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ORIGINAL ARTICLE

External Carbon Concentration and pH Effects on Nitrate and Nitrite Removal from Nutrient Media by *Pseudomonas aeruginosa*

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ABSTRACT
The effect of pH and carbon source concentration in nitrate and nitrite removal from nutrient media by immobilized and free cells of Pseudomonas aeruginosa was carried-out. The pH of 6, 8, 10 and external carbon source from sodium acetate at concentrations of 10 g/L, 15 g/L and 20 g/L under a shaking condition of 120 rev/sec, temperature of 37 ^{o}C were used. Estimation of nitrate and nitrite concentrations was carried-out using standard methods. The results revealed significant nitrate removal at pH 8 and 10. At a pH of 6, no remarkable decrease in nitrate concentration was observed in presence of the respective cells. For nitrite concentration, in presence of respective cells, significant decreases was observed after 24 h and 48 h, at pH 8 and 10, respectively. These decreases were observed to be consistent throughout the period of incubation. At a pH of 6, no remarkable decrease in the concentration of nitrite in the media was observed in presence of the respective cells. These observations were irrespective of the cells used for inoculation. Moreover, a significant reduction in nitrate and nitrite concentrations was observed when different sodium acetate concentrations were used irrespective of the cells used for inoculation.
***KEYWORDS**: Nitrate, nitrite, carbon source, immobilized cells, free cells and Pseudomonas aeruginosa.*

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INTRODUCTION

Safe drinking water and adequate sanitation are essential in sustaining life. The presence of nutrients, such as nitrates and nitrites in high concentration in receiving water bodies is known to cause eutrophication and various health impacts. Nitrate is known to exist naturally in water, soil, atmosphere and the environment [1]. The presence of pollutants in water may be due to industrial, agricultural or domestic activities, which can lead to a variety of health and environmental impacts. The presence of nitrate and nitrite in water in excess of the acceptable limits could create conditions that favour the growth of algae and cyanobacteria [2, 3].

Because of its ease of conversion to nitrate, nitrite is rarely found in drinking water providers. About 80 % of total contact to nitrite is caused by the decrease of nitrate consumed by oral bacteria. Because nitrate and nitrite are non- volatile nutrients, contact from drinking water is associated to only consumption [4]. Although the presence of nitrate and nitrite is useful to aquatic life when present in small amounts, when in excess, it causes eutrophication and degradation of receiving water bodies [5]. The degradation of receiving water bodies like streams, rivers and lakes is accountable for the quality of wastewater effluents. Some of the impacts of eutrophication include decrease in the level of dissolved oxygen, toxic substance release, biomagnification in aquatic life and increase in the level of nutrient load [2].

The presence of nitrate and nitrite in excess amounts in irrigation water and water for livestock can be harmful to both farmers and consumers [6]. Summarily, nutrient induced production of aquatic plants in receiving water bodies leads to several harmful consequences. Because the presence of nitrate water in concentration that is excess of the acceptable limits is known to cause several health and environmental impacts, extensive research has concentrated its removal. Although the processes for nitrate removal from water can either be physical, chemical and biological, in recent years, biological processes is advocated. This is because biological processes are indicated to have less drawbacks than other processes. Although several traditional biological treatment procedures have the ability of eliminating voluminous segments of biodegradable organic compounds residing in wastewater, several harmful compounds are rarely extracted due to their toxicity[5] In addition, they impose harmful effects on the composition and activities of microorganism populations in activated sludge flocs, thereby reducing the

Akpor OB and Babarindetotal performance of these amenities. The uptake of these compounds is an existent problem for waste treatment engineers and scientists [7].
One of the advantages of immobilized microbes is the cost effectiveness, since they depict no substantial loss of activity even after repeated use [8]. The use of immobilized microbial technology is therefore indicated as promising tool for wastewater treatment in the past few years and in the forthcoming years ahead of us. The most increasing areas of the application of immobilized cells is their use in the decrease of environmental pollutions through biodegradation of several detrimental compounds[9].
It is opined that immobilized microbial cells are suitable for the treatment of waste by transforming the harmful compounds into nutrient, carbon dioxide and biomass by biodegradation through their intermediates. Enhanced biodegradation level was detected in immobilized cells owing to the absence of internal and external mass transfer resistance. Other benefits of immobilized cells are improved microbial cell stability, which permits incessant process operation and avoids the biomass-liquid separation condition[10]. This study was carried-out so as to evaluate the relationships of pH and external carbon source concentration on nitrate and nitrite removal by immobilized and free cells of *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

The media used for the study had the following composition dissolved in distilled water: sodium acetate (5 g/L), peptone (5 g/L), yeast extract (5 g/L), magnesium sulphate (0.5 g/L), potassium dihydrogen phosphate (0.5 g/L), sodium nitrate (0.5 g/L) and sodium nitrite (0.5 g/L). After preparation, the media was dispensed in 200 mL quantities into 250mL capacity conical before sterilization using an autoclave at 121°C for 15 min.
The external carbon source used for the study was sodium acetate while the test bacteria used for the study was *Pseudomonas aeruginosa* ATCC 9027 P-1. Before using for the study, the pure culture of the bacteria was grown in sterile nutrient broth for 24 h, after which it was streaked in nutrient agar plate to ascertain its purity. To obtain the free cells, the pure culture was cultured in nutrient broth for 24h at 37°C, after which the broth culture was centrifuged at 500rpm for 30min to separate the cells from the culture media. After centrifugation, the supernatant was discarded while the cells were suspended in sterile normal saline (0.9 % NaCl solution). The suspended cells were vortexed and dispensed in sterile universal bottles and stored in a refrigerator until when needed.
Immobilization of the test isolate in the sodium alginate-calicum chloride mixture, agarose and agar-agar were carried out as described earlier [11, 12]. For immobilization in the sodium alginate and calcium chloride mixture, 5 % and 2.5 % of the solutions were used; respectively while 2.5 % and 5 % the agar-agar and the agarose solutions were used for immobilization.
Nitrate and nitrite removal study in the media in presence of the cells was carried out in batches at different pH (6, 8 and 10) and different sodium acetate concentrations (10 g/L, 15 g/L and 20 g/L). The experimental setup was as follows: in 250 mL capacity conical flasks, 200 mL volume of media was prepared and sterilized. After sterilization, a known population of the respective free or immobilized cells were inoculated into each flask and incubated at the required temperatures for the study. Just immediately after inoculation and every 24 h for 120 h, aliquot samples (10 mL) were taken from each flask for the determination of nitrate and nitrite concentrations in the media and the pH of the media, using standard procedures [13]. Nitrate, nitrite and pH were determined using the salicylate and the Strickland and Parsons Methods and pH meter, respectively.
Nitrate analysis was carried out using the salicylate method. In brief, to a 100mL glass beaker, 2mL aliquot of the sample was pipetted, after which 2mL of 0.5 % sodium salicylate was added and evaporated to dryness on a hot plate. 2mL of concentrated H_2SO_4 was added to the beaker after cooling while tilting the beaker to wet the bottom and lower edge of the wall completely and allowed to stand for about 10minutes. 30mL of distilled water was cautiously added which was followed by 8mL of 50 % (w/v) sodium hydroxide (NaOH) solution for colour development and made up to the mark by distilled water. The absorbance was measured after 10minutes after addition of NaOH at a wavelength of 410 nm using a 6705 UV/VIS spectrophotometer. A reagent blank was also run with the samples to help the spectrophotometer to zero. A standard nitrate solution was prepared using different concentrations of sodium nitrate. All standards were treated like the samples as above. A calibration curve drawn was by plotting absorbance (y-axis) versus standard concentration (x-axis). Nitrate concentration was calculated as:

$$mg - nitrate/L = concentration\ from\ calibration\ curve\ X\ 20XD$$

Where D= dilution factor

For nitrite determination, 2mL aliquot of the sample was pipetted into a 100mL glass beaker, after which 1.0mL of 5 % sulphanilamide solution using a micropipette, mixed and allowed to react for 3-5 min, after which 1.0mL of 0.5 % N-(1-Naphthyl)-ethylenediamine dihydrochloride solution was added for colour development and mixed immediately. The solution was shaken thoroughly and made up to the mark with distilled water. Prior to the measurement, the wavelength of the spectrophotometer was set at 543 nm and then set to zero with distilled water and the reagents without the sample. The absorbance samples were then measured using a 6705 UV/VIS spectrophotometer.

A standard nitrite solution was prepared using different concentrations of sodium nitrite. All standards were treated like the samples as above. A calibration curve drawn was by plotting absorbance (y-axis) versus standard concentration (x-axis). Nitrate concentration was calculated as:

$$mg - nitrite/L = concentration\ from\ calibration\ curve\ X\ 20\ X\ D$$

Where D= dilution factor

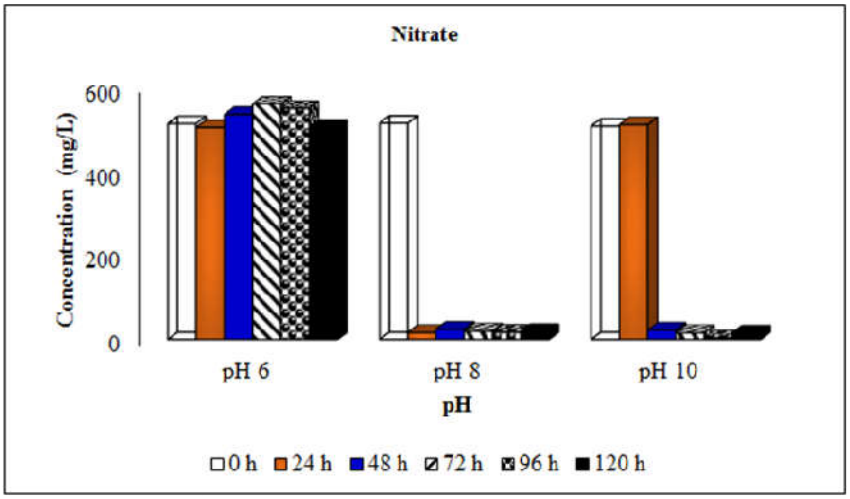
All reagents used for the study were of analytical grade while all experimental setups were carried out in duplicate. Statistical analyses were carried out using the SPSS statistical software package. The test for the comparison of means was done using the one-way variance (ANOVA). All statistics were run at a probability level of 0.05.

RESULTS

As revealed in Fig. 1, in presence of the alginate immobilized cells, significant nitrate removal was observed at pH after 24 h of incubation at pH 8. At pH 10, significant nitrate reduction was observed after 48 h of incubation. These reductions at pH 8 and 10 were consistent throughout the period of incubation. At a pH of 6, no remarkable decrease in nitrate concentration was observed in presence of the alginate immobilized cells. At the end of the 120 h incubation period, nitrate levels in the media were observed to decrease from 614.91 mg/L to 505.90 mg/L at pH 6, from 466.10 mg/L to 19.31 mg/L at pH 8 and from 508.78 mg/L to 15.88 mg/L at pH 10. Nitrate removals at pH 8 and 10 were observed to be significantly higher than that at pH 6 ($p \leq 0.05$).

For nitrite concentration, in presence of the alginate immobilized cells, as was observed for nitrate, significant decreases was observed after 24 h and 48 h at pH 8 and 10, respectively. These decreases were observed to be consistent throughout the period of incubation. At a pH of 6, no remarkable decrease in the concentration of nitrite in the medium was observed in presence of the alginate immobilized cells. This observation was irrespective of the period of incubation. At the expiration of the incubation time, nitrite level in the media showed a decrease from 193.80 mg/L to 129.36 mg/L (Fig. 1). Although the decreases in nitrite concentration at pH 8 and 10 were not observed to be significantly different, they were however significantly higher than that at pH 6 ($p \leq 0.05$).

When the agarose immobilized cells were used for investigation at the different pH, significant decreases in nitrate concentration in the media were observed after 24 h of incubation and after 96 h incubation at pH 8 and 10, respectively. These decreases were consistent throughout the period of incubation. At the end of the incubation time, nitrite concentration in presence of the agarose immobilized cells showed decreases from 614.91 mg/L to 372.52 mg/L, from 420.50 mg/L to 24.00 mg/L and from 508.78 mg/L to 17.69 mg/L, at pH 6, 8 and 10, respectively (Fig. 2). The reduction in nitrite concentration in presence of the agarose immobilized cells was observed to be significantly higher at pH 8 than at pH 6 and 10 ($p \leq 0.05$).



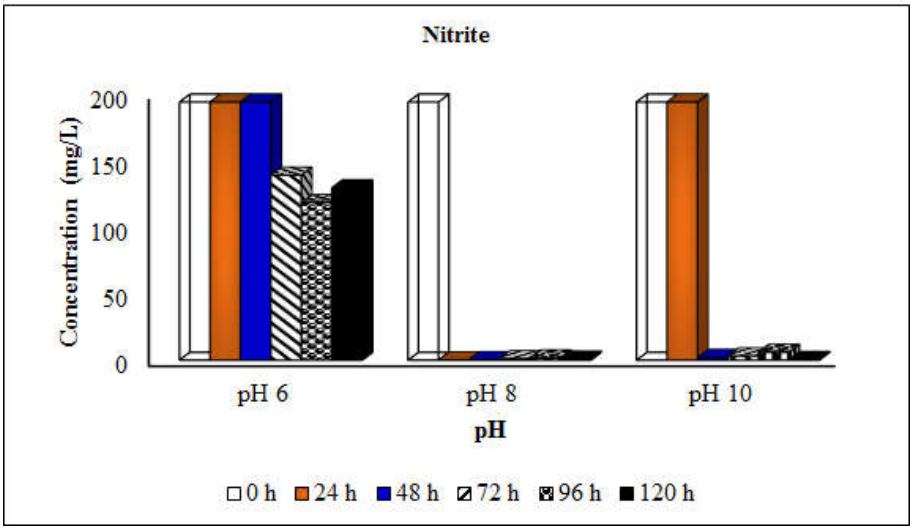


Fig. 1: Nitrate and nitrite concentrations in the media in presence of the alginate immobilized cells at the different pH

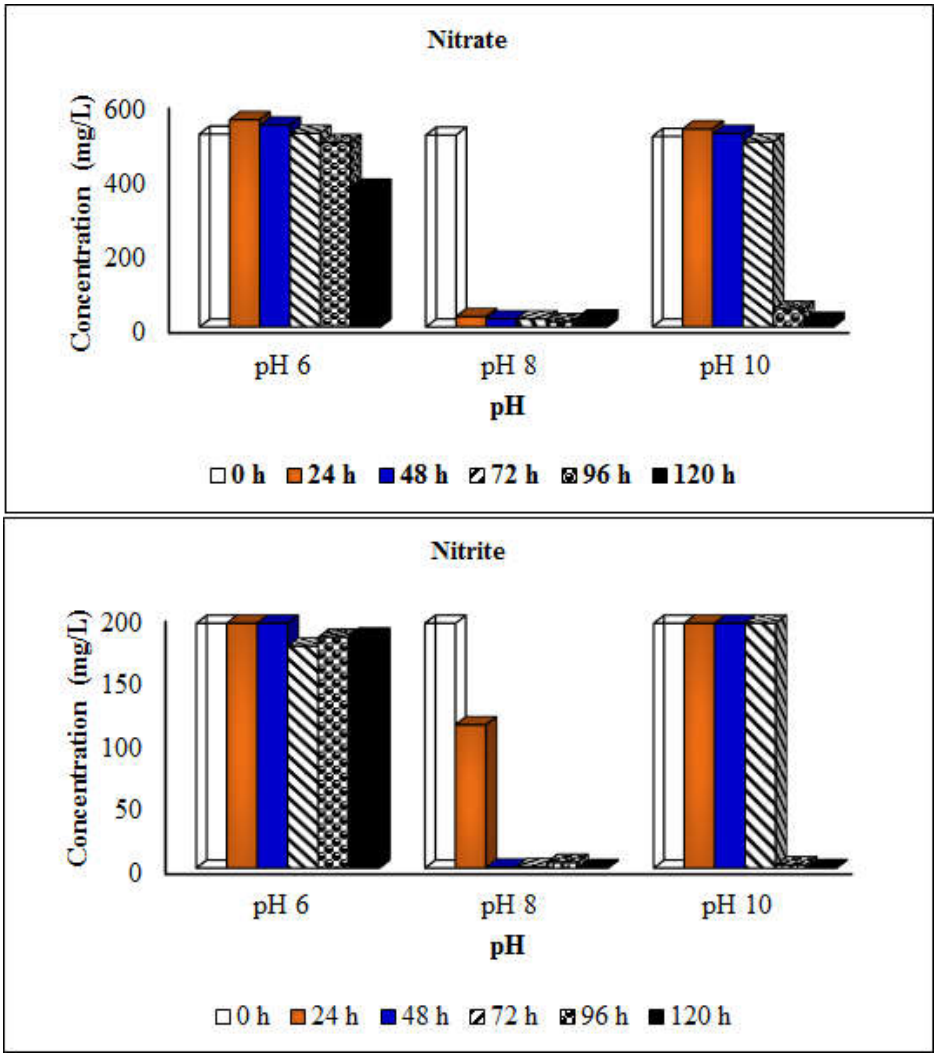


Fig. 2: Nitrate and nitrite concentrations in the media in presence of the agarose immobilized cells at the different pH

In the case of nitrite levels in the media, remarkable decrease in concentration was observed at pH 8 and 10 in presence of the agarose immobilized cells, with no remarkable decrease was observed at pH 6. Significant reduction in concentration was observed from 48 h of incubation at pH 8 and from 96 h of incubation at pH 10. After the 120 h incubation period, nitrite levels showed a decrease from an initial concentration of 193.8 mg/L to final levels of 183.6 mg/L, 0.23mg/L and 0.26 mg/L at pH 6, 8 and 10, respectively (Fig. 2). Generally, nitrite reduction in presence of the agarose immobilized cells was observed to be significantly higher than those at pH 6 and 10 ($p \leq 0.05$).

As revealed in Fig. 3, in presence of the agar immobilized cells, decrease in nitrate concentration was observed in the media after 24 h of incubation at pH 8. This decrease was consistent throughout the period of incubation. At a pH of 6 and 8, no remarkable decreases in nitrate concentrations were observed in presence of the agar immobilized cells. At the expiration of the 120 h incubation period, nitrate levels in the media were observed to decrease from 614.91 mg/L to 642.34 mg/L at pH 6, from 466.1 mg/L to 27.25 mg/L at pH 8 and from 508.78 mg/L to 445.07 mg/L at pH 10. Although there was no significant between the nitrate decreases at pH 6 and 10, there was a significant difference between removal at pH 8 and those at pH 6 and pH 10 ($p \leq 0.05$).

For nitrite concentration, in presence of the agar immobilized cells, a significant decrease was observed in the media after 48 h, at pH 8. This decrease was observed to be consistent throughout the period of incubation. At pH of 6 and 10, no remarkable decrease in the concentration of nitrite in the medium was observed in presence of the agar immobilized cells. This observation was irrespective of the period of incubation. At the expiration of the incubation time, nitrite level in the media showed a decrease from 193.80 mg/L to 167.64 mg/L (Fig. 3). Although the decrease in nitrite concentration at pH 8 was not observed to be significantly different, it was however significantly higher than those at pH 6 and 10 ($p \leq 0.05$).

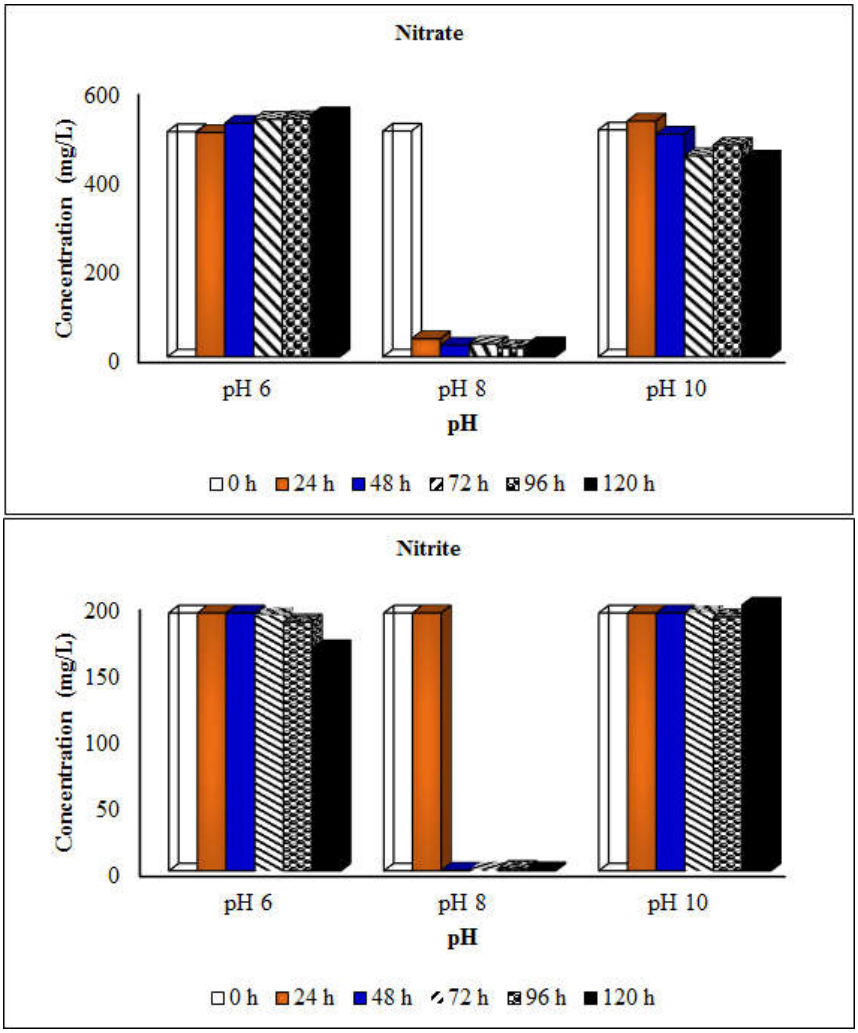


Fig. 3: Nitrate and nitrite concentrations in the media in presence of the agar immobilized cells at the different pH

As indicated in Fig. 4, in the presence of the free cells suspended in normal saline, nitrate levels in the media was observed to display substantial decreases after 24 h and 72 h incubation at pH 8 and 10, respectively. This decrease was consistent till incubation was terminated. At a pH of 6, no remarkable decrease in nitrate concentration was observed in presence of the alginate immobilized cells. This observation was also consistent with incubation time. At the end of the 120 h incubation period, nitrate levels in the media revealed decreases from initial concentrations of 550.915 mg/L to 530.8 mg/L at pH 6, 466.1 mg/L to 29.06 at pH 8, 508.78 mg/L to 20.21 mg/L (Fig. 4). The decrease in nitrate concentration at pH 8 was observed to be significantly higher than that at pH 10. Also, nitrate removals at pH 8 and 10 were observed to be significantly higher than that at pH 6 ($p \leq 0.05$).

In the case of nitrite levels in the media, remarkable decrease in concentration was observed at pH 8 in presence of the free cells, no remarkable decrease was observed at pH 6 and 10. After the 120 h incubation period, nitrite levels showed decrease from initial concentrations of 193.8 mg/L to 210.23 mg/L at pH 6, 193.8 mg/L to 1.23 mg/L at pH 8 and 193.8 mg/L to 19.38 mg/L at pH 10 (Fig. 4). There was no significant difference between nitrite levels at pH 6 and 8. However, nitrite level at pH 8 was observed to be significantly higher than those observed at pH 6 and 8 ($p \leq 0.05$).

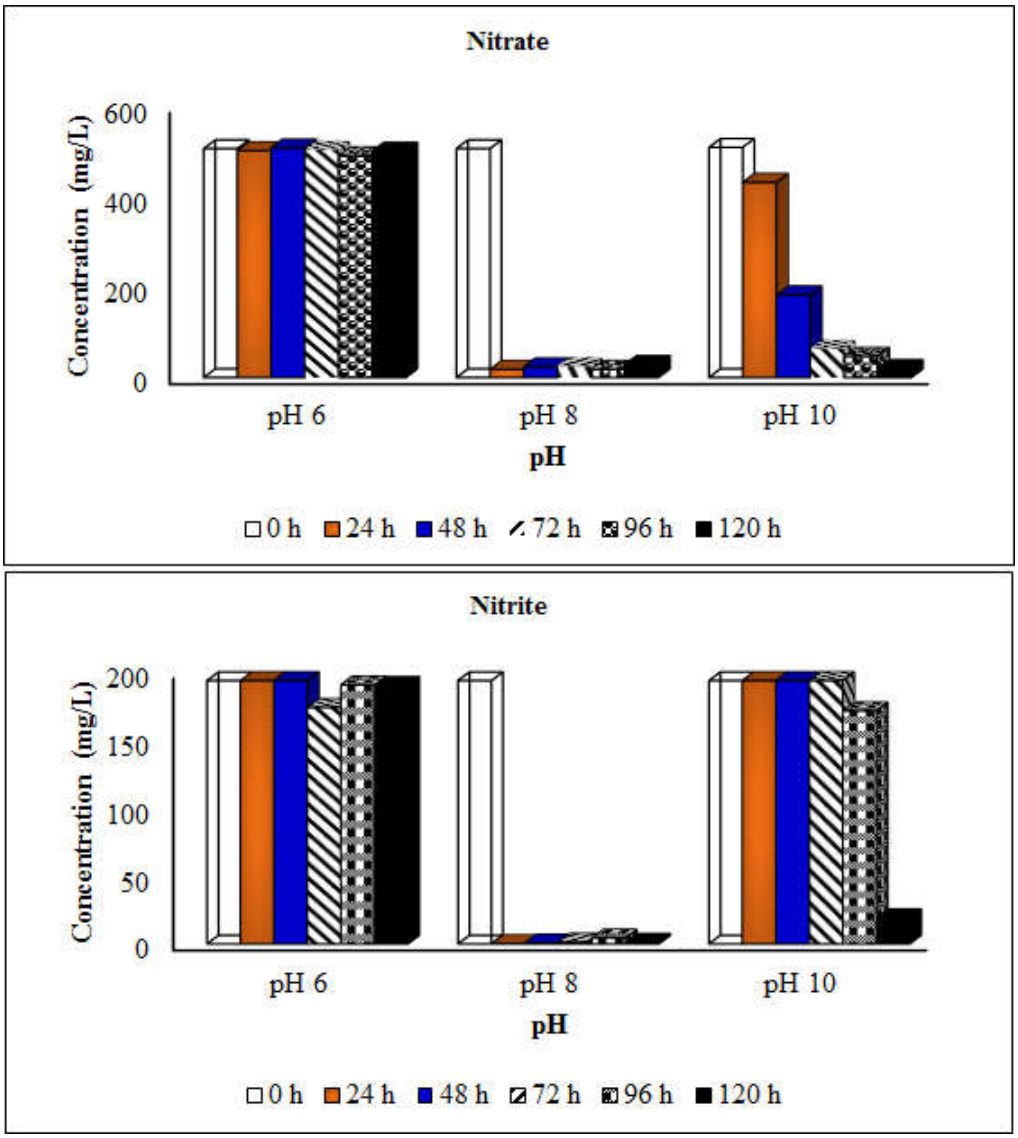


Fig. 4: Nitrate and nitrite concentrations in the media in presence of the free cells suspended in normal saline at the different pH

As presented in Table 1, in the presence of the respective immobilized and free cells used for inoculation, pH of the media was not observed to follow any pattern, showing minute increases and decreases at the different initial pH. At initial pH of 6, a change of 2.7 % increase, 0.7 % decrease, 2.6 % increase and 2.2 % decrease were observed in presence of the alginate immobilized, agarose immobilized, agar immobilized and free cells, respectively (Table 1).

Table 1: pH profile of the media at the different initial pH

pH	Initial	Final	% change
Alginate immobilized cells			
pH 6	5.96	5.80	2.7
pH 8	8.17	8.32	-1.9
pH 10	9.99	9.44	5.5
Agarose immobilized cells			
pH 6	5.98	6.02	-0.7
pH 8	8.17	8.38	-2.6
pH 10	9.99	9.10	8.9
Agar immobilized cells			
pH 6	5.96	6.12	-2.6
pH 8	8.17	8.27	-1.2
pH 10	9.99	9	10
Free cells			
pH 6	5.96	5.82	2.2
pH 8	8.17	8.36	-2.3
pH 10	9.99	8.10	18.9
Uninoculated control			
pH 6	5.96	6.02	-1.0
pH 8	8.17	8.27	-1.2
pH 10	9.99	9.10	8.9

Initial and final represent pH of the medium at 0 h and 120 h, respectively. All values are averages of triplicate analysis. All % values were decreases, except negative values that represent increases.

As illustrated in Fig. 5, at the different sodium acetate concentrations in the media, significant reduction in nitrate and nitrite concentrations were observed in presence of the alginate immobilized cells. This was irrespective of the sodium acetate concentration used in the media. After the end of incubation, nitrate levels in presence of the alginate immobilized cells varied from 480.99 mg/L, 470.71 mg/L and 478.79 mg/L to 25.09 mg/L, 24.37 mg/L and 25.09 mg/L, at sodium acetate concentrations of 10 g/L, 15 g/L and 20 g/L, respectively. As for nitrite levels in the media, concentration in presence of the alginate immobilized cells varied from 290.70 mg/L to 0.71 mg/L, from 273.98 mg/L to 0.52 mg/L and from258.86 mg/L to 0.36 mg/L, at sodium acetate concentrations of 10 g/L, 15 g/L and 20 g/L, respectively (Fig. 5). The decreases in nitrate and nitrite concentrations in presence of the alginate immobilized cells did not vary significantly with the different sodium acetate concentrations used ($p \leq 0.05$).

In presence of the agarose immobilized cells, significant decrease in nitrate and nitrite concentrations were observed after 72 h incubation and was consistent till the end of the incubation period. This observation was similar at the different sodium acetate concentrations used for investigation. Nitrate levels at the end of the period of incubation were observed to decrease from 480.99 mg/L to 31.04 mg/L at 10 g/L of sodium acetate, from 470.52 mg/L to 30.86 at 15 g/L of sodium acetate and from 478.79 mg/L to 25.45 mg/L at 20 g/L of sodium acetate. In the case of nitrite concentrations in the media, decreases from 290.70 mg/L to 0.94 mg/L, from 273.98 mg/L to 0.68 mg/L and from 285.86 mg/L to 0.87 mg/L were observed at sodium acetate concentrations of 10 g/L, 15 g/L and 20 g/L, respectively (Fig. 6). The concentrations of nitrate and nitrite in the media at the different sodium acetate concentrations were not observed to differ significantly in presence of the agarose immobilized cells ($p \leq 0.05$).

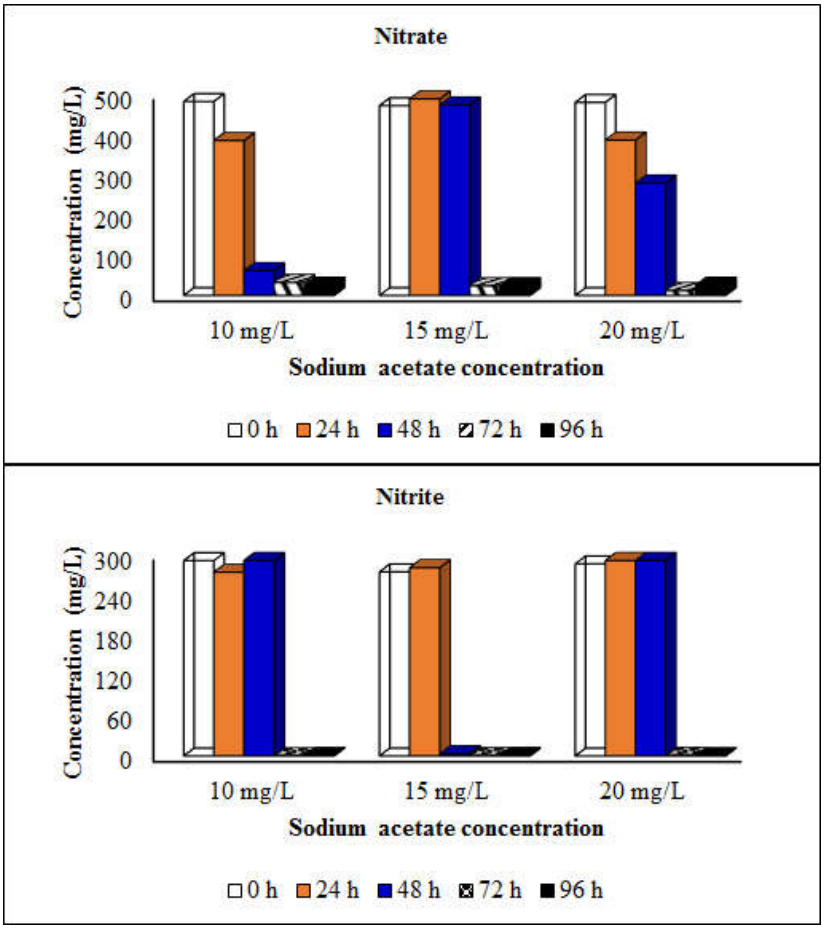


Fig. 5: Nitrate and nitrite concentrations in the media in presence of the alginate immobilized cells at the different sodium acetate concentrations

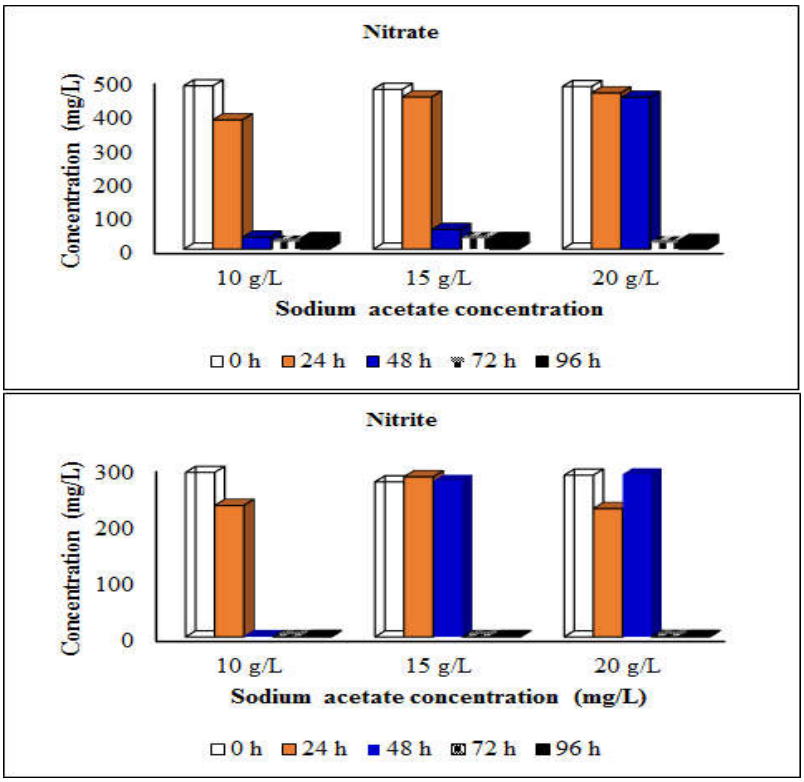


Fig. 6: Nitrate and nitrite concentrations in the media in presence of the agarose immobilized cells at the different sodium acetate concentrations

As shown in Fig. 7, in presence of the agar immobilized cells, nitrate and nitrite concentrations in the media were observed to decrease significantly from 72 h of incubation. This observation was irrespective of the concentration of sodium acetate used in the media. After the end of incubation, the concentrations of nitrate and nitrite when 10 g/L of sodium acetate was used varied from 480.99 mg/L to 32.67mg/L and from 290.70 mg/L to 0.39 mg/L, respectively. When 15g/L of sodium acetate was used, the concentrations of nitrate and nitrite in the media reduced from 470.52mg/L to 30.14mg/L and from 273.98 mg/L to 0.52mg/L, respectively. A decrease in nitrate and nitrite concentrations from 478.79 mg/L to 22.74 mg/L and from 285.56 mg/L to 0.78 mg/L were observed when 20 g/L of sodium acetate was used (Fig. 7). Generally, the decrease in nitrate and nitrite levels in presence of the agar immobilized cells were not observed to differ significantly between the different acetate concentrations used for investigation ($p \leq 0.05$).

When the media was inoculated with the free cells suspended normal saline, nitrate and nitrite concentrations at the different sodium acetate concentrations were observed to show significant decreases in concentration from 72 h and 48 h of incubations, respectively. Nitrate levels in the media in presence of the free cells showed a decrease from 480.99 mg/L to 24.91 mg/L, from 470.71 mg/L to 35.92 mg/L and from 478.79 mg/L to 38.17 mg/L at sodium acetate concentrations of 10 g/L, 15 g/L and 20 g/L, respectively (Fig. 8). In the case of nitrite in presence of the free cells, concentrations in the media showed a decrease from 290.70 mg/L to 1.07 mg/L, from 273.98 mg/L to 0.97 mg/L and from 285.86 mg/L to 0.87 mg/L, at sodium acetate concentrations of 10 g/L, 15 g/L and 20g/L, respectively (Fig. 8). Although there were variations in nitrate and nitrite concentrations in the media at the respective sodium acetate concentrations, these differences were not significant ($p \leq 0.05$).

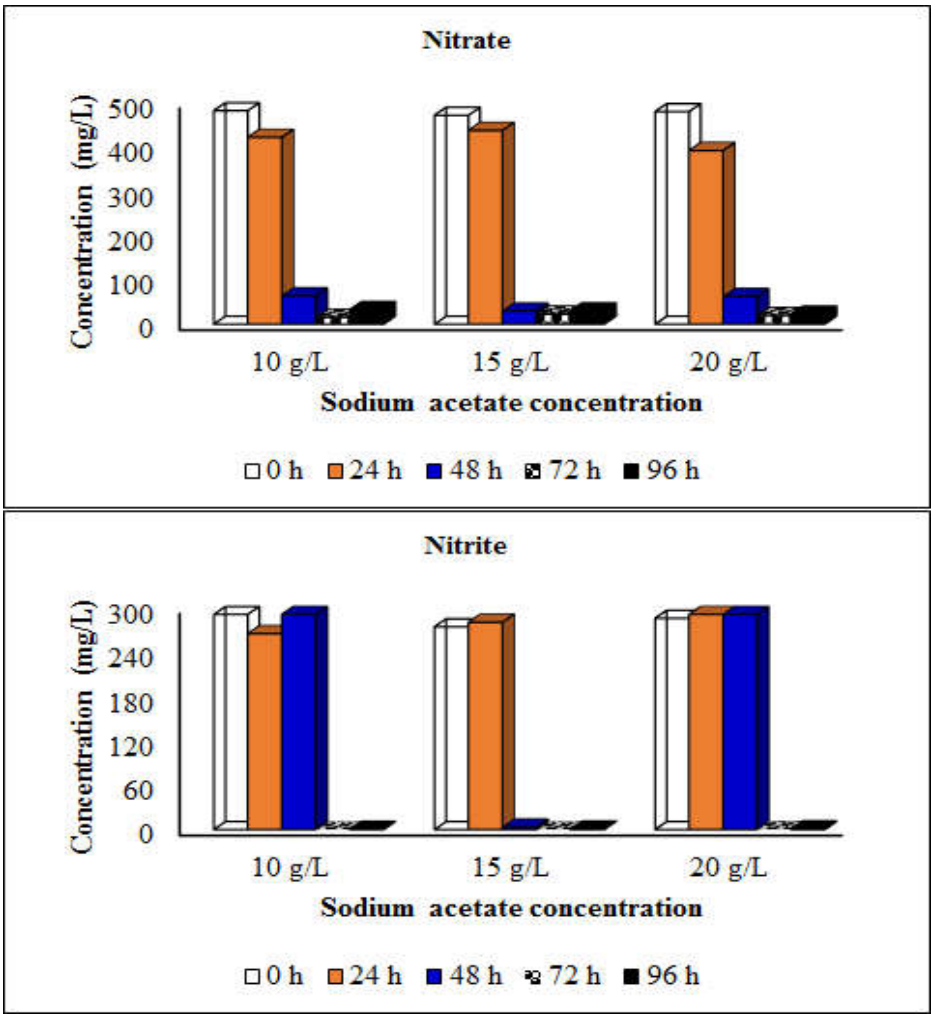


Fig. 7: Nitrate and nitrite concentrations in the media in presence of the agar immobilized cells at the different sodium acetate concentrations

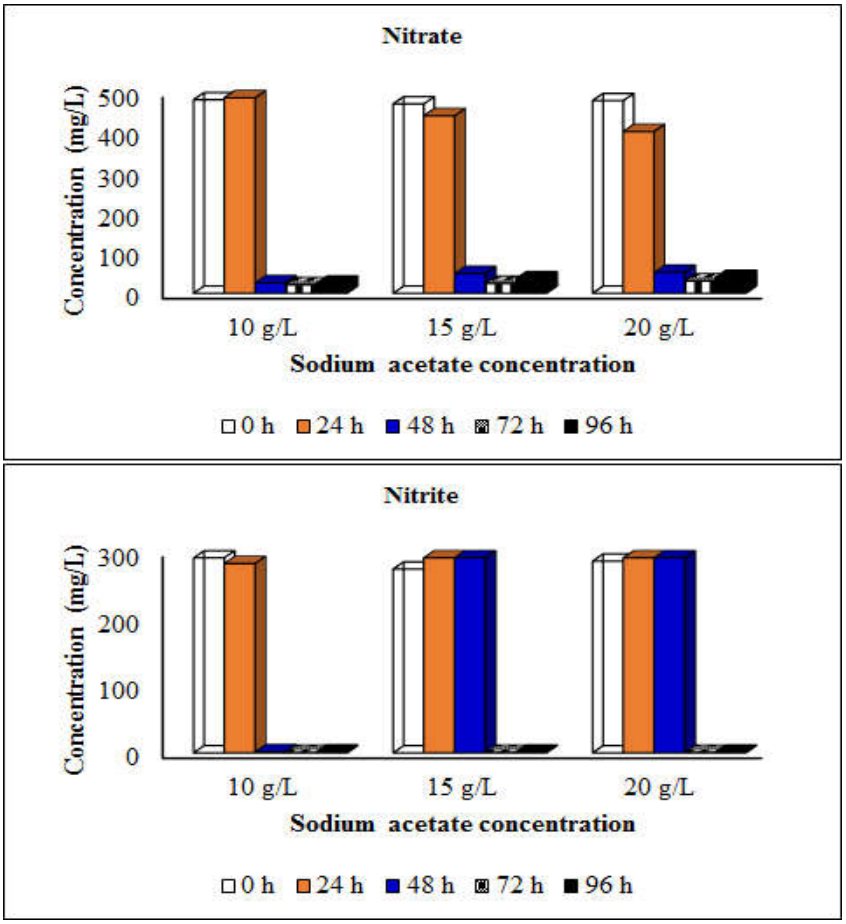


Fig. 8: Nitrate and nitrite concentrations in the media in presence of the free cells suspended in normal saline at the different sodium acetate concentrations

As presented in Table 2, in presence of the respective immobilized or free cells, pH of the media was observed to increase remarkable at the end of incubation. This trend was evident in the media with the respective sodium acetate concentrations.

Table 2: pH profile of the media at the different sodium acetate concentrations

pH	Initial	Final	% change
Alginate immobilized cells			
10 g/L	6.77	8.68	28.2
15 g/L	6.80	8.70	27.9
20 g/L	6.84	8.72	27.5
Agarose immobilized cells			
10 g/L	6.77	8.75	29.3
15 g/L	6.80	8.69	27.8
20 g/L	6.84	8.78	28.4
Agar immobilized cells			
10 g/L	6.77	8.85	30.7
15 g/L	6.80	8.70	27.9
20 g/L	6.84	8.72	27.5
Free cells			
10 g/L	6.77	8.69	28.4
15 g/L	6.80	8.62	26.8
20 g/L	6.84	8.68	26.9

Initial and final represent pH of the medium at 0 h and 120 h, respectively. All values are averages of triplicate analysis. All % values are increases

DISCUSSION

With respect to pH, optimum pH for nitrate and nitrite removal by the cells was observed to be between 8 and 10. At pH6, no significant decrease in the nitrate and nitrite levels was observed. Earlier investigators have indicated an optimum pH of 7 in similar studies [14]. The pH of wastewater is an essential factor that affects biological nutrient removal from wastewater. pH is also said to affect the stability of a wastewater treatment process [15, 16]. The removal of nutrients from aqueous solution and wastewater by adsorption is highly dependent on the pH solution. Chen *et al*., [17], showed that the rate of nitrification would be reduced when alkalinity is below 40 g m^{-3}. Gujer and Boler, [18], reported that to sustain maximum nitrification rate, nitrifying biofilters used in community wastewater treatment, alkalinity level is usually.

Zhu and Chen, [19], indicated that the optimum pH for nitrification can range from 7 to 9, while optimum pH range for *Pseudomonas* species that possess denitrifying capacity is between 8 and 10. It is reported that reduced nitrification activity occurs at lower pH levels, which may result indirectly from substrate limitation since the fraction of ammonia to nitrogen in total ammonia nitrogen decreases with the decrease of pH [20]. In a study on the removal of nitrate from municipal wastewater sludge by *Chlorella vulgaris, Spirulina platensis* and *Scenedesmus quadricauda* remarkable decrease in nitrate levels was observed at pH 6 [1].

Furthermore, Flora and co-workers [21] have opined that nitrification amounts may be enhanced by increasing bulk pH to alkaline values. Betlach and Tiedje, [22], when exploring the build-up of denitrification intermediates in *Pseudomonas* species indicated that that nitrite accumulation is dependent on relative rates of nitrate and nitrite decrease. Some earlier workers have hypothesized that enzyme activity is retarded when pH range is outside the range of 6-8, which may disparity in nitrate utilization and nitrite build-up among pH treatments over time [23]. Other studies have suggested that the optimal pH for denitrification in cultures of *Pseudomonas* species should be within neutral and alkaline conditions [24].

In this present study, significant decreases in nitrate and nitrite concentration in the media were observed in the presence of different sodium acetate concentrations. Decreases in concentrations of the nitrate and nitrite did not differ significantly between the sodium acetate concentrations used in the media.

The presence of carbon source in biological nutrient removal studies is vital in enhancing the growth of the microorganisms due to its electron donor ability [25]. The external carbon source used in this study was sodium acetate. Earlier investigators have indicated sodium acetate as an ideal external carbon source in nutrient removal studies[26-28].

When investigating the influence of carbon source on nitrate removal of contaminated groundwater in a denitrifying submerged filter, Gomez *et al*., [29] reported increases in nitrate decrease with increasing concentration of carbon source. In the case of nitrite, with increase in carbon concentration, initial increase in concentration was first observed before decreases with time [29]. However, as observed by Wang *et al*., [7], phosphate uptake and associated denitrification rate increased in presence of sodium acetate concentration of 200 mg/L but decreased when sodium acetate concentration was increased to 300 mg/L.

When and co-workers [30] have suggested that the rate of nitrate and nitrite removal may be greatly affected by the composition of the microbial population, as well as the quantity of the available organic matters. In a study on the limitations on nitrogen removal by treatment wetlands under maritime climatic conditions, small and average carbon concentrations levels were observed to amount to equal nitrate removal efficiencies while high carbon concentrations gave rise to maximum nitrate removal capacities [31]. An increase in consumption of carbon sources has also been indicated to increase the population of denitrifying bacteria more than nitrate reducing bacteria [32].

CONCLUSION

This study which was aimed at investigating the effects pH and carbon concentrations on nitrate and nitrite removal ability of immobilized and free cells of *Pseudomonas aeruginosa* revealed

that optimum pH for nitrate and nitrite removal in presence of the cells ranges between 8 and 10. At pH 6, only minute or no decrease in nitrate and nitrite concentrations was observed in presence of the cells. This trend was irrespective of the amount of cells used for inoculation.

In addition, remarkable nitrate and nitrite removal was observed at the different concentrations of sodium acetate used in the media. This observation was irrespective of the sodium acetate concentration used.

The study has been able to provide valuable information on the effects of the parameters investigated on nitrate and nitrite removal under the experimental conditions used for the study.

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