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Research Article

ASSESSMENT OF THE EFFECTS OF TEMPERATURE, PH, CARBON AND NITROGEN SOURCES ON PHOSPHATE REMOVAL FROM WASTEWATER BY TWO SELECTED FUNGAL SPECIES

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ABSTRACT
The aim of the study was to investigate the optimum conditions (temperature, pH, and external carbon and nitrogen sources) for phosphate removal from wastewater by *Aspergillus flavus* and *Aspergillus niger* under shake flask conditions. Before inoculation with the respective test isolates, the wastewater used was first filtered and supplemented with magnesium sulphate (0.5 g/L), a known concentration of an external carbon and nitrogen source, dispensed in 200 mL quantities into 250 mL capacity conical flasks and sterilized in an autoclave. After inoculation, aliquot wastewater samples were taken from each flask prior inoculation and every 24 h, for 96 h for the estimation phosphate concentration, using standard procedures. After the 96 h incubation period, phosphate levels in the wastewater were observed to show increases of 16.51 %, 14.31 % and 10.95 % in the presence of the *Aspergillus flavus* and 16.09 %, 13.76 % and 18.10 % in the presence of the *Aspergillus niger* at incubation temperatures of at 25^{o}C, 35^{o}C and 45^{o}C, respectively. At the different pH investigated, the highest decrease of 13.57 % for *Aspergillus flavus* and 20.40 %, *for Aspergillus niger* was observed at pH 6 and 10, respectively. At the different concentrations of sodium acetate that were used for investigation, highest phosphate removal was observed at 5 g/L and 20 g/L, for the *Aspergillus flavus* and *Aspergillus niger*, respectively. Apart from glucose, all the carbon sources investigated (lactose, sucrose or methanol) were shown to enhance phosphate removal by the isolates. Although phosphate removal was observed at the different concentrations of peptone, highest removal was observed at 20 g/L. The study was able to provide information on the optimum temperature, pH, external carbon and nitrogen sources, sodium acetate and peptone concentrations that will enhance phosphate removal by the test isolates.

Keywords: *Phosphate, Uptake, Wastewater, Fungi*

INTRODUCTION
The world is faced with formidable challenges in meeting rising demands of clean water due to extended droughts, population growth, more stringent health based regulations and competing demands from a variety of users (US Bureau of Reclamation and Sandia National Laboratories, 2003). In recent time, poor sanitation and lack of drinking water of high quality is estimated to be the main global cause of approximately 4,000 deaths per day (Bartram *et al.*, 2005) Since daily human activities, ranging from domestic chores to industrial activities, produces wastewater, wastewater treatment is inevitable. The pressing need to treat wastewater is solely for two reasons: preventing environmental pollution and safeguarding water supplies in order to protect public health. To achieve this purpose, organic matter, nitrogen, phosphorus and heavy metal in wastewater must be reduced to minimal level. Wastewater problem arises from extensive industrialization, increasing population density and a highly urbanized society (EPA, 2002; McCasland *et al.*, 2008). Anthropogenic impact on natural environment especially on aquatic ecosystems is currently a topic of increasing concern. The effluents generated from domestic and industrial activities give rise to effluent, which when discharge indiscriminately into water bodies without treatment, can introduce a wide range of chemical pollutants and microbial contaminants to water sources (Eikelboom and Draaijer, 1999). When these contaminant are in high quantity more than the critical values stipulated by regulatory bodies, it can impact the health of humans and animals negatively (EPA,

2000; CDC, 2002). Nitrogen and phosphorus are present in aquatic bodies which aid the growth of plant and other microorganism but when in excess, can be detrimental. Phosphorus exists in three forms: as organophosphate, orthophosphate and polyphosphate. Municipal wastewater contains about 50% to 70% orthophosphate (Meganck and Faup, 1988). The EU-directive (91/271/EEC) sets the limit for total phosphorus in effluent wastewater in sensitive areas to 1 or 2 mg P/l, depending on the size of the plant, or a minimum of 80% reduction of the influent concentration (Tykesson, 2005). It has been generally understood that phosphorus is the limiting nutrient in lakes and waterways. The main sources of phosphorus released into the environment include fertilizers, detergents, cleaning preparations, and boiler waters to which phosphates are added for treatment (Pradyot, 1997).

Wastewater can be treated by chemical method, biological method or both. Chemical removal involves the addition of lime, aluminum and ferric chloride to wastewater. This method is expensive together with the accumulation of large quantities of chemical waste sludge (Fuhs and Chen, 1975). Biological phosphate removal from wastewater is a generally accepted; less cost effective to chemical phosphate removal and has been widely investigated.

Microorganisms like bacteria, fungi and protozoa play important roles in biological nutrient removal. These organisms have ability to store phosphate intracellularly, thereby reducing the quantity in the environment. Previous Studies by previous workers shows that fungi produce a large variety of extracellular proteins, organic acids, and other metabolites and are able to resist inhibitory chemicals in wastewater making them highly promising and efficient in nutrient removal studies (Guest *et al.*, 2002). Although the role of fungi in phosphate removal in wastewater is widely reported, there is optimum conditions for removal by specific species is still a continuous investigation. This study was therefore aimed at investigating the optimum conditions for phosphate removal in wastewater by two fungal species.

MATERIALS AND METHODS

Two species of *Aspergillus* (*niger* and *flavus*) were used for this investigation. The isolates were maintained on Sabouraud dextrose Agar (Biolab) slants at 4^{o}C until usage. Prior to use, the test isolates were plated out in petri dishes containing sterile sabouraud dextrose agar and incubated at 25^{o}C for 72 h.

The wastewater used for this investigation was collected from the Landmark University Commercial Farm. The collected wastewater was allowed to settle and filtered, using Whatman No. 1 filter paper. The filtered wastewater was then supplemented with a known concentration of a carbon source, a nitrogen source and magnesium sulphate (0.5 g/L). The different supplements were weighed and first dissolved separately in distilled water before mixing together. The prepared wastewater was then dispensed in 200 mL quantity in 250 mL capacity Erlenmeyer's flasks before sterilizing in an autoclave at 121^{o}C and 1.05 kg/cm^{2} for 15 min.

To ascertain the effectiveness of the sterilization, the sterilized flasks were left on the work bench for 24 h, after which aliquot samples were taken from them and plated on nutrient and sabouraud dextrose agar to check for bacterial and fungal growths, respectively. Only flasks whose contents showed no growth on the media were used for phosphate removal studies.

For phosphate removal study**,** to each sterile wastewater, a known inoculums size of the respective test fungal species suspended in sterile normal saline (0.85 % NaCl w/v) was inoculated. After inoculation, the flasks were incubated at a known temperature in a shaking incubator at a shaking speed of 150 rpm. Just before inoculation and after every 24 h for the next 96 h, aliquot wastewater samples were taken from each flask for the estimation of the phosphate content, pH. Phosphate concentration in the wastewater was estimated using the ascorbic acid method, as described in standard methods (APHA, 2012).

In this investigation, the parameters investigated were temperature, pH, sodium acetate concentration, external carbon source, peptone concentration and external nitrogen sources. Three different incubation temperatures (25 ^{o}C, 35 ^{o}C and 45 ^{o}C), with sodium acetate (5 g/L) and peptone (5 g/L) as the carbon and nitrogen sources in the wastewater, respectively at a pH of 7. For pH, the study was carried out using pH 6, 8 and 10. The pH of the wastewater was adjusted using 1 M HCl and 1 M NaOH. The carbon and

nitrogen sources used for this phase of the study were sodium acetate (5 g/L) and peptone (5 g/L), respectively, at a temperature of 30^{o}C.

In the case of sodium acetate concentration, 5 g/L, 10 g/L and 15 g/L were used for the study, with the pH of the wastewater adjusted to 7 while incubation in the wastewater and nitrogen source were. The nitrogen source was nitrogen at a concentration of 5 g/L while incubation was at 30^{o}C. The different external carbon sources used for the study were glucose, lactose, sucrose and methanol at respective concentrations of 5 g/L. With respect to nitrogen sources were peptone, yeast extract and meat extract at respective concentrations of 5 g/L.

During the various phases of the experiment, pH of the test wastewater was adjusted to 7 while incubation at 30^{o}C, except in the temperature and pH variations studies, respectively. Except for the nitrogen sources experiment setups, the nitrogen source used in each investigation was peptone at 5 g/L. Similarly, apart from the carbon source experiment setup, the carbon source used in all phases of the study was sodium acetate at a concentration of 5 g/L. All experimental procedures and analysis were carried out in triplicates. All reagents that were used were of analytical grades.

RESULTS AND DISCUSSION

Results

As shown in Figure 1, phosphate concentration in the wastewater at the different incubation temperatures in presence of the *Aspergillus flavus* was observed to show increases from initial levels of 43.20 mg/L, 44.24 mg/L and 43.73 mg/L at 0 h to final levels after 96 h incubation, of 51.74 mg/L, 51.63 mg/L and 49.11 mg/L at 25^{o}C, 35^{o}C and 45^{o}C, respectively. In the presence of the *Aspergillus niger*, phosphate levels in the wastewater were also observed to show increases from 44.49 mg/L to 53.02 mg/L at 25^{o}C, from 43.48 mg/L to 50.42 mg/L at 35^{o}C and from 42.23 mg/L to 51.56 mg/L at temperature at 45^{o}C, respectively (Figure 1).

Generally, at the end of the 96 h incubation time, phosphate increases of 16.51 %, 14.31 % and 10.95 % were observed in the presence of the *Aspergillus flavus*, at incubation temperatures of 25^{o}C, 35^{o}C and 45^{o}C, respectively. In the presence of the *Aspergillus niger*, phosphate increases of 16.09 %, 13.76 % and 18.10 % were observed after 96 h incubation time, at 25^{o}C, 35^{o}C and 45^{o}C, respectively.

When investigating the effect of pH on phosphate concentration in the wastewater in the presence of the *Aspergillus flavus*, the results revealed changes after 96 h from 41.27 mg/L to 35.67 mg/L, from 39.78 mg/L to 38.09 mg/L and 40.21 mg/L to 44.86 mg/L at pH 6, pH 8 and pH 10 respectively. In the presence of the *Aspergillus niger*, phosphate levels were observed to change from initial concentrations of 46.83 mg/L, 46.97 mg/L and 45.45 mg/L to final concentrations after 96 h of 39.88 mg/L, 38.74 mg/L and 36.18 mg/L at pH 6, pH 8 and pH 10 respectively (Figure 2). In the presence of the *Aspergillus flavus*, a highest decrease in phosphate concentration of 13.57 % was observed after the 96 h incubation time at pH 6. In the case of the *Aspergillus niger*, decreases of 14.84 %, 17.52 % and 20.40 % at pH 6, 8 and 10, respectively.

Figure 3 shows the trend in phosphate concentration at different concentrations of sodium acetate as external carbon source in presence of the test isolates. In the presence of the *Aspergillus flavus*, phosphate levels were observed to show decreases in concentration after 96 h incubation from 44.44 mg/L to 35.39 mg/L at 5 g/L of acetate, from 45.49 mg/L to 44.79 mg/L, at 10 g/L of sodium acetate and from 43.55 mg/L to 34.91 mg/L at 15 g/L of sodium acetate. Similarly, concentrations in the presence of the *Aspergillus niger*, at the expiration of incubation showed decreases from 45.04 mg/L to 37.50 mg/L, from 46.32 mg/L to 42.47 mg/L and from 44.20 mg/L to 36.08 mg/L at 5 g/L, 10 g/L and 15 g/L of sodium acetate, respectively (Figure 3). The decreases in phosphate concentrations in the wastewater translates to 20.37 %, 1.50 % 19.84 % at 5 g/L, 10 g/L and 15 g/L sodium acetate, respectively in the presence of the *Aspergillus flavus.* In the presence of the *Aspergillus niger*, the decreases in concentration translates to 16.74 %, 15.06 % and 18.37 % at 5 g/L, 10 g/L and 15 g/L of sodium acetate, respectively.

At the different carbon sources, phosphate concentration in the wastewater in the presence of the *Aspergillus flavus* showed an increase from 21.77 mg/L to 24.23 mg/L with glucose as carbon source and

decreases from 23.40 mg/L to 10.89 mg/L, from 23.99 mg/L to 17.59 mg/L and from 26.40 mg/L to 13.69 mg/L with lactose, sucrose and methanol as carbon sources, respectively, after the 96 h incubation time.

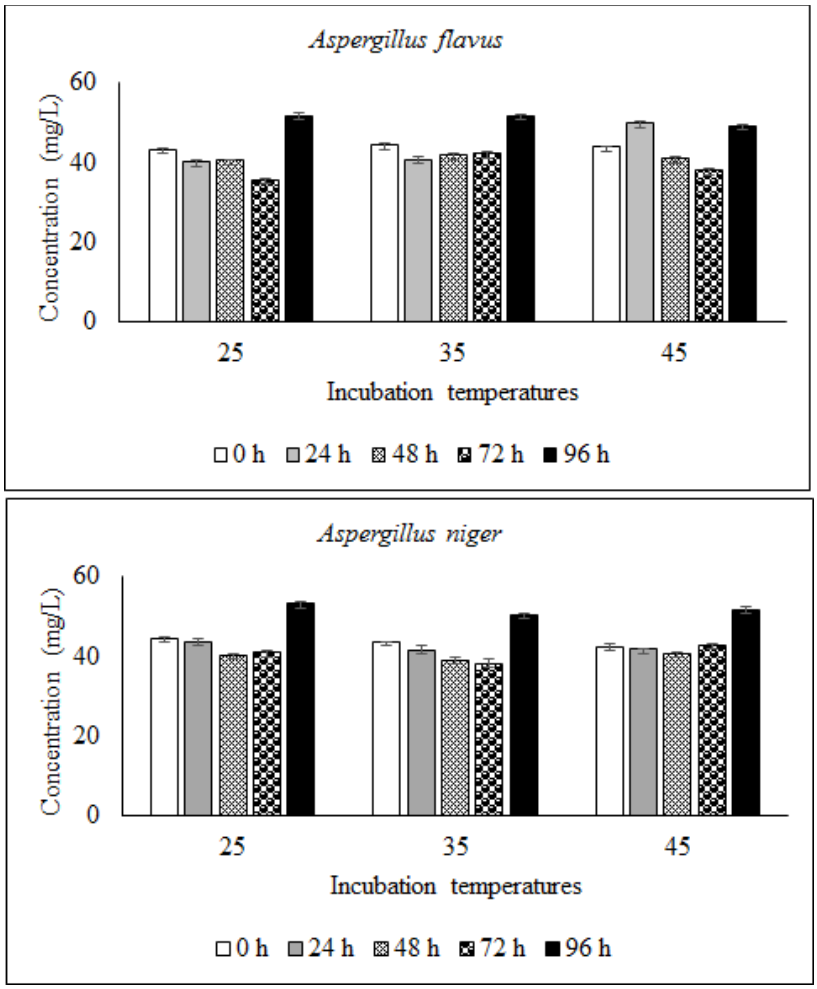

Figure 1: Phosphate concentrations in the wastewater at the different incubation temperatures in presence of the test fungal species

Similarly, in the presence of the *Aspergillus niger*, phosphate concentrations showed a slight increase from 23.40 mg/L to 25.19 mg/L with glucose as carbon source and decreases from 22.98 mg/L to 20.22 mg/L, from 24.05 mg/L to 8.29 mg/L and from 25.51 mg/L to 14.58 mg/L with lactose, sucrose and methanol as carbon sources, respectively (Figure 4). In the presence of the two test isolates, decreases in concentration after 96 h incubation were observed when lactose, sucrose or methanol was used as carbon source. With lactose, sucrose or methanol as carbon source, decreases in phosphate concentration of 53.46 %, 26.68 % and 481.4 %, respectively were observed in the presence of the *Aspergillus flavus*. In the presence of the *Aspergillus niger*, decreases in phosphate concentration of 12.01 %, 65.53 % and 42.85 % were observed with lactose, sucrose or methanol as carbon source, respectively.

At the different concentrations of peptone in the wastewater, phosphate levels after the 96 h incubation time in the presence of the *Aspergillus flavus* was observed to vary from 61.52 mg/L to 50.71 mg/L, from 66.18 mg/L to 52.32 mg/L, from 71.82 mg/L to 50.94 mg/L and from 70.36 mg/L to 43.89mg/L at peptone concentrations of 5 g/L, 10 g/L, 15 g/L and 20 g/L, respectively. When *Aspergillus niger* was used as the inoculum, phosphate levels showed decreases after the 96 h incubation period from 62.44 mg/L to 48.25 mg/L, from 63.18 mg/L to 57.02 mg/L, from 68.08 mg/L to 49.73 mg/L and from 63.50

mg/L to 54.57 mg/L at 5 g/L, 10 g/L, 15 g/L and 20 g/L of peptone, respectively (Figure 5). Generally, the highest decrease in phosphate concentration in the wastewater was observed in the with peptone concentrations of 20 g/L and 15 g/L, in the presence of the *Aspergillus flavus*, respectively. At the end of the 96 h incubation period, phosphate decreases of 29.11 %, 27.51 %, 29.07 % and 37.62 % were observed in the presence of the *Aspergillus flavus*, at peptone concentrations of 5 g/L, 10 g/L, 15 g/L and 20 g/L, respectively. In the presence of the *Aspergillus niger*, phosphate decreases of 22.73 %, 9.75 %, 22.39 % and 14.06 %, were observed at peptone concentrations of 5 g/L, 10 g/L, 15 g/L and 20 g/L, respectively.

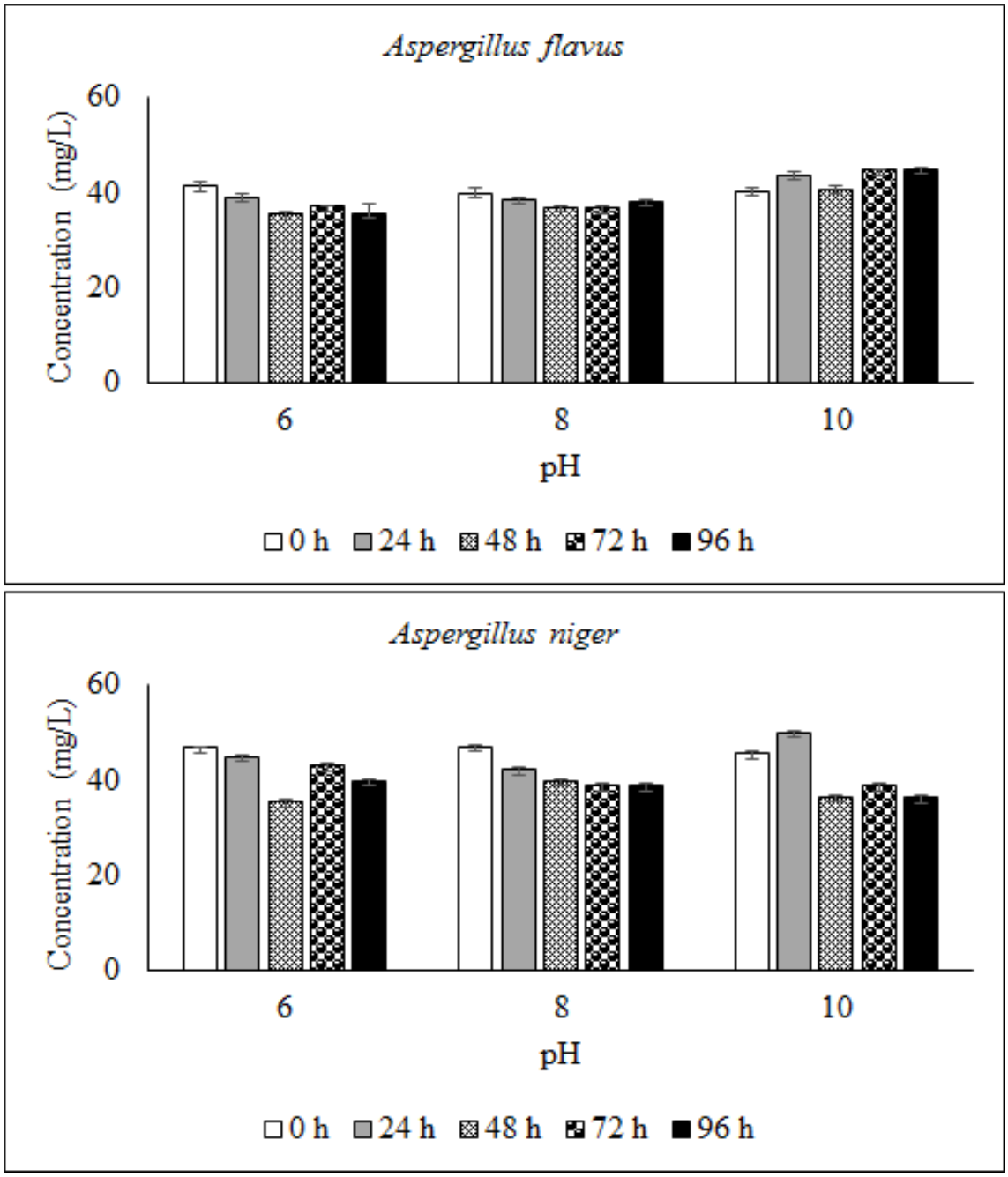

Figure 2: Phosphate concentrations in the wastewater at the pH in presence of the test fungal species

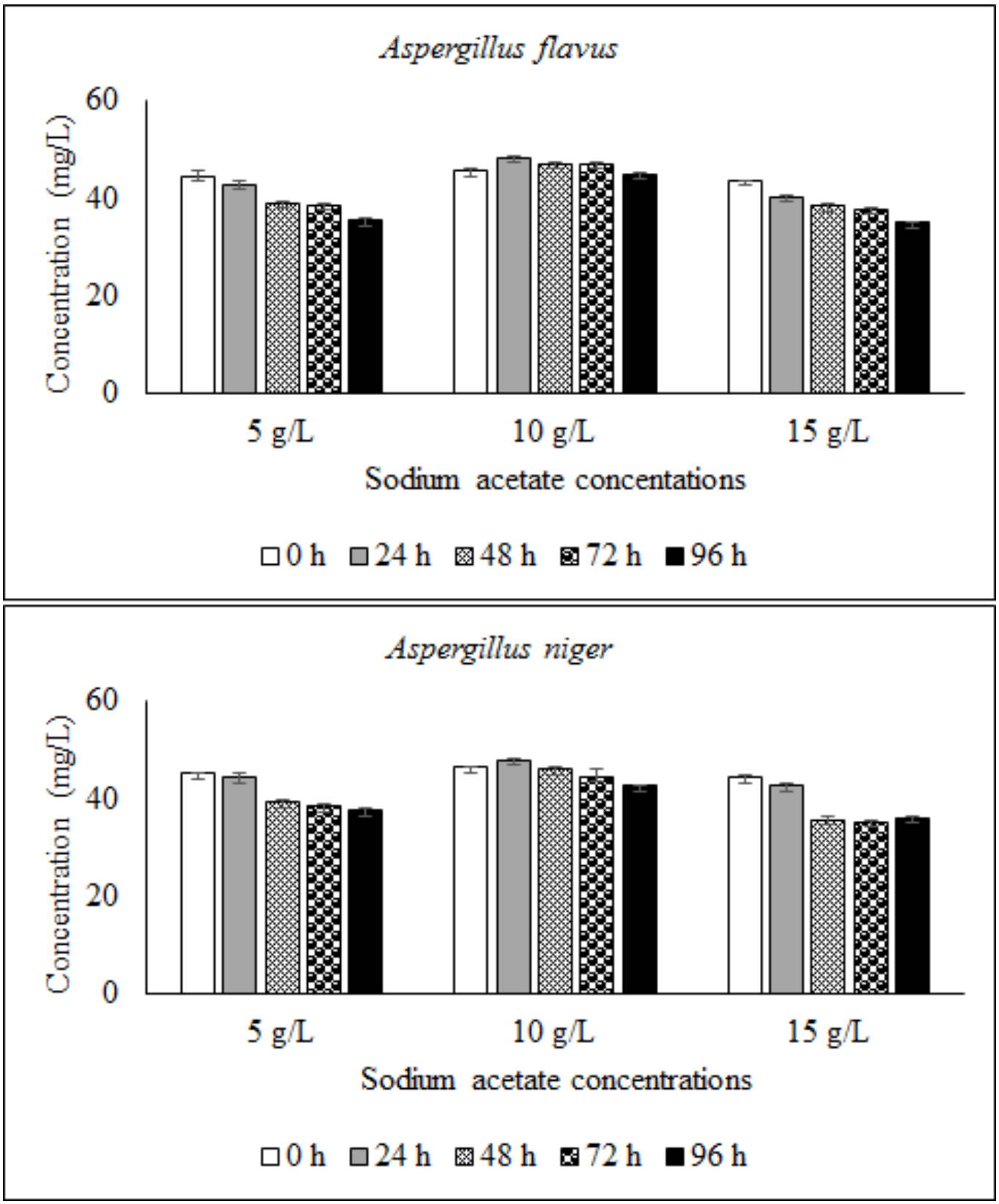

Figure 3: Phosphate concentrations in the wastewater at the concentrations of sodium acetate in presence of the test fungal species

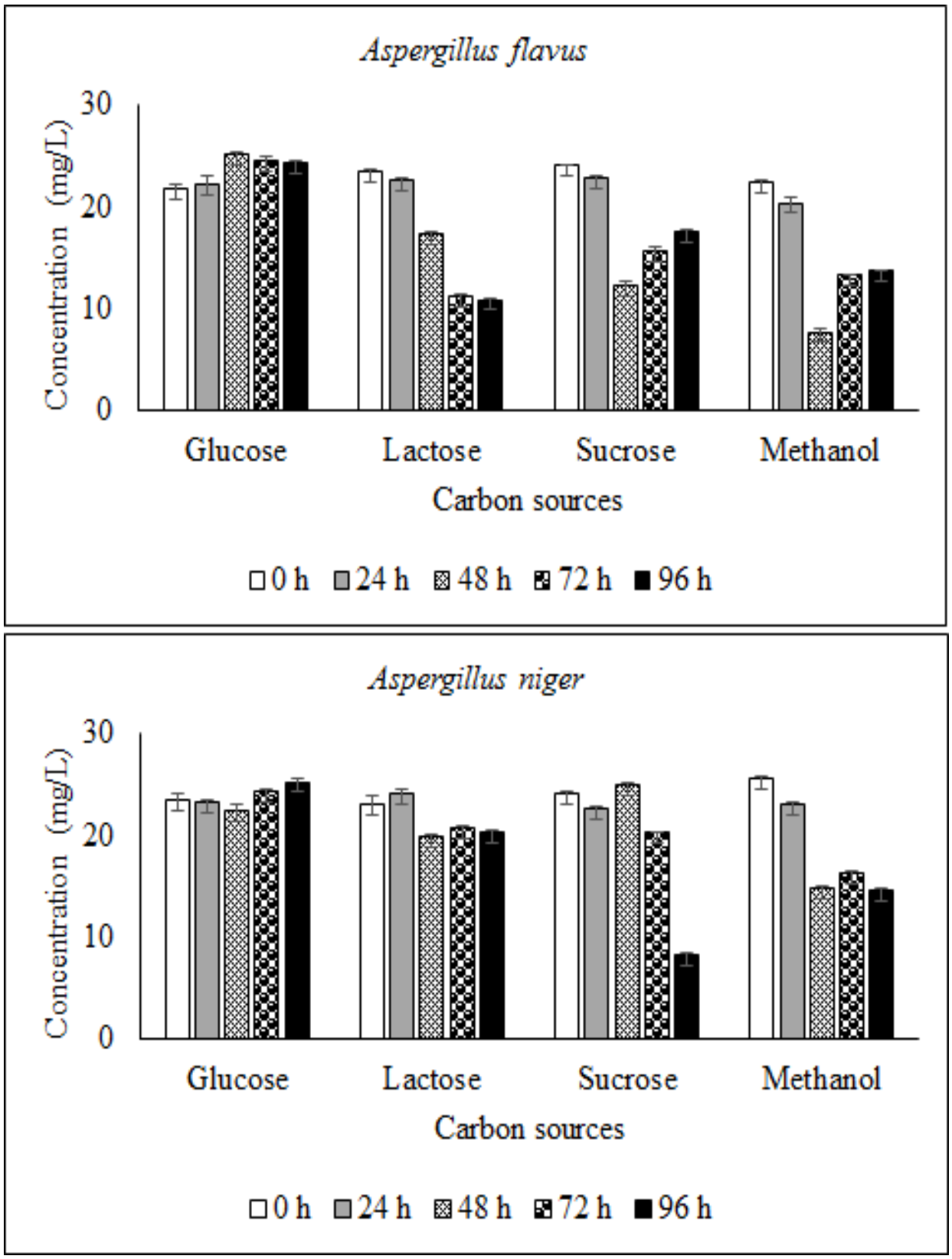

Figure 4: Phosphate concentrations in the wastewater at the different carbon sources in presence of the test fungal species

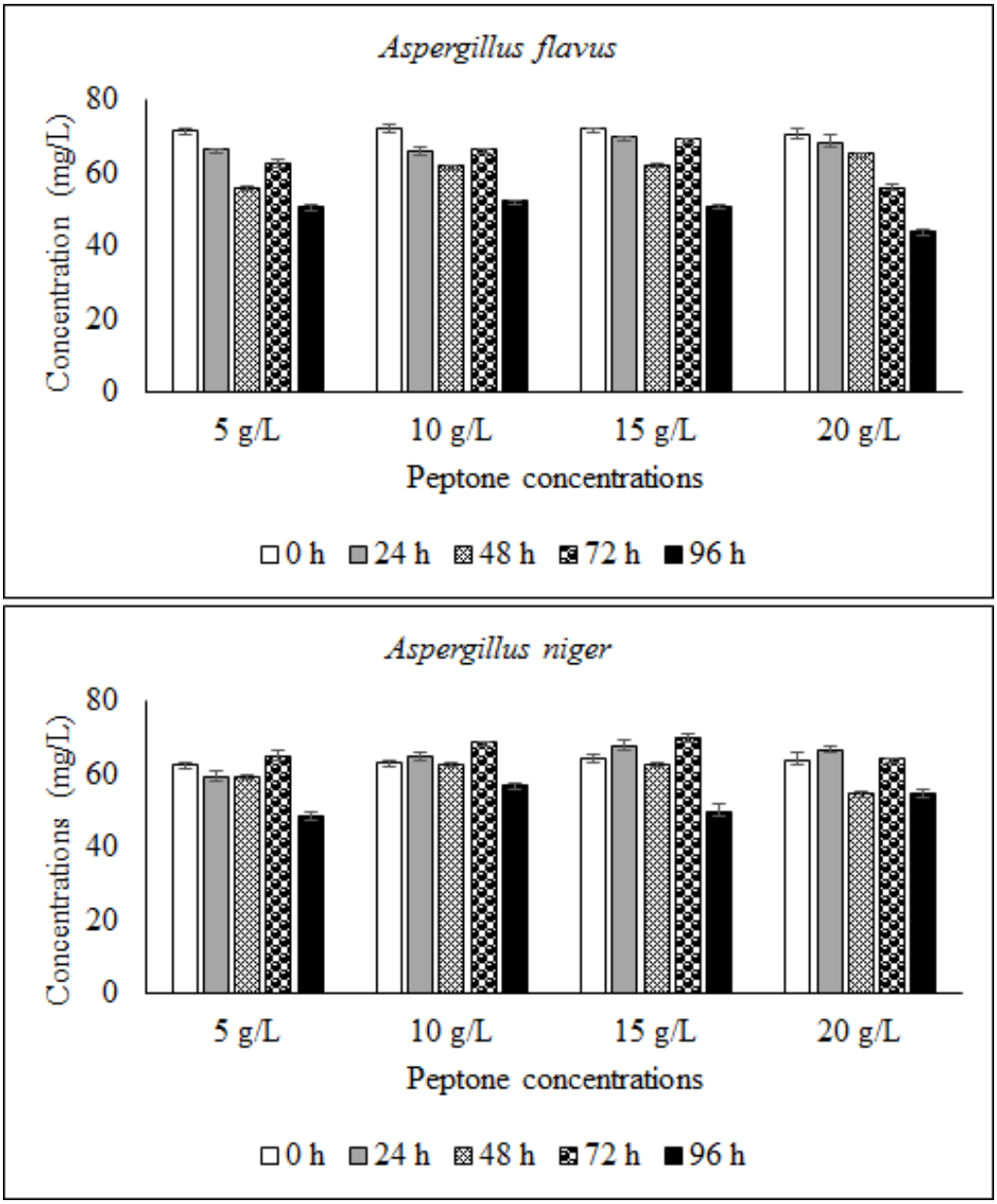

Figure 5: Phosphate concentrations in the wastewater at the concentrations of peptone in presence of the test fungal species

With different nitrogen sources in the wastewater, phosphate concentration in the wastewater in the presence of the *Aspergillus flavus* showed variation from after the 96 h incubation period from 48.01 mg/L to 28.51 mg/L, from 49.57 mg/L to 32.41 mg/L and from 48.00 mg/L to 27.08 mg/L with peptone, yeast extract and meat extract, as nitrogen sources, respectively. In the presence of the *Aspergillus niger*, phosphate concentrations showed decreases from 46.62 mg/L to 29.27 mg/L, from 43.91 mg/L to 33.79 mg/L and from 49.02 mg/L to 33.12 mg/L for peptone, yeast extract and meat extract, as nitrogen sources, respectively (Figure 6). At the end of the 96 h incubation time, decreases of 40.01 %, 34.62 % and 43.58 % in phosphate levels in the wastewater in the presence of the *Aspergillus flavus* were observed with peptone, yeast extract and meat extract, as nitrogen sources, respectively. In the presence of the *Aspergillus niger*, decreases after 96 h incubation of 37.22 %, 23.05 % and 28.14 % were observed with peptone, yeast extract and meat extract, as nitrogen sources, respectively.

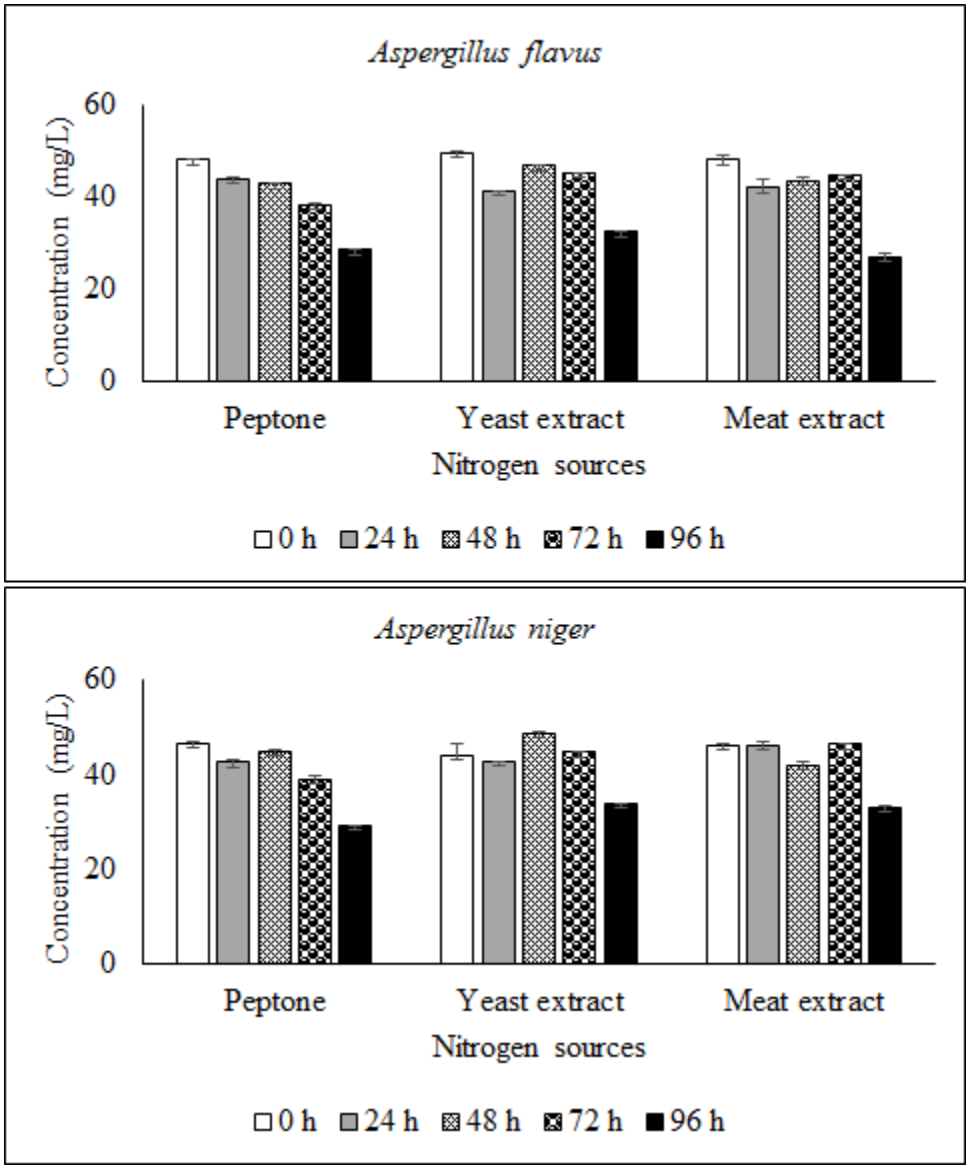

Figure 6: Phosphate concentrations in the wastewater at the different nitrogen sources in presence of the test fungal species

Discussion

The present study made use of two fungal isolates. A variety of fungi species (*Rhizopus, Aspergillus, Neurospora, Fusarium, Saccharomyces* and *Penicillium*) have been implicated in biological wastewater treatment systems (Donmez and Aksu, 1999). In the present study the optimum temperature for phosphate removal by the test fungal species was observed to be 25^{o}C. In a study carried out by Mamais and Jenkins (1992), optimum operating temperature for enhanced nutrient removal was indicated to range from 28^{o}C to 33^{o}C. In the study by Brdjanovic *et al.,* (1997) on the short-term effect of temperature on nutrient removal, optimum temperature was observed to be 20^{o}C. Gonzales-Martines and Wilderer (1991), in a laboratory scale experiment with synthetic wastewater in a fixed film bio reactor, found that phosphate release increased with decreasing temperature.

Wastewater treatment can be affected by temperatures in many ways, both directly and indirectly affecting the biomass. The concentration of biological nutrients in wastewater is known to depend very much on temperature. Mulkerrins *et al.,* (2004) indicates that temperature not only have influence on the

metabolic activities of the microbial population but also affect factors, such as gas transfer rates and the settling characteristics of biological solids. The ability of fungi in biological nutrient removal from wastewater has been investigated by earlier workers.

Although there is an optimal temperature range for any organism, earlier investigators have reported that optimal temperature for growth may not necessarily be the same as the optimal temperature for substrate oxidation and reduction (Charley *et al.*, 1980; Saito *et al.*, 2004). In biological phosphate removal, polyphosphate accumulating organisms in anaerobic condition are known to convert readily available organic matter to carbon compounds using the energy derived from the process to create hydroxyalkanoates, resulting in phosphorus release, leading to uptake by aerobic condition subsequently (Jeyanayagam, 2005). Temperature have been indicated to affect the reaction performance and kinetics of biological nutrient removal systems, although there are conflicting reports on its effects on enhanced biological nutrient removal systems (Erdal *et al.*, 2003; Thongchai *et al.*, 2003)

With respect to pH, the present study revealed an optimum of 6 in presence of the test isolates. pH is said to play a major role in nutrient removal by affecting different factors, which in turn affects growth and activity of the organisms involved. Studies carried out by earlier workers have shown that pH affect enzymes of interest, affinity for the substrate, substrate availability, effects of inhibitory compounds, and substrate or product production (Prosser, 1989; Antoniou *et al.*, 1990; Groeneweg *et al.*, 1994). At low pH in anoxic conditions, microbial activity solubilizes phosphate compounds. As the pH increases, phosphate is precipitated and incorporated in the sludge which explains the decrease in the phosphate removal ability in the experiment. Phosphorus precipitation can also be induced by the increase in phosphate concentration resulting from phosphorus release, from the polyphosphate pool under anaerobic conditions and denitrification, as the denitrification process produces alkalinity.

In the present study, the effect of varying concentrations of sodium acetate on phosphate removal ability of the isolates was investigated. In the present study, sodium acetate was used as the carbon source in concentrations of 5 g, 10 g, and 15 g respectively. All the isolates used for the investigation showed remarkable phosphate removal ability with the different concentrations of sodium acetate, with the highest removal at concentration of 5 g. It is hypothesized that carbon concentration is an important factor to the efficiency of polyphosphate accumulating organisms. The choice of acetate was deliberate because several earlier workers have indicated acetate as an ideal external carbon source in nutrient removal studies (Smolders *et al.*, 1995; Hood and Randall, 2001; Randall and Liu 2002; Chen *et al.*, 2005).

To investigate the effect of external carbon source on phosphate removal by the test fungal isolates, glucose, lactose, sucrose and methanol were investigated. In this study, both the *Aspergillus flavus* and *Aspergillus niger* showed remarkable phosphate removal in the presence of lactose, sucrose and methanol. In the presence of glucose, an increase in phosphate levels was observed at the end of incubation. There is conflicting reports on the effect of glucose as carbon source in biological nutrient removal. Some earlier investigators have reported that glucose is detrimental for enhanced biological phosphate removal (Mino *et al.*, 1998), while other studies have reported enhanced nutrient removal when glucose was used as carbon source (Jeon and Park, 2000; Wang *et al.*, 2002; Kumar and Chaudhari, 2003). Mino *et al.*, (1998) explained that the poor results with glucose as carbon source may be due to rapid proliferation from glycogen accumulating organisms in the biomass.

Wang *et al.*, (2002) reported that EBPR with glucose was enhanced by a long anaerobic retention time and high initial glucose concentration. Randall *et al.*, (1997) compared different carbon sources in batch tests with biomass from a serial batch reactor fed with volatile fatty acids produced by pre-fermentation of glucose. In their report they observed that methanol had no significant effect on enhanced biological phosphate removal. Also, some studies with different carbon sources have revealed that during nutrient removal, some substrates are utilized directly while others are first transformed first, before they are taken up. According to Johansson (1994), during nutrient removal, glucose must first be fermented volatile fatty acid before it can be utilized for metabolism.

When different nitrogen sources (peptone, yeast extract and meat extract) were used in this investigation, remarkable phosphate removal was observed in the presence of peptone. During biological nutrient

removal, nitrogen source is said to increase enzyme and hydrogen production for efficient removal (Al-Saleh and Zahran, 1999). Nitrogen is important in the growth and development of microorganisms and also forms a major part in their nucleic acid.

Rathi *et al.*, (2001) reported that among the various nitrogen sources utilized as additives for *Burkholderia cepacia*, none affected lipase production significantly. Also, a study by Schär-zammaretti *et al.*, (2005) indicated that the morphology and structure of microbial cell wall changes based on the media composition revealed both peptone and yeast extract have a major influence on the physicochemical properties of the cell wall, particularly the membrane-bound proteins.

To test the effect of nitrogen concentration on phosphate removal, different concentrations (5 g/L, 10 g/L, 15 g/L and 20 g/L) of peptone used for investigation. The study revealed optimum concentration of peptone to be 15 g/L. It is reported that carbon to nitrogen (C/N) ratio is important in biological removal process. The C/N ratio has been shown to affect fermentative hydrogen by mixed microflora fed with sucrose with an optimal ratio of 47. During nutrient removal, peptone is said to undergo anaerobic phosphorus uptake through biochemical pathways other than the traditional polyphosphate accumulating organisms (Carucci *et al.*, 1999).

Conclusion

This study, which was aimed at investigating the role of *Aspergillus flavus* and *Aspergillus niger* in the removal of phosphate in wastewater, was able to reveal the following:

- An increase in sulphate concentration was observed at the different incubation temperatures investigated. This trend was irrespective of the test isolate used for investigation.
- With respect to pH, remarkable phosphate removal was only observed at 6 in the presence of the *Aspergillus flavus* while in the presence of the *Aspergillus* niger, remarkable removal was observed at the different pH (6, 8 and 10) investigated.
- Phosphate removal was observed at all concentrations of sodium acetate that were used for investigation, although maximum removal was observed at 5 g/L. This trend was irrespective of the test isolate used for investigation.
- All the external carbon sources were observed to enhance phosphate removal by the isolates, although highest removal was observed for lactose and sucrose, in the presence of *Aspergillus flavus* and *Aspergillus niger*, respectively.
- Decrease in phosphate concentration were observed at the different concentrations of peptone concentrations and nitrogen sources used for investigation. This trend was also irrespective of the est isolates.

The findings of this investigation given an insight to the optimum conditions for phosphate removal from wastewater by the test fungal isolates under the experiment conditions investigated.

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REFERENCES

Al-Saleh AA and Zahran AS (1999). Synthetic of extracellular lipase by strain of *Pseudomonas fluorescens* isolated from raw camel milk. *Food Microbiology* **16** 149-156.

Antoniou P, Hamilton J, Koopman B, Jali R, Holloway B, Lybertos G and Svoronos SA (1990). Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. *Water Research* **24**(1) 97-101.

APHA (2012). *Standard Methods for the Examination of Water and Wastewater*, 22nd edition (APHA, Washington D.C.).

Bartram JK, Lewis R, Lenton P and Wright A (2005). Focusing on improved water and sanitation for health. *Lancet* **365** 810-812.

Brdjanovic D, Van Loosdrecht MCM, Hooijmans CM, Alaerts GJ and Heijnen JJ (1997). Temperature effects on physiology of biological phosphorus removal. *Journal of Environmental Engineering* **123**(2) 144-154.

Carucci A, Lindrea M and Majone M (1999). Different mechanisms for the anaerobic storage of organic substrates and their effect on enhanced biological phosphate removal (EBPR). *Water Science and Technology* **39**(6) 21-28.

Charley RC, Hooper DG and McLee AG (1980). Nitrification kinetics in activated sludge at various temperatures and dissolved oxygen concentrations. *Water Research* **14** 1387-1396.

Chen Y, Chen Y-S, Xu Q, Zhou Q and GU G (2005). Comparison between acclimated and unacclimated biomass affecting anaerobic-aerobic transformations in the biological removal of phosphorus. *Process Biochemistry* **40** 723-732.

Donmez G and Aksu Z (1999). The effect of Copper (II) ions on the growth and bioaccumulation properties of some yeast. *Process Biochemistry* **35** 135-142.

Eikelboom DH and Draaijer A (1999). Activated sludge information system (ASIS). Available: http://www.asissludge.com.

EPA (2000). Wastewater technology fact sheet (trckling filter). EPA 382-F-00-014, USEPA, Washington. Available: http://www.nesc.wwu.edu/nsfc/pdf/eti/TFgen

EPA (2002). Onsite wastewater treatment systems manual. EPA/625/R-00/008/2002. Available: http:// www.epa.gov/owmitnet/mtbfact.htm.

Erdal UG, Erdal ZK and Randall CW (2003). A thermal adaptation of bacteria to cold temperatures in an enhanced biological phosphorus removal system. *Water Science Technology* **47**(11) 123-128.

Fuhs GW and Chen M (1975). Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. *Microbiology Ecology* **2** 119-138.

Gonzales-Martinez S and Wilderer PA (1991). Phosphate removal in a biofilm reactor. *Water Science Technology* **23** 1405-1415.

Groeneweg J, Sellner B and Tappe W (1994). Ammonia oxidation in *Nitrosomonas* at NH_3 concentrations near $K_{m:}$ effect of pH and temperature. *Water Research Technology* **28**(12) 2561-2566.

Hood CR and Randall AA (2001). A biochemical hypothesis explaining the response of enhanced biological phosphorus removal biomass to organic substrates. *Water Research* **35**(11) 2758-2766.

Jeon CO and Park JM (2000). Enhanced biological phosphorus removal in a sequencing batch reactor supplied with glucose as a sole carbon source. *Water Research* **34**(7) 2160-2170.

Jeyanayagam S (2005). True confessions of the biological nutrient removal process. *Florida Water Resources Journal* 37-46.

Johannes RE (1965). Influence of marine protozoa on nutrient regeneration. *Limnology Oceanography* **10** 434-442.

Kumar BM and Chaudari S (2003). Evaluation of sequencing batch reactor (SBR) and sequencing batch biofilm reactor (SBBR) for biological nutrient removal from simulated wastewater containing glucose as carbon source. *Water Science Technology* **48**(3) 73-79.

Mamais D and Jenkins D (1992). The effects of MCRT and temperature on enhanced biological phosphorus removal. *Water Science Technology* **26**(5/6): 955-965.

McCasland M, Trautmann N, Porter K and Wagenet R (2008). Health effects of nitrate in drinking water. Available: http://pmep.cee.comell.edu/facts.slides–self/facts/nit-heef-grw85.html

Meganck MTL and Faup GM (1988). Enhanced biological phosphorus removal from waters. *In*: *Biotreatment Systems*, 3rd edition, edited by Wise DL (CRC Press: Roca Raton).

Mulkerrins D, Dobson ADW and Colleran E (2004). Parameters affecting biological phosphate removal from wastewaters. *Environmental International* **30** 249 -259.

Pradyot P (1997). *Handbook of Environmental Analysis, Chemical Pollutants in Air, Water, Soil and Solid Wastes* (Lewis Publishers, CRC Press Incorporation) 233-237.

Prosser JI (1989). *Autotrophic Nitrification in Bacteria, in Advances in Microbial Physiology*, edited by Rose AH and Tempest DW (Academic Press Inc: New York) **30.**

Randall AA and Liu Y-H (2002). Polyhydroxyalkanoates form potentially a key aspect of aerobic phosphorus uptake in enhanced biological phosphorus removal. *Water Research* **36** 3473-3478.
Randall AA, Benefield LD and Hill WE (1997). Induction of phosphorus removal in an enhanced biological phosphorus removal population. *Water Research* **31**(11) 2869-2877.
Saito T, Brdjanovic D and van Loosdrecht MCM (2004). Effect of nitrite on phosphate uptake by phosphate accumulating organisms. *Water Research* **3** 3760-3768.
Schär-Zammaretti P, Dillmann ML, D'Amico N, Affolter M and Ubbink J (2005). Influence of fermentation medium composition on physicochemical surface properties of *Lactobacillus acidophilus*". *Application of Environmental Microbiology* **71** 8165-8173.
Smolders GJF (1995). A metabolic model of the biological phosphorus removal; stoichiometry, kinetics and dynamic behavior. Doctoral Dissertation, Department of Biogeochemical Engineering, Delft, University of Technology, the Netherlands.
Thongchai P, Aspiradee D and Jin A (2003). Effect of temperature shock on activities of phosphorus-accumulating organisms. *Science Asia* 29 365-370.
Tykesson E (2005). Enhanced biological phosphorus removal. Doctoral Thesis, Department of Water and Environmental Engineering, Lund Institute of Technology, Lund University, Sweden.
U.S. Bureau of Reclamation and Sandia National Laboratories (2003). Desalination and water purification technology roadmap. A Report of the Executive Committee Water Purification.
Wang N, Peng J and Hill G (2002). Biochemical model of glucose induced enhanced biological phosphorus removal under anaerobic condition. *Water Research* **36** 49-58.