

Original Research Article

Effect of Temperature on Sulphate Removal from Wastewater by Selected Bacterial and Fungal Species

Abstract

The aim of this study was to investigate the effect of temperature on sulphate removal from wastewater by selected bacterial (*Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Lysinibacillus* spp.) and fungal (*Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp. and *Aspidium* spp.) isolates. The study was carried out under shake flasks conditions at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C at shaking speed of 150 rpm. After inoculation with the respective isolates, aliquot wastewater samples were aseptically removed from each flask prior inoculation and every 24 h, for 96 h for the estimation of sulphate levels in the wastewater and pH, using standard procedures. The results revealed optimum temperatures for sulphate removal to range from 30 °C -35 °C and 30 °C – 40 °C, for the bacterial and fungal isolates, respectively. Also observed was a consistent increase in pH of the wastewater throughout the period of incubation. This trend was irrespective of the test microbial isolates and the incubation temperature. The study was able to reveal the role of temperature in sulphate removal ability of the test isolates under the experimental conditions used for investigation.

Introduction

The release of acidic, sulphate and metal-containing wastewater is an important environmental problem. The discharge of enormous amounts of wastewater that contain high sulphate and dissolved metal concentrations is a main source of pollution in receiving water bodies. The presence of high sulphate levels in water cause is known to cause many environmental Problems, such as taste, odor and laxative effect which can lead to dehydration [1].

Although various methods exist for sulphate removal from wastewater, recently, biological removal processes have gained increasing interest. This is mainly due to the ability of biological processes in the production of effluents that are suitable for discharge into the environment. In addition, biological sulphate removal process is said to have developed over the past few years that it is indicated that it can compete successfully with other sulphate removal technologies for full-scale treatment of mine and other industrial effluents. In addition to metal removal and neutralisation, biological sulphate removal process can be used in the treatment of industrial effluents[2].

It is hypothesised that when sulphate is present in the wastewater stream, biogenic sulphide produced by sulphate-reducing bacteria is an important alternative sulphide source for metal precipitation. Sulphate reducing bacteria are facultative anaerobes that use sulphate ions as the terminal electron acceptor for metabolism of organic substrates The removal mechanism is based on the fact that, under anaerobic conditions, sulphate-reducing bacteria have the ability of oxidizing simple organic compounds, using sulphate as terminal electron acceptor, which is reduced to sulphide[2, 3].

In the presence of organic matter and sulphate, sulphate-reducing bacteria can reduce sulphate to sulphide. A major disadvantage of biological sulphate removal by the addition of organic carbon sources as substrate is indicated to be the high residue organic carbon content or the release of coloured water, which requires downstream treatment. In comparison with chemical treatment with respect to costs is said to be far more economical [4, 5].

It is reported that thermal adaptation and flexibility of microorganisms to function effectively at different temperature regimes is related to underlying molecular mechanisms[6]. Previous studies on on extracellular enzymes isolated from marine bacteria report the possibility that the temperature optimum of the initial step of organic carbon remineralization may exceed the temperature optima of terminal steps of remineralization [7]. Although there was reports on terminal processes of organic carbon remineralization[8], such as sulphate reduction and oxygen consumption measured in environments which experience massively different temperature regimes, as well as the specific temperature dependence of sulphate reduction[9], there is not much information on the role of temperature on sulphate uptake by individual bacterial and fungal species. This study was therefore aimed at investigating the effect of temperature on sulphate removal from wastewater by selected bacterial and fungal strains

Materials and Methods

Test microorganisms

A total of eight microorganisms, consisting of four fungal and four bacteria species were used for this investigation. The fungal isolates were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* spp. and *Absidia* spp., while the bacterial isolates were *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Lysinibacillus* spp. The isolates were obtained from the laboratory stock of the Department of Biological Sciences, Landmark University, OmuAran, Kwara State, Nigeria. The isolates have previously been used for nitrate and phosphate removal studies in synthetic wastewater (Akpor et al., 2013; Olaolu et al., 2013).

Wastewater media

The wastewater used for the study was collected from a waste stabilization pond in the Landmark University Commercial Farms located in OmuAran, Kwara State, Nigeria. The wastewater was filtered in 200 mL quantity into 250 mL capacity Erlenmeyer's flasks, using Whatman No. 1 filter paper and then supplemented with sodium acetate (5 g/L) and magnesium sulphate (0.15 g/L) and then sterilised in an autoclave for 15 min at 121 °C at 15 psi.

Sulphate removal studies

Before inoculation with a test isolate, all sterilised flasks were left in the incubator for 24 h, after which samples were taken from them and plated on nutrient and sabourand dextrose agar to observe for bacterial and fungal growth, respectively. This was done to ascertain the efficiency of the sterilisation. Only flasks that showed no growth on the respective media were used for sulphate removal studies.

To each sterile flask containing the wastewater media, one mL of an overnight broth culture of the test isolate was inoculated before incubating in a shaking incubator at 30 °C at a shaking speed of 150 rpm. Prior inoculation and every 24 h for 96 h, aliquot wastewater samples (30 mL) were withdrawn from each incubated flask for the estimation of pH and sulphate concentration in the wastewater.

All experimental analyses were carried out in triplicate. The reagents that were used were of analytical grade

Results

In the presence of the *Aspergillusniger*, sulphate concentration at the different temperatures showed a decrease from 5944.79 mg/L to 4712.71 mg/L, from 5880.51 mg/L to 4764.04 mg/L, from 5862.65 mg/L to 4425.22 mg/L and from 5944.79 mg/L to 5277.41 mg/L, at incubation temperatures of 25 °C, 30°C, 35°C and 40°C, respectively (Fig. 1). These decreases translate to 20.72 % at 25 °C, 18.99 % at 30°C, 24.52 % at 35°C and 11.23 % at 40°C.

The pH variation of the wastewater in the presence of the *Aspergillusniger* revealed an increase at the end of the 96 h incubation period. At the expiration of the period of incubation, percent increases in the pH were found to be 13.75 %, 19.47 %, 12.10 % and 15.04 %, at incubation temperatures of 25 °C, 30°C, 35°C and 40°C, respectively (Table 1).

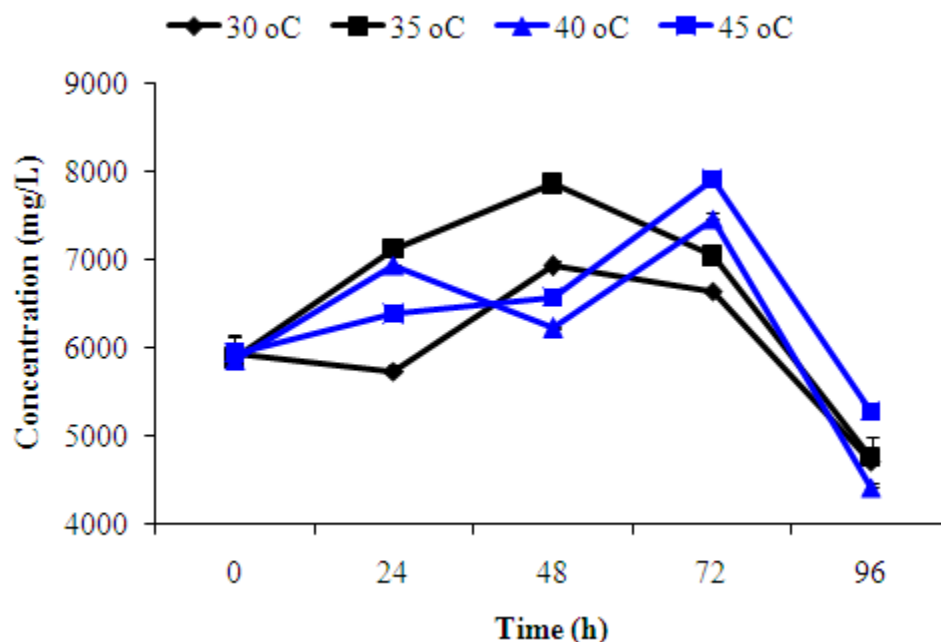


Fig. 1: Sulphate concentrations in the presence of the *Aspergillusniger* at the different incubation temperatures

Table 1: Variation in pH of the wastewater at the different temperatures in presence of the test fungal species

Time	Incubation Temperatures			
	30 °C	35 °C	40 °C	45 °C
<i>Aspergillusniger</i>				
0 h	6.4	6.4	6.4	6.4
24 h	6.8	6.3	7.0	6.8
48 h	6.6	7.2	7.0	7.2
72 h	6.8	7.8	7.1	7.3
96 h	7.5	8.0	7.3	7.6
% increase	13.75	19.47	12.10	15.0
<i>Aspergillusflavus</i>				
0 h	6.4	6.4	6.4	6.4
24 h	7.0	6.8	6.9	6.9
48 h	7.2	7.6	7.0	7.2
72 h	7.3	7.3	7.6	7.6
96 h	8.2	8.0	7.0	8.3
% increase	21.75	19.67	8.57	22.6
<i>Fusariumspp.</i>				
0 h	6.4	6.4	6.4	6.4
24 h	6.8	6.6	6.9	6.9
48 h	7.5	8.2	7.0	7.6
72 h	8.3	8.0	7.4	7.4
96 h	8.4	7.9	7.1	7.8
% increase	22.94	18.93	9.17	17.5
<i>Absidiaspp.</i>				
0 h	6.4	6.4	6.4	6.4
24 h	8.2	7.5	7.0	8.8
48 h	8.5	8.0	7.5	8.6
72 h	8.1	7.9	7.8	8.4
96 h	8.2	7.9	8.3	8.4
% increase	21.84	18.69	22.50	23.00

All values are averages of triplicate analysis.

As shown in Fig. 2, in the presence of the *Aspergillusflavus*, sulphate concentration in the wastewater at the end of the 96 h incubation was observed to show decrease from 5944.79 mg/L to 4897.52 mg/L at 30 °C, from 5780.51 mg/L to 5739.44 mg/L at 35 °C, from 5862.65 mg/L to 5215.81 mg/L at 40 °C, from 5944.79 mg/L to 5277.41 mg/L at 45 °C (Fig. 2).

With respect to pH of the wastewater in the presence of the *Aspergillusflavus*, there was a steady increase with time at the different temperatures. After the 96 h incubation time, pH levels were

found to increase from the initial of 6.4 to 8.2, 8.0, 7.0 and 8.3, at 30 °C, 35 °C, 40 °C and 45 °C, respectively (Table 1).

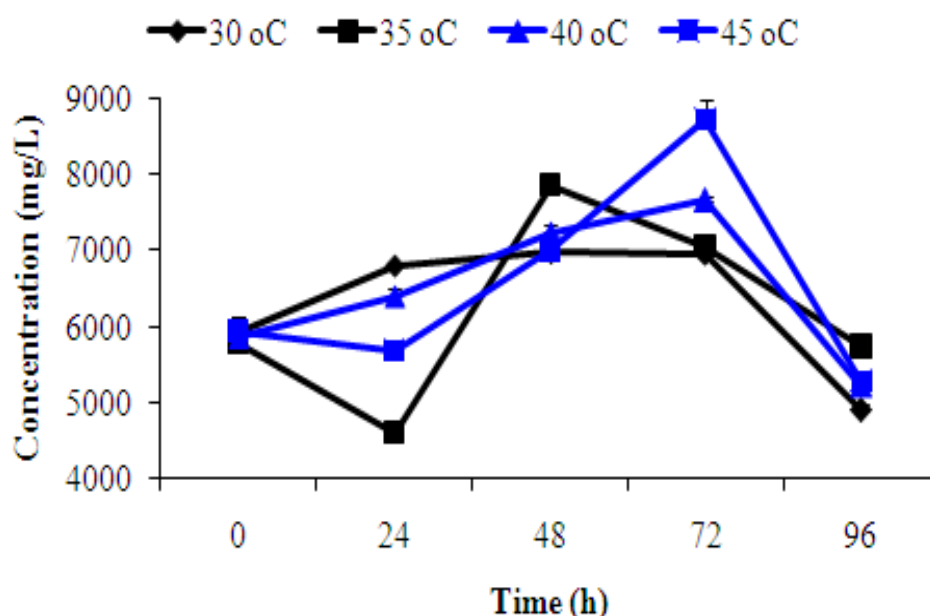


Fig. 2: Sulphate concentrations in the presence of the *Aspergillusflavus* at the different incubation temperatures

When inoculated with *Fusarium*spp., sulphate concentration in the wastewater was observed to show an increase after 96 h at incubation temperatures of 35 °C and 45 °C. Only slight decreases in concentration were observed at incubation temperatures of 30 °C and 40 °C. At the expiration of the 96 h incubation period, sulphate levels in the wastewater were observed to change from 5944.79 mg/L to 5493.02 mg/L, from 5780.51 mg/L to 5913.99 mg/L, from 5862.65 mg/L to 5421.15 mg/L and from 5944.79 mg/L to 5965.32 mg/L, at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively (Fig. 3).

From an initial value of 6.4, the pH of the wastewater in the presence of the *Fusarium*spp. was observed to increase to 8.4, 7.9, 7.1 and 7.8, after the 96 h incubation period at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively. This translates to increases of 22.94 %, 18.93 %, 9.17 % and 17.54 %, respectively (Table 1).

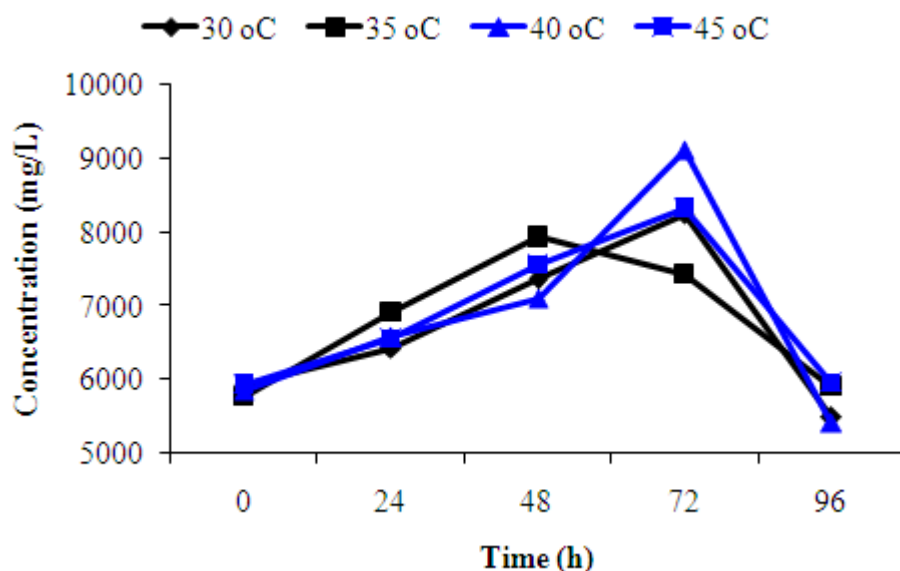


Fig. 3: Sulphate concentrations in the presence of the *Fusarium* spp. at the different incubation temperatures

In the presence of the *Absidia* spp., remarkable sulphate was removed from the wastewater, at incubation temperatures of 30 °C and 35 °C, decreasing from concentrations of 5944.79 mg/L to 4137.74 mg/L and from 5780.51 mg/L to 4599.77 mg/L, respectively. At incubation temperatures of 40 °C and 45 °C, sulphate level at the end of the 96 h incubation period changed from initial levels of 5862.65 mg/L and 5944.79 mg/L to final levels of 6201.47 mg/L and 5677.84 mg/L, respectively (Fig. 4).

In terms of pH of the wastewater in the presence of the *Absidia* spp., although there was an increase at the expiration of the incubation period, the change in pH did not follow any pattern with incubation. This trend was irrespective of the incubation temperatures. At the end of incubation, pH was observed to increase from the initial value of 6.4 to 8.2, 7.9, 8.3 and 8.4, at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively (Table 1).

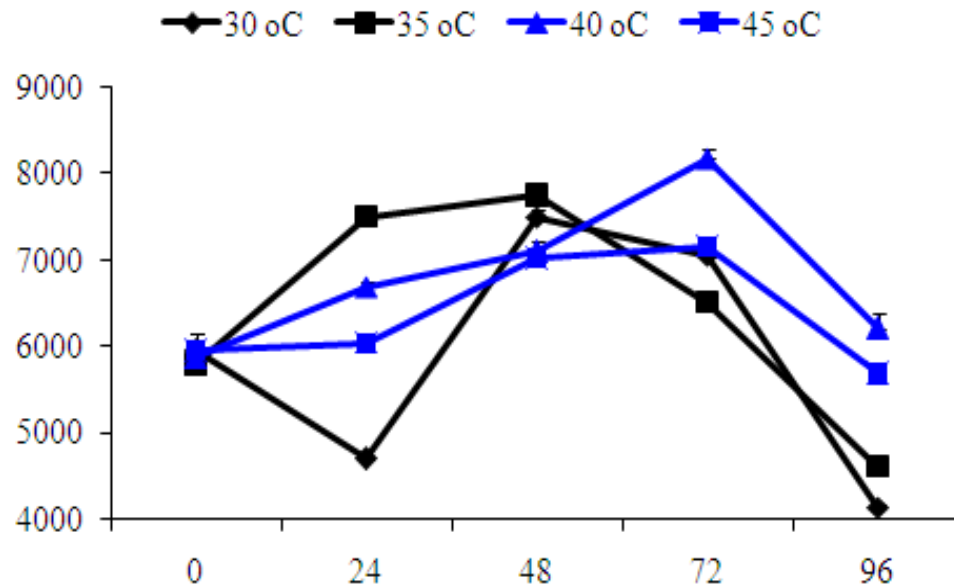


Fig. 4: Sulphate concentrations in the presence of the *Absidia* spp. at the different incubation temperatures

Sulphate concentration in the wastewater in the presence of the *Pseudomonas* spp. showed decreases after 96 h incubation at incubation temperatures of 30 °C and 35 °C. At 40 °C and 45 °C incubation temperatures sulphate levels at the end of incubation were observed to increase. From the initial concentrations of 5842.11 mg/L, 5780.51 mg/L, 5831.85 mg/L and 5944.79 mg/L, sulphate levels were found to be 5102.86 mg/L, 4733.24 mg/L, 6263.07 mg/L and 7885.31 mg/L, at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively (Fig. 5).

With respect to pH of the wastewater in the presence of the *Pseudomonas* spp., there was an observed consistent increase with time. This trend was irrespective of the incubation temperatures. At the expiration of the 96 h incubation time, pH levels increased from the initial 6.4 to 7.4, at 30 °C, 8.5 at 35 °C, 7.5 at 40 °C and 6.9 at 45 °C (Table 2).

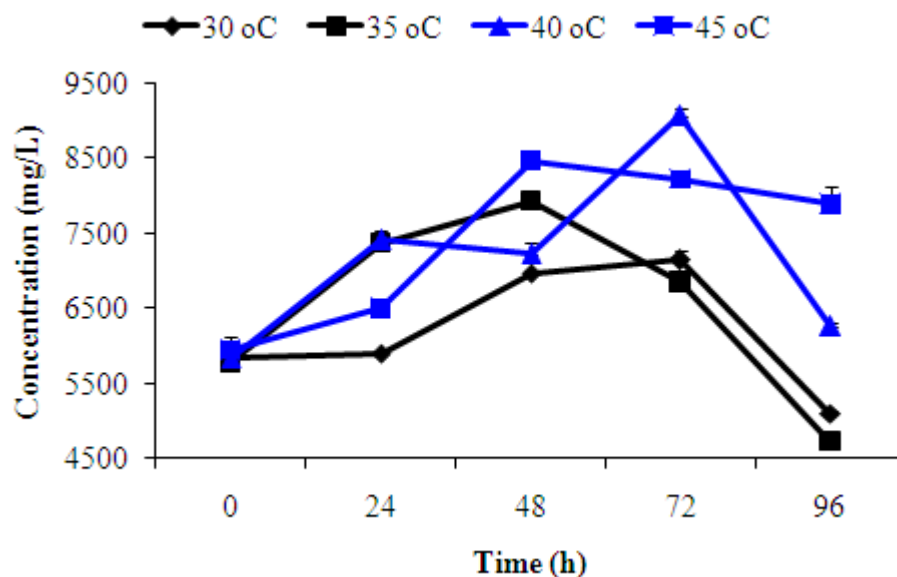


Fig. 5: Sulphate concentrations in the presence of the *Pseudomonas* spp. at the different incubation temperatures

When inoculated with the *Klebsiella* spp., sulphate levels in the wastewater after the end of the incubation period showed remarkable decrease at incubation temperature of 35 °C. At incubation temperatures of 40 °C and 45 °C, increases in concentrations were observed at the end of the incubation time. After the 96 h incubation period, sulphate levels were observed to change from 599.79 mg/L to 4205.54 mg/L, at 30 °C, from 5780.51 mg/L to 3583.30 mg/L at 35 °C, from 5862.64 mg/L to 6632.70 mg/L at 40 °C and from 5831.85 mg/L to 6109.06 mg/L at 45 °C (Fig. 6).

An increase in the pH of the wastewater inoculated with the *Klebsiella* spp. was observed at the expiration of incubation. This observation was irrespective of the different incubation temperatures. From the initial value of 6.4, pH of the wastewater was found to increase to 8.3, 8.2, 7.9 and 7.2, at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively (Table 2).

Table 2: Variation in pH of the wastewater at the different temperatures in presence of the test bacterial species

Time	Incubation Temperatures			
	30 °C	35 °C	40 °C	45 °C
<i>Pseudomonas spp.</i>				
0 h	6.4	6.4	6.4	6.4
24 h	7.0	6.3	7.2	6.7
48 h	7.4	7.3	7.4	6.9
72 h	7.6	7.5	7.5	6.9
96 h	7.4	8.5	7.7	6.9
% increase	13.17	24.06	16.51	6.30
<i>Klebsiella spp.</i>				
0 h	6.4	6.4	6.4	6.4
24 h	6.9	6.8	7.1	6.7
48 h	7.1	7.2	7.1	7.7
72 h	7.9	7.4	7.1	7.4
96 h	8.3	8.2	7.9	7.7
% increase	22.19	21.64	18.82	16.87
<i>Staphylococcus spp.</i>				
0 h	6.4	6.4	6.4	6.4
24 h	7.0	6.8	7.0	6.9
48 h	7.3	7.2	7.2	7.8
72 h	7.3	7.3	5.0	7.4
96 h	7.7	7.5	7.2	7.7
% increase	16.51	14.63	10.47	16.11
<i>Lysinibacillus spp.</i>				
0 h	6.4	6.4	6.4	6.4
24 h	6.9	6.8	7.0	6.9
48 h	7.0	7.0	7.1	7.3
72 h	7.2	7.2	7.1	7.2
96 h	7.4	7.3	7.5	7.4
% increase	12.86	12.30	13.79	13.05

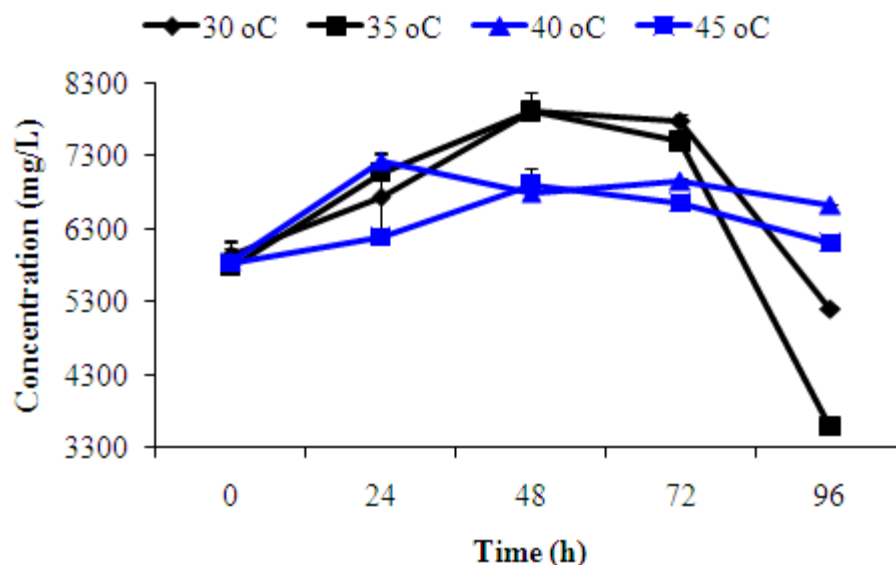


Fig. 6: Sulphate concentrations in the presence of the *Klebsiella* spp. at the different incubation temperatures

As shown in Fig. 7, remarkable sulphate was removed in the presence of the *Staphylococcus* spp. only at incubation temperatures of 30 °C and 35 °C. After the 96 h incubation time, sulphate levels at 30 °C and 35 °C showed a decrease from 5944.77 mg/L to 4743.51 mg/L and from 5780.51 to 4230.14 mg/L, respectively. At incubation temperatures of 40 °C and 45 °C, sulphate levels varied from the initial concentrations of 5862.65 mg/L and 5944.79 mg/L to 6492.04 mg/L and 5872.92, respectively (Fig. 7).

In the case of pH of the wastewater in the presence of the *Staphylococcus* spp., there was consistent a steady increase with time at the different incubation temperatures. At the expiration of the 96 h incubation period, pH showed increases from the initial 6.4 to 7.7, 7.5, 7.2 and 7.7 at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively. This translates to increases of 16.51 %, 14.51 %, 10.47 % and 16.11 %, respectively (Table 2).

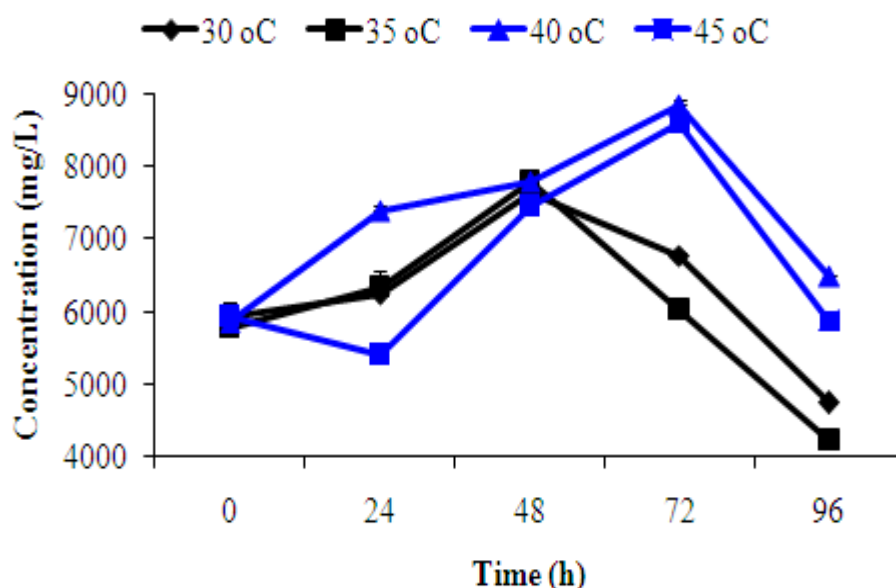


Fig. 7: Sulphate concentrations in the presence of the *Staphylococcus* spp.at the different incubation temperatures

In the presence of the *Lysinibacillus*spp., sulphate concentration in the wastewater was observed to vary from initial levels of 5944.77 mg/L, 5780.51 mg/L, 5862.65 mg/L, 5944.77 mg/L to final levels of 4538.16 mg/L, 4599.77 mg/L, 7094.73 mg/L, 5996.12 mg/L at incubation temperatures of 30 °C, 35 °C, 40 °C and 45 °C, respectively (Fig. 8).

With respect to pH of the wastewater in the presence of the *Lysinibacillus*spp., there was a steady increase with time. This trend was irrespective of the incubation temperatures. From the initial pH of 6.4, there was an increase of 12.86 % at 30 °C, 12.30 % at 35 °C, 13.79 % at 40 °C and 13.05 at 45 °C (Table 2).

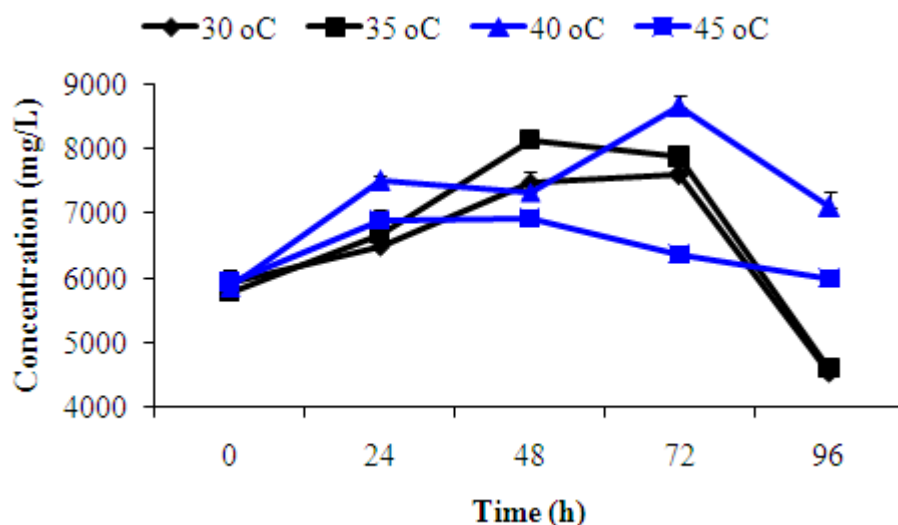


Fig. 8: Sulphate concentrations in the presence of the *Lysinibacillus* spp. at the different incubation temperatures

As shown in Table 3, at incubation temperature of 30 °C, sulphate removal among the isolates ranged from 7.60 % to 30.40 %, with the lowest and highest observed in the presence of the *Fusarium* spp. and *Absidia* spp., after the 96 h incubation period. At incubation temperatures of 40 °C and 45 °C, at the expiration of incubation, the majority of the test isolates showed either an increase in concentration of very minute removal (Table 3).

Table 3: Percent sulphate removed by the isolates at the different incubation temperatures after the 96 h incubation period

Test isolates	30 °C	35 °C	40 °C	45 °C
<i>Aspergillus niger</i>	20.73	18.99	24.52	11.23
<i>Aspergillus flavus</i>	17.62	0.71	11.03	11.23
<i>Fusarium</i> spp.	7.60	-2.31	7.53	-0.35
<i>Absidia</i> spp.	30.40	20.43	-5.78	4.49
<i>Pseudomonas</i> spp.	12.65	18.12	-7.39	-32.64
<i>Klebsiella</i> spp.	12.44	38.01	-13.13	-4.75
<i>Staphylococcus</i> spp.	20.21	26.82	-10.74	1.21
<i>Lysinibacillus</i> spp.	23.66	20.42	-21.02	-0.86

All values represent percent decreases except negative values that indicate increases

Discussion

In the present study, the optimum temperature range for sulphate removal by the test isolates was observed to be 30 to 35 °C for the bacteria and 30 – 40 °C for the fungi. In a study on phosphate and nitrate uptake from wastewater using the same test bacterial isolates by, by Akpor and co-workers, the optimum temperature for nutrient removal by the test isolates was observed to vary

for phosphate and nitrate removals. Their study revealed that slight phosphate removal by the isolates at temperature range of 30 °C and 35 °C. In the case of nitrate, optimum range for removal was observed to range from 25 °C to 35 °C (Akpor et al., 2014). For the fungal isolates, in a previous study on nutrient removal, optimum temperature for phosphate removal was observed at 30 °C -40 °C while nitrate highest removal nitrate was obtained at 35°C in the presence of the *Fusarium*spp. and *Aspergillusflavus*. In the presence of the *Absidias*spp. and *Aspergillusniger* maximum removal was obtained at 25 °C (Akpor et al., 2013). Brdjanovicet al., (1996)reported an optimum temperature of 20°C during the evaluation of the short-term effects of temperature on phosphate removal in biological systems, in the same vein, Jones and Stephenson, (1996)observed a optimum temperature of 30°Cfor phosphate removal in wastewater where high removalrates were also observed at two extreme temperatures of (5°C and 40°C).

In addition, a temperature of 25°C has been indicated as the optimum temperature for phosphate and nitrate removal in a study that evaluated the effect of temperature on the nutrient removal efficiency of three protozoan isolates in activated sludge mixed liquor (Akpor et al., 2008). However, despite the importance of temperature as a major factor affecting the growth and metabolic processes of microorganisms, few contradictory reports on its effects on nutrient removal in the presence of different organisms have been reported (Mino, 2000; Seviour et al., 2003).These inconsistencies in temperature findings among different investigators have been attributed to varying substrates, diverse system configurations, use of different analytical procedures and the application of different operational/optimization conditions (Larsdotter, 1994).Besides, a major factor contributing to this phenomenon is the great difference existing between the optimal temperature for bacterial growth and that needed for nutrient removal (Sabalowsky, 1999; Saito, 2004).

It is indicated that although the majority of sulphate-reducing bacteria are mesophilic organisms, they have the ability to still perform within a temperature range of 10-50 °C. It is hypothesied that as the temperaturerises, chemical and enzymatic reactions in the cell proceed at more rapid rates andbacterial growth becomes faster. In any organism, there exists a minimum temperature, below which growth no longer occurs and an optimum temperature at which growth is most rapid, and a maximum temperature above which growth is not possible (Madigan et al., 1997; Greben et al., 2002). Study by Greben et al. (2002) on biological sulphate removal, although sulphate was reduced at 15 °C, it was observed that the reduction rate was lowerthan at 25 °C.In a study on biological sulphate removal using hydrogen as theenergy source, at the different temperature that were used for investigation, the amount of sulphate that was removed by was observed to amount to 950 mg/L, 750 mg/L and 610 mg/L, at 23 °C, 20 °C and 18 °C, respectively (Eloff et al., 2003).

Conclusion

In evaluating the effect of temperature on the sulphate removal ability of the test isolates, the study revealed optimum temperature ranges for removal to be 30 -35 °C and 30 – 40 °C, for the bacteria and fungi isolates, respectively. At the temperature of 45 °C the sulphate removal ability of the test isolates was observed to be drastically limited a trend that was uniform among the bacterial and fungal isolates. Also observed in this study wasa consistent increase in pH of the

wastewater throughout the period of incubation. This trend was irrespective of the test microbial isolates and the incubation temperatures.

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.

References

Akpor OB, Adelani-Akande TA and Aderiye BI.2013. The effect of temperature on nutrient removal from wastewater by selected fungal species. *International Journal of Current Microbiology and Applied Sciences*, 2(9): 328-340

Akpor OB, Olaolu TD and Okolie EC. 2014. The effect of temperature on nitrate and phosphate uptake from synthetic wastewater by selected bacteria species. *British Microbiology Research Journal* 4(3): 328-342

Akpor OB, Momba MNB and Okonkwo JO.2008.The effects of pH and temperature on phosphate and nitrate uptake by wastewater protozoa. *African Journal of Biotechnology*, 7(13): 2221-2226

Arnosti C, Jorgensen BB, Sagemann J, Thamdrup B. 1998. Temperature dependence of microbial degradation of organic matter in marine sediments: polysaccharide hydrolysis, oxygen consumption, and sulfate reduction. *Marine Ecology Progress Series*, 165: 59-70

Brdjanovic D, Hooijmans CM, van Loosdrecht MCM, Alaerts GJ and Heeijen JJ. 1996. The dynamic effect of potassium limitation on biological phosphorus removal. *Water Research* 30(1): 303-313

Dill D, Du Preez L, Graff M and Maree J. 1994. Biological sulphate removal from acid mine drainage using produce gas as carbon and energy source: process limitations and their resolution. *International Mine Water Association (IMWA) Proceedings*

Eloff E, Greben HA, Maree JP, BV Radebe BV and RE Gomes RE. 2003. Biological sulphate removal using hydrogen as the energy source. 8th International Congress on Mine Water & the Environment, Johannesburg, South Africa

Elsgaard L, Isaksen MF, Jorgensen BB, Alayse AM, Jannasch HW. 1994. Microbial sulfate reduction in deep-sea sediments at the Guaymas Basin hydrothermal vent area: influence of temperature and substrates. *Geochim Cosmochim Acta* 58:3335-3343

Greben HA, Bologo H and J.P Maree JP. 2002. The 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 (WISA) 19 – 23 May 2002, Durban, South Africa

Jenkins D, Richard M and Daigger G. 2003. *Manual on the Causes and Control of Activated Sludge Bulking and Foaming*, 2nd edition. Lewis publishers, Boca.

Jones M and Stephenson T. 1996. The effect of temperature on enhanced biological phosphorus removal. *Environmental Technology* 17: 965-976

- 493 Jorgensen BB. 1982. Mineralization of organic matter in the sea bed-the role of sulphate
494 reduction. *Nature* 296: 643-645
495
- 496 Larsdotter K. 2006. Microalgae for phosphorus removal from wastewater in a Nordic climate. A
497 Doctoral Thesis from the School of Biotechnology, Royal Institute of Technology, Stockholm,
498 Sweden
- 499 Madigan MT, Martinko JM and Parker J. 1997. *Biology of Microorganisms*. Eight ed. Prentice-
500 Hall, Inc.
501
- 502 Maree JP, Netshidaulu I, Strobos G, Nengovhela R and Geldenhuys AJ. 2004. Integrated process
503 for biological sulphate removal and sulphur recovery. *Proceedings of the 2004 Water Institute of*
504 *Southern Africa (WISA) Biennial Conference, Cape Town, South Africa*
505
- 506 Mino T. 2000. Review: Microbial selection of polyphosphate-accumulating bacteria in
507 activated sludge wastewater treatment processes for enhanced biological removal. *Biochemistry*
508 63(3): 341-348
- 509 Olaolu TD, Akpor OB and Aderiye BI. 2013. Investigation of the effect of initial biomass on
510 nitrate and phosphate removal from synthetic wastewater by selected bacteria isolates. *Journal of*
511 *Natural Sciences Research*, 3(11): 39-49
512
- 513 Papirio S. 2012. Fluidized-bed bioreactor applications for the treatment of metal, sulfate and
514 nitrate-contaminated mine waters. A Doctoral Thesis of the Department of Chemical
515 Engineering, Università degli Studi di Cassino e del Lazio Meridionale, Italy
516
- 517 Sabalowsky AR. 1999. An investigation of the feasibility of nitrification and denitrification of a
518 complex industrial wastewater with high seasonal temperatures. Masters Thesis from Virginia
519 Polytechnic Institute and State University. Blacksburg.
- 520 Saito T, Brdjanovic D and van Loosdrecht MCM. 2004. Effect of nitrite on phosphate uptake by
521 phosphate accumulating organisms. *Water Research*, 3: 3760-3768
- 522 Sawicka JE, Jorgensen BB, and Bruchert V. 2012. Temperature characteristics of bacterial
523 sulfate reduction in continental shelf and slope sediments. *Biogeosciences Discuss*, 9: 673–700
524
- 525 Samee M. 2007. Biological sulfate reduction of reverse osmosis brine concentrate: process
526 modeling and design. A Doctoral Dissertation of the Department of Environmental Engineering,
527 Faculty of the Graduate School University of Southern California, USA
528
- 529 Seviour RJ, Mino T and Onuki M. 2003. The microbiology of biological phosphorus removal in
530 activated sludge systems. *FEMS Microbiological Review* 27, 99-127
531
- 532 Thongchai P, Aspiradee D and Jin A.. 2003. Effect of temperature shock on activities of
533 phosphorus-accumulating organisms. *Science Asia* 29, 365-370
534