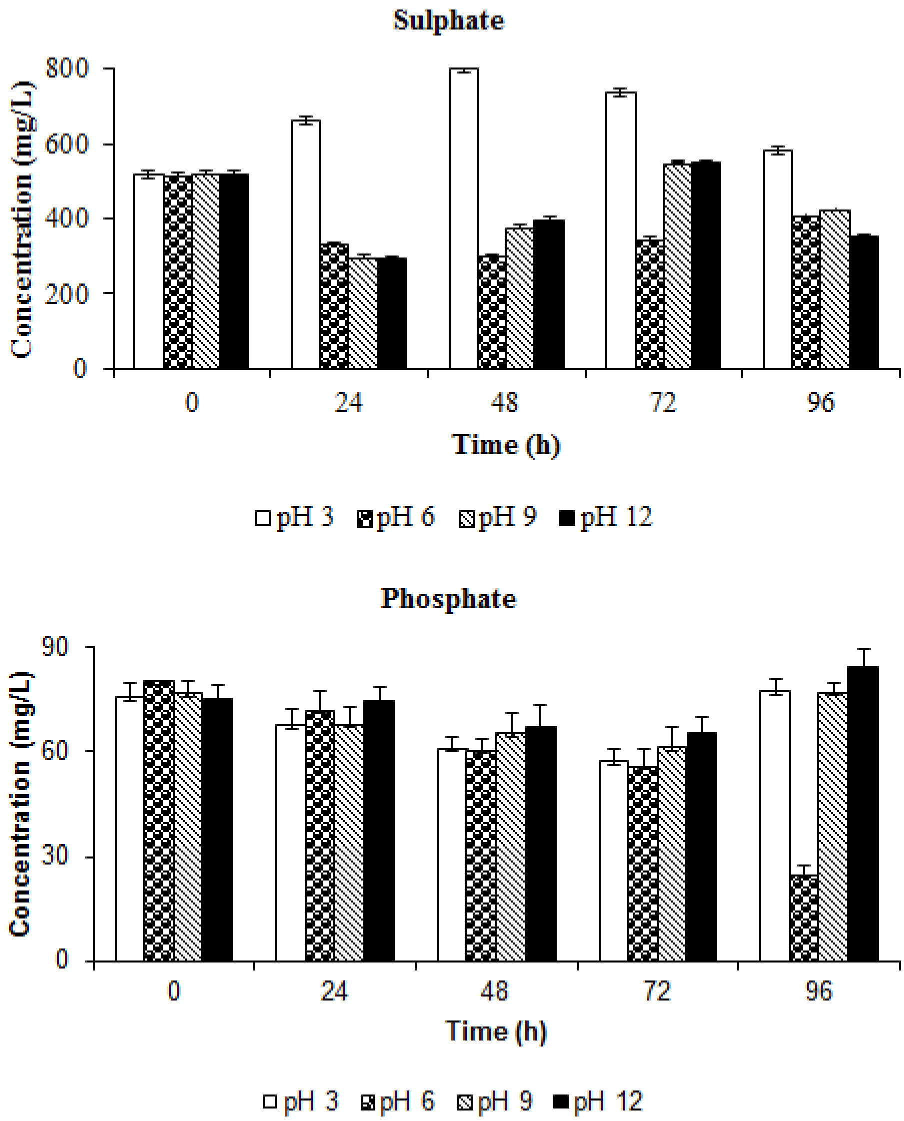


**Fig. 5: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Klebsiella* sp.**

In the presence of the *Pseudomonas* sp., apart from pH 3, where there was remarkable increase in sulphate concentration (from 517.07 to 738.68 mg/L), remarkable decreases in concentration were observed for all others. At the end of the 96 h incubation period, sulphate levels for pH, 6, 9 and 12 decreased from 515.86 to 388.96 mg/L, 519 to 332.40 mg/L and from 515.14 to 181.21 mg/L, respectively (Fig. 6).

For phosphate concentration in the wastewater in the presence of the *Pseudomonas* sp., slight decreases were only observed between 24 and 72 h incubation, after which there were increase which was irrespective of the pH used in the investigation. At the expiration of the incubation period, the concentration of phosphate was observed to vary from 75.60 to 68.01 mg/L, 80.39 to 87.60, 76.83 to 84.51 mg/L and 75.36 to 79.25 mg/L for pH, 3, 6, 9 and 12 respectively (Fig. 6).

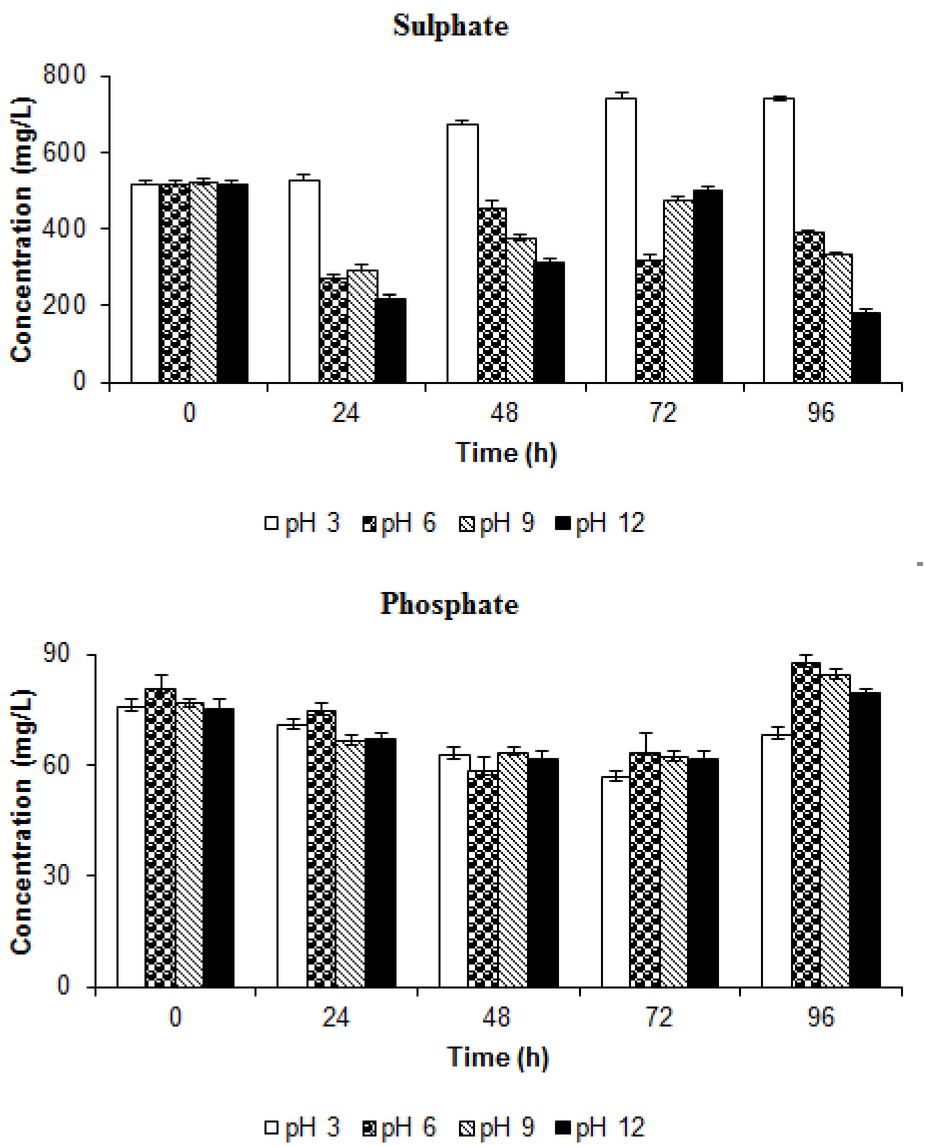


**Fig. 6: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Pseudomonas* sp.**

At the end of the 96 h incubation period, sulphate concentration in the wastewater in the presence of the *Staphylococcus* sp was found to be 704.05 mg/L, 324.32 mg/L, 515.92 mg/L and 304.48 mg/L, for pH 3, 6, 9 and 12, respectively. During the period of incubation, remarkable decreases in concentrations were observed between 24 and 48 h for pH 6, 9 and 12. For pH 3, there was consistent increase in sulphate concentration with time up till 72 h, after which a slight decrease was observed (Fig. 7).

In the case of phosphate levels in the wastewater, after the 96 h incubation period, concentrations were observed to change from initial levels of 75.60 mg/L, 80.39 mg/L, 76.85 mg/L and 75.36 mg/L to final levels of 76.74 mg/L, 76.07 mg/L, 81.67 mg/L and 82.43 mg/L, for pH 3, 6, 9 and 12, respectively. The lowest phosphate values of 49.94 mg/L and 61.65 were observed after 72 h for pH 3 and 12 respectively while lowest levels of 51.93 mg/L and 56.38 mg/l were observed after 48 h (Fig. 7)

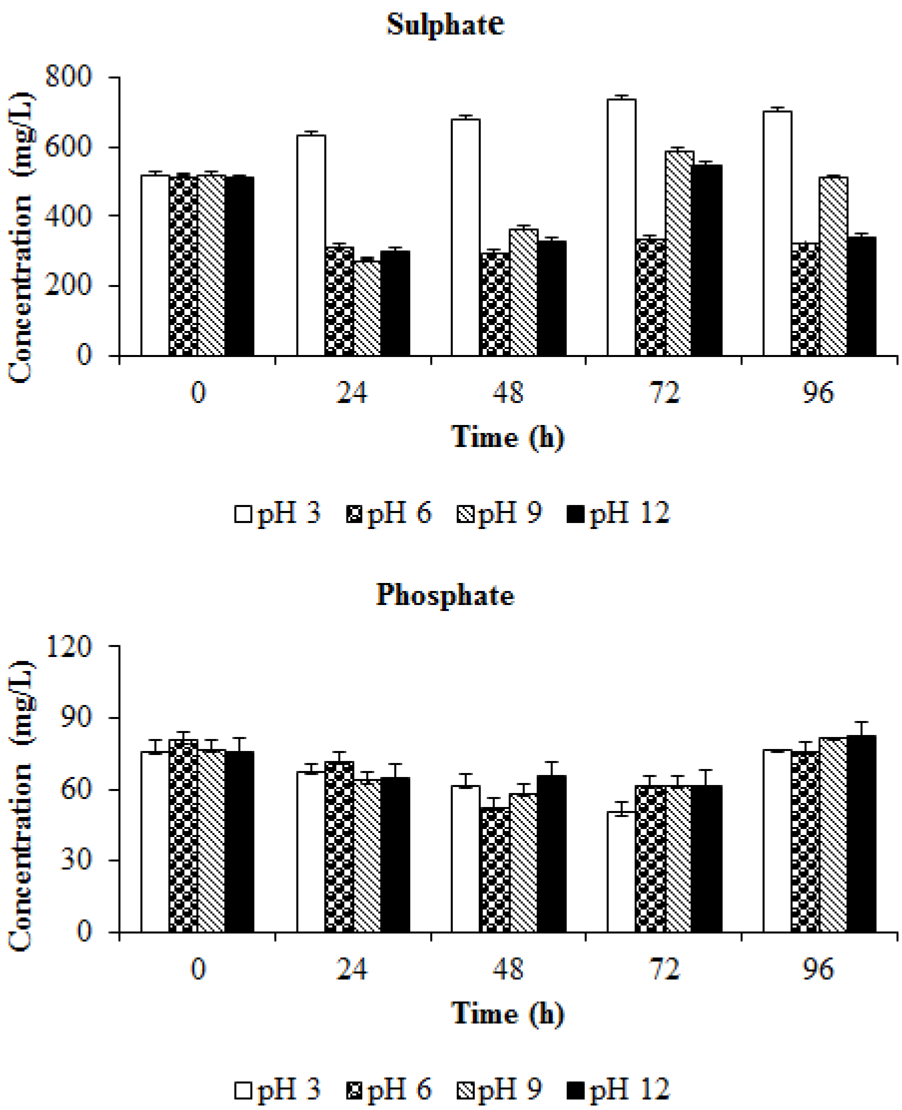


**Fig. 7: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Staphylococcus* sp.**

As shown in Fig. 8, in the presence of the *Lysinibacillus* sp., sulphate levels in the wastewater were observed to vary from initial values of 517.07 mg/L, 515.86 mg/L, 519.38 mg/L and 510.14 mg/L, to final values of 662.50 mg/L, 365.88 mg/L, 407.43 mg/L and 231.99 mg/L, for pH 3, 6, 9 and 12, respectively after the 96 h incubation. For pH 6, 9 and 12, remarkable decreases in sulphate concentrations were observed between 24 and 48 h incubation (Fig. 8).

For phosphate levels in the presence of the *Lysinibacillus* sp., no remarkable decrease in concentration was observed with time. This trend was irrespective of the pH used in the investigation. At the end of the 96 h incubation time, phosphate concentrations were observed to vary from initial levels of 75.60 mg/L, 80.39 mg/L, 76.83 mg/L and 75.36 mg/L to final levels of 62.03 mg/L, 85.46 mg/L, 75.27 mg/L and 89.54 mg/L, for pH 3, 6, 9 and 12, respectively (Fig. 8).

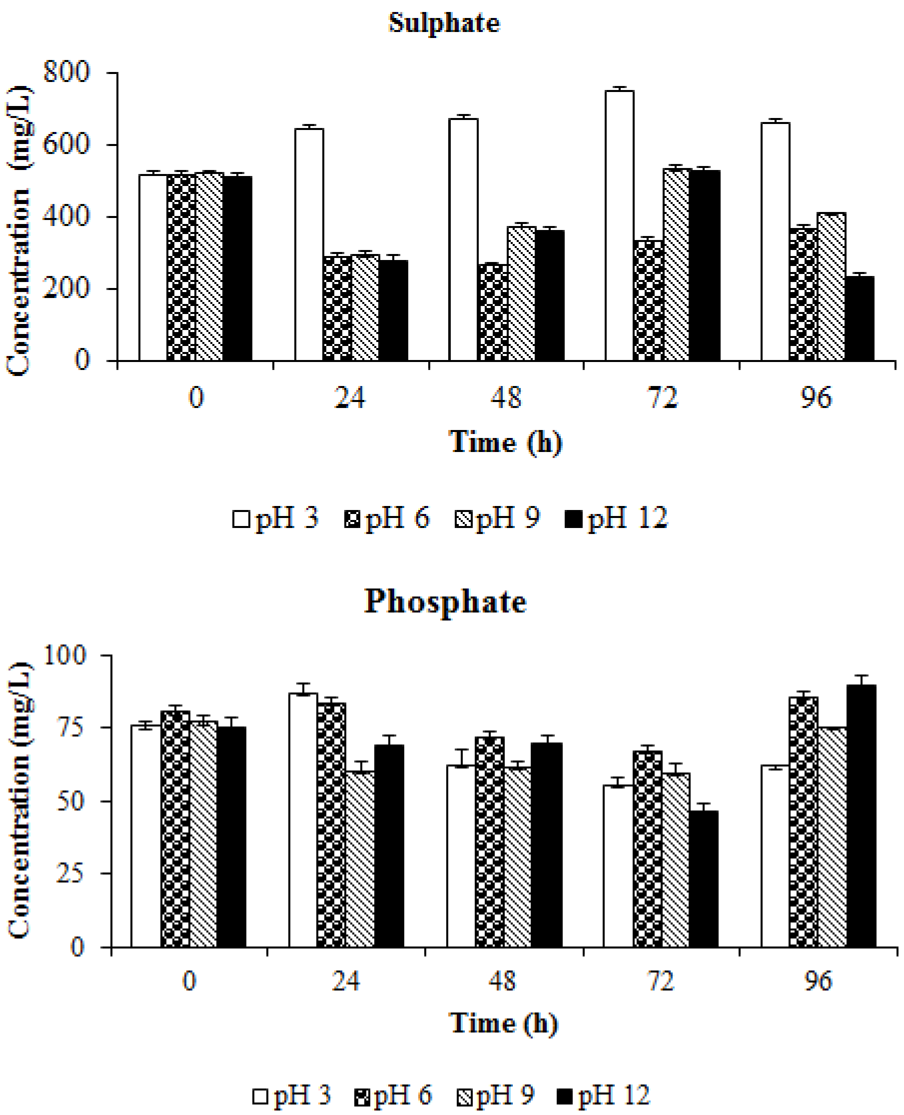


**Fig. 8: Variation in sulphate and phosphate concentration in the wastewater at the different pH by the *Lysinibacillus* sp.**

## 4. Discussion

The wastewater used for this study was supplemented with sodium acetate, to serve as external carbon source. The choice of acetate was due to its extensive and effective usage in most studies on the effect of pH on biological phosphate removal [16]. Also, it has been reported by various authors [2, 8] that synthetic small molecular weight organic compounds like lactate, acetate, propionate, pyruvate and butyrate, ethanol and other alcohols usually serves as good electron donors during biological sulphate removal.

Among the different test isolates used in this study, the optimum pH ranges for sulphate removal was observed to be 9 and 12, with highest removal observed at pH 12. This result contradicts the findings of [5], who reported optimum pH of 4.5 for sulphate removal from wastewater using a bioelectrochemical system. This finding also disagrees with previous reports by [6] and [4], which indicated that sulphate-reducing bacteria are usually suitable for growth in the neutral conditions of pH 6–8 and sensitive to pH changes.

The present study revealed a higher sulphate removal at alkaline pH and little or no sulphate decrease in the wastewater at acidic pH. Although the growth rate of the isolates was not investigated during the course of this investigation, in a study on sulfate inhibition effect on sulfate reducing bacteria by[13] an acidic media (pH 6 and 4) resulted in negligible growth in the sulphate reducing bacteria. It is indicated that pH play an important role, when sulfate is reduced to sulfide. Since sulfide can be present in different forms like $H_2S$, $HS^-$ and $S^{2-}$, its state is solely dependent on the pH of the environment. At a pH of 7.0 most of the sulfide concentration is said to be in the hydrogen sulfide form while at low pH, hydrogen sulfide exists in undissociated form. An increase in pH is indicated to cause a dissociation of the $HS^-$ and $S^{2-}$. In general, sulphate reducing bacteria are reported to be less sensitive to total sulfide at high pH and more sensitive to undissociatedsulphide concentration. At increased pH, less concentration of undissociated $H_2S$ is needed to inhibit the growth [12, 13].

In the case of phosphate, under the experimental conditions used for the investigation, none of the isolates showed remarkable decrease in concentration at the expiration of incubation, except in the presence of the *Klebsiella* sp. At the end of the incubation period, remarkable phosphate decrease was observed at pH 6. During biological phosphate removal, pH is said to play an important role. It has been indicated that a higher pH could lead to greater phosphate release in wastewater containing acetate, which may be due to an increase in the energy requirement for the transportation of acetate. It is therefore hypothesized that the optimal initial pH for remarkable phosphate removal efficiency should be controlled between 6.4 and 7.2 [16]. Earlier workers have indicated the impact of pH on biological phosphate removal. An investigation by [14], on the effect of pH on phosphate removal from wastewater by electro-coagulation with iron plate electrodes indicated optimum phosphate removal at pH range of 7 to 9. In a study by [16] on the effect of various initial pH values on anaerobic and aerobic transformations of soluble ortho-phosphate, phosphate release was observed to decrease when initial pH increased from 6.4 – 6.8, but increased as pH was raised from 6.8 – 8.0. An increase in the pH of wastewater to 8 during biological treatment has been reported to lead to a reduction in phosphate concentration [10].

## 5. Conclusion

This investigation, which was based on the role of pH in sulphate and phosphate uptake by the test microbial isolates, was able to reveal optimum pH for sulphate uptake to be between 9 and 12, after 24 and 48 h incubation. Although all the isolates showed remarkable sulphate uptake ability, only one of the bacteria (*Klebsiella* sp.) showed notable phosphate removal ability under the experimental conditions. The study was able to provide an insight to the role of pH on removal of sulphate and phosphate in wastewater by the test microbial species.

## References

[1] A. Luptakova, I. Kotulicova, E. Macingova, J. Jencarova. "Bacteria elimination of sulphates from mine waters". *Chemical Engineering Transactions*", vol. 35, pp. 853-858, 2013.

[2] B. Bharati, G.P. Kumar. "A study on efficiency of five different carbon sources on sulfate reduction". *Journal of Environmental Research and Development*, vol. 7(1), pp. 416-420, Sep. 2012.

[3] C.T. Benatti, C.R.G. Tavares, E. Lenzi. "Sulphate removal from waste chemicals by precipitation". *Journal of Environmental Management*, vol. 90, pp. 504-511. Jan. 2009.

[4] C.Q. Zhao, Q.H. Yang, W.Y. Chen, H. Li, H. Zhang. "Isolation of a sulfate reducing bacterium and its application in sulfate removal from tannery wastewater". *African Journal of Biotechnology*, vol. 10(56), pp. 11966–11971, Sep. 2011.

[5] F. Liang, Y. Xiao, F. Zhao. "Effect of pH on sulphate removal from wastewater using a bioelectrical system". *Chemical Engineering Journal*, vol. 218, pp. 147-153, Dec. 2013.

[6] F. Widdel, Microbiology and ecology of sulfate-and sulfur-reducing bacteria, In: A.J.B. Zehnder (Ed.), Biology of Anaerobic Microorganisms, Wiley Interscience, New York, 1988

[7] H.H. Chou, J.S. Huang, S.K. Chen, M.C. Lee."Process kinetics of an expanded granular sludge bed reactor treating sulphate-containing wastewater". *Chemical Engineering Journal*, vol. 170, pp. 233-246., May 2011.

[8] H. Harada, S. Uemura, K Momonoi. "Interaction between sulfate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing different levels of sulphate". *Water Research*, vol. 28(1), pp. 355-367, Feb. 1994.

[9] J.G. Kuene, L.A. Robertson. (1992). "The use of natural bacterial populations for the treatment ofsulphur-containing wastewater". *Biodegradation*, vol. 3(2-3), pp. 239-254, 1992.

[10] J. Zhu, A. Luo, P. Ndegwa. "Raising pH by low-level aeration for soluble phosphorus removal of swine manure". *American Society of Agriculture, Biology and Engineering*, vol. 44(2), pp. 391-396, 2001.

[11] O. Korostynska, A. Mason, A. Al-shamma'a.."Monitoring of nitrates and phosphate in wastewater: current technologies and further challenges". *International Journal on Smart Sensing and Intelligent Systems*, vol. 5(1), pp. 149-176, Mar. 2012.

[12] R.H. Perry, D. Green, Perry's Chemical Engineer's Handbook, 6th edn. McGraw-Hill, Singapore, 1994

[13] S. Al-Zuhair, M. El-Nass, H. Al-Hassani. "Sulfate inhibition effect on sulphate reducing bacteria". *Journal of Biochemistry and Technology,* vol. 1(2), pp. 39-44. Sep. 2008.

[14] S. Irdemez, N. Demircioğlu, Y.S. Yildiz."The effect of pH on phosphate removal from wastewater by electro-coagulation with iron plate electrode". *Journal of Hazardous Materials*, vol. B137, pp. 1231-1235, Apr. 2006.

[15] S. Zub, T. Kurisso, A. Menert, V. Blonskaja."Combined biological treatment of high-sulphate wastewater from yeast production". *Water and Environment Journal*, vol. 22, pp. 274-286, Feb. 2008.

[16] Y. Liu, Y. Chen, Q. Zhou. "Effect of initial pH control on enhanced biological phosphorus removal from wastewater containing acetic and propionic acids". *Chemosphere*, vol. 66, pp. 123-129, Jan. 2007.