



The Effect of Temperature on Nitrate and Phosphate Uptake from Synthetic Wastewater by Selected Bacteria Species

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Authors' contributions

This work was carried out in collaboration between all authors. Author OBA designed the study, carried out the laboratory analysis, performed the statistical analysis and wrote the first draft of the manuscript. Author TDO carried out the laboratory analysis, performed the statistical analysis, carried out the analysis of results and proof read the first draft manuscript and author ECO managed the literature search, carried out the laboratory analysis and proof read the first draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to ascertain the effect of temperature on nutrient uptake ability of four bacterial species.

Methodology: A total of four bacterial species (*Klebsiella* sp., *Pseudomonas* sp., *Lysinibacillus* sp. and *Staphylococcus* sp.) were used for the study. The media used for the investigation was synthetic wastewater. Four different temperatures (25°C, 30°C, 35°C and 40°C) were used for the investigation. The study was carried out under shake flasks conditions. Immediately after inoculation with the respective test bacterial species and every 24 h for a 96 h incubation time, aliquot wastewater samples were removed from the flasks for the estimation of total phosphate, nitrate, pH and growth rate, using standard procedures.

Results: The results revealed phosphate and nitrate removal ranges of 10.84 % to 55.55 % and 90.67 % to 97.27 %, respectively in the presence of the *Klebsiella* sp. In the presence of the *Pseudomonas* sp., *Lysinibacillus* sp. and *Staphylococcus* sp., phosphate removals

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ranges of 0.36 % to 46.98 %, 11.89 % to 50.80 % and 2.74 % to 51.21 % were observed, respectively. For nitrate concentrations, removal levels that ranged from 2.19 % to 92.95 %, 0.97 % to 23.12 % and 7.56 % to 91.66 % were observed in the presence of *Pseudomonas* sp, *Lysinibacillus* ssp. and *Staphylococcus* sp., respectively. All the test bacterial species showed some measure of efficiency in phosphate removal. For nitrate removal, the *Lysinibacillus* sp. did not exhibit remarkable nitrate removal ability at any of the temperatures. In addition, the optimum temperatures for phosphate removals were observed to be 30°C to 40°C for the *Klebsiella* sp. and *Pseudomonas* sp; and 30°C to 35°C for the *Lysinibacillus* sp. and *Staphylococcus* sp. For nitrate removal, optimum temperatures for removal were observed to be 25°C to 40°C, for the *Klebsiella* sp and 25°C to 35°C, for the *Pseudomonas* sp. and *Staphylococcus* sp.

Conclusion: The study was able to reveal the optimum temperatures for phosphate and nitrate uptake in synthetic wastewater by the test bacterial species.

Keywords: Bacterial species; nutrient uptake; wastewater.

1. INTRODUCTION

It is reported that nutrient-linked causes are the basis for approximately 25 % of all water body impairments. Some of these impairments include oxygen depletion, algal growth, ammonia, harmful algal blooms, biological integrity, and turbidity [1]. Although phosphorus (used in the cells mainly for production of phospholipids, ATP and nucleic acid) and nitrogen (second most important nutrient after carbon, and may comprise more than 10% of a biomass) are important nutrients for living organisms, their presence in excess amount in receiving water bodies is the leading cause of eutrophication [2,3].

The eutrophication of receiving water bodies is due to the presence of nutrients in excessive amounts. Eutrophication could lead to the depletion of oxygen, death of aquatic life and other hazards to human population and the environment [4,5]. Owing to the negative impacts of eutrophication, there is the need to treat wastewater effluents to reduce nutrient limits that complies with standards set by regulatory bodies [1].

The most commonly used treatment methods used in the elimination of phosphorus and nitrogen from polluted wastewater are chemical and biological [6]. Because of its simplicity, economy and various other environmental benefits, biological removal processes is advocated over chemical methods in recent [7]. Chemical methods are also not encouraged because of the high cost of the chemical process and volume of sludge produced during treatment [8]. Over the few decades, biological nutrient removal processes have been employed for the removal of total nitrogen and phosphorus from wastewater by using different microorganisms under different environmental conditions [9].

Although other microorganisms such as fungi, protozoa and microalgae have been implicated in nutrient removal from wastewater, nutrient removal in wastewater treatment systems have been attributed mainly to bacteria [10]. Bacteria are known to be of great numerical importance in the activated sludge system and are responsible for the stabilization of wastes coming into a treatment plant. A number of studies have revealed the involvement and efficiency of bacteria in nutrient removal from wastewater. A number of bacteria, such as *Pseudomonas*, *Klebsiella*, *Acinetobacter*, *Escherichia coli*, *Bacillus* and *Enterobacter* have

been reported by several investigators as important in biological nutrient removal [5,11,12, 13,14].

In biological nutrient removal systems, an optimum temperature condition is essential for the efficiency of the treatment processes. This is because temperature is known to have influence on water chemistry and biological activities. Also, microbial growth is known to be strongly influenced by temperature, with optimal temperatures for microbial activity indicated to range from 20-25°C [15]. This investigation was therefore aimed at ascertaining the role of temperature in nutrient uptake abilities of four bacteria species *Pseudomonas* sp., *Klebsiellasp.*, *Lysinibacillus* sp. and *Staphylococcus* sp.

2. MATERIAL AND METHODS

A total of four bacterial species (*Pseudomonas* sp., *Klebsiell asp*, *Lysinibacillu* ssp. and *Staphylococcus* sp.) were used for this study. The isolates were obtained from the Department of Microbiology, Ekiti State Univesrity, Ado Ekiti, Ekiti State, Nigeria. Prior use, the isolates were maintained in nutrient agar slants and stored at 4°C till needed. Before usage, all isolates were first streaked in nutrient agar plates to ascertain their purity.

For nutrient removal studies, the isolates were cultured in nutrient broth and incubated for 18 h to 24 h in an incubator at 37°C. The population of the bacteria in the nutrient broth was estimated, using standard plating procedures [17]. For this study, the population of cells that were used for inoculation was 2.91×10^8 cfu/mL, 6.31×10^8 cfu/mL, 1.75×10^8 cfu/mL and 7.1×10^8 cfu/mL for the *Klebsiellasp.*, *Pseudomonas* sp, *Lysinibacillus* sp. and *Staphylococcus* sp, respectively.

For the nutrient removal studies, the media that was used was synthetic wastewater. The synthetic wastewater was composed of 5.0 g/L sodium acetate, 0.5 g/L magnesium sulphate, 0.5 g/L potassium nitrate, 0.5 g/L potassium dihydrogen phosphate, 1.0 g/L meat extract, 1.0 g/L peptone and 0.5 g/L sodium chloride. The different components of the media were weighed and dissolved separately in small quantities of deionised water before combining everything together as one media and making up to the desired mark.

Initially, the media was dispensed in 200 mL quantity in 250 mL Erlenmeyer flasks, which were plugged with non-adsorbent cotton wool and sterilised in an autoclave at 121 °C at 2.07 kg/cm^2 for 15 min. After sterilisation, aliquot samples from each of the flasks were taken and cultured in nutrient agar plates and then incubated. This was to ascertain the efficiency of the sterilisation conditions. Only flasks whose samples did not show any growth in the culture plates were used for the nutrient removal studies.

After sterilisation, a known population of the respective bacterial species were inoculated into each flask and incubated at the required temperatures for the study. Just immediately after inoculation and every 24 h for 96 h, aliquot samples of 20 mL were taken from each flask for the determination of the phosphate and nitrate concentrations, growth rate and pH, using standard procedures [16]. Phosphate, nitrate and pH were determined using the ascorbic acid method, salicylate method and pH meter, respectively.

In this study, all reagents used were of analytical grade. Also, all experimental setups were carried out in triplicate.

Generally, all statistical analysis was carried out using the PAST (paleontological statistics software package for education and data analysis [17]). The test for the comparison of means was done using the one-way analysis of variance (ANOVA). All statistics were run at probability level of 0.05.

3. RESULTS AND DISCUSSION

In the presence of the *Klebsiellasp.*, although there were decreases in phosphate concentration in the wastewater at the different temperatures, significant decreases were only observed at incubation temperatures of 30°C, 35°C and 40°C. The significant decreases were observed from 48 h to the end of incubation. At the expiration of the 96 h incubation period, phosphate concentration in the wastewater was observed to decrease from an initial value of 191.6 mg/L to 170.8 mg/L at 25°C, 77.1 mg/L at 30°C, 83.0 mg/L for 35°C and 85.2 mg/L, at 40°C (Fig. 1). The decreases in phosphate concentrations at 30°C, 35°C and 40°C were observed to be significantly different from that at 25°C ($P \leq 0.05$).

For nitrate concentration in the presence of the *Klebsiellasp.*, significant decreases were observed from the 24 h of incubation at incubation of concentration 25°C, 30°C and 35°C. However at the 96 h incubation period, significant decreases in concentration were observed at the different temperatures. From an initial concentration of 256.3 mg/L, nitrate levels were observed after 96 h to be 23.9 mg/L, 11.2 mg/L, 7.0 mg/L and 18.4 mg/L, at 25°C, 30°C, 35°C and 40°C, respectively (Fig. 2). Generally, the decrease in nitrate concentration in the presence of the *Klebsiellasp.* was not observed to be significantly different at the different incubation temperatures ($P \leq 0.05$).

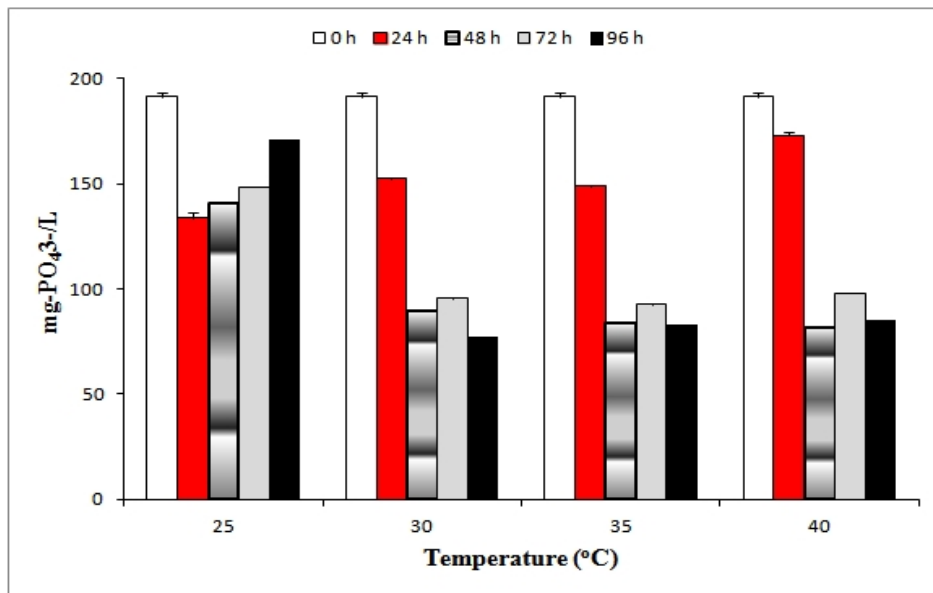


Fig. 1. Trend in phosphate removal at the different temperatures in presence of the *Klebsiella sp*

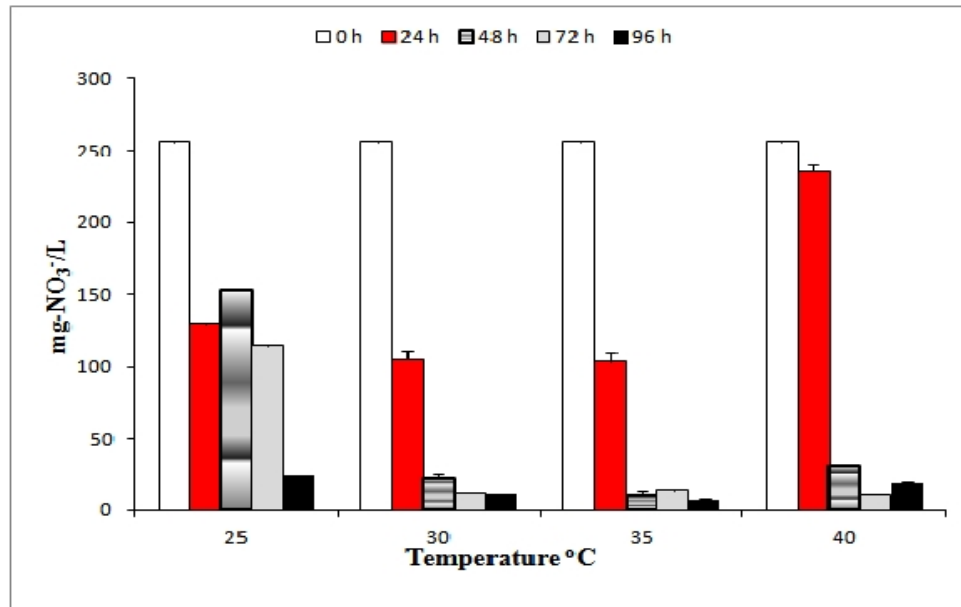


Fig. 2. Trend in nitrate removal at the different temperatures in presence of the *Klebsiella* sp.

As shown in figure 5, in the presence of the *Lysinibacillus* sp., remarkable decreases in phosphate concentrations were only observed at temperature of 30°C and 35°C. No remarkable decreases were observed at temperatures of 25°C and 40°C. At 30°C and 35°C, remarkable decreases were observed from 48 h till the end of the incubation period. From an initial concentration of 191.5 mg/L, phosphate concentrations were observed to be 191.6 mg/L at 25°C, 94.3 mg/L at 30°C, 104.9 mg/L, at 35°C and 168.8 mg/L, at 40°C (Fig. 3). The phosphate levels at temperatures of 30°C and 35 °C were observed to be significantly lower than those at 25°C and 40°C ($P \leq 0.05$).

For nitrate removal in presence of the *Lysinibacillus* sp., no significant decreases in concentration were observed at the different temperatures. Slight decreases in concentration were however observed 25°C. From an initial concentration of 256.3 mg/L, nitrate levels after the 96 h incubation period were found to be 197.0 mg/L, 252.9 mg/L, 239.2 mg/L and 249.9 mg/L, at 25°C, 30°C, 35°C and 40°C, respectively (Fig. 4). Generally, no significant differences in nitrate levels were observed between the different temperatures ($P \leq 0.05$).

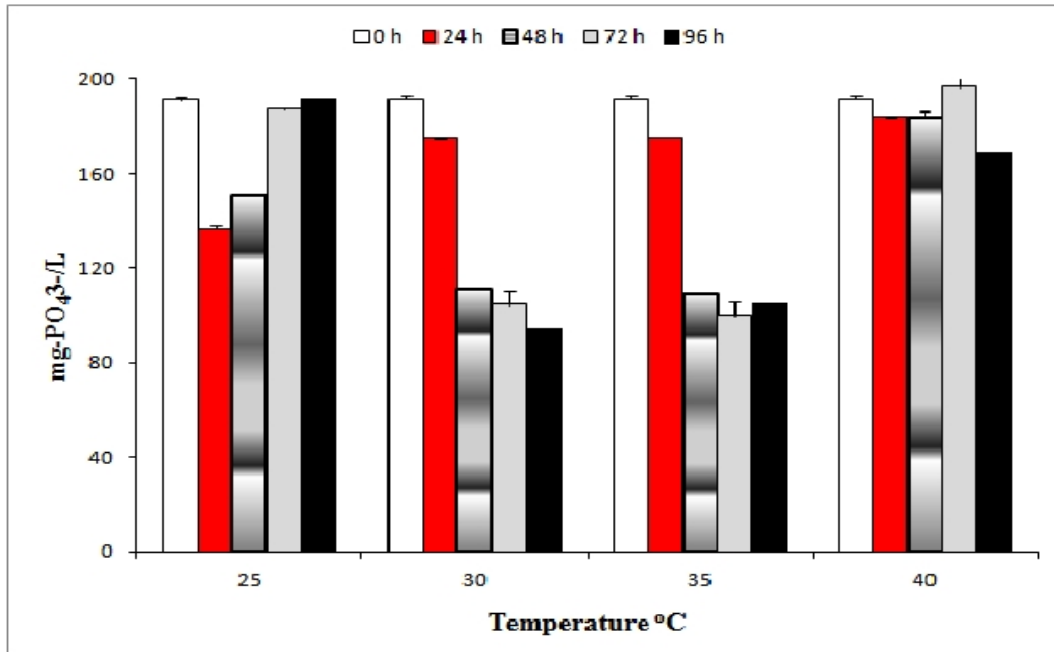


Fig. 3. Trend in phosphate removal at the different temperatures in presence of the *Lysinibacillus* sp.

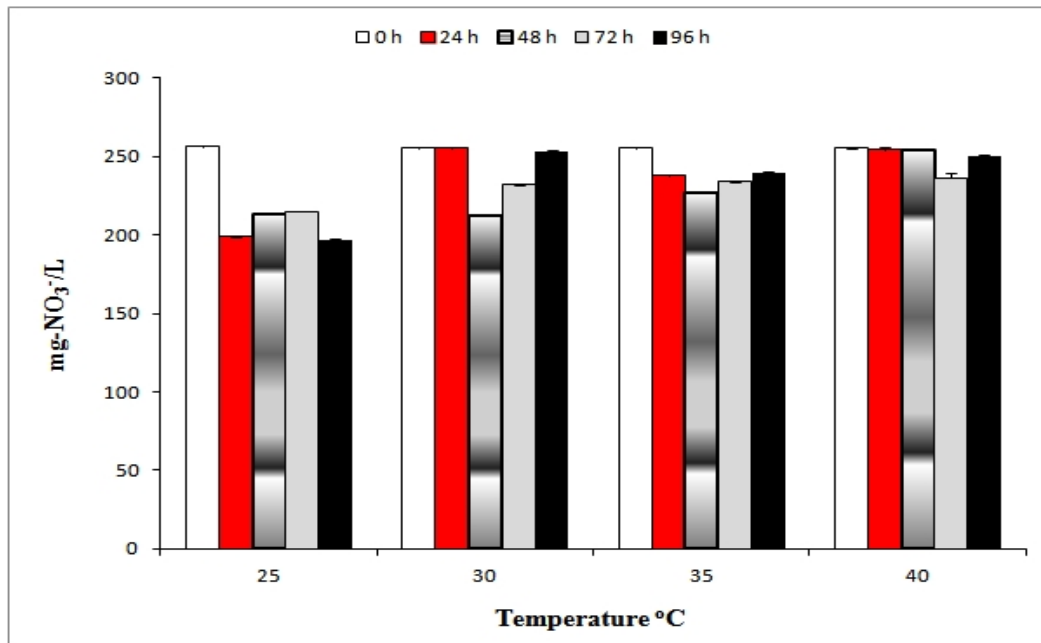


Fig. 4. Trend in nitrate removal at the different temperatures in presence of the *Lysinibacillus* sp.

When the *Pseudomonas* sp. was used for inoculation, only small decreases in phosphate concentration were observed between 24 h and 72 h incubation, after which there was a sharp increase with time at 25°C. At incubation temperatures of 30°C, 35°C and 40°C, although no decreases in concentrations were observed within the first 24 h of incubation, remarkable decreases were observed from 48 h to the end of incubation. At the expiration of the incubation periods, phosphate levels at the different temperatures were found to be 190.8 mg/L, 78.6 mg/L, 88.5 mg/L and 101.1 mg/L, at incubation temperatures of 25°C, 30°C, 35°C and 40°C, respectively (Fig. 5). At the expiration of the incubation period, phosphate levels at temperatures of 30°C, 35°C and 40°C were observed to be significantly lower than levels at 25°C ($P \leq 0.05$).

In the case of nitrate concentration in presence of the *Pseudomonas* sp., significant removals were observed from 24 h to the end of incubation at temperatures of 25°C, 30°C, and 35°C. At 40°C, no remarkable decreases in concentration were observed. From an initial concentration of 267.70 mg/L, nitrate concentrations after the end of the incubation period were observed to be 11.5 mg/L, 20.8 mg/L, 18.6 mg/L and 261.0 mg/L, at temperature of 25°C, 30°C, 35°C and 40°C, respectively (Fig. 6). At the end of incubation, nitrate concentrations at 25°C, 30°C and 35°C were observed to be significantly lower than concentration at 40°C ($P \leq 0.05$).

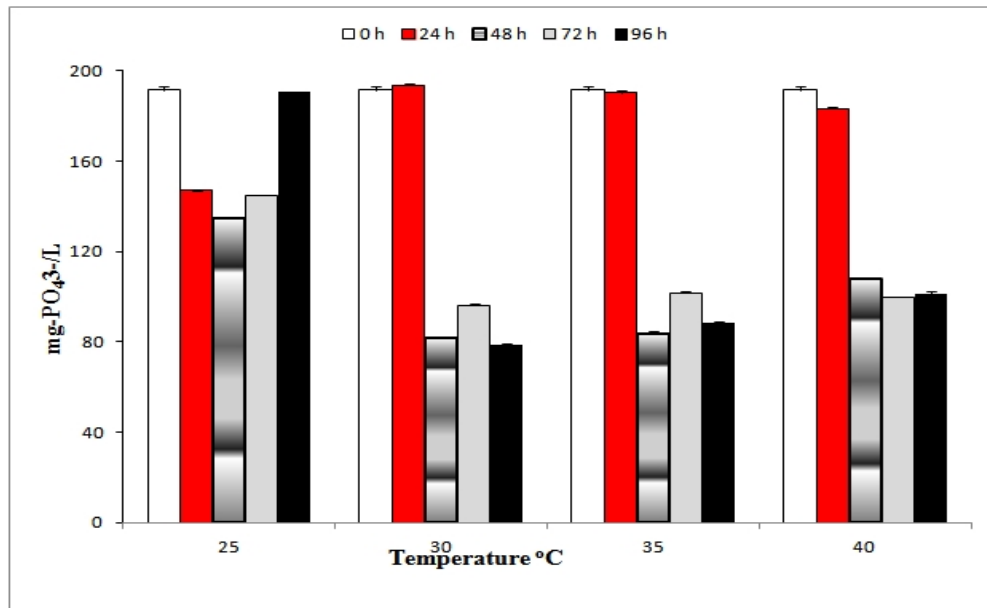


Fig. 5. Trend in phosphate removal at the different temperatures in presence of the *Pseudomonas* sp.

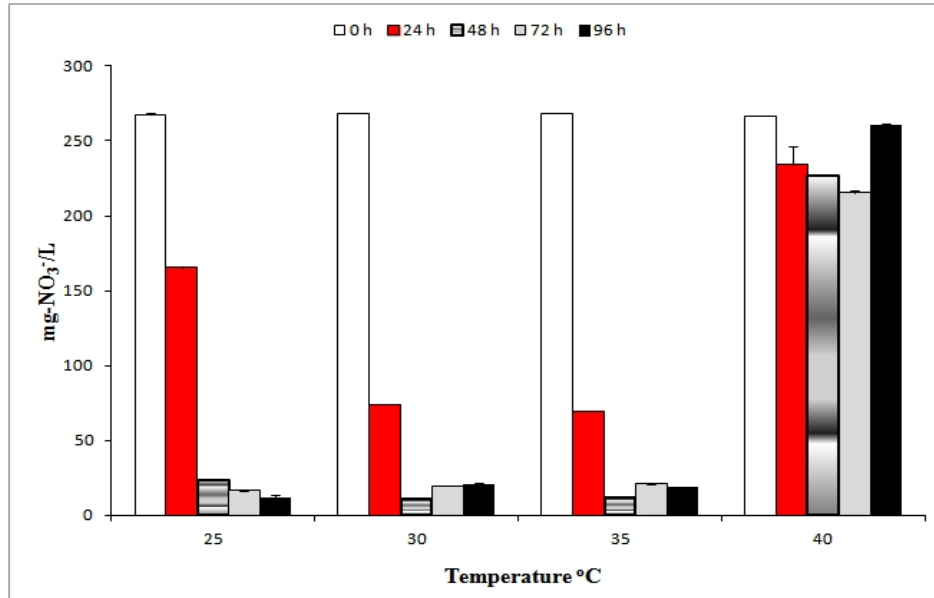


Fig. 6. Trend in nitrate removal at the different temperatures in presence of the *Pseudomonas* sp.

In the presence of the *Staphylococcus* sp., significant phosphate removals were only observed at 30°C and 35°C. At 25°C, slight decreases in concentration were observed between 24 h and 48 h. At 40°C, no notable decreases in concentration were observed within the first 72 h of incubation. From an initial concentration of 191.50 mg/L, phosphate levels after the 96h incubation period were found to be 186.2 mg/L at 25°C, 93.4 mg/L at 30°C, 94.6 mg/L at 35°C and 121.0 mg/L at 40°C (Fig. 7). The phosphate decreases at temperatures of 30°C and 35°C were observed to be significantly lower than levels at 25°C and 40°C ($P \leq 0.05$).

For nitrate removals in presence of the *Staphylococcus* sp were observed to decrease significantly from 48 h to the end of the incubation period at incubation temperatures of 25°C, 30°C and 35°C. At 40°C, no remarkable decreases in nitrate levels were observed with incubation time. At end of the incubation period, nitrate levels were found to decrease from initial levels of 256.3 mg/L to 33.9 mg/L, 21.4 mg/L and 27.6 mg/L and 236.1 mg/L at temperatures of 25°C, 30°C, 35°C and 40°C, respectively (Fig. 8).

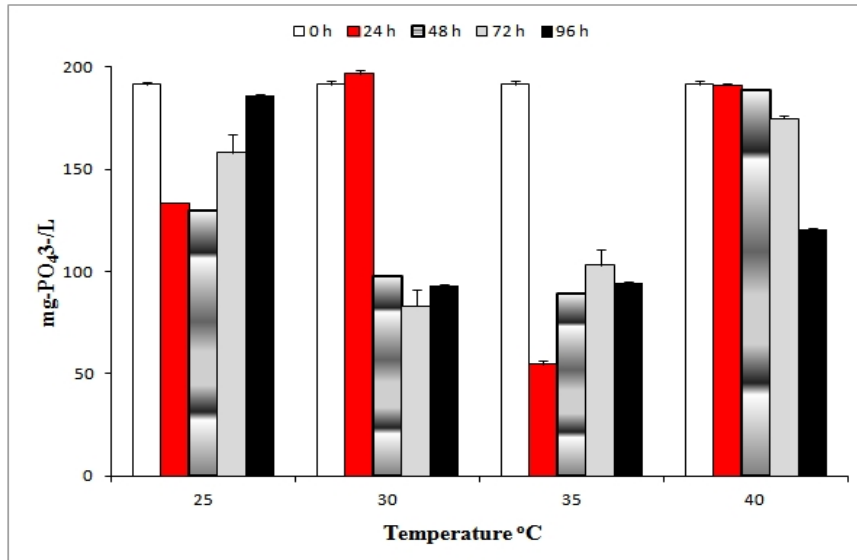


Fig. 7. Trend in phosphate removal at the different temperatures in presence of the *Staphylococcus* sp.

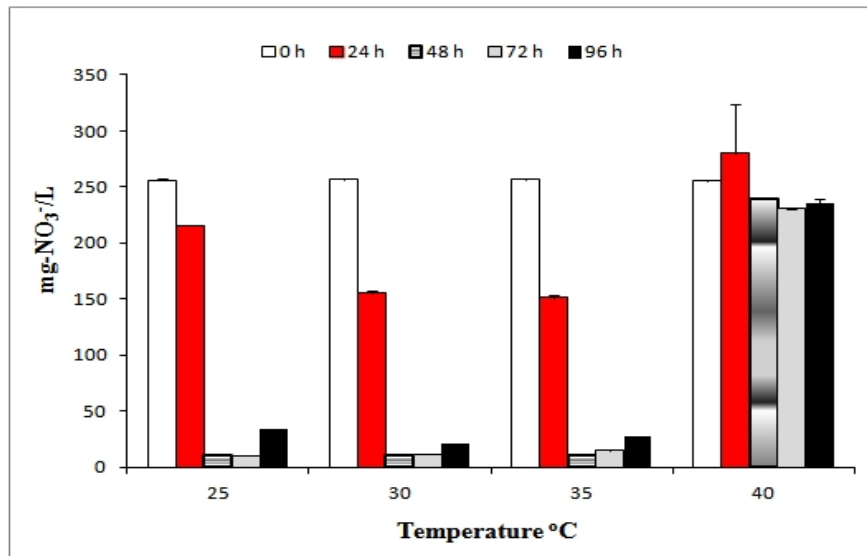


Fig. 8. Trend in nitrate removal at the different temperatures in presence of the *Staphylococcus* sp.

With respect to percentage nutrient removal at the different temperatures, maximum phosphate and nitrate removals of 59.8% and 97.3% were observed at 30 °C and 35 °C, respectively in presence of the *Klebsiella* sp. In the presence of the *Pseudomonas* sp., maximum phosphate and nitrate removals of 59.0% and 92.8% were observed at 30 °C and 35 °C, respectively. Also, the highest phosphate removal levels of 50.8% and 51.2% were observed at 30 °C in presence of the *Lysinibacillus* sp. and *Staphylococcus* sp., respectively.

For nitrate removal, the highest levels were observed at 25°C and 35°C in the presence of the *Lysinibacillus* ssp. and *Staphylococcus* sp., respectively (Table 1).

Table 1. Percentage change in phosphate and nitrate concentrations at the different temperatures in presence of the test bacterial species

Temperature	Initial (mg/L)	Final (mg/L)	% removed	Initial mg/L	Final mg/L	% removed
	Phosphate			Nitrate		
<i>Klebsiella</i> sp.						
25°C	191.6	170.8	10.8	256.1	23.9	90.7
30°C	191.6	77.1	59.8	256.9	11.2	95.6
35°C	191.6	83.0	56.7	256.9	7.0	97.3
40°C	191.6	85.2	55.6	256.9	18.4	92.9
<i>Lysinibacillus</i> sp.						
25°C	191.5	191.6	-0.1	256.3	197.0	23.1
30°C	191.6	94.2	50.8	255.4	252.9	1.0
35°C	191.6	104.9	45.2	256.1	239.2	6.6
40°C	191.6	168.8	11.9	255.2	249.6	2.2
<i>Pseudomonas</i> sp.						
25°C	191.5	190.8	0.4	267.7	11.5	91.6
30°C	191.5	78.6	59.0	268.4	20.8	92.3
35°C	191.5	88.5	53.8	256.0	18.6	92.8
40°C	191.5	101.5	47.0	266.8	260.9	2.2
<i>Staphylococcus</i> sp.						
25°C	191.5	186.2	2.7	256.3	33.9	86.8
30°C	191.5	93.4	51.2	256.9	21.4	91.7
35°C	191.6	94.6	50.6	256.0	27.6	89.2
40°C	191.5	121.0	36.8	255.4	236.1	7.6

Initial and final represent concentrations at 0 h and 96 h, respectively. Negative values represent % increases. P values are less than 0.05.

The trend in pH in the presence of the test bacterial isolates during the nutrient removal study is displayed in Table 2. As shown in the Table, there was a general increase in pH with incubation time. This trend was irrespective of the bacterial species or the incubation temperature. There seemed to be a general gradual increase in pH from 5.9 towards neutral and alkalinity. In presence of the isolates, pH at the end of incubation was observed to range from 6.8 to 7.3, from 6.2 to 7.2, from 6.4 to 7.6, and from 6.5 to 6.6, in the presence of the *Klebsiella* sp., *Lysinibacillus* sp., *Pseudomonas* sp., and *Staphylococcus* sp., respectively (Table 2).

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

Time	25°C	30°C	35°C	40°C
<i>Klebsiellasp.</i>				
0h	5.9	6.6	5.9	6.6
24h	6.4	6.5	6.4	6.4
48h	6.9	6.6	6.9	6.6
72h	7.4	7.0	7.4	6.7
96h	7.3	7.3)	7.3	6.8
<i>Lysinibacillus sp.</i>				
0h	5.9	6.5	5.9	6.6
24h	6.3	6.3	6.3	6.4)
48h	6.7	6.3	6.7	6.3
72h	7.1	6.4	7.1	6.2
96h	7.2	6.5	7.2	6.2
<i>Pseudomonas sp.</i>				
0h	5.9	6.5	5.9	6.6
24h	6.3	6.3	6.3	6.4
48h	6.9	6.4	7.0	6.3
72h	7.5	6.6	7.5	6.4
96h	7.6	6.9	7.6	6.4
<i>Staphylococcus sp.</i>				
0h	5.9	6.5	5.9	6.6
24h	6.2	6.3	6.2	6.6
48h	6.4	6.4	6.4	6.6
72h	6.5	6.5	6.5	6.5
96h	6.6	6.5	6.6	6.6

All values are average of triplicate analysis.

As shown in Table 3, growth rates of the test bacterial species during the nutrient removal studies were observed to range from 1.871 d⁻¹ to 1.987 d⁻¹, 1.839 d⁻¹ to 1.970 d⁻¹, from 1.850 d⁻¹ to 1.942 d⁻¹ from and from 1.837 d⁻¹ to 1.942 d⁻¹ in the presence of the *Klebsiellasp.*, *Lysinibacillus sp.*, *Pseudomonas sp.* and *Staphylococcus sp.*, respectively. In all the bacterial species, highest growth rates were observed at 25 °C (Table 3).

In the present study, the carbon source was acetate. The choice of acetate was deliberate. Previous investigators have indicated acetate as a preferred carbon source during nutrient removal studies [18,19]. The study was carried out using four bacterial isolates. The test bacterial species were isolated from domestic wastewater sources and have previously been implicated as having ability to degrade dietary oil substrate in wastewater [20]. The choice was of the isolates was deliberate. This was because since they have been reported to have oil degradation ability, exploring their efficiency in the removal of eutrophic nutrients in wastewater could reveal their bioremediation potential.

The study showed optimum temperature for nutrient removal by the test isolates to vary for phosphate and nitrate removals. The observation for phosphate indicates some measure of removals in presence of all the test isolate at temperature range of 30°C and 35°C. In the case of nitrate, optimum range for removal in presence of the three isolates (*Klebsiella sp.*, *Pseudomonas sp.* and *Staphylococcus sp.*) that displayed uptake efficiencies was 25°C to 35°C. In a study on the effect of temperature on the nutrient removal efficiency of three

protozoan isolates, 25°C was indicated as the optimum temperature for phosphate and nitrate removal in activated sludge mixed liquor [21].

Table 3. Growth rate (d^{-1}) of the test bacterial species at the different temperatures during the nutrient removal studies

Time	25°C	30°C	35°C	40°C
<i>Klebsiella</i> sp				
24 h	1.543	1.952	1.905	1.856
48 h	1.954	1.958	1.910	1.862
72 h	1.974	1.958	1.925	1.868
96 h	1.987	1.967	1.927	1.871
<i>Lysinibacillus</i> sp				
24 h	1.940	1.832	1.893	0.000
48 h	1.951	1.834	1.896	1.933
72 h	1.961	1.834	1.898	1.933
96 h	1.970	1.839	1.903	1.935
<i>Pseudomonas</i> sp				
24 h	1.936	1.837	1.830	1.857
48 h	1.939	1.839	1.841	1.858
72 h	1.940	1.846	1.845	1.859
96 h	1.942	1.853	1.850	1.860
<i>Staphylococcus</i> sp				
24 h	1.936	1.835	1.896	0.000
48 h	1.939	1.836	1.898	1.856
72 h	1.940	1.836	1.900	1.857
96 h	1.942	1.837	1.903	1.859

All values are average of triplicate analysis.

Despite the fact that temperature is indicated as one of the factors that affect the growth and metabolic processes of microorganisms, there seems to be contradictory reports on its effects on nutrient removal in presence of difference organisms [22, 23]. It is also specified that temperature is a key parameter which affects reaction kinetics and performance of biological nutrient removal systems. There are also, conflicting observations on the role of temperature on enhanced biological nutrient removal systems [24, 25]. It is suggested that the inconsistencies in temperature findings among different investigators may be as a result of varying substrates, diverse system configurations, use of different analytical techniques and the application of different operational conditions, hence making it challenging to compare results [3,26].

An observation in this study was that although maximum growth rates were obtained at incubation temperature of 25°C by the different isolates during the nutrient removal studies, this was not the optimum temperature for nutrient removal by the majority of the isolates. It is indicated that while there may be optimal ranges temperature for growth by different organisms, such optimal ranges may not necessarily be the same for nutrient removal [27, 28]. Although all the temperatures investigated during the course of this study were within the mesophilic ranges, available reports suggests that biological treatment activity is reported to accelerate in warm temperatures and to slow in cool temperatures, but extremely hot or cold temperatures can stop treatment processes [29]. According to Mamais and Jenkins [30], the optimum operating temperature for an efficient biological nutrient process should range between 28°C and 30°C.

In the by Brdjanovic [31], during the investigation of the short-term effects of temperature on phosphate removal in biological systems, an optimum temperature 20°C was observed. Similarly, Jones and Stephenson [32] indicated that although a temperature of 30°C was observed as optimum for phosphate removal in wastewater, remarkable removals were also observed at extreme temperatures of 5°C and 40°C.

The study revealed a slow but steady increase in pH throughout the period of incubation, a trend that was observed irrespective of the isolate used for investigation or incubation temperature. Steady increases in pH during nutrient removal studies have been reported by earlier workers [21,28]. The increase in pH during biological nutrient removal is suggested to be due to the utilization of residual oxygen as a result of nutrient uptake, which simultaneously consumes H⁺ in reactors [28].

4. CONCLUSION

The study which investigated the effect of temperature on the nutrient removal ability of the test bacterial species was able to reveal the following:

- The Optimum temperatures for nutrient removal were observed to range from 30°C to 35 °C for phosphate removal and 25°C to 35°C for nitrate removal
- The optimum temperature for the highest growth rate was not observed to be the optimum temperature for nutrient removal by the majority of the test bacterial species
- All the test bacterial species showed remarkable nitrate removal were efficient at incubation temperature of 25°C to 35°C. At 40°C, only the *Klebsiella* sp still exhibited nitrate removal ability.
- Between 30°C and 35°C, all the four isolates showed remarkable phosphate removal
- During nutrient removal studies in presence of the test bacterial species, there were consistent but slow increases in pH of the wastewaters.

The study was able to provide relevant information on the effect of temperature on the nutrient removal efficiency of the isolates in synthetic wastewaters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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