

## Original Research Article

### The Effect of Temperature on Nutrient Removal from Wastewater by Selected Fungal Species

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#### ABSTRACT

The main goal of wastewater treatment is to safeguard the environment by preventing the pollution of receiving water bodies. This study was aimed at investigating the effect of temperature in nutrient removal by four test fungal species (*Fusarium* sp, *Absidia* sp, *Aspergillus niger* and *Aspergillus flavus*) in synthetic wastewater at different incubation temperatures of 25°C, 30°C, 35°C and 40°C. After inoculation with each test isolate, aliquot samples were taken prior inoculation and every 24 h, for 96 h for the estimation of phosphate, nitrate and pH in the wastewater, using standard procedures. After 96 h incubation, the percentage phosphate concentration removal ranged between 29.17 %-36.17 %, 26.17 %-35.75 %, 8.01 %-37.94 % and 11.19 %-42.65 %, in the presence of the *Fusarium* sp, *Absidia* sp, *Aspergillus niger* and *Aspergillus flavus*, respectively. Optimum temperature for phosphate removal was observed at 30°C -40°C. After 96 h incubation at 25°C, the phosphate concentration was very high with most of the isolates. In the case of nitrate, highest removals of 42.89 % and 82.07 % were obtained at 35°C in the presence of the *Fusarium* sp and *Aspergillus flavus*. In the presence of the *Absidia* sp and the *Aspergillus niger*, maximum removals of 21.21 % and 30.59 %, respectively were observed at 25°C, after 96 h incubation. The study was revealed the effect of temperature in the removal of phosphate and nitrate from wastewater by the test fungal species.

#### Keywords

Temperature,  
nitrate and  
phosphate  
removal;  
wastewater;  
fungi

#### Introduction

The two fundamental reasons for treating wastewater are to safeguard public health and prevent the pollution of receiving water bodies. These objectives can be achieved by reducing the concentrations of phosphate, nitrate, as well as heavy metals. The entrance of water with excessive

damage to the ecosystem (Rocca *et al.*, 2007). Despite the fact that phosphorus, which is common in fertilizers, detergents, human and animal waste is a nutrient needed in the soil for plant growth, when in excess amount, it results in algal blooms and huge amounts of aquatic growth. This

could result in the depletion of water quality and may also cause harm to fish and other aquatic organisms (Myers, 2008).

Although both chemical and biological processes have been implemented in the treatment of wastewater, but due to many drawbacks of chemical processes, biological processes are preferred in recent years. Among living organisms in wastewater treatment systems, microbes, such as bacteria, fungi, and protozoa are said to play important roles in nutrient removal, in the treatment of wastewater. These microbes assist in carrying out biochemical reactions and transformations that take place in the system as a part of the treatment process (Andersson et al., 2005). For example, to combat the release of phosphate into the environment a group of microorganisms was added to wastewater, which had the ability to accumulate and metabolise phosphate intracellularly (Korostynska et al., 2012). These organisms were also reported to have the ability to store phosphate, thereby reducing the concentration that is released into the environment.

Similarly, the presence of nitrate in drinking water is indicated to raise health concerns particularly because of its link to disorder in blood, thus affecting infants and other susceptible individuals (REF?). A high level of nitrates in drinking water is also known to cause an increase in risk of specific cancers and adverse reproductive outcomes. To protect infants against the risk of methemoglobinemia, which results when exposed to excessive nitrate concentration, the World Health Organisation (WHO) has given a guideline of 11mg/L nitrate-N (equivalent to 50mg/L as nitrate) in particular as prescription for protection against infant methemoglobinemia (Jalal et al., 2011).

Several microorganisms (bacteria, fungi and algae) have been investigated for their possible role in nutrient removal from wastewaters, with findings revealing different optimum conditions for different organisms. It is argued that because of the fastidious nature of nitrifying bacteria, which has resulted in many operational problems for wastewater treatment plants employing some form of biological nutrient removal, a lot of research have been focused mainly on meeting the requirements of this group of bacteria. Of recent, attention has shifted to the role of fungi in denitrification and at greater rates than bacteria. Besides, their having nitrification potential, fungi are also reported to possess resistance to a variety of inhibitory chemicals, hence their potential for nutrient removal is highly promising (Guest et al., 2002).

In addition, during biological nutrient removal, temperature was considered to be very important when assessing the overall efficiency of the treatment process. This is because temperature is indicated not to only have influence on the metabolic activities of the microbial population but also factors, such as gas transfer rates and the settling characteristics of biological solids (Mulkerrins et al., 2004).

This study was therefore aimed at investigating the effect of temperature on phosphate and nitrate removal from synthetic wastewater by four fungal species.

## Materials and Methods

### Test isolates

Four fungal isolates (*Fusarium sp*, *Absidia sp*, *Aspergillus niger* and *Aspergillus*

*flavus*) obtained from the Microbiology Department of the Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria were used in this study. The isolates were obtained as pure cultures and stored in Malt Extract agar slants at 4°C until usage. Prior to use, the isolates were plated out in Petri dishes containing sterile malt extract agar and incubated at 25°C for 72 h.

### Media for the study

The media for nutrient removal study was synthetic wastewater. The synthetic wastewater was composed of 5 g/L sodium acetate, 0.5 g magnesium sulfate, 1 g/L peptone, 1 g/L yeast extract, 0.5 g/L potassium dihydrogen phosphate, 0.5 g/L potassium nitrate and 0.5 g/L sodium chloride. The different components of the media were first weighed and dissolved separately in deionized water before mixing together as a single media.

Before usage, 200mL of the synthetic wastewater was dispensed into 200 mL quantity in 250 mL Erlenmeyer flasks. All the flasks containing the media were sterilized in an autoclave at 121 °C and 1.05kg/cm<sup>2</sup>for 15 min.

### Nutrient removal study

Sterile growth media were inoculated with a known volume of the spore suspension of each test isolate. Prior to inoculation a suspension of each of the test isolates was prepared in normal saline [(0.85%; w/v) sodium chloride in deionized water]as described by Aderiye *et al.* (1996). In this experiment, the inoculum size used was 2.80 x 10<sup>2</sup>spores/mL, 1.16 x 10<sup>3</sup>spores/mL, 7.50 x 10<sup>2</sup>spores/mL and 3.16 x 10<sup>3</sup>spores/mL for *Fusarium* sp., *Absidia* sp., *Aspergillus niger* and *Aspergillus flavus* respectively..

After inoculation with each isolate, replica flasks were incubated at temperatures of 25°C, 30°C, 35°C and 40°C. Prior inoculation with a test isolate and after every 24h, aliquot samples were taken from each of the flasks for the estimation of total phosphate, nitrate-nitrogen and pH in the wastewater, using standard methods (APHA, 2012). Phosphate and nitrate contents were determined using the ascorbic acid and salicylate methods respectively while the pH was determined using a pH meter. At each of the incubation temperatures, an uninoculated control was also set up. All the reagents used were of analytical grades. All experiments were carried out in triplicates and repeated twice.

For statistical analyses, the PAST: Paleontological statistics software package for education and data analysis as described by Hammer *et al.*, (2001) was used. The One-Way Analysis of Variance (ANOVA) was used for comparison of differences in means of samples whilethe Pearson Correlation Index was used to test for relationship. All the statistical analyses were done at a confidence interval of 95%.

### Results

Phosphate concentration in the wastewater in the presence of *Fusarium* sp. showed remarkable decreases after 96h at incubation temperatures of 30°C, 35°C and 40°C. Meanwhile, at 25°C, there was an increase in the phosphate concentration after 96h incubation. Following 96h incubation, the phosphate concentration in the wastewater was 180.69mg/L, 110.03mg/L, 106.13mg/L and 98.32mg/L, at 25°C, 30°C, 35°C and 40°C respectively (Fig. 1). The phosphate concentrations at 30°C, 35°C and 40°C were found to be

significantly lower than the value obtained at 25°C ( $p \leq 0.05$ ).

In the presence of *Fusarium* sp., the nitrate concentration in the wastewater showed significant decreases after 72h incubation. This trend was observed at the different incubation temperatures. After the 96 h incubation period, the concentrations of nitrate at the different temperatures were found to change from an initial level of 178.35 mg/L at 0 h to final concentrations of 125.73mg/L, 175.43mg/L, 101.86mg/L and 108.05mg/L, at incubation temperatures of 25°C, 30°C, 35°C and 40°C respectively (Fig. 2). Although the nitrate concentrations during incubation at the different temperatures were observed to be different, these differences were not observed to differ significantly ( $p \leq 0.05$ ).

In the case of the *Absidia* sp., the phosphate concentration in the wastewater after 96h incubation showed a slight increase from 155.34mg/L to 157.96mg/L at 25°C. At 30°C, 35°C and 40°C, the amount of phosphate was observed to decrease to 99.81 mg/L, 107.56 mg/L and 114.68 mg/L respectively (Fig. 3). At the end of incubation, the concentration of phosphate in the wastewater at 30°C was significantly lower than the values recorded at other temperatures ( $p \leq 0.05$ ).

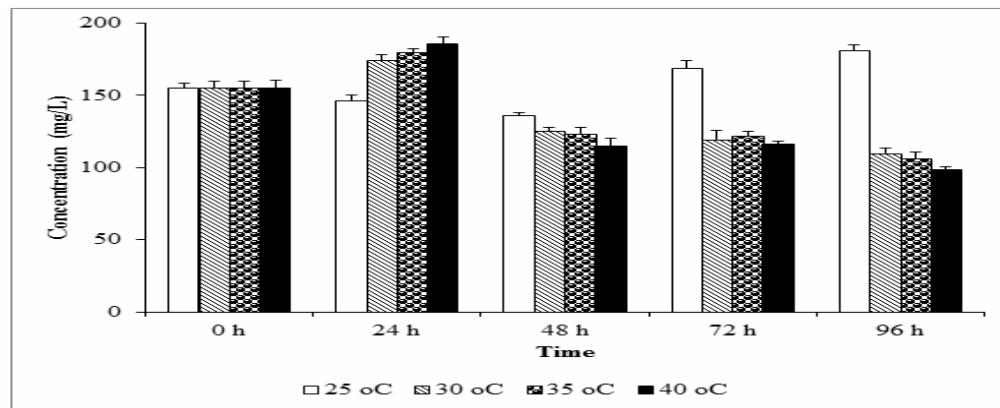
Nitrate concentrations in the wastewater in the presence of the *Absidia* sp. showed the least values at 72h. Within the same incubation period, the concentration of nitrate decreased from 178.35 mg/L at 0h to 32.04mg/L, 68.19mg/L, 51.84mg/L and 26.99mg/L at 25°C, 30°C, 35°C and 40°C

respectively. After 96h, drastic increase in the amount of nitrate was however observed at the different temperatures; 140.52mg/L at 25°C, 186.65mg/L at 30°C, 188.08mg/L at 35°C and 183.73mg/L at 40°C (Fig. 4). Despite the differences in nitrate concentrations at different temperatures in the presence of the *Absidia* sp., these differences were not observed to be significantly different ( $p \leq 0.05$ ).

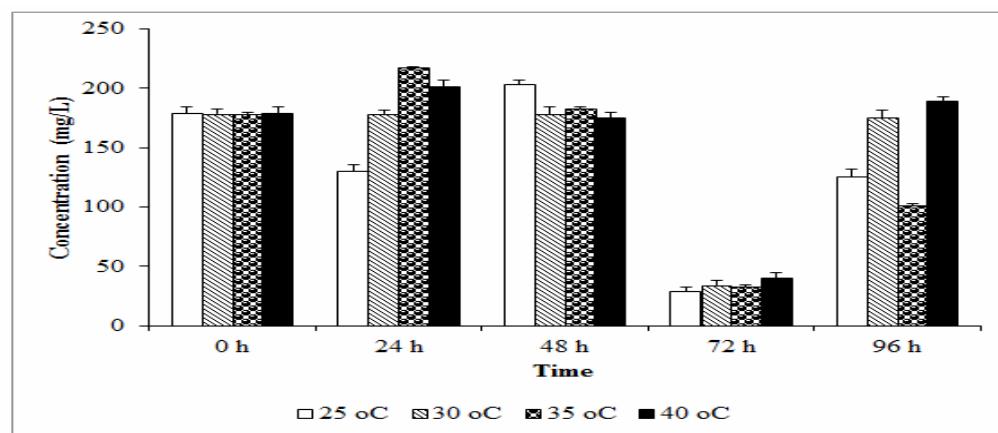
In the presence of the *Aspergillus niger*, the phosphate concentration in the wastewater showed significant decreases between 48h and 96h, at 30°C, 35°C and 45°C. After 96h, the amount of phosphate decreased from 155.34mg/L to 142.89mg/L, 98.37mg/L, 96.64mg/L and 96.40mg/L, at 25°C, 30°C, 35°C and 40°C respectively (Fig. 5). Throughout the incubation period, the phosphate concentration of the wastewater at 25°C when treated with *Aspergillus niger* was observed to be significantly higher than those obtained at other temperatures ( $p \leq 0.05$ ).

The nitrate concentration of the wastewater in the presence of the *Aspergillus niger* significantly decreased after 72h to 27.70mg/L, 25.01mg/L, 43.01mg/L and 53.26mg/L at 25°C, 30°C, 35°C and 40°C respectively. But after 96h the nitrate levels in the wastewater were 123.79mg/L at 25°C, 138.18mg/L, 148.18mg/L and 184.90mg/L (Fig. 6). Generally, nitrate levels at incubation temperatures of 25°C, 30°C and 35°C were found to be significantly lower than level at 40°C ( $p \leq 0.05$ ).

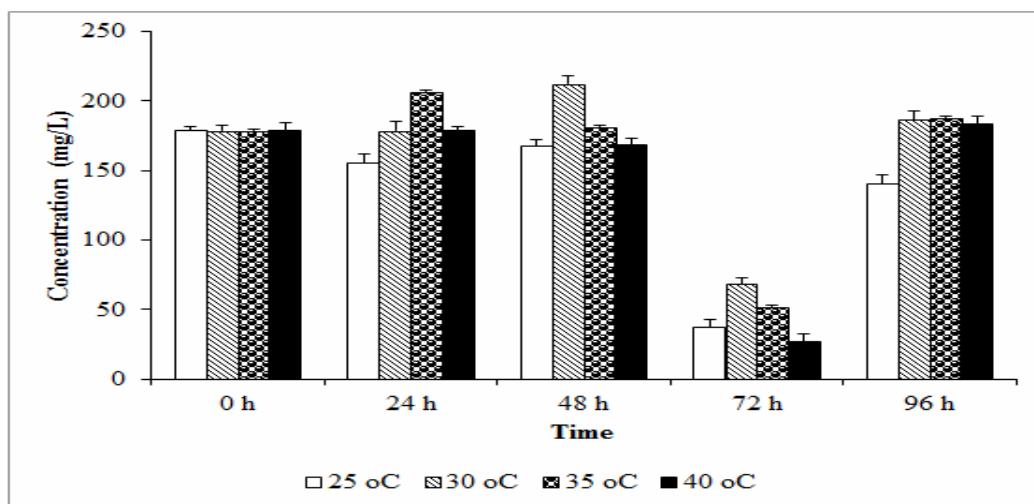
**Fig.1** Phosphate concentration in the wastewater at the different temperatures, when inoculated with the *Fusarium* sp



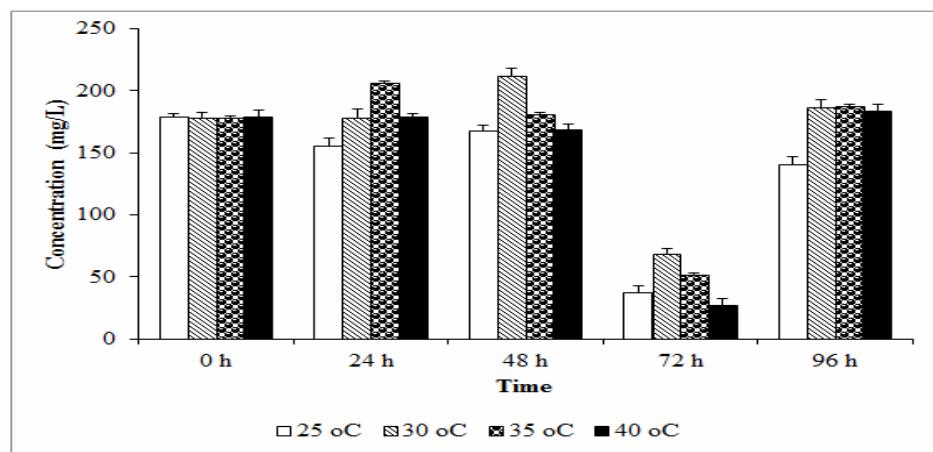
**Fig.2** Nitrate concentration in the wastewater at the different temperatures, when inoculated with the *Fusarium* sp



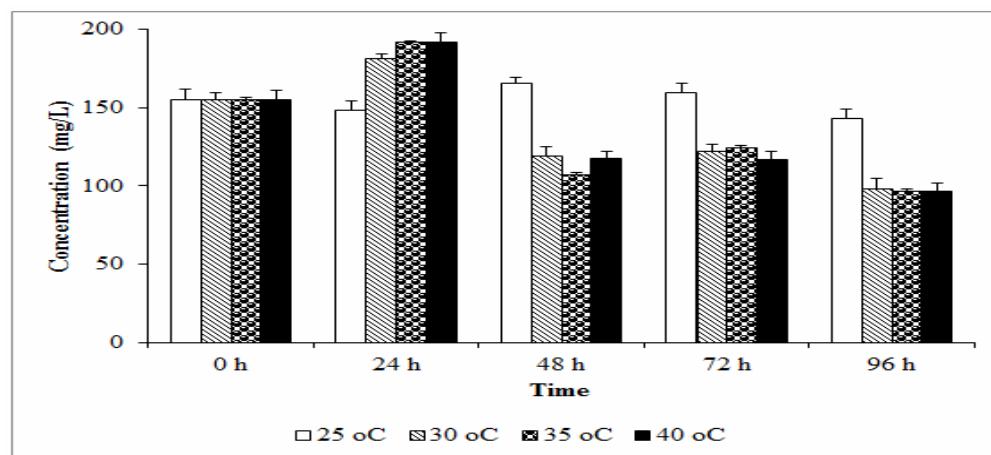
**Fig.3** Phosphate concentration in the wastewater at the different temperatures, when inoculated with the *Absidia* sp



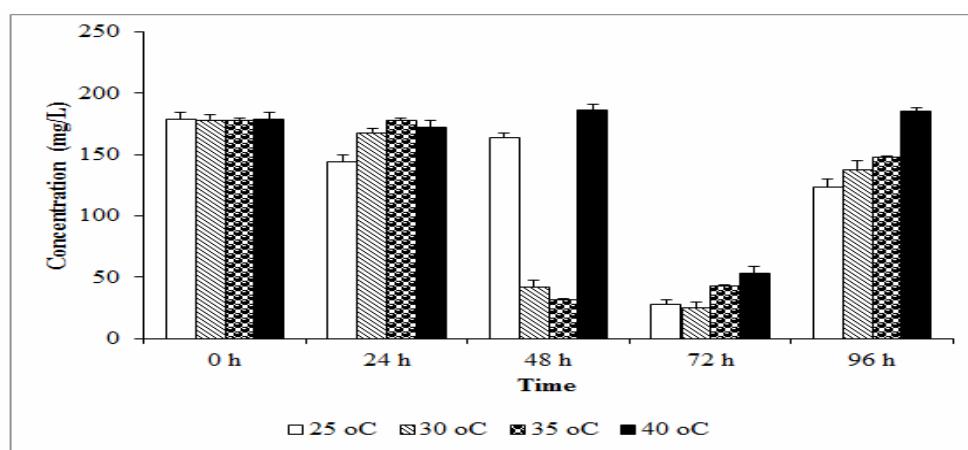
**Fig.4** Nitrate concentration in the wastewater at the different temperatures, when inoculated with the *Absidia* sp



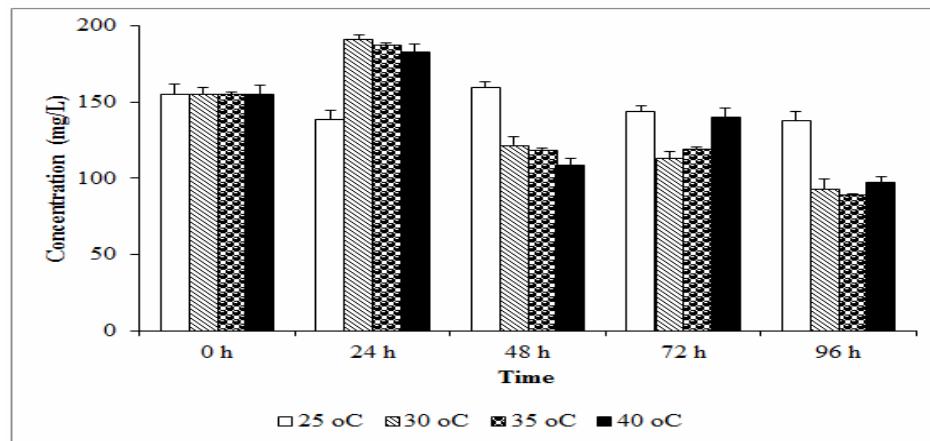
**Fig.5** Phosphate concentration in the wastewater at the different temperatures, when inoculated with the *Aspergillus niger*



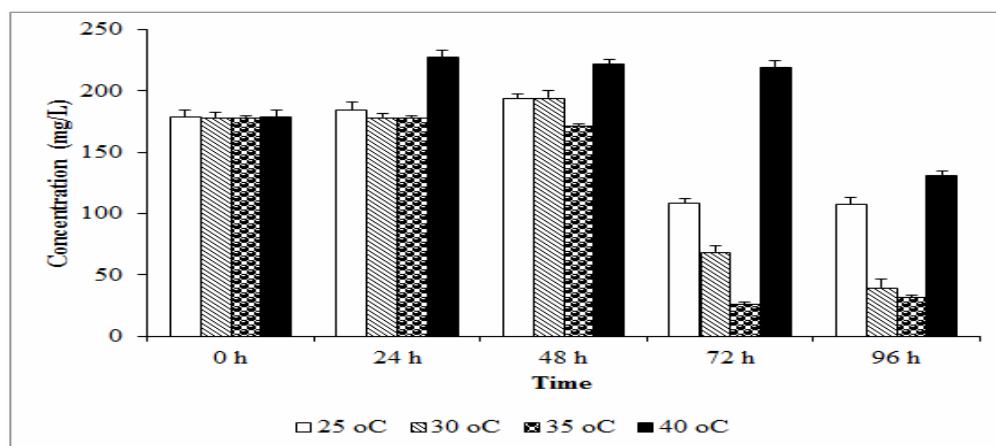
**Fig.6** Nitrate concentration in the wastewater at the different temperatures, when inoculated with the *Aspergillus niger*



**Fig.7** Phosphate concentration in the wastewater at the different temperatures, when inoculated with the *Aspergillus flavus*



**Fig.8** Nitrate concentration in the wastewater at the different temperatures, when inoculated with the *Aspergillus flavus*



As shown in Fig. 7, phosphate concentrations at the different temperatures in the presence of the *Aspergillus flavus* showed significant decreases at incubation temperatures of 30°C, 35°C and 40°C after 96 h incubation. At all the different temperatures, decreases in phosphate concentrations were recorded at the end of incubation. At the end of

incubation, the amount of phosphate in the wastewater decreased from 155.34mg/L at time 0h to 137.95mg/L, 92.84mg/L, 89.08mg/L and 97.34mg/L at incubation temperatures of 25°C, 30°C, 35°C and 40°C respectively (Fig. 7). Although the phosphate concentrations at different temperatures differed, the values were not observed to be significantly different ( $p \leq 0.05$ ).

**Table.1** Percent phosphate<sup>1</sup> change at the different incubation temperatures in presence of the test fungal species

<b>Incubation Temperatures</b>	<b>PO<sub>4</sub><sup>3-</sup> concentration at 0 h (mg/L)</b>	<b>PO<sub>4</sub><sup>3-</sup> concentration at 96 h (mg/L)</b>	<b>Percent change in concentration</b>
<b><i>Fusarium sp</i></b>			
25°C	155.34	180.69	14.03*
30°C	155.34	110.03	29.17
35°C	155.34	106.13	31.68
40°C	155.34	98.32	36.71
<b><i>Absidia sp</i></b>			
25 °C	155.34	157.96	1.66*
30 °C	155.34	99.81	35.75
35 °C	155.34	107.56	30.76
40 °C	155.34	114.68	26.17
<b><i>Aspergillus niger</i></b>			
25 °C	155.34	142.89	8.01
30 °C	155.34	98.37	36.67
35 °C	155.34	96.64	37.79
40 °C	155.34	96.4	37.94
<b><i>Aspergillus flavus</i></b>			
25 °C	155.34	137.95	11.19
30 °C	155.34	92.84	40.23
35 °C	155.34	89.08	42.65
40 °C	155.34	97.34	37.34

<sup>1</sup>All values are average of triplicate analyses;

\*Values are percent increases

**Table.2** Percent nitrate change at the different incubation temperatures in presence of the test fungal species

Incubation Temperature	NO <sub>3</sub> <sup>-</sup> concentration at 0 h (mg/L)	NO <sub>3</sub> <sup>-</sup> concentration at 96 h (mg/L)	Percent change in concentration
<b><i>Fusarium</i> sp</b>			
25 °C	178.35	125.73	29.50
30 °C	178.35	175.43	1.64
35 °C	178.35	101.86	42.89
40 °C	178.35	189.05	5.66*
<b><i>Absidia</i> sp</b>			
25 °C	178.35	140.53	21.21
30 °C	178.35	186.65	4.45*
35 °C	178.35	188.08	5.17*
40 °C	178.35	183.73	2.93*
<b><i>Aspergillus niger</i></b>			
25 °C	178.35	123.79	30.59
30 °C	178.35	148.18	16.92
35 °C	178.35	148.18	16.92
40 °C	178.35	184.90	3.54*
<b><i>Aspergillus flavus</i></b>			
25 °C	178.35	107.37	39.80
30 °C	178.35	39.70	77.74
35 °C	178.35	31.98	82.07
40 °C	178.35	130.96	26.57

<sup>1</sup>All values are average of triplicate analyses;

\*Values are percent increases

**Table.3** Variation in the pH of the wastewater treated with fungi at the different temperatures

Temperature	0 h	24 h	48 h	72 h	96 h
<i>Fusarium sp</i>					
25 °C	6.0	6.3	6.3	6.4	6.5
30 °C	6.0	6.3	6.3	6.4	6.5
35 °C	6.0	6.1	6.1	6.2	6.5
40 °C	6.0	6.2	6.2	6.3	6.5
<i>Absidia sp</i>					
25 °C	6.0	6.3	6.5	6.5	6.5
30 °C	6.0	6.1	6.2	6.2	6.2
35 °C	6.0	6.1	6.2	6.2	6.2
40 °C	6.0	6.1	6.2	6.2	6.2
<i>Aspergillus niger</i>					
25 °C	6.0	6.3	6.5	6.6	6.6
30 °C	6.0	6.1	6.1	6.2	6.3
35 °C	6.0	6.0	6.3	6.3	6.4
40 °C	6.0	6.1	6.1	6.1	6.1
<i>Aspergillus flavus</i>					
25 °C	6.0	6.3	6.4	6.4	7.1
30 °C	6.0	6.3	6.2	7.0	6.6
35 °C	6.0	6.2	6.3	6.9	6.4
40 °C	6.0	6.1	6.1	6.3	6.1

All values are average of triplicate analyses

For nitrate concentration in the presence of *Aspergillus flavus*, significant decreases were observed at 72h and 96h, with very high decreases observed at 30°C and 35°C. The observed decreases in phosphate concentrations at 72h and 96h were irrespective of the different incubation temperatures. Following incubation, the nitrate levels in the wastewater were 107.37mg/L at 25°C, 39.70mg/L at 30°C, 31.98mg/L at 35°C and 130.96mg/L at 40°C (Fig. 8). Generally, the nitrate levels at 30°C and 35°C were observed to be significantly lower than those of 25°C and 40°C ( $p \leq 0.05$ ).

When the wastewater was treated with *Absidia* sp. and *Fusarium* sp. at 25°C, about 1.66 and 14.01% of phosphate was released into the medium. After 96h, 36.71% of the phosphate was removed at 40°C in the presence of the *Fusarium* sp. while with *Absidia* sp., *Aspergillus niger* and *Aspergillus flavus* higher phosphate removals of 35.75 %, 37.94 % and 42.65 % were recorded at 30°C, 40°C and 35°C respectively (Table 1).

With *Absidia* sp. and *Aspergillus niger*, the highest nitrate removal of 21.21% and 30.59% was observed at 25°C after 96h while about 42.89% and 82.07% of the nitrates were removed when the wastewater was treated with *Fusarium* sp. and *Aspergillus flavus* respectively at 35°C (Table 2).

There were slight increases in the pH values of the wastewater incubated at different temperatures with time. This trend was irrespective of the isolate used or the incubation temperature (Table 3). There was no significant difference between the pH values of the wastewater at the different incubation temperatures

( $p \leq 0.05$ ) neither were these values different when treated with the fungal isolates.

## Discussion

The present study revealed the optimum temperature range for phosphate removal in the presence of the fungal isolates to be 30°C-40°C. For nitrate removal, the optimum temperature of 25°C was required by *Absidia* sp. and *Aspergillus niger* and 35°C by *Fusarium* sp. and *Aspergillus flavus*. It is indicated that in biological nutrient removal systems, the nitrification process is a rate limiting procedure, with nitrifiers growing slower (15-20 times) than heterotrophic organisms and they work faster as temperatures increase but become inhibited at temperatures above 30°C. The concentration of biological nutrients in wastewater is known to depend very much on temperatures. Earlier reports have suggested that at low temperatures of between 10-12°C the rate of nitrification diminishes by 50 %, while that of denitrification reduced by 80 % (Baltic Marine Environment Protection, 1990).

In this study all the isolates had significant nitrate removal ability at the different temperatures, although the degree of removal differed at varied temperatures. In most cases, bacteria have been reported as the commonest microorganisms involved in nutrient removal in wastewater. All the microbes (bacteria, protozoa and fungi) reported in biological nutrient removal system will remove carbon, nitrogen and phosphorus via the waste activated sludge or effluent (Grote, 2010).

In other reports, temperature has been implicated as an important factor in determining the rate of nitrification,

although it is a factor that operators have little control over. Nitrifying organisms are indicated to be slower and less active at lower temperatures, hence the rate of nitrification is said to decrease at reduced temperatures (Klebs, 2005).

At 30°C, 35°C and 40°C, the test isolates demonstrated both phosphate and nitrate removal abilities, although the extent of nitrate removal was far higher. Simultaneous phosphate and nitrate removal by protozoa has been reported (Akpor et al., 2008). In anaerobic conditions, polyphosphate accumulating organisms are known to convert readily available organic matter to carbon compounds and use the energy generated through the breakdown of polyphosphate molecules to create polyhydroxyalkanoates, which results in the release of phosphorus after breakdown that is taken up during subsequent aerobic conditions (Jeyanayagam, 2005; EPA, 2009)

Although temperature is indicated to be a key parameter that affects the reaction performance and kinetics of biological nutrient removal systems, there are conflicting reports on its effects on enhanced biological nutrient removal systems (Erdal et al., 2003; Thongchai et al., 2003). Mamais and Jenkins (1992) have indicated that the optimum operating temperature for enhanced nutrient removal is likely to range from 28°C to 33°C. It is however argued that the optimum temperature for growth may not necessarily be the same for nutrient removal (Saito et al., 2004). In some studies, biological phosphate removal efficiency was indicated to improve at temperatures of 20°C to 37°C. Some other studies have indicated better phosphorus removal at lower temperatures of 5°C - 15°C. Some reports have indicated that polyphosphate accumulating organisms are found to be lower-range mesophiles or

perhaps psychrophiles at predominantly 20°C or possibly lower (Panswadet et al., 2003; Mulkerrinset al., 2004).

This study showed that the different fungal species have varying optimum temperature for nutrient removal. The optimum temperature for nitrate removal was found to be 35°C for *Fusarium* sp. and *Aspergillus niger*. For the *Aspergillus flavus* and the *Absidia* sp., the optimum temperature for significant nitrate removal was found to be 25°C.

All the isolates used for the investigation showed significant nitrate removal ability at all the incubation temperatures. In the case of phosphate, no remarkable removal was observed at 25°C but there were increases in the phosphate concentration in the presence of the isolates. In addition, the study revealed only slight increases in the pH values during the period of incubation, a trend that was observed irrespective of the test isolates used or the incubation temperature. However, this study has provided valuable insight to the effect of temperature on the nutrient removal abilities of the fungal isolates.

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