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INVESTIGATION OF THE ROLE OF TWO BACTERIAL SPECIES IN THE REMOVAL OF SULPHATE FROM WASTEWATER

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ABSTRACT

The aim of this study was to investigate the optimal conditions for sulphate from wastewater by Escherichia coli *and* Psendomonas aeruginosa *under shake flask conditions. Before inoculating with the respective isolates, the wastewater was first filtered in 200 mL quantity into 250 mL capacity and then sterilized in an autoclave. After inoculation with the test isolates, aliquot wastewater samples was aseptically removed from each flask, every 24 h, for 96 h, for the estimation of sulphate concentration, using procedures. The results revealed sulphate removal at 35 °C and 45 °C while at 25 °C, an increase in sulphate level in the wastewater was observed at the end of the 96 h incubation period. This trend was irrespective of the test bacterial species. At the different pH, sulphate decreases of 50.31 %, 26.67 % and 18.14 % at pH 6, 8 and 10 were observed in the presence of the Escherichia coli while in the presence of the Pseudomonas aeruginosa, sulphate decreases of 31.53 % and 21.52 % were observed at pH 6 and 8, respectively. With the different concentrations of sodium acetate that were used for investigation, maximum sulphate removal was observed at 10 g/L. This trend was also irrespective of the test isolates. In presence of the different external carbon sources, sulphate concentrations in the wastewater after the 96 h incubation period were observed to increases at the expiration of incubation. Yeast and meat extracts were observed to enhance sulphate removal in the presence of the Escherichia coli while in presence of the Pseudomonas aeruginosa, remarkable sulphate removal was only observed when yeast extract was used as the nitrogen source. There was no decrease in sulphate level at the different concentrations of peptone that were investigated. This trend was common in presence of both isolates. The study was able to reveal the roles of temperature, pH, carbon and nitrogen concentrations and carbon/nitrogen sources on sulphate removal from wastewater by the test is bacterial species under the experimental conditions used for investigation.*

Keywords: Sulphate, Wastewater, PH, Carbon, Nitrogen, Temperature.

1. INTRODUCTION

Wastewater, which and is generated from domestic residences, industries and agriculture is

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water that contains a mixture of liquid and solid materials from domestic or industrial sources that adversely degrade water quality (EPA, 2008). Industrial effluents of mining, food processing, soap making and petroleum refining industries are rich in sulphates, acids and metals, which may lead to mineralization and pollution of potential of receiving waters (Du Preez and Maree, 1994). Because, mineralization and pollution of water bodies can negatively affect the aquatic environment and even human being, there is therefore the need for the treatment of wastewater effluents. This will help to prevent the deterioration of receiving water bodies (Prigione *et al.*, 2008; Akpor, 2011).

In recent times, most of the techniques used in the treatment of wastewater are not able to completely degrade all pollutants present in the effluents (Spina *et al.*, 2012). These techniques, especially the chemical process, cause an increased sludge volume which often results in sludge with poor settling and dewatering characteristics and the depression of the pH. These drawbacks have increased the preference for biological wastewater treatment. (Vander Post and Schutte, 2003).

Microorganisms in water are known to play important roles in nutrient recycling nutrient, such as phosphate, nitrogen and heavy metals apart from contributing to various outbreaks of waterborne diseases. These microbes do this by carrying out biochemical reactions and transformations as part of the treatment process (Andersson *et al.*, 2005). Sulphate reducing bacteria are have been shown to have the best potential for sulphate removal from wastewater (Al- Zuhair *et al.*, 2008) but this project takes potential and role of naturally occurring bacteria , *Escherichia coli* and *Pseudomonas aeruginosa*, in the removal of suphate from wastewater. The aim of this study was to assessing the effects of temperature, pH, sodium acetate concentration, peptone concentration, carbon and nitrogen sources on sulphate concentration in wastewater in the presence of *Escherichia coli* and *Pseudomonas aeruginosa*.

2. MATERIALS AND METHODS

The test bacterial species used for this experiment were *Escherichia coli* and *Pseudomonas aeruginosa*. Before usage, the isolates were first streaked on nutrient agar plates and incubated for 24 h at a temperature of 35 °C to ascertain their purity. The pure isolates were then aseptically suspended in sterile normal saline (0.85 % NaCl, w/v).

Throughout the investigation period, the wastewater used was collected from the Landmark University Commercial Farms. The wastewater was collected in 10 L plastic containers and transported to the laboratory, where it was filtered using Whatman No. 1 filter paper. The filtered wastewater was then supplemented with magnesium sulphate (0.05 g/L) and known concentrations of an external carbon and nitrogen source before dispensing in 200 mL quantities into 250 mL capacity conical flasks and sterilized in an autoclave for 15 min at 121 °C at 15 psi.

For sulphate removal study, to each sterile flask (except those that were used as controls), a known inoculum size of the respective test bacteria isolate suspended in sterile normal saline was aseptically inoculated, using sterile micropipettes. The flasks were then incubated in a rotary shaking incubator and agitated at a speed of 150 rpm. Aliquot wastewater samples (10 mL) were aseptically taken from each flask, prior to inoculation and every 24 h for the next 96 h, for the estimation of sulphate concentration, using standard procedures (APHA, 2012).

In this study, the effects of temperature, pH, sodium acetate concentration, external carbon and nitrogen sources; and peptone concentration on sulphate levels in presence of the test bacterial species were investigated. The varying temperature and pH that were investigated were 25 °C, 35 °C and 45 °C; and 6, 8 and 10, respectively. The sodium acetate concentrations that were used for investigation were 5 g/L, 10 g/L and 15 g/L while concentrations of peptone that were used for investigation were 5 g/L, 10 g/L, 15 g/L and 20 g/L. The external carbon sources that were used for investigation were glucose, sucrose, lactose and methanol while the external nitrogen sources were peptone, yeast extract and meat extract.

In all experimental setups, incubation was at a temperature of 30 °C (except in the temperature variation studies) while the pH of the wastewater was adjusted to 7.0 (except in the pH variation studies). Also, except in the carbon and nitrogen sources variation studies, the external carbon and nitrogen sources that were used to supplement the wastewater were sodium acetate and yeast extract, respectively at concentrations of 5 g/L.

All experimental setups were carried out in triplicates. All the reagents used were of analytical grades.

3. RESULTS

As revealed in Fig. 1, in the presence of the *Escherichia coli*, sulphate level in the wastewater was observed to vary after 96 incubation period from 3865.45 mg/L to 4226.94 mg/L, from 3877.03 mg/L to 3778.03 mg/L and from 3891.56 mg/L to 3445.89mg/L, at incubation temperatures of 25 °C, 35 °C and 45 °C, respectively (Fig. 1). This variation translates to percentage decreases of 2.54 % and 11.45 % at 35 °C and 45 °C and an increase of 9.35 % at 25 °C. In the presence of the *Pseudomonas aeruginosa*, after the 96 h incubation time, sulphate levels in the wastewater was observed show variations from 3676.81 mg/L to 4326.53 mg/L at 25 °C, from 3629.06 mg/L to 3399.18 mg/L at 35 °C and from 3697.71 mg/L to 4167.25mg/L at 45 °C. This trend translates to an increases of 17.67 % and 12.70 % at 25 °C and 35 °C, respectively and a decrease of at 6.33 % (Fig. 1).

At the different pH, sulphate levels in the wastewater at the expiration of incubation showed variation in the presence of the *Escherichia coli* from 6516.36 mg/L to 3237.90 mg/L, from 6529.06 mg/L to 4787.970 mg/L and from 6565.89 mg/L to 5474.64mg/L at pH 6, 8 and 10, respectively. This trend revealed decreases of 50.31 %, 26.67 % and 18.14 % at pH 6, 8 and 10 (Fig. 2). Also, when the *Pseudomonas aeruginosa* was used as the inoculum, sulphate concentration after the end of incubation showed variation from 6467.43 mg/L to 4428.40 mg/L at pH 6, from 6462.82 mg/L to 5071.85 mg/L at pH 8 and from 6437.21 mg/L to 7276.09 mg/L at pH 10.

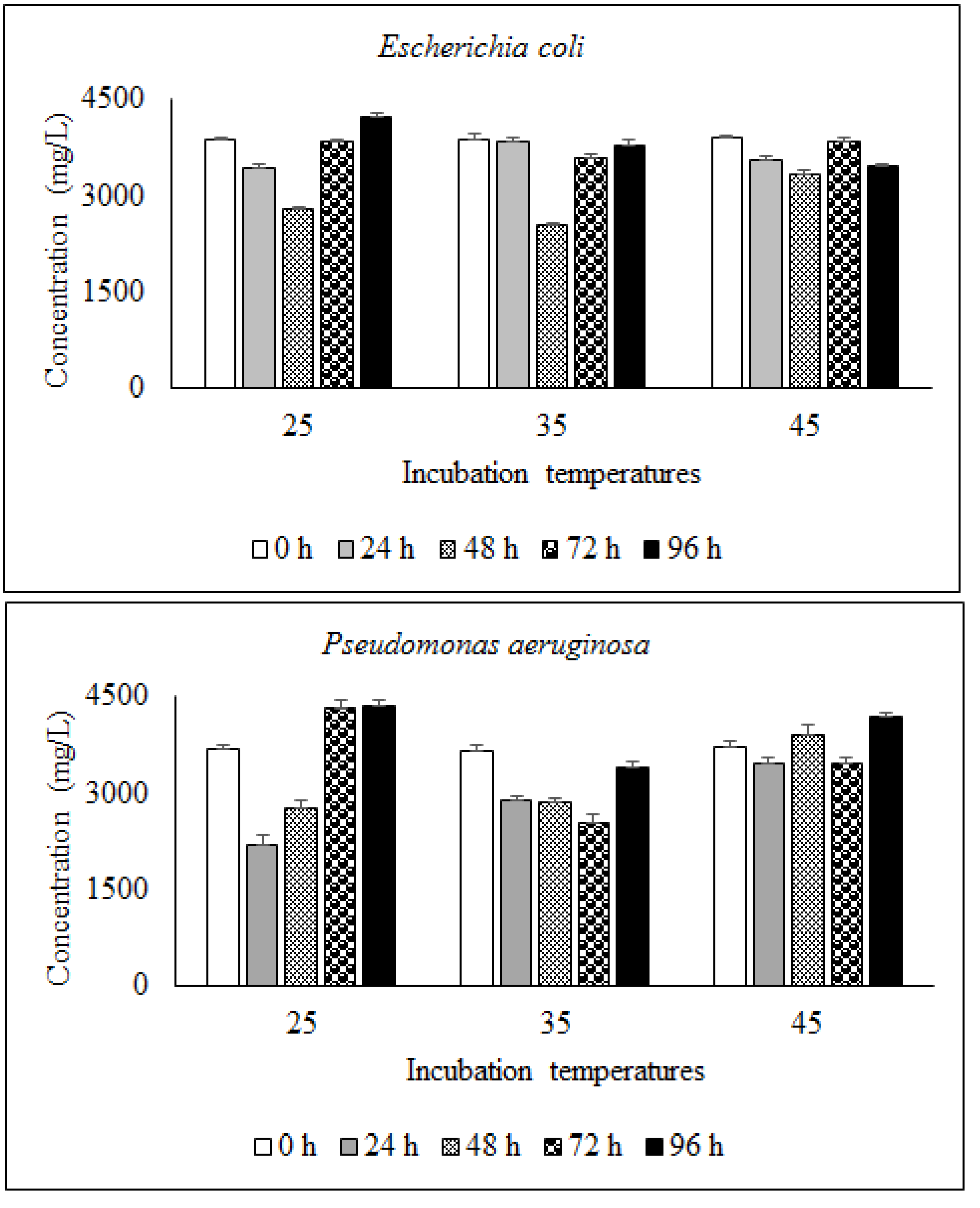

Fig-1. Sulphate levels in the wastewater at the different temperatures in presence of the test bacterial species

This variation is a decrease of 31.53 % and 21.52 % at pH 6 and 8, respectively and an increase of 13.03 % at pH 10 (Fig. 2).

Figure 3 shows the effect of sodium acetate concentration on sulphate levels during the period of incubation. As shown in the figure, in the presence of the *Escherichia coli*, sulphate levels in wastewater was observed to increase from 2353.34 mg/L to 3575.38 mg/L (51.93 % increase) and from 2389.49 mg/L to 3489.06 mg/L (46.02 % increase) at sodium acetate concentrations of 5 g/L and 15 g/L, respectively. At sodium acetate concentration of 10 g/L, remarkable decrease in sulphate level was observed, decreasing from 2352.05 mg/L to 1120.83 mg/L (52.35 %

decrease). When *Pseudomonas aeruginosa* was used for inoculation, sulphate concentration in the wastewater was found to increase from 3469.52 mg/L to 3593.80 mg/L(3.58 % increase) at 5 g/L of sodium acetate and from 3406.89 mg/L to 3700.81 mg/L (8.63 % increase) at 15 g/L of sodium acetate. A decrease from 3469.04 mg/L to 883.78 mg/L (74.52 % decrease) was observed at sodium acetate concentration of 10 g/L (Fig. 3).

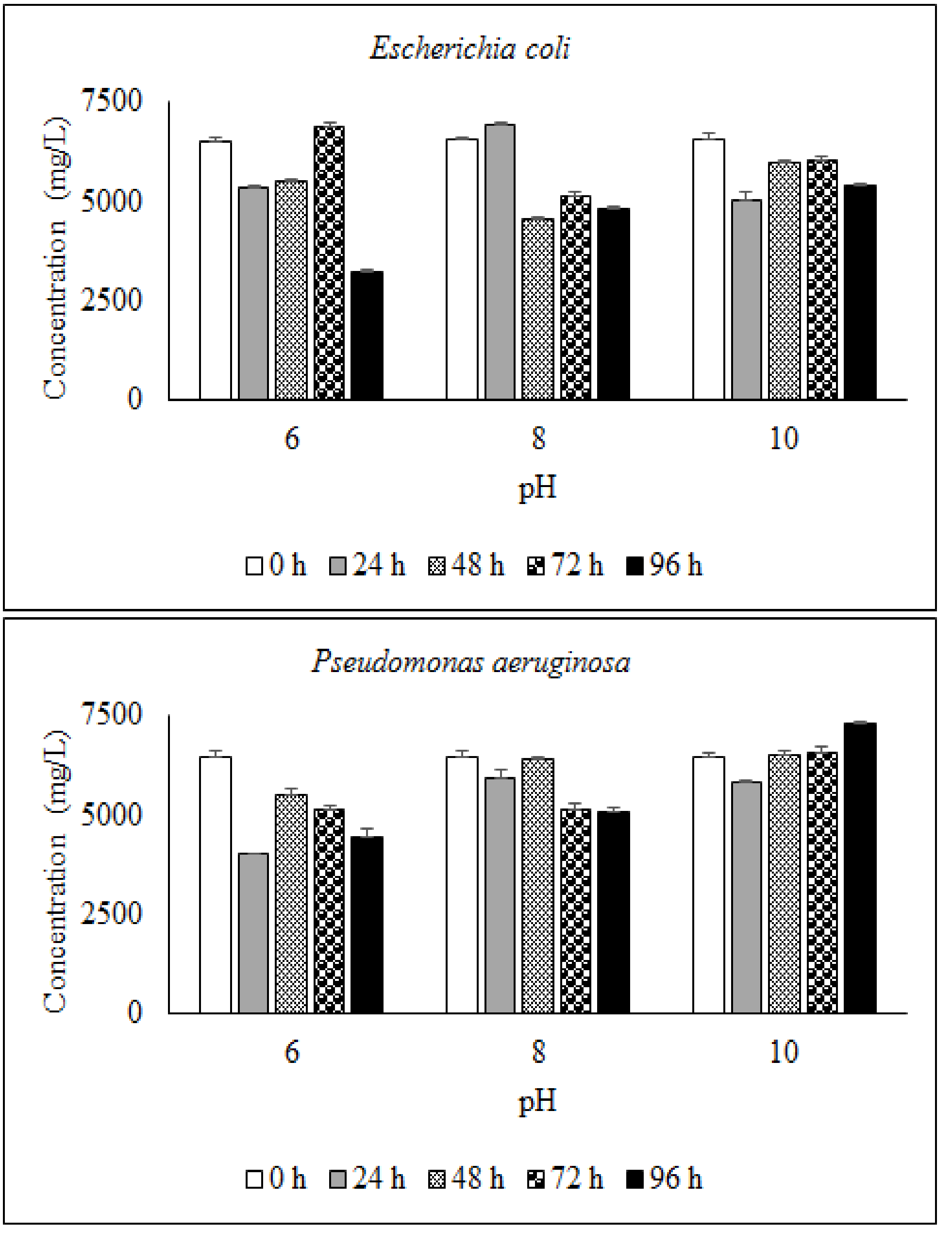

Fig-2. Sulphate levels in the wastewater at the different pH in presence of the test bacterial species

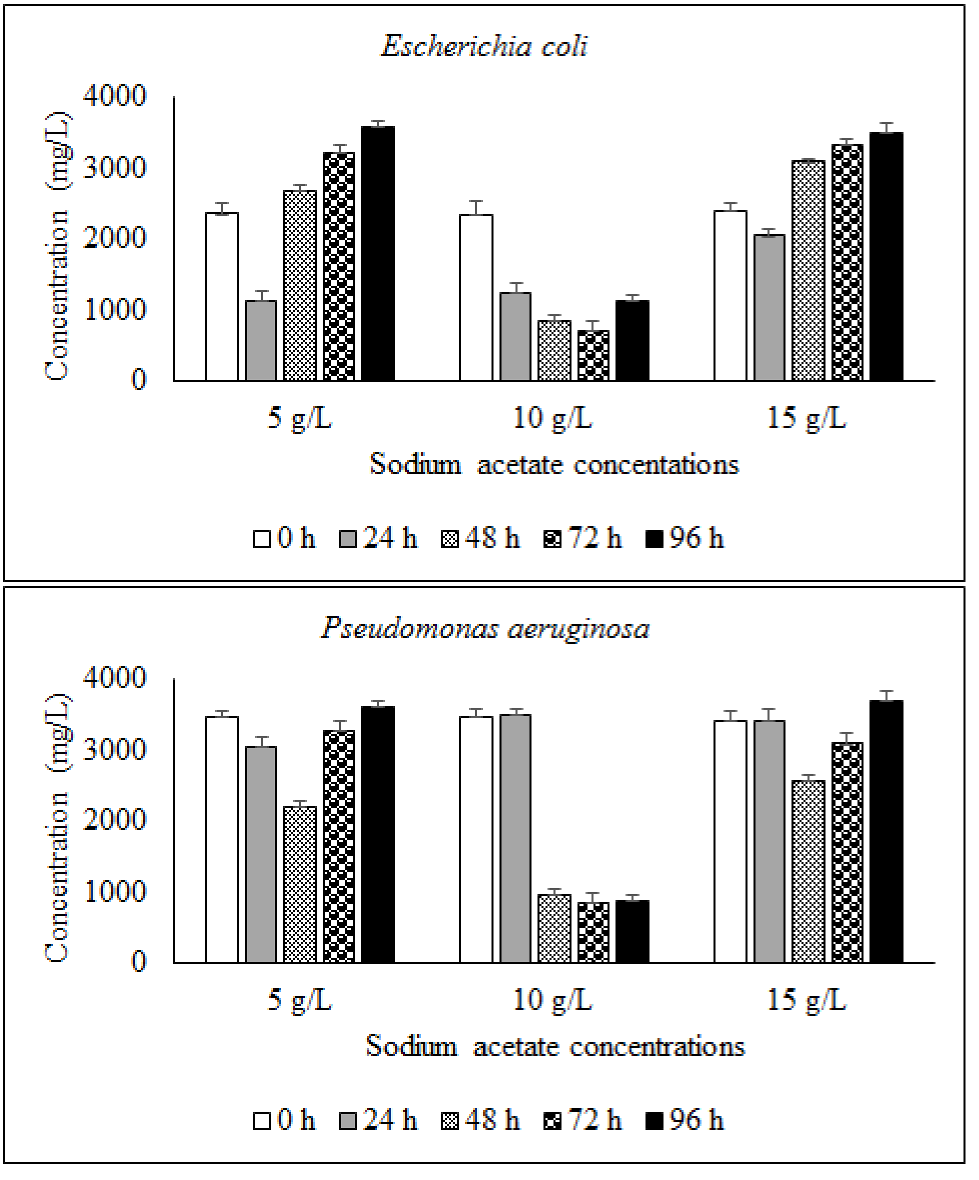

Fig-3. Sulphate levels in the wastewater at the different concentrations of sodium acetate in presence of the test bacterial species

At the different external carbon sources, sulphate concentrations in the wastewater after the 96 h incubation period in the presence of the *Escherichia coli* showed increases from 2393.56 mg/L to 2827.86 mg/L (18.15 % increase), 2332.92 mg/L to 3208.78 mg/L (37.54 increase), from 2352.94 mg/L to 3371.15 mg/L (43.27 % increase) and from 2358.00 mg/L to 2752.59 mg/L(16.73 % increase) when glucose, lactose, sucrose and methanol were used as carbon sources, respectively (Fig. 4). In the presence of the *Pseudomonas aeruginosa*, sulphate concentrations in the wastewater at the expiration of incubation varied from 2680.83 mg/L to 3458.79 mg/L (29.02 % increase), with glucose as carbon source, from 2605.30 mg/L to 3425.27

mg/L (31.47 % increase), with lactose as carbon source, 2643.73 mg/L to 2211.35 mg/L (16.35 % increase), with sucrose as carbon source and from 2645.80 mg/L to 3193.30 mg/L (20.69 % increase), with methanol as carbon source (Fig. 4).

With respect to the external nitrogen sources, at the end of the 96 h incubation time, the concentration of sulphate in the wastwater in the presence of the *Escherichia coli* showed remarkable decreases of 36.32 % (decreasing from 1068.56 mg/L to 680.42 mg/L) and 28.51 % (decreasing from 1092.43 mg/L to 780.93 mg/L) when yeast extract and meat extract were used, respectively. In the presence of peptone as the external nitrogen source, sulphate level at the end of incubation showed a remarkable increase from 1062.03 mg/L to 2309.19 mg/L (Fig. 5). In the case of the *Pseudomonas aeruginosa*, the concentration of sulphate in the wastewater supplemented with the different nitrogen sources was observed to show increases after the 96 h incubation period from 1095.51 mg/L to 17956.20 mg/L and from 1035.57 mg/L to 1755.16 mg/L in the presence of peptone and meat extract, respectively. This translates to an increase of 78.57 % and 69.49 %, respectively. A remarkable decrease in sulphate levels was however observed in the presence of the yeast extract, decreasing from 1020.62 mg/L to 363.42 mg/L (Fig. 5).

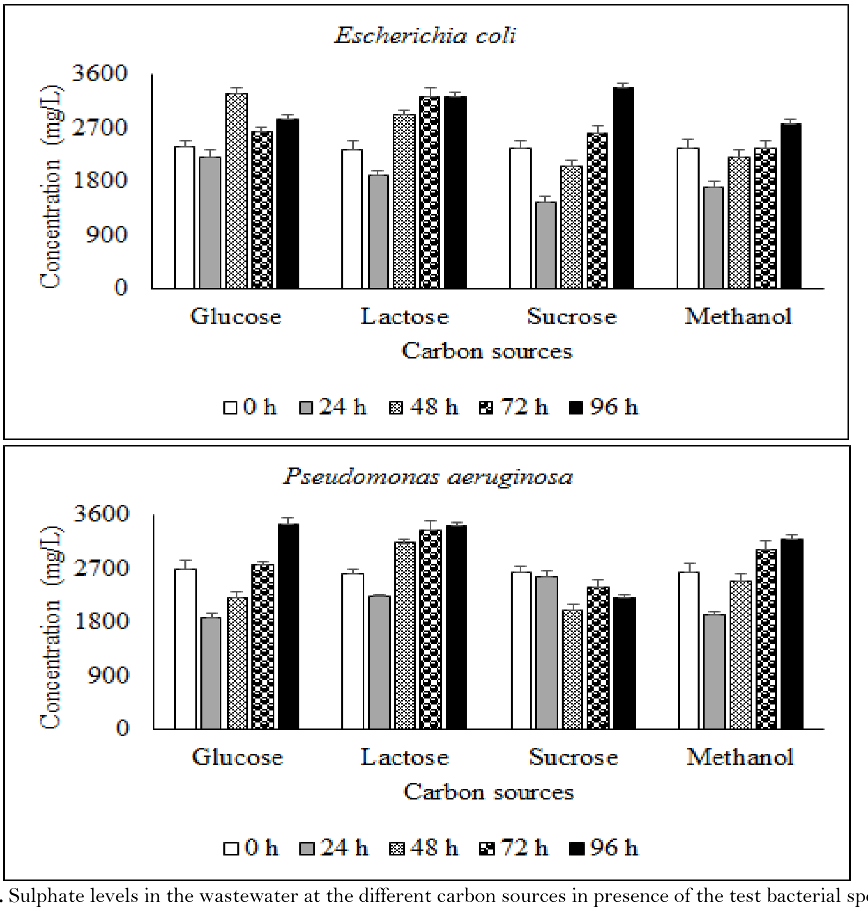

Fig-4. Sulphate levels in the wastewater at the different carbon sources in presence of the test bacterial species

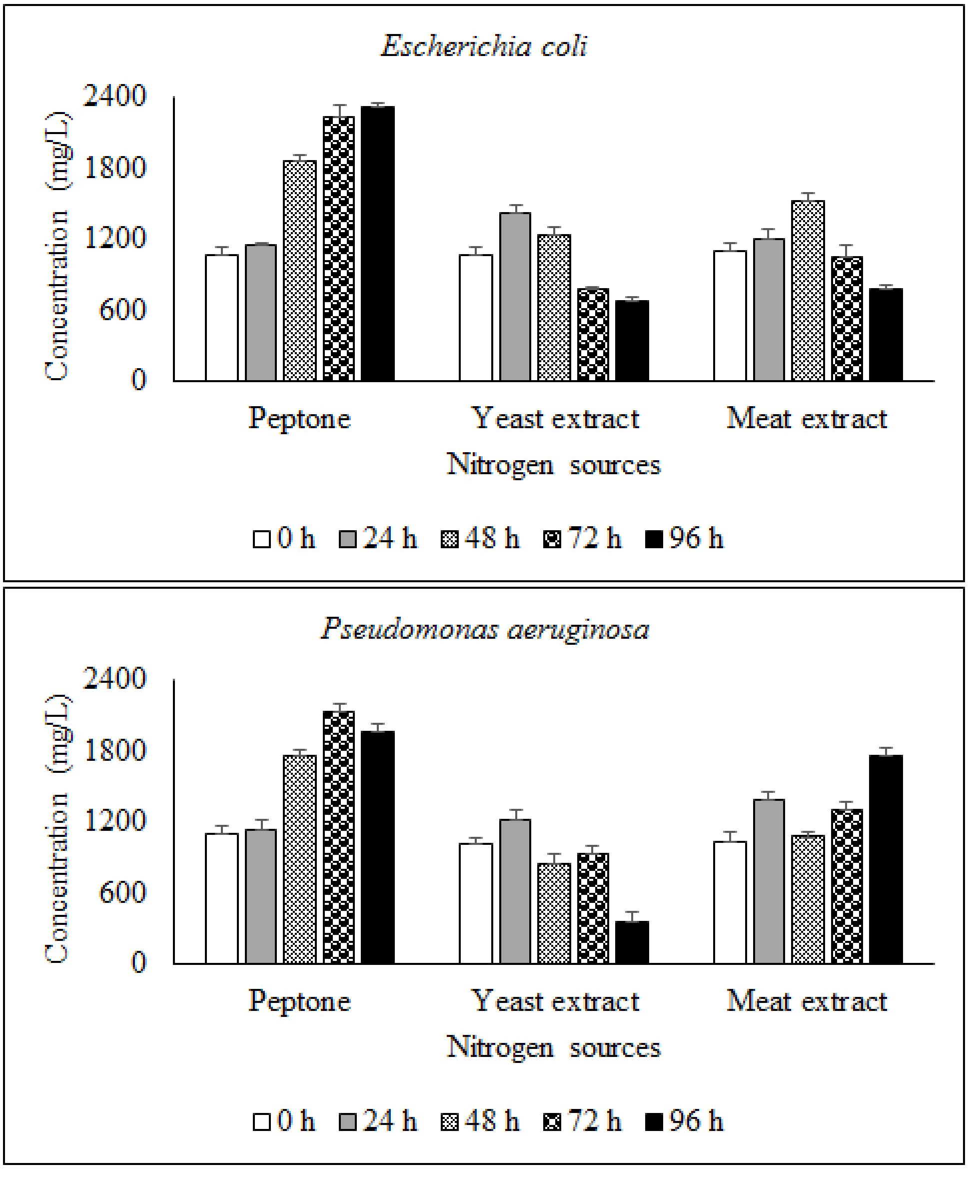

Fig-5. Sulphate levels in the wastewater at the different nitrogen sources in presence of the test bacterial species

As shown in Figure 6, at the different concentrations of peptone used for investigation, increases in sulphate levels were observed in the wastewater at the end of the 96 h incubation time. This trend was irrespective of the test isolate used for investigation. In the presence of *Escherichia coli*, sulphate concentration in the wastewater was observed to increase from 1603.10 mg/L to 1848.44 mg/L, from 1677.84 mg/L to 2741.01 mg/L, from 1808.76 mg/L to 2868.94 mg/L and from 1621.14mg/L to 3141.44 mg/L at peptone concentrations of 5 g/L, 10g/L, 15 g/L and 20 g/L, respectively. This shows increases of 14.68 %, 62.77 %, 58.61 % and 93.78 %, respectively (Fig. 6). When the *Pseudomonas aeruginosa* was used as the inoculum, the level of sulphate in the wastewater after the 96 h incubation period increased from 1319.18 mg/L to

1585.42mg/L, from 1323.71, mg/L to 2464.57mg/L, from 1339.69 mg/L to 43116.67 mg/L and from 1354.97mg/L to 3209.75mg/L at 5 g/L, 10 g/L, 15 g/L and 20 g/L, respectively. This shows increases of 20.18 %, 86.19 %, 132.64 % and 136.89 %, respectively (Fig. 6).

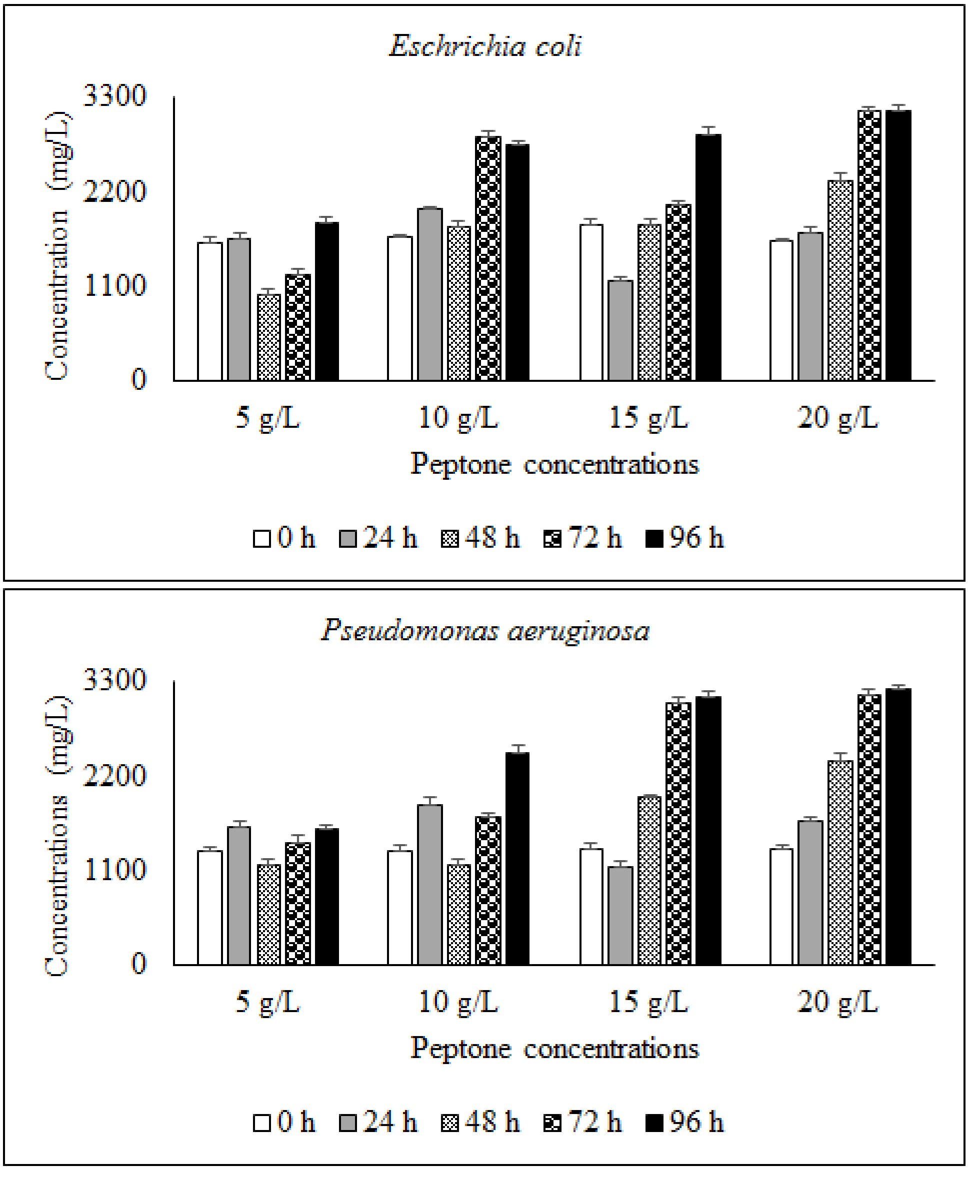

Fig-6. Sulphate levels in the wastewater at the different concentrations of peptone in presence of the test bacterial species

4. DISCUSSION

In this study, optimum temperature for sulphate uptake by the test bacteria species was observed at 45 °C and 35 °C, for the *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Al-Zuhair *et al.* (2008), indicated in his investigation that optimum temperature for sulphate removal was at 35°C when he used sulphate reducing bacteria. The temperature of the wastewater is considered an important factoring that determines the nutrient removal capabilities of bacteria. This is due to the fact that biological treatment activity has been reported to accelerate in warm temperatures and to slow in cool temperatures, but extremely hot or cold temperatures can stop treatment processes (Metcalf and Eddy, 2003). 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 settling characteristics of biological solids (Mulkerrins *et al.*, 2004; Akpor *et al.*, 2013).

The present investigation observed optimum pH for sulphate removal by the test isolates to be 8. At pH 6 and 10, only slight decreases in sulphate levels were observed at the end of incubation in the presence of the test bacterial species. Earlier investigators have indicated an optimum pH of 7 in similar studies (Al- Zuhair *et al.*, 2008). The pH of the wastewater is an important factor that affects the nutrient removal from wastewater by the test bacterial isolates because it affects the stability of the wastewater treatment process (O'Flaherty and Colleran, 1998; Zub *et al.*, 2008). It is indicated for effective wastewater treatment processes, it is vital to maintain pH of wastewater between 6 and 9.

At the different concentrations (5 g/L, 10 g/L and 20 g/L), of sodium acetate, sulphate uptake was not observed at the end of incubation. This trend was irrespective of the test isolate used for inoculation. This observation may be due to the possible effect of a build-up of organics of several degrees, hence a significant concentration of the carbon will first need to be hydrolyzed before nutrient removal can be achieved (Heymann, 1985; Kristensen and Jorgensen, 1990).

The findings of this study revealed sodium acetate as the most ideal carbon source in the presence of the *Pseudomonas aeruginosa*. In a study by Bharati and Kumar (2012), lactate and acetate were reported as an ideal carbon sources that enhance sulphate removal by sulfur reducing bacteria. Similarly, White and Gadd (1996) have indicated the use of ethanol and sodium acetate as effective carbon sources in nutrient removal studies. In biological nutrient removal processes, the presence of a carbon source is essential in promoting the growth of microbes, since it serves as an electron donor. Numerous experiments have revealed the significance of carbon sources in nutrient removal systems (Carucci *et al.*, 1997; Tasli *et al.*, 1997; Kargi and Uygur, 2003). In this study, the effect of lactose, sucrose, methanol, glucose and sodium acetate as external carbon sources on the sulphate removal ability of the test isolates was investigated. The use of these carbon sources in nutrient removal studies have been reported in earlier studies (Isaacs and Henze, 1995; Jeon and Park, 2000). The choice of some of the carbon sources is indicated to be due to their low cost and presence in industrial wastewaters, thus making make them cheap potential sources of carbon for the biological treatment of wastewater with a high nutrient content (Guadalupe *et al.*, 1998).

It this experiment, malt extract, yeast extract and peptone were used as external nitrogen sources in the wastewater. The results of the study revealed yeast extract as the most ideal nitrogen source in the *Escherichia coli* while in the presence of the *Pseudomonas aeruginosa*, highest sulphate increase was observed when peptone was used as the nitrogen source. This result corroborates the findings of White and Gadd (1996) who reported that both cornsteep and yeast extract had positive effects on the sulphate reduction potential of bacteria; with the strongest effect showed by cornsteep.

5. CONCLUSION

This study, which was aimed at investigating the effect of temperature, pH, carbon and nitrogen concentrations and carbon and nitrogen sources on sulphate removal ability of the two bacterial species, was able to reveal the following:

- Optimum temperature for sulphate removal was observed to be range from 35 ^oC to 45 ^oC, with maximum removal observed at 45 ^oC and 35 ^oC in the presence of the *Escherichia coli* and *Pseudomonas* aeruginosa, respectively.
- Although sulphate removal was observed at the different pH, maximum removal was observed at pH 6 in the presence of the *Escherichia coli.* With the *Pseudomonas aeruginosa* as the test inoculum, sulphate removal was only observed at pH 6 and 8, with the optimum at pH 6.
- At the different concentrations of sodium acetate, remarkable removal was observed at 10 g/L, a trend that was similar in both test bacterial species.
- In the presence of the different external carbon sources (lactose, sucrose, methanol, and glucose), an increase in sulphate level was observed at the end of incubation. This trend was irrespective of the isolate used.
- With respect to the nitrogen sources, remarkable decrease in sulphate concentration was observed in the presence of *Escherichia coli* when either yeast or meat extract was used. In the presence of the *Pseudomonas aeruginosa*, remarkable removal was only observed in the presence of the yeast extract.
- Increase in sulphate concentration was observed at the different concentrations of peptone that was used for investigation. This trend was irrespective of the two test bacterial species.

Although the study cannot be considered to be exhaustive, as there is need for further scale-up investigations, it has provided information on the optimum conditions for sulphate uptake by the test bacterial species. Knowledge of this could help in effective biological wastewater treatment processes and decision-making that is science-based.

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