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

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 and designed the study, was involved in the laboratory analysis and wrote the first draft of the manuscript. Author SOD was involved in the laboratory analysis, carried out literature search, assisted in the interpretation of some of the results and proof read the first draft of the manuscript. Author RA was involved in the design of the experiment, carried out laboratory analysis and proof read the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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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 *Absidia* spp.) isolates. The study was carried out under shake flasks conditions at incubation temperatures of 30ºC, 35ºC, 40ºC and 45ºC 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 30ºC -35ºC and 30ºC – 40ºC, 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.

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

Keywords: Temperature; sulphate removal; wastewater; bacteria; fungi.

1. 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 were 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 study was therefore aimed at investigating the effect of temperature on sulphate removal from wastewater by selected bacterial and fungal strains.

2. MATERIALS AND METHODS

2.1 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 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, Omu Aran, Kwara State, Nigeria. The isolates have previously been used for nitrate and phosphate removal studies in synthetic wastewater [10,11].

2.2 Wastewater Media

The wastewater used for the study was collected from a waste stabilization pond in the Landmark University Commercial Farms located in Omu Aran, 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 121ºC at 15 psi.

2.3 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 30ºC 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, using standard procedures [12].

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

3. RESULTS AND DISCUSSION

In the presence of the *Aspergillus niger*, 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 25ºC, 30ºC, 35ºC and 40ºC, respectively (Fig. 1). These decreases translate to 20.72 % at 25ºC, 18.99 % at 30ºC, 24.52 % at 35ºC and 11.23 % at 40ºC.

The pH variation of the wastewater in the presence of the *Aspergillus niger* 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 25ºC, 30ºC, 35ºC and 40ºC, respectively (Table 1).

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

Time	Incubation Temperatures			
	30ºC	**35ºC**	**40ºC**	**45ºC**
	Aspergillus niger			
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
	Aspergillus flavus			
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
	***Fusarium* spp.**			
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
	***Absidia* spp.**			
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 *Aspergillus flavus*, 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 30ºC, from 5780.51 mg/L to 5739.44 mg/L at 35ºC, from 5862.65 mg/L to 5215.81 mg/L at 40ºC, from 5944.79 mg/L to 5277.41 mg/L at 45ºC (Fig. 2).

With respect to pH of the wastewater in the presence of the *Aspergillus flavus*, 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 30ºC, 35ºC, 40ºC and 45ºC, respectively (Table 1).

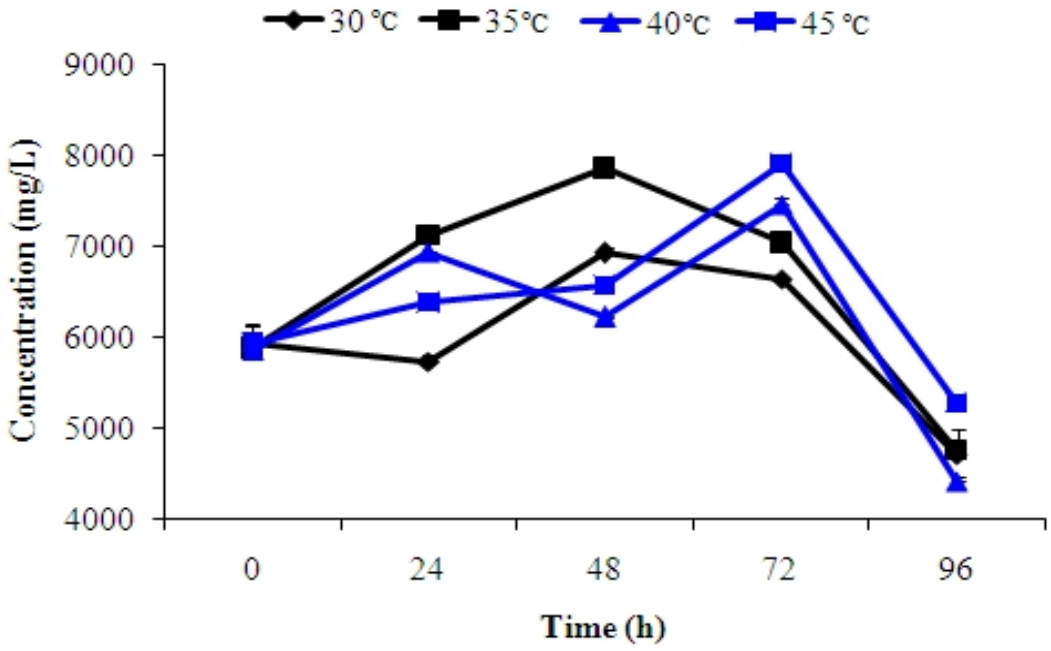

Fig. 1. Sulphate concentrations in the presence of the *Aspergillus niger* at the different incubation temperatures

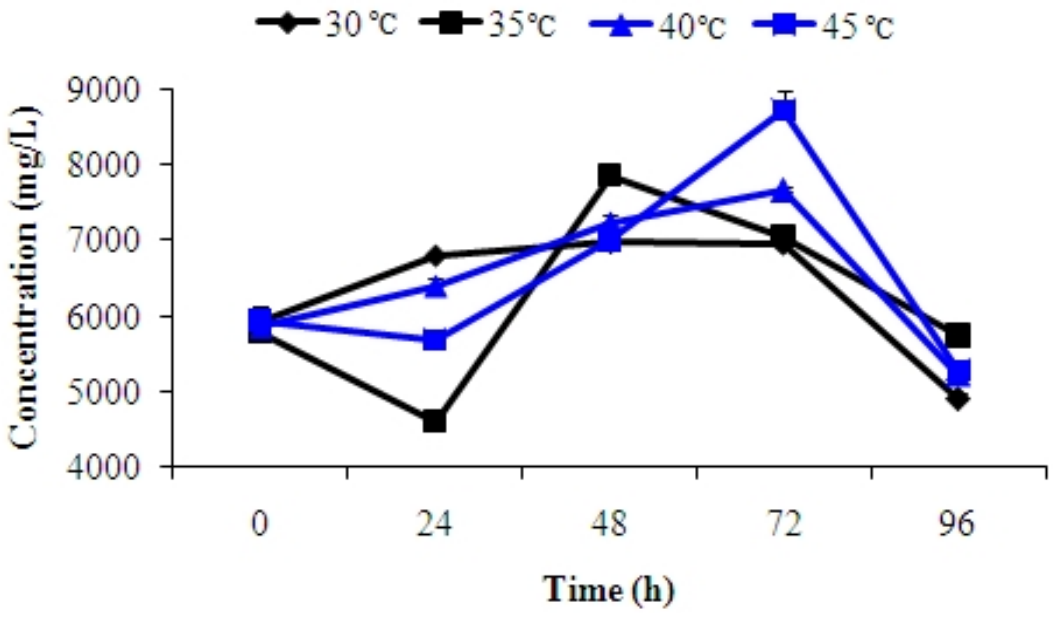

Fig. 2. Sulphate concentrations in the presence of the *Aspergillus flavus* at the different incubation temperatures

When inoculated with *Fusarium* spp., sulphate concentration in the wastewater was observed to show an increase after 96 h at incubation temperatures of 35ºC and 45ºC. Only

slight decreases in concentration were observed at incubation temperatures of 30ºC and 40ºC. 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 30ºC, 35ºC, 40ºC and 45ºC, respectively (Fig. 3).

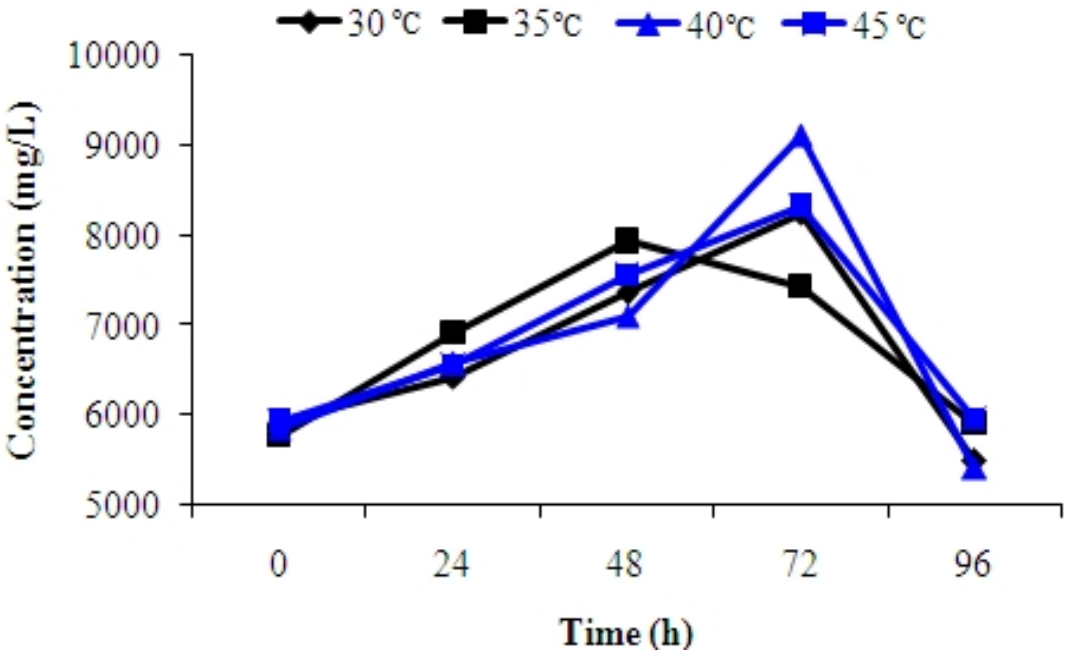

Fig. 3. Sulphate concentrations in the presence of the *Fusarium* spp. at the different incubation temperatures

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 30ºC, 35ºC, 40ºC and 45ºC, respectively. This translates to increases of 22.94%, 18.93%, 9.17% and 17.54%, respectively (Table 1).

In the presence of the *Absidia* spp., remarkable sulphate was removed from the wastewater, at incubation temperatures of 30ºC and 35ºC, 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 40ºC and 45ºC, 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 30ºC, 35ºC, 40ºC and 45ºC, respectively (Table 1).

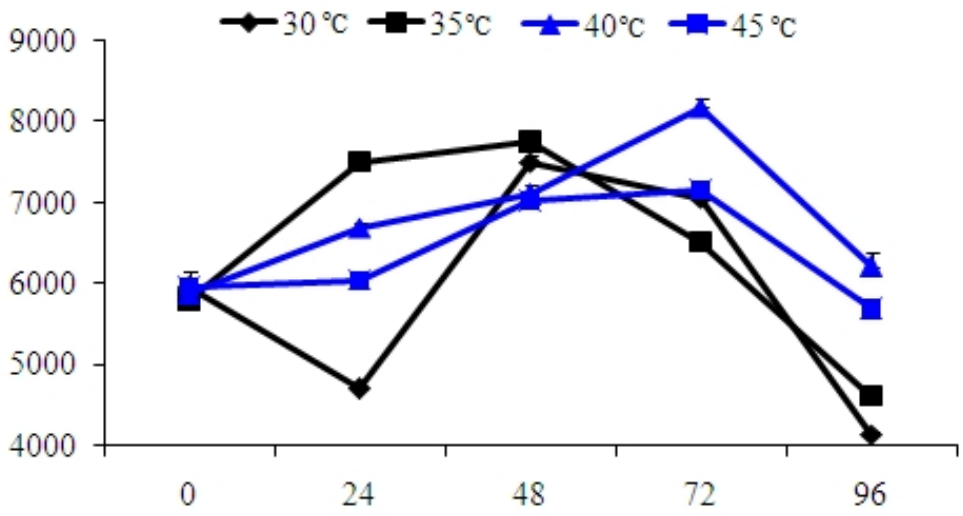

Fig. 4. Sulphate concentrations in the presence of the *Absidia* spp. at the different incubation temperatures

Sulphate concentration in the wastewater in the presence of the *Pseudomonas* spp**.** showed decreases after 96 h incubation at incubation temperatures of 30ºC and 35ºC. At 40ºC and 45ºC 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 30ºC, 35ºC, 40ºC and 45ºC, respectively (Fig. 5).

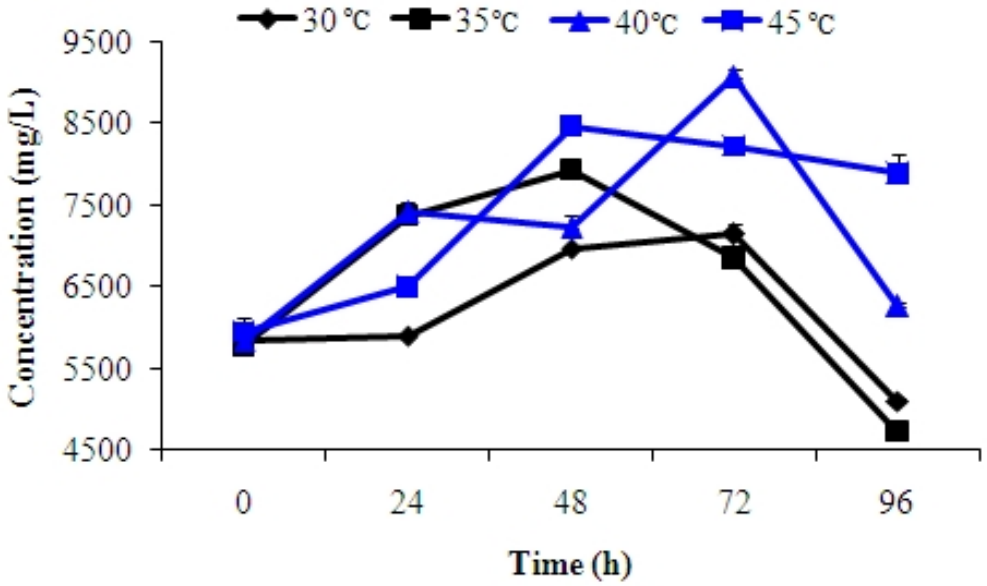

Fig. 5. Sulphate concentrations in the presence of the *Pseudomonas* spp. at the different incubation temperatures

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 30ºC, 8.5 at 35ºC, 7.5 at 40ºC and 6.9 at 45ºC (Table 2).

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

Time	Incubation Temperatures			
	30ºC	**35ºC**	**40ºC**	**45ºC**
	***Pseudomonas* spp.**			
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
	***Klebsiella* spp.**			
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
	***Staphylococcus* spp.**			
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
	***Lysinibacillus* spp.**			
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

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 35ºC. At incubation temperatures of 40ºC and 45ºC, 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 30ºC, from 5780.51 mg/L to 3583.30 mg/L at 35ºC, from 5862.64 mg/L to 6632.70 mg/L at 40ºC and from 5831.85 mg/L to 6109.06 mg/L at 45ºC (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 30ºC, 35ºC, 40ºC and 45ºC, respectively (Table 2).

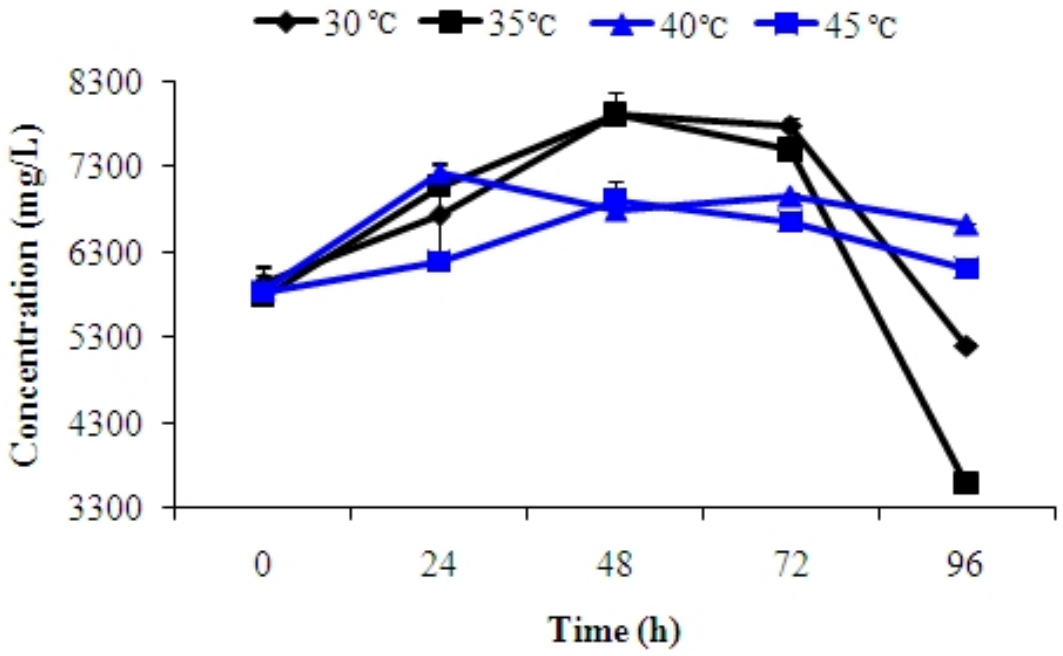

Fig. 6. Sulphate concentrations in the presence of the *Klebsiella* spp. at the different incubation temperatures

As shown in Fig. 7, remarkable sulphate was removed in the presence of the *Staphylococcus* spp. only at incubation temperatures of 30ºC and 35ºC. After the 96 h incubation time, sulphate levels at 30ºC and 35ºC 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 40ºC and 45ºC, 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 30ºC, 35ºC, 40ºC and 45ºC, respectively. This translates to increases of 16.51%, 14.51%, 10.47 % and 16.11 %, respectively (Table 2).

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 30ºC, 35ºC, 40ºC and 45ºC, 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 30ºC, 12.30% at 35ºC, 13.79% at 40ºC and 13.05 at 45ºC (Table 2).

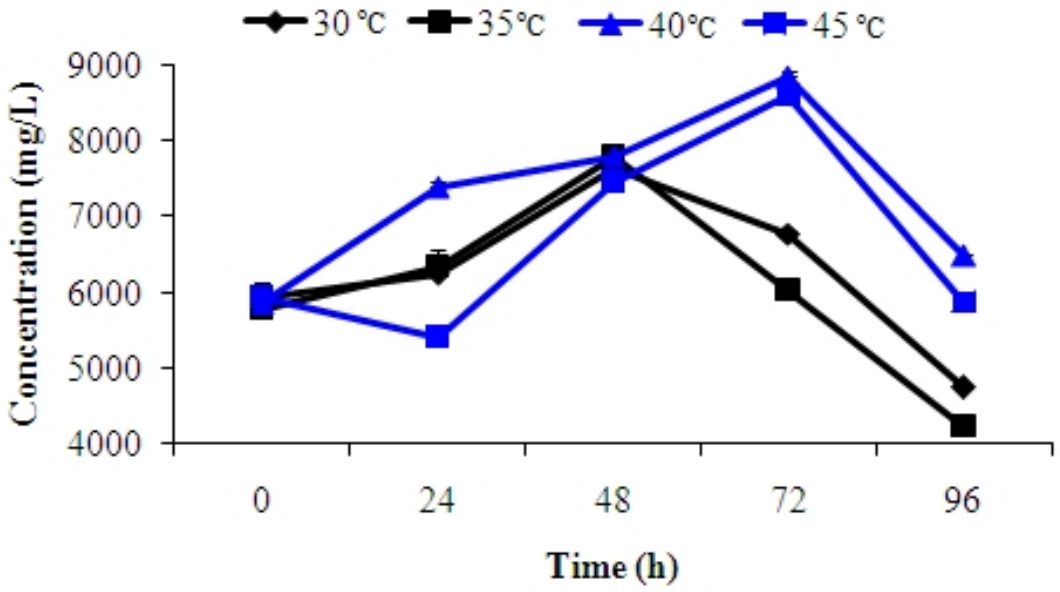

Fig. 7. Sulphate concentrations in the presence of the *Staphylococcus* spp. at the different incubation temperatures

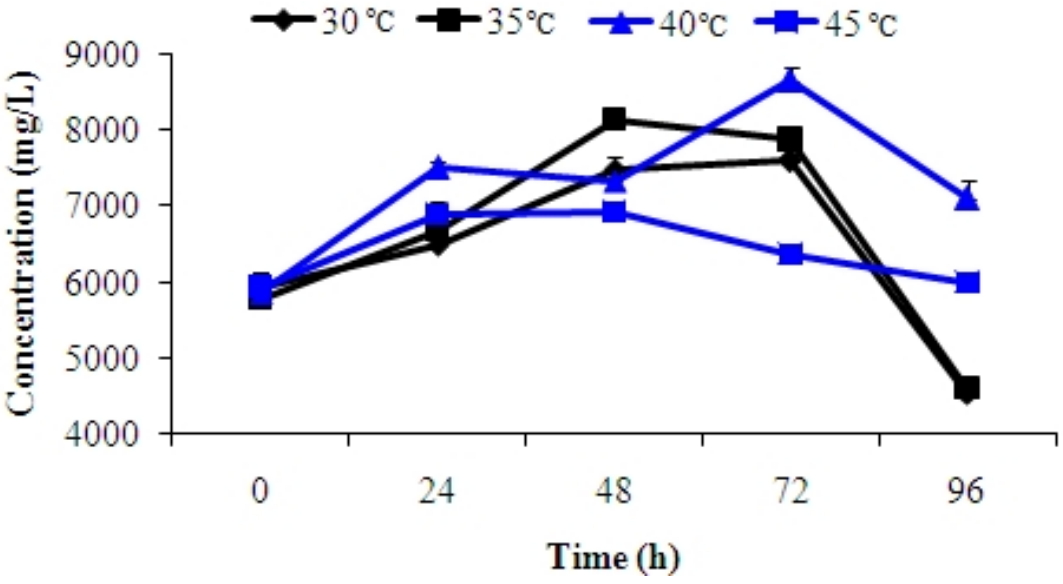

Fig. 8. Sulphate concentrations in the presence of the *Lysinibacillus* spp. at the different incubation temperatures

As shown in Table 3, at incubation temperature of 30ºC, 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 40ºC and 45ºC, 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	30ºC	35ºC	40ºC	45ºC
Aspergillus niger	20.73	18.99	24.52	11.23
Aspergillus flavus	17.62	0.71	11.03	11.23
Fusarium spp.	7.60	-2.31	7.53	-0.35
Absidia spp.	30.40	20.43	-5.78	4.49
Pseudomonas spp.	12.65	18.12	-7.39	-32.64
Klebsiella spp.	12.44	38.01	-13.13	-4.75
Staphylococcus spp.	20.21	26.82	-10.74	1.21
Lysinibacillus spp.	23.66	20.42	-21.02	-0.86

All values represent percent decreases except negative values that indicate increases

In the present study, the optimum temperature range for sulphate removal by the test isolates was observed to be 30 to 35ºC for the bacteria and 30 – 40ºC for the fungi. In a study on phosphate and nitrate uptake from wastewater using the same test bacterial isolates by, Olaolu and co-workers [13], 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 30ºC and 35ºC. In the case of nitrate, optimum range for removal was observed to range from 25ºC to 35ºC [11]. For the fungal isolates, in a previous study on nutrient removal, optimum temperature for phosphate removal was observed at 30ºC -40ºC while nitrate highest removal nitrate was obtained at 35ºC in the presence of the *Fusarium* spp. and *Aspergillus flavus*. In the presence of the *Absidia* spp. and *Aspergillus niger* maximum removal was obtained at 25ºC [10]. Brdjanovic et al. [14] reported an optimum temperature of 20ºC during the evaluation of the short-term effects of temperature on phosphate removal in biological systems. In the same vein, Jones and Stephenson, [15] observed a optimum temperature of 30ºC for phosphate removal in wastewater where high removal rates were also observed at two extreme temperatures of (5ºC and 40ºC).

In addition, a temperature of 25ºC 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 [13]. 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 [16,17]. 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 [18]. 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 [19,20].

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 10-50ºC. It is hypothesied that as the temperature rises, chemical and enzymatic reactions in the cell proceed at more rapid rates and bacterial 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 [5,21]. In the study by Greben et al. [5] on biological sulphate removal, although sulphate was reduced at 15ºC, it was observed that the reduction rate was lower than at 25ºC. In a study on biological sulphate removal using hydrogen as the energy 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 23ºC, 20ºC and 18ºC, respectively [22].

4. 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 30-35ºC and 30–40ºC, for the bacteria and fungi isolates, respectively. At the temperature of 45ºC 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 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 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. Knowledge of this could help in effective biological wastewater treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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