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Research Article

ROLE OF *ASPERGILLUS NIGER* AND *ASPERGILLUS FLAVUS* IN THE MINERALIZATION OF SULPHATE IN WASTEWATER

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ABSTRACT
The objective of this study was to investigate the role of temperature, pH, carbon and nitrogen sources in the mineralization of sulphate in wastewater by *Aspergillus niger and Aspergillus flavus*. The study was carried out under shake flask conditions. Before inoculation with the respective isolates, the wastewater was filtered in 200 mL quantity into 250 mL capacity conical flasks before sterilization in an autoclave. After inoculation with the respective isolates, aliquot wastewater samples were removed from each flask, prior inoculation and every 24 h, for 96 h, for the estimation of sulphate concentrations, using standard methods. The study revealed remarkable sulphate removal at 25 °C and 35 °C in presence of the test isolates. A pH of 6, 5 g/L and 10 g/L of peptone and sodium acetate were observed to enhance sulphate removal by the isolates. Glucose, sucrose, lactose and methanol were observed to enhance sulphate removal in presence of the *Aspergillus niger* while in the presence of the *Aspergillus flavus*, lactose was not observed to enhance sulphate removal. The study was able to give an insight to the optimum conditions for sulphate removal from wastewater by the test fungal isolates under the experimental conditions investigated.

Keywords: *Sulphate, Wastewater, Fungi, Temperature, pH, Carbon Source*

INTRODUCTION
Sulphate is a common constituent of many natural waters and wastewaters and it is sometimes present in high concentrations (Lens *et al*., 1998). Sulphate is produced in the environment from the oxidation of elemental sulphur, sulphide minerals or organic sulphur. Soils are thought to have about 850 mg of sulphate/kg and about 885 mg of sulphate/L (Field, 1972).
Generally, most public water supplies contain sulphate concentrations of less than 500 mg/L (EPA, 2011). Sulphate levels in water around 250 mg/L and above are usually detected by an off odour or taste. This has led to people switching to bottled water as a source of drinking water, whereas, others have adapted to the high level of sulphate in water. Extremely high sulphate concentrations in water have been recorded; for example, 1,200 mg/L in a coal mine in Pennsylvania and 63,000 mg/L in a zinc mine in Idaho (Moore, 1991).
Sulphate could be from various sources. They could be leached from the soil which is commonly found in water supplies. Other sources include, decaying plant and animal matter which release sulphate into water, numerous chemical products including ammonium sulphate fertilizers which contain sulphate in a variety of forms. Also, industrial sulphates could be as a result of burning of sulphur containing fossil fuels, household wastes (example, detergents), and effluents from tanneries, steel mills, sulphate pulp mills and textile plants (EPA, 1990). Worldwide, the effluents discharged from wastewater treatment systems represent one of the largest sources of pollution. These effluents have given rise to negative impacts on aquatic ecosystems and to humans due to the harmful substances they contain. Various of these impacts range from death of aquatic life, algal blooms, habitat destruction from sedimentation, debris, and increased water flow and other short and long term toxicity from chemical contaminants; in addition with chemical accumulation and magnification at higher levels of the food chain (Canada, 2010). Although chemical and biological processes have been used in the treatment of wastewater, but due to the limitations of chemical processes such as high cost, biological processes for treatment of wastewater has been favoured. Microorganisms such as bacteria, fungi, algae and protozoans have been proposed to have

great importance in the removal of these nutrients, in the treatment of wastewater. These assist in carrying out biochemical reactions and transformations that occur in the system as part of the treatment process (Andersson *et al*., 2005).

Investigations have been carried out on these organisms (bacteria, fungi, algae and protozoans) on the roles they play in nutrient removal from wastewaters and various findings have shown that different organisms have different optimum conditions. A number of studies have indicated the preference of fungi over bacteria in the removal of pollutants from wastewater. The reason is due to the fact that, fungi have been reported to possess resistance to a variety of inhibitory chemicals which implies that they have great ability to remove nutrients from wastewaters (Guest *et al*., 2002). They also have the ability to adapt to extreme environmental conditionsand a number of them, such as *Aspergillus niger* are indicated possess the ability to produce homologous and heterologous proteins (Grewal and Kalra, 1995). The objective of this study was to investigate the role of temperature, pH, carbon and nitrogen sources in the mineralization of sulphate in wastewater by *Aspergillus niger and Aspergillus flavus*.

MATERIALS AND METHODS

Two fungi species used for this study were *Aspergillus niger and Aspergillus flavus*. The isolates were part of the laboratory stock at the Department of Biological Sciences, Landmark University, Omu Aran, Kwara State, Nigeria. Before using the test isolates for the investigation, they were first plated out on sterilizedsaboraud dextrose agar, after which the pure cultures were then suspended in sterile normal saline (0.85 % NaCl w/v).

The wastewater used for this study was obtained from the Landmark University Commercial Farm, located in Omu-aran, Kwara State, Nigeria. The collected wastewater was filtered, using Whatman No. 1 filter paper and supplemented with 0.5 g/L of magnesium sulphate (0.5 g/L),5 g/L of sodium acetate (external carbon source) and 5 g/L of peptone (external nitrogen source). The supplemented wastewater was later dispensed into 250mL capacity conical flaks in 200 mL quantities and then sterilized in an autoclave for 15 min at 121 ^{o}C at 15 psi. The sterilized wastewater was allowed to cool before inoculating with the respective test isolates. Just before inoculation and every 24 h, for 96 h, aliquot (10 mL) wastewater samples were removed from each flask for the estimation of sulphate concentration, using standard procedures (APHA, 2012).

Sulphate mineralization studies in presence of the test fungal species were carried out at different incubation temperatures (25 ^{o}C, 35 ^{o}C and 45 ^{o}C), pH (6, 8 and 10), sodium acetate concentrations (5 g/L, 10 g/L and 15 g/L), external carbon sources (glucose, lactose, sucrose and methanol), external nitrogen sources (peptone, yeast extract and meat extract) and pep[tone concentrations (5 g/L, 10 g/L, 15 g/L and 20 g/L).

Except for the temperature and pH variation studies, where temperature and pH were varied, all experimental flasks were incubated at 30 ^{o}C while the pH of the wastewater was adjusted to 7. All experimental setups were carried out in triplicates. All reagents that were used were of analytical grades.

RESULTS AND DISCUSSION

Results

As shown in Figure 1, in the presence of the *Aspergillus niger*, decreases in sulphate concentration in the wastewater were observed at incubation temperatures of 25 ^{o}C and 35 ^{o}C; decreasing from 3959.10 mg/L to 2376.84 mg/L (39.97 % decreases) and from 4451.36 mg/L to 4027.13 mg/L (0.98% decrease), respectively. At 45 ^{o}C, an increase in sulphate concentration from 4082.45 mg/L to 4618.74 mg/L (13.13 % increases) was observed at the end of the 96 h incubation. In the presence of the *Aspergillus flavus* was observed to decrease from 3988.00 mg/L to 3264.26 mg/L, from 4000.06 mg/L to 2841.31mg/L and from 3912.12mg/L to 2171.85mg/L at incubation temperatures of 25^{o}C, 35^{o}C and 45^{o}C, respectively. This translates to decreases of 22.57 %, 28.97 % and 44.40 %, respectively (Figure 1).

At the different pH, remarkable sulphate removal was only observed at pH 6. This trend was irrespective of the test isolate used for investigation. At the expiration of the 96 h incubation period, sulphate

decreases of 23.41 % and 30.58 % were observed at the end of the 96 h incubation time in the presence of the *Aspergillus niger* and *Aspergillus flavus*, respectively. After the period of incubation, sulphate levels in the wastewater varied from 1562.84 mg/L to 1196.94 mg/L (pH 6), from 1578.26 mg/L to 1702.21 mg/L (pH 8) and from 1564.69 mg/L to 1490.65 mg/L (pH 10) in the presence of the *Aspergillus niger*. In the presence of the *Aspergillus flavus*, sulphate levels at the end of incubation showed variation from 1600.25 mg/L to 1110.84 mg/L, from 1591.63 mg/L to 1549.30 mg/L and from 1607.69 mg/L to 1575.42 mg/L at pH 6, 8 and 10, respectively (Figure 2).

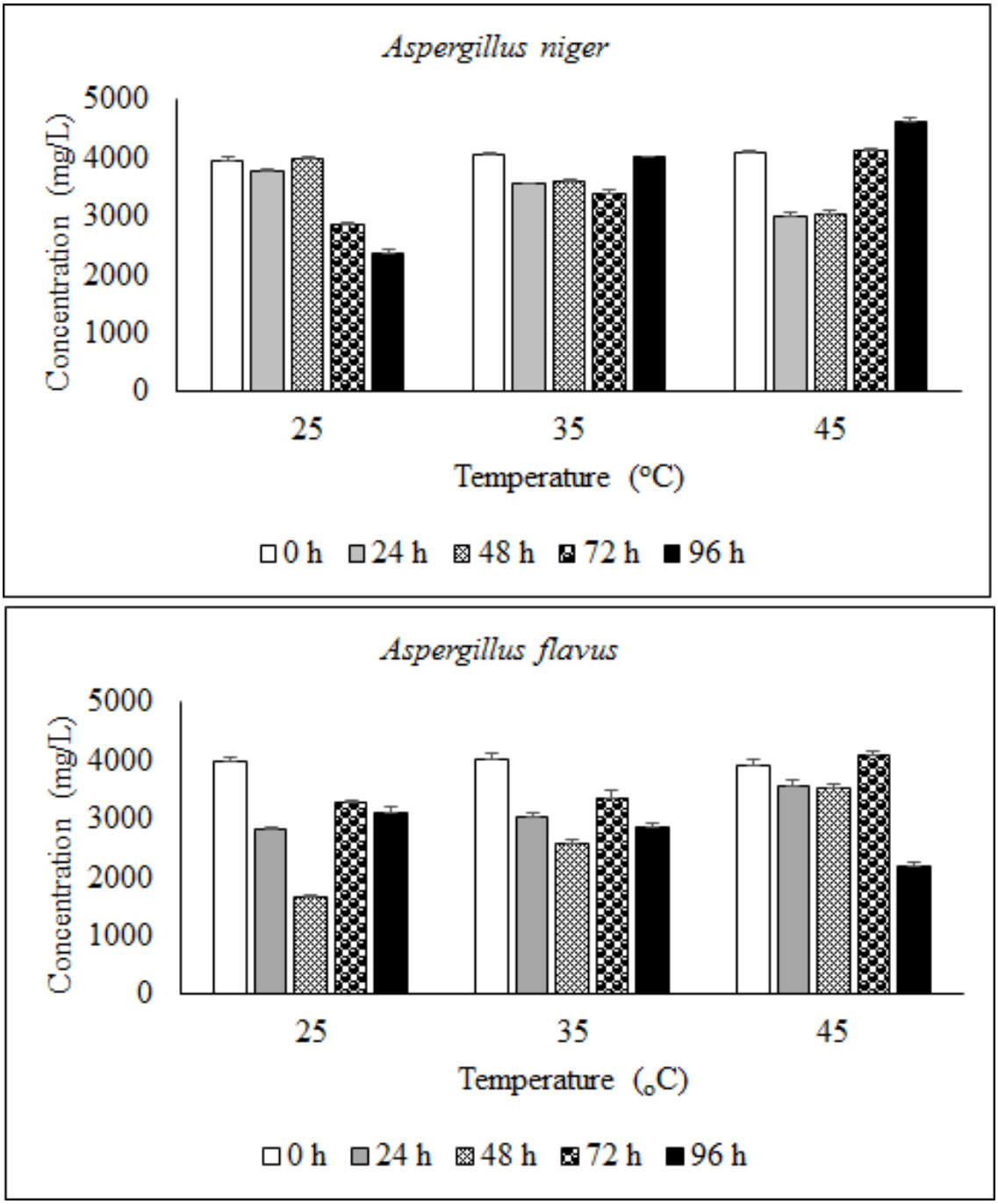

Figure 1: Sulphate concentrations in the wastewater at the different incubation temperatures in presence of the test fungal species

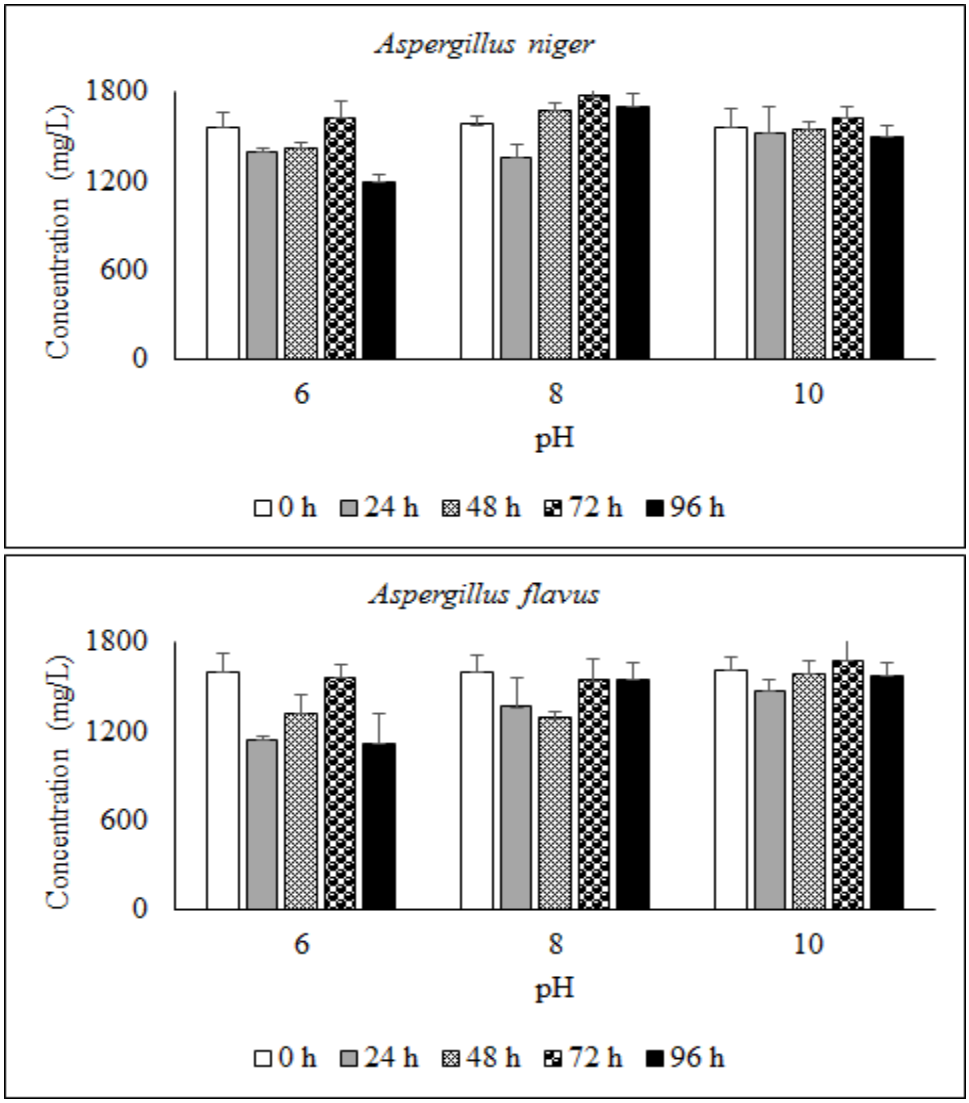

Figure 2: Sulphate concentrations in the wastewater at the pH in presence of the test fungal species

As shown in Figure 3, at the different concentrations of peptone, remarkable sulphate removal was only observed at 10 g/L. At the end of incubation, sulphate levels in the wastewater at peptone concentration of 10 g/L, sulphate level decreased from 3386.83 mg/L to 1067.90 mg/L and from 3469.04 mg/L to 883.78 mg/L, in the presence of the *Aspergillus niger and Aspergillus* flavus, respectively. This translates to decreases of 68.47 % and 74.52 %, respectively. At peptone concentration of 5 g/L and 15 g/L, sulphate concentration in the presence of the *Aspergillus niger* was observed to vary from initial concentrations of 3346.39 mg/L and 3340.09 mg/L to final concentrations of 3111.63 mg/L and 3194.48 mg/L, respectively. Similarly, in the presence of the *Aspergillus flavus*, sulphate concentration at the expiration of incubation varied from 3469.52 mg/L to 3593.80 mg/L and from 3406.89 mg/L to 3700.81 mg/L, at peptone concentrations of 5 g/L and 15 g/L, respectively (Figure 3).

At the different external carbon sources in the wastewater, remarkable sulphate removal was observed in the presence of the *Aspergillus niger*. In the presence of *Aspergillus flavus*, remarkable removal was only observed for glucose, sucrose and methanol. In the presence of the *Aspergillus niger*, sulphate levels at the end of incubation varied from 1602.72 mg/L to 1226.43 mg/L, from 1679.43 to 1319.43 mg/L, from 1590.05 to 1259.00 mg/L and from 1641.53 mg/L to 1155.28 mg/L, for glucose, lactose, sucrose and methanol, respectively. This trend traslates to decreases of 23.48 %, 21.44 %, 20.82 % and 29.62 %,

respectively. When *Aspergillus flavus* was used as the test isolate, sulphate levels showed a variation from 1551.44 mg/L to 1244.83 mg/L, from 1561.65 mg/L to 1438.78 mg/L, from 1584.47 mg/L to 1361.53 mg/L and from 1553.29 mg/L to 1230.98 mg/L, for glucose, lactose, sucrose and methanol, respectively. This translate top decreases of 19.76 %, 7.87 %, 14.07 % and 20.75 %, respectively (Figure 4).

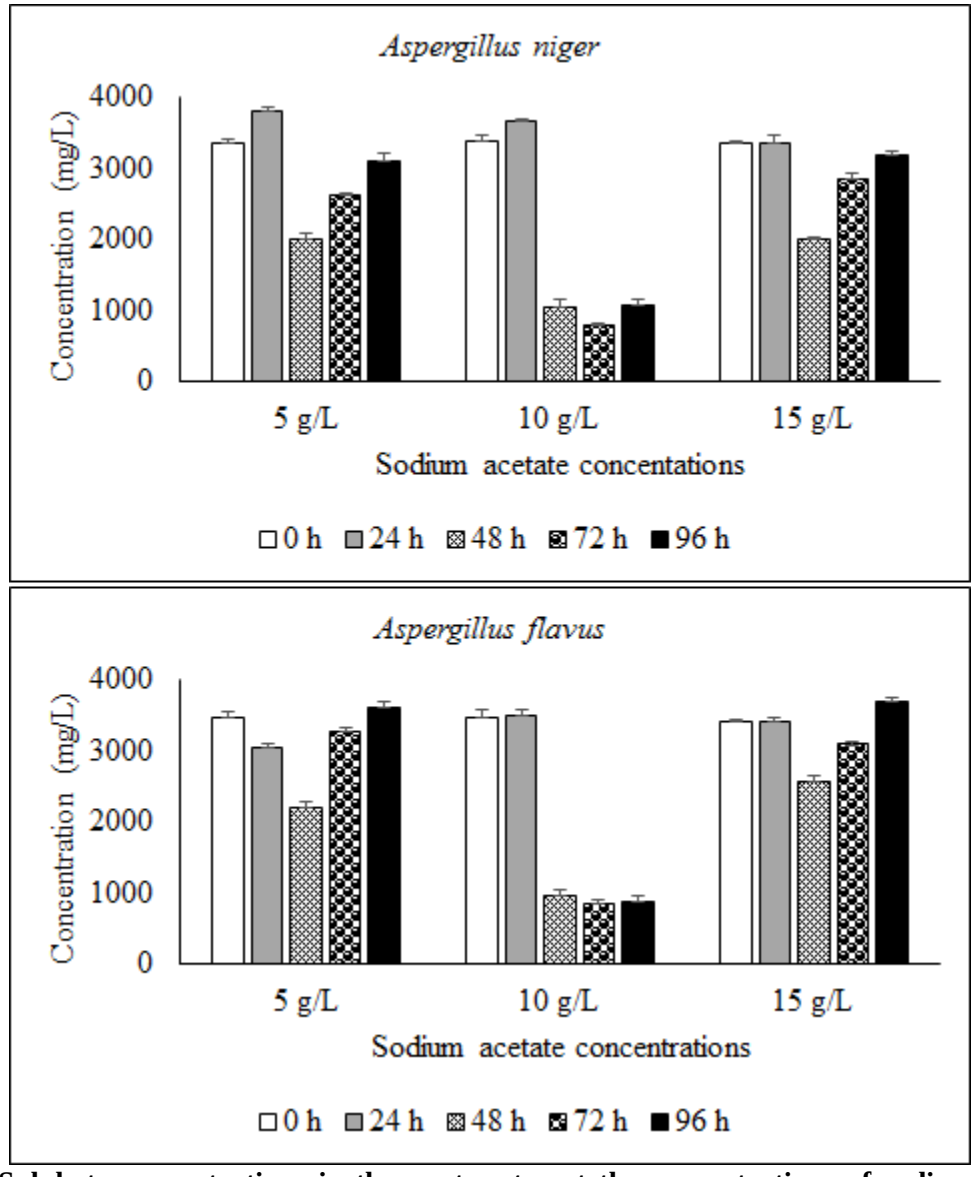

Figure 3: Sulphate concentrations in the wastewater at the concentrations of sodium acetate in presence of the test fungal species

With the different nitrogen sources in the wastewater, sulphate concentrations at the expiration of incubation time the presence of the *Aspergillus niger*was observed to vary from initial levels of 1176.15 mg/L, 1196.88 mg/L and 1120.13 mg/L, to final levels after the 96 h incubation period of 2719.20 mg/L, 2696.03 mg/L and 2263.43 mg/L, when peptone, yeast extract and meat extract were used as external nitrogen sources, respectively. Also, in the presence of the *Aspergillus flavus*, the concentration of sulphate in the wastewater varied 1173.88 mg/L to 2811.43 mg/L, from 1137.70 mg/L to 1691.78 mg/L and from 1188.80 mg/L to 2252.23 mg/L, for peptone, yeast extract and meat extract, respectively (Figure 5).

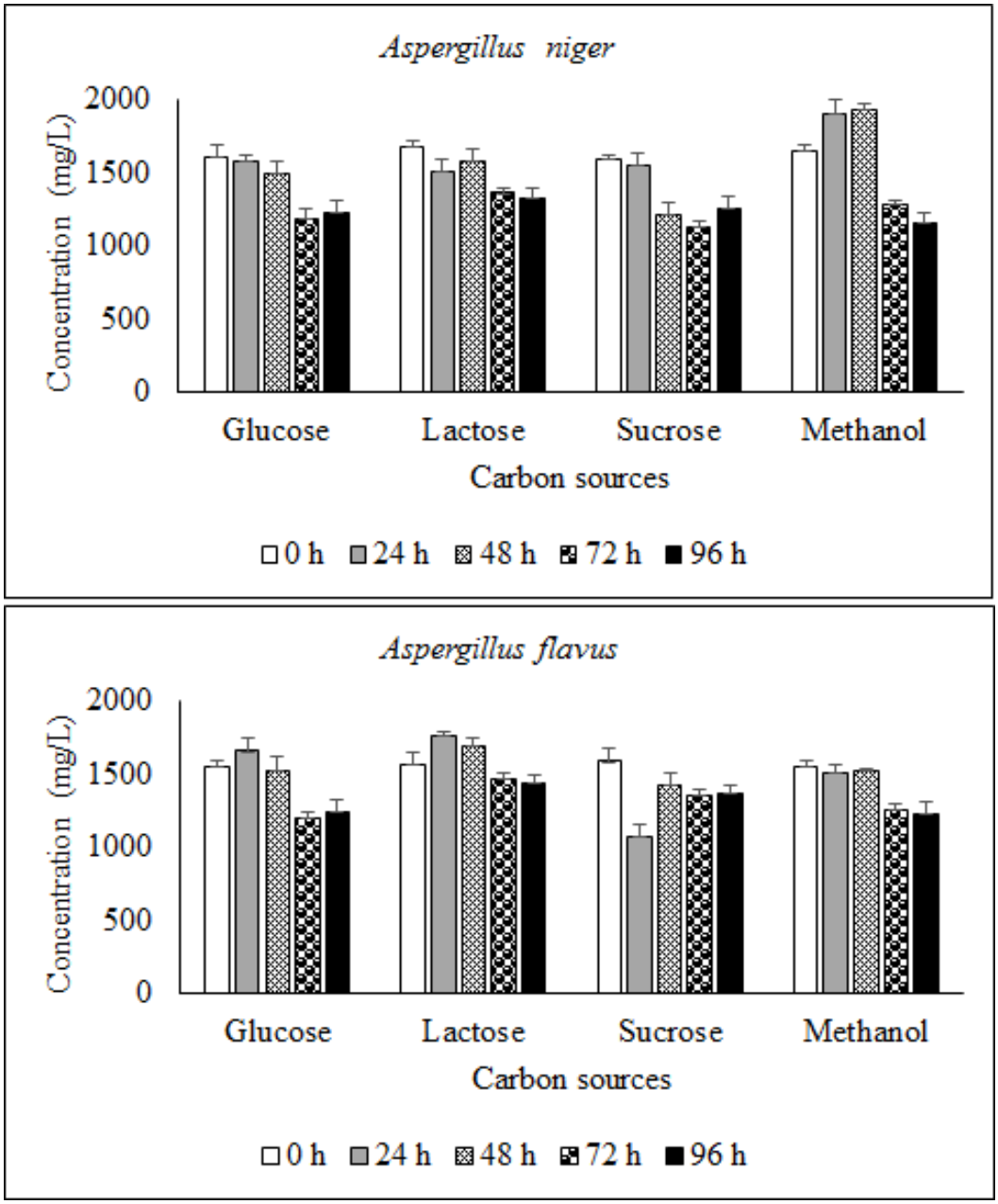

Figure 4: Sulphate concentrations in the wastewater at the different carbon sources in presence of the test fungal species

At the different peptone concentrations, remarkable decreases (56.68 % in presence of the *Aspergillus niger* and 41.99 % in presence of the *Aspergillus* flavus) in sulphate levels were only observed at peptone concentration of 5 g/L. sulphate levels in the wastewater in the presence of the *Aspergillus niger*, showed a variation from 1757.50 mg/L, to 761.43 mg/L, from 1758.33 to 1410.23 mg/L, from 1587.48 to 2971.28 mg/L and from 1595.60 mg/L to 2971.28 mg/L, at peptone concentrations of 5 g/L, 10 g/L, 15 g/L and 20 g/L, respectively. In the presence of the *Aspergillus flavus*, sulphate levels were observed to vary from 1467.75 mg/L to 851.48 mg/L, from 1482.58 mg/L to 2086.08 mg/L, from 1459.27 mg/L to 2593.43 mg/L and from 1492.45 mg/L to 2596.03 mg/L 33 to 1410.23 mg/L, from 1587.48 to 2971.28 mg/L and from 1595.60 mg/L to 2971.28 mg/L, at peptone concentrations of 5 g/L, 10 g/L, 15 g/L and 20 g/L, respectively.

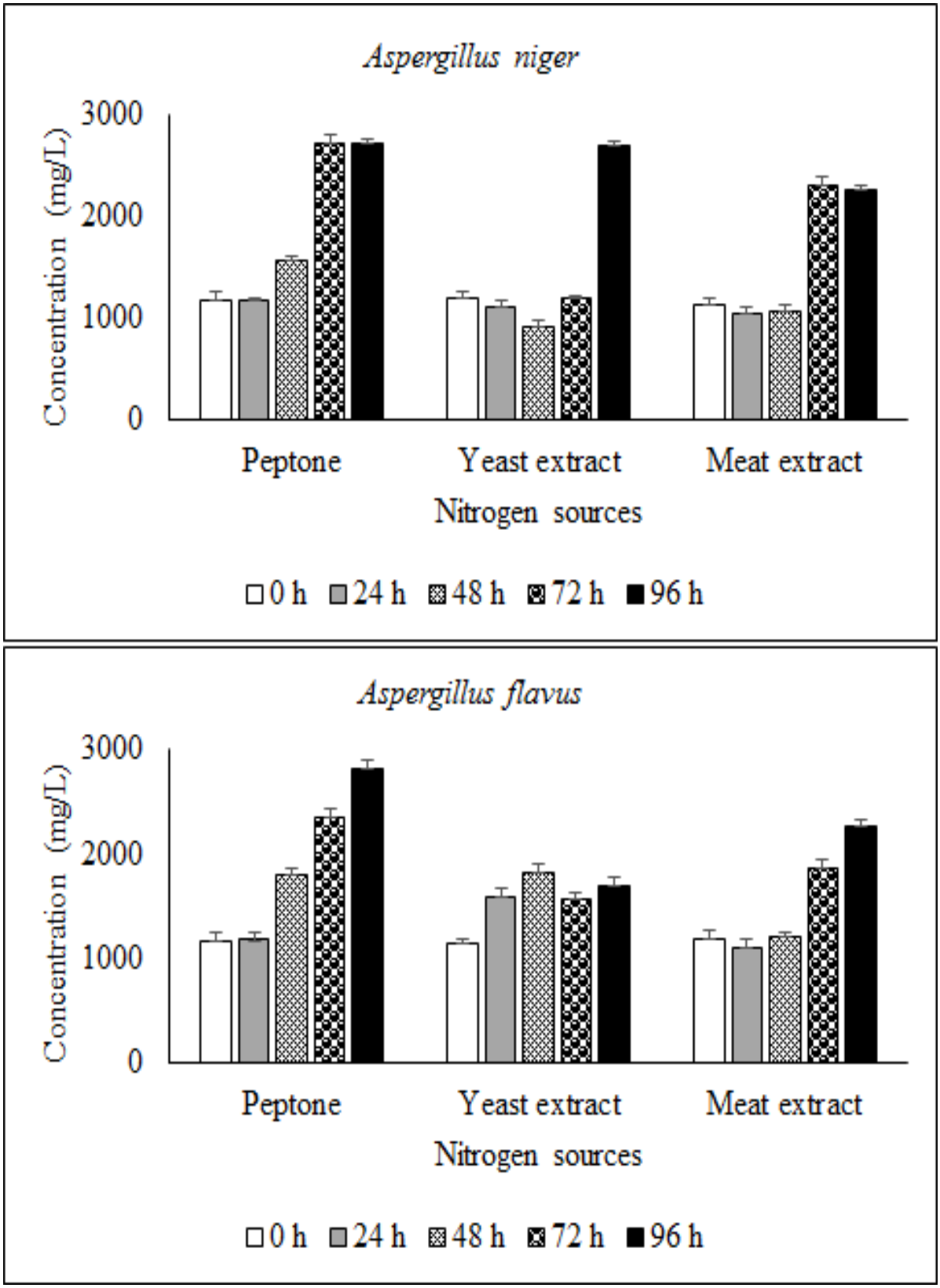

Figure 5: Sulphate concentrations in the wastewater at the different nitrogen sources in presence of the test fungal species

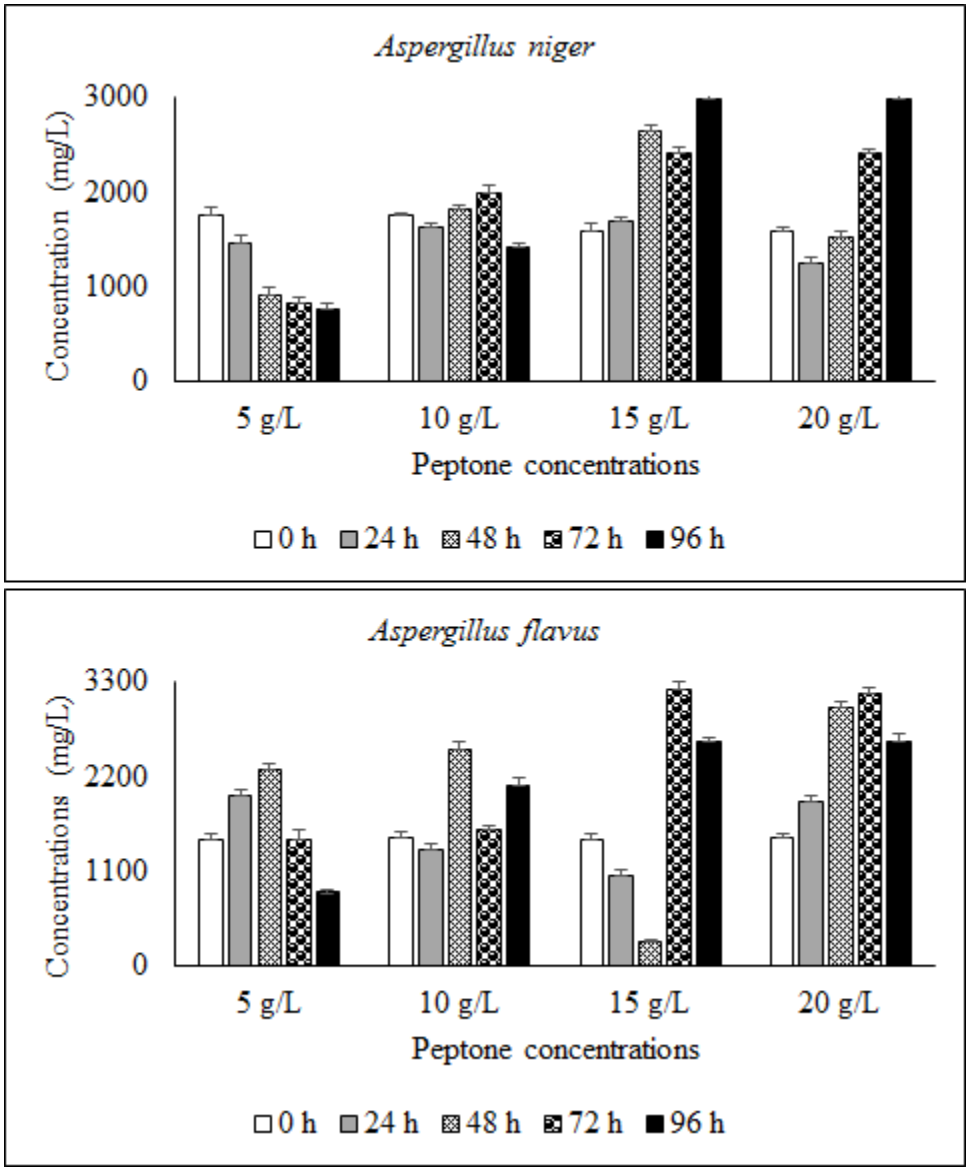

Figure 6: Sulphate concentrations in the wastewater at the different peptone concentrations in presence of the test fungal species

Discussion

The present study made use of two fungi species. The choice of the isolates was deliberate. Apart from the fact that they have been implicated in biological nutrient removal in previous studies, fungi are indicated to be highly effective in industrial fermentation and bioremediation. Fungi are said to be preferred over other organisms in bioremediation because they are easier to remove from liquid substrates (Akthar and Mohan, 1995; Bosshard *et al*., 1996; Adelani-Akande *et al*., 2014).

In the present investigation, the optimum temperature range for sulphate removal by the test fungi isolates was observed to be between 35 ^{o}C and 45 ^{o}C for the *Aspergillus flavus* and between 25 ^{o}C and 35 ^{o}C for the *Aspergillus niger*.In a study by Mamais and Jenkins (1992), the optimum temperature for nutrient removal was reported to range between 28 ^{o}C and 33 ^{o}C. Temperature is reported to be one of the parameters used in assessing the efficacy of a treatment process. This is because, temperature does not only have effects on the metabolic activities of microbial population, but also on factors, such as gas transfer rates and the settling characteristics of biological solids (Mulkerrins *et al.*,2004).

With respect to pH, the optimum for sulphate removal by the isolate was observed to be 6. This observation corroborates the report of Moore-Landecker (1990) that showed that the optimum pH for most fungi is within the acidic range. The effects of pH on fungal metabolism include availability of metal ions, cell permeability and enzymatic activity. A low pH is said to increase iron availability while a higher pH increases enzymatic activity (Moore-Landecker, 1990). In a study on bioremediation of hydrocarbons, using *Penicillium*, an alkaline pH was said to be more favourable than an acidic pH (Norris, 1994). In a previous study on the role of pH on sulphate removal by selected bacterial and fungal species by Akpor *et al*., (2014), optimum pH was observed to be 12. In their findings, high sulphate removal was achieved at alkaline pH while an acidic pH reduced the rate at which sulphate was removed. It is reported that pH plays an important role when sulphate is reduced to sulphide. Sulphate can be present in various forms such as H_2S, HS^- and S^{2-} and these various states are dependent on the pH of the environment (Perry and Green, 1994; Al-Zuhair *et al*., 2008).

In investigating the effect of carbon concentration on sulphate removal ability of the, sodium acetate was used as the external carbon source. Sodium acetate has been reported as an ideal carbon source in biological nutrient removal studies by earlier workers (Akpor *et al.,* 2008). At the various concentrations (5 g/L, 10 g/L and 15 g/L) of sodium acetate, the study revealed at 10 g/L and 15 g/L, in the presence of the *Aspergillus niger* and *Aspergillus flavus*, respectively. Some reports have shown that the concentration of a biodegradable carbon source affects the biological nutrient removal rate. Various authors have proposed different concentrations of readily biodegradable carbon source in wastewater that could enhance nutrient removal (Ekama *et al*., 1986; Morales *et al*., 1991). At the different external carbon sources (glucose, lactose, sucrose, methanol and acetate), this study revealed remarkable sulphate removal in the presence of the different carbon sources at the expiration of incubation. With methanol as carbon source, an increase in sulphate level was however observed in the presence of the *Aspergillus flavus*. In a previous study by Greben *et al*., (2000), methanol was indicated as a suitable carbon and energy source for sulphate reduction. Among the different carbon sources, maximum sulphate removal was observed in the presence of glucose and lactose, for the *Aspergillus flavus* and *Aspergillus niger*, respectively. Previous studies on the effect of carbon source on sulphate reducing bacteria have indicated lactate as an ideal carbon source that favours sulphate removal in wastewater (Kaksonen, 2004). With the different nitrogen sources (yeast extract, meat extract and peptone), this study revealed enhanced sulphate removal in the presence of the yeast extract and meat extract. This trend was irrespective of the test fungal species used. According to the findings of Korda *et al*.,(1997), on the bioremediation of petroleum oil, using *Penicillium*, yeast extract was observed to be an ideal nitrogen source.

Conclusion

This present study showed remarkable sulphate removal at 25 ^oC and 35 ^oC, in presence of the *Aspergillus niger* while in the presence of the *Aspergillus flavus*, removal was observed at all incubation temperatures investigated. With respect to pH, sulphate decreases in the wastewater were only observed at 6. This trend was irrespective of the isolate used. At the different concentrations of sodium acetate, decreases in sulphate levels in the wastewater were only observed at 10 g/L. All the carbon sources investigated were observed to enhance sulphate removal by the isolates, with the exception of lactose in the presence of the *Aspergillus flavus*. In the case of peptone concentration investigated, increases in sulphate concentration were observed at 10 g/L and 15 g/L. This trend was similar for both test isolates. Finally, none of the nitrogen sources investigated was observed to enhance sulphate removal by the isolates, as increases in concentration were observed at the end of the period of incubation. The study was to provide information on the effects of the parameters investigated on sulphate concentration in wastewater in presence of the test fungal species.

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