Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.11, 2013

Investigation of the Effect of Initial Biomass on Nitrate and Phosphate Removal from Synthetic Wastewater by Selected Bacteria Isolates

Olaolu TD[1], Akpor OB[1*] and Aderiye BI[1,2]
1.Department of Biological Sciences, Landmark University, Omu Aran, Kwara State, Nigeria
2. Department of Microbiology, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria
* Corresponding author: akpor.oghenerobor@lmu.edu.ng

The research is financed by the College of Science and Engineering, Landmark University, Omu Aran, Kwara State, Nigeria

Abstract
Although nitrogen and phosphorus are important nutrients to the existence of living organisms, their presence in excessive amounts in wastewater could have detrimental effects to humans and other living organisms. The present investigation was aimed at ascertaining the effect of initial biomass concentration on the nutrient removal efficiency of four bacterial species in synthetic wastewater under shaking flasks conditions. Four different initial biomasses [2.91 x 10^8 cfu/mL, 5.82 x 10^8 cfu/mL, 8.73 x 10^8 cfu/mL and 1.16 x 10^9 cfu/mL (*Klebsiella* sp.); 6.31 x 10^8 cfu/mL, 1.26 x 10^9 cfu/mL, 1.89 x 10^9 cfu/mL and 2.52 x 10^9 cfu/mL (*Pseudomonas* sp.); 1.75 x 10^8 cfu/mL, 3.49 x 10^8 cfu/mL, 5.24 x 10^8 cfu/mL and 6.98 x 10^8 cfu/mL (*Lysinibacillus* sp.), and 7.1 x 10^8 cfu/mL, 1.42 x 10^9 cfu/mL, 2.13 x 10^9 cfu/mL and 2.84 x 10^9 cfu/mL (*Staphylococcus* sp.)] were used for the study. For nutrient removal studies, the respective initial biomasses of the test isolates were inoculated into flasks containing the wastewater media. Aliquot samples were taken at the beginning of the study and every 24 h for 96 h for the estimation of growth rate, pH, phosphate and nitrate concentrations in the wastewater, using standard methods. The results revealed only slight phosphate decreases in the wastewater after the expiration of incubation. All the test isolates showed significant nitrate removal ability except the *Lysinibacillus* sp. After 96 h incubation 68.36 to 90.67 %, 91.80 to 95.29 %, 3.20 to 11.48 % and 86.77 to 94.33 % of nitrate was removed in the presence of the *Klebsiella* sp., *Pseudomonas* sp., *Lysinibacillus* sp. and *Staphylococcus* sp., respectively. The study was able to reveal the phosphate and nitrate removal ability of the isolates at the different initial biomasses used for the investigation.
Keywords: Bacteria, wastewater, nutrient removal, phosphate, nitrate

1. Introduction

Nitrogen and phosphorus are known to be two the most important nutrients to living organisms; however an excess of these nutrients causes several water-quality problems. Some of these problems include the degradation of aquatic habitat, pollution of drinking water and the depletion in the economic and recreational value of water (USGS, 1996). The presence of these nutrients in high amounts in receiving water bodies is also known to lead to eutrophication. Eutrophication results because of the explosive thrive of photosynthetic algae, thereby reducing the amount of dissolved oxygen available to other aquatic organisms, thus leading to their death. The presence of excessive amount of algae due to eutrophication could also lead to the massive contamination of water due to the production of phycotoxins.

In wastewater treatment, nitrate and phosphate are considered priority targets for removal (EPA, 2002; Ward *et al*., 2005; Jalal *et al*., 2011). The possible entry of nitrate and phosphate ions into aquatic environment is through household sewage water and industrial effluents-particularly fertilizer and soap industries. It is postulated that only orthophosphate can be chemically precipitated. During biological treatment, most of the organic phosphorus and polyphosphates are converted to the orthophosphate form (Krishnaswamy *et al*., 2011). There is the indication that nitrate contamination is a global problem, which is reported to stand as the second most dangerous pollutant after pesticides. At high concentrations, nitrate is a threat to infants and other susceptible individuals. Besides, its carcinogenic effects have also been reported. Similarly, phosphate, which in water is generally present as polyphosphate and orthophosphate, is indicated to cause digestive problems in humans when present in extremely high concentrations. At concentrations above 1.0 mg/L, it is indicated to interfere with coagulation in water treatment plants (DebRoy *et al*., 2012).

Conventionally, chemical and biological processes have been employed for the removal of nutrients from wastewaters. Because, biological treatment processes are known to be cost-effective methods for wastewater, microbial strategies for the removal of environmental pollutants from waste streams or contaminated sites can provide attractive alternatives to other traditional methods (Krishnaswamy *et al*., 2011). In recent years, because

of the many negative impacts, such as increased volume of sludge, production of sludge with poor settling and dewatering characteristics and the depression of the pH, biological treatment processes is being advocated (Gray, 2002; Akpor, 2011).

Because biological nutrient removal techniques involving the use of microorganisms have shown to offer great potential in the removal of nutrients from wastewater; it is advocated for use in recent times. Some of the advantages of biological treatment processes include, low operating costs, eco-friendly and cost-effective alternative of conventional techniques and efficiency at lower levels of contamination (Srivastavia and Majumder 2008). During treatment, the nutrients are used by the microorganisms for the construction of cell membranes, cellular maintenance, synthesis of nucleic acids and chemical energy transfer reactions within cells. In some instances, some of the nutrients are also stored for future use by the cells (Krishnaswamy *et al*., 2011).

A number of microorganisms (bacteria, fungi, algae and protozoa) have been implicated in the remediation of nitrogen and phosphorus polluted wastewaters. It is however argued that the extent of removal is dependent on the net production of living biomass ((Bitton, 1994; Ramothokang *et al*., 2006; Mbwele, 2006, Krishnaswamy *et al*., 2011). This study was therefore aimed at investigating the nutrient removal efficiency of four bacterial species (*Pseudomonas* sp*., Klebsiella* sp*., Lysinibacillus* sp. and *Staphylococcus* sp.) using initial biomass concentrations for inoculation.

2. Materials and Methods

2.1 Test bacterial species

Four bacterial (*Pseudomonas* sp.., *Klebsiella* sp*., Lysinibacillus* sp. and *Staphylococcus* sp.) were used for this investigation. They were obtained from the Microbiology Department of the Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. Each of the test isolates were stored in nutrient agar slants and incubated at 4 ^{o}C until needed. Prior use, each isolate was first streaked in nutrient agar slants and incubated at 37 ^{o}C for 24 h to ascertain their purity, after which the isolates were sub-cultured into nutrient broth. For each experiment, only 18-24 h old broth cultures of the isolates were used for nutrient removal study.

2.2 Synthetic wastewater

The composition of the synthetic wastewater used for the study was as follows: sodium acetate (5 g/L), magnesium sulfate (0.5 g/L), potassium nitrate (0.2 g/L), potassium dihydrogen phosphate (0.5 g/L), meat extract (1 g/L), peptone (1 g/L) sodium chloride(0.5 g/L). Each of the components of the synthetic wastewater was first dissolved separately in 50 mL of deionized water, before mixing with the other components and making up to the mark.

Before usage, the synthetic wastewater was first dispensed in 200 mL quantity in 250 mL Erlenmeyer's flasks. All flasks containing the synthetic wastewater were cotton-plugged and sterilized at 121^{o}C for 15min at 15 psi in an autoclave. After sterilization, flasks were first incubated for 24 h to ascertain that there was no growth from any contaminant.

2.3 Nutrient removal study

To the sterile synthetic wastewater in Erlenmeyer's flasks, a known population of the respective isolates was inoculated. In this study, four different initial biomasses [2.91 x 10^{8} cfu/mL, 5.82 x 10^{8} cfu/mL, 8.73 x 10^{8} cfu/mL and 1.16 x 10^{9} cfu/mL (*Klebsiella* sp.); 6.31 x 10^{8} cfu/mL, 1.26 x 10^{9} cfu/mL, 1.89 x 10^{9} cfu/mL and 2.52 x 10^{9} cfu/mL (*Pseudomonas* sp.); 1.75 x 10^{8} cfu/mL, 3.49 x 10^{8} cfu/mL, 5.24 x 10^{8} cfu/mL and 6.98 x 10^{8} cfu/mL (*Lysinibacillus* sp.), and 7.1 x 10^{8} cfu/mL, 1.42 x 10^{9} cfu/mL, 2.13 x 10^{9} cfu/mL and 2.84 x 10^{9} cfu/mL (*Staphylococcus* sp.)] were used for investigation. The estimation of the initial biomasses was carried out using standard plating techniques (APHA, 2012).

After inoculation, the inoculated flasks were incubated in a rotary shake at 120 revolutions per minute (rpm) at 25 ^{o}C. Immediately after inoculation (referred to as 0 h in this study) and every 24 h for 96 h, aliquot samples were aseptically taken from each flask for the estimation of the pH, growth rate, phosphate and nitrate concentrations in the wastewater, using standard procedures. In all cases, phosphate and nitrate concentrations in the wastewater were analysed using the ascorbic acid method and the salicylate methods, respectively (APHA, 2012).

All experiment analyses were carried out in triplicate. Also, all reagents used were of analytical grades. In all cases, an uninoculated control was also set up for each batch of experiment.

2.4 Statistical analysis

For statistical analysis, the PAST (paleontological statistics software package for education and data analysis) was used (Hammer *et al*., 2001). The test for the comparison of means was done using the one-way analysis of variance (ANOVA) test while the tests for relationships were carried out using the Pearson correlation index.

3. Results

3.1 Phosphate removal in presence of the test bacterial species

Figure 1 shows the trend in the phosphate concentration of the synthetic wastewater inoculated with the *Klebsiella* sp. As revealed in the figure, there were slight decreases in the concentration of phosphate in the synthetic wastewater between 24 h and 48 h incubation period, after which increases were observed with time. This trend was irrespective of the initial biomass used for investigation. At the end of the 96h incubation period, phosphate concentration in presence of the *Klebsiella* sp. was observed to change from 154.40mg/L at 0 h to 141.70mg/L, 132.70mg/L, 158.11 mg/L 132.71mg/L, at for biomass concentrations at 2.91 x 10^8 cfu/mL, 5.82 x 10^8 cfu/mL, 8.73 x 10^8 cfu/mL and 1.16 x 10^9 cfu/mL, respectively (Fig. 1). Despite the observed differences in the phosphate concentrations at the different initial biomasses during the incubation period, these differences were not observed to be significant ($p \leq 0.05$).

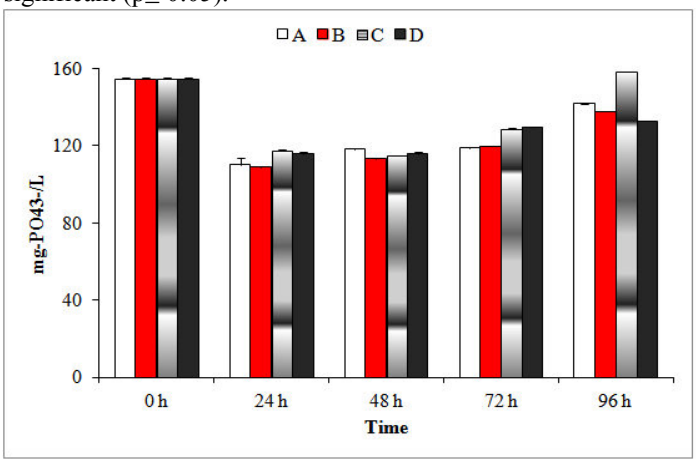

Fig. 1: Variation in phosphate concentration in presence of the different initial biomasses of the *Klebsiella* sp. (A, B, C and D represent initial biomass concentrations at 2.91 x 10^8 cfu/mL, 5.82 x 10^8 cfu/mL, 8.73 x 10^8 cfu/mL and 1.16 x 10^9 cfu/mL, respectively)

Phosphate levels in the wastewater in presence of the *Pseudomonas* sp. showed steady decreases in concentration between 0h and 48h incubation, after which there were steady increases with time. This trend was irrespective of the initial biomass used for inoculation. At the end of the 96 h incubation time, the concentration of phosphate at initial biomass of 6.31 x 10^8 cfu/mL was observed to decrease from 154.4mg/L to 144.7mg/L.At initial biomasses of 1.26 x 10^9 cfu/mL, 1.89 x 10^9 cfu/mL and 2.52 x 10^9 cfu/mL, phosphate concentrations were observed experiences increases from 154.40 mg/L at 0 h to 168.73 mg/L, 158.45 mg/L and 171.73 mg/L (Fig. 2), respectively. Throughout the period of incubation there were no significant decreases in phosphate concentrations in the wastewater at the different initial biomasses. Also, despite the differences in phosphate concentrations at the different initial biomasses, these differences were not observed to be significant ($p \leq 0.05$).

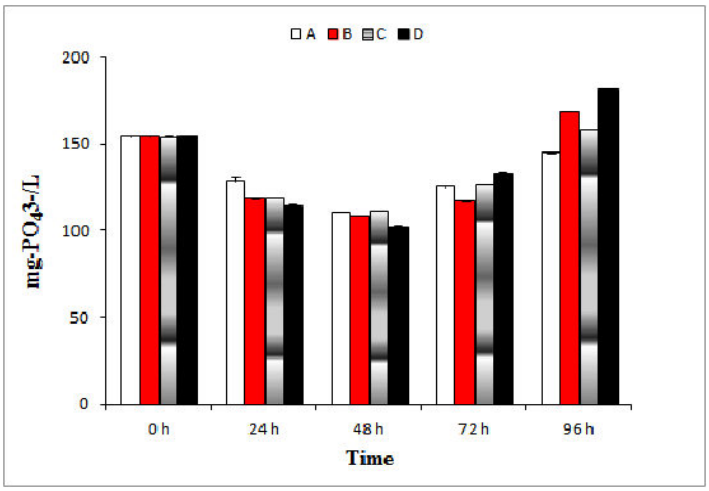

Fig. 2: Variation in phosphate concentration in presence of the different initial biomasses of the *Pseudomonas* sp. (A, B, C and D represent initial biomass concentrations at.6.31 x 10^8 cfu/mL, 1.26 x 10^9 cfu/mL, 1.89 x 10^9 cfu/mL and 2.52 x 10^9 cfu/mL, respectively)

In the presence of the *Lysinibacillus* sp., only slight decreases in phosphate concentration were observed with the first 48 h of incubation, after which there were steady increases in concentration with time. This trend was irrespective of the different initial biomasses. At the expiration of the 96 h incubation period, phosphate concentration increased from initial level of 154.40 mg/L to 164.57 mg/L, 154.50 mg/L, 182.02 mg/L and 196.70 mg/L, at initial biomasses of 1.75 x 10^8 cfu/mL, 3.49 x 10^8 cfu/mL, 5.24 x 10^8 cfu/mL and 6.98 x 10^8 cfu/mL, respectively (Fig. 3). Throughout the period of incubation, no significant decrease in phosphate concentration was observed at the different initial biomasses. Also, the phosphate concentration in the wastewater was not observed to differ significantly between the different initial biomasses ($p \leq 0.05$).

The trend in phosphate concentration in the wastewater in the presence of the *Staphylococcus* sp showed only slight decreases within the first 48 h incubation, after which there were steady increases with incubation time. This trend was irrespective of the initial biomass used for inoculation. After the expiration of the 96 h incubation period, phosphate concentration was found to be 159.74 mg/L, 150.20 mg/L, 141.11 mg/L and 141.70 mg/L, at initial biomasses of 7.1 x 10^8 cfu/mL, 1.42 x 10^9 cfu/mL, 2.13 x 10^9 cfu/mL and 2.84 x 10^9 cfu/mL, respectively (Fig. 4). Throughout the period of incubation, phosphate levels at the different initial biomasses did not differ significantly. Also, no significant decrease in phosphate concentration was observed at the different initial biomasses ($p \leq 0.05$).

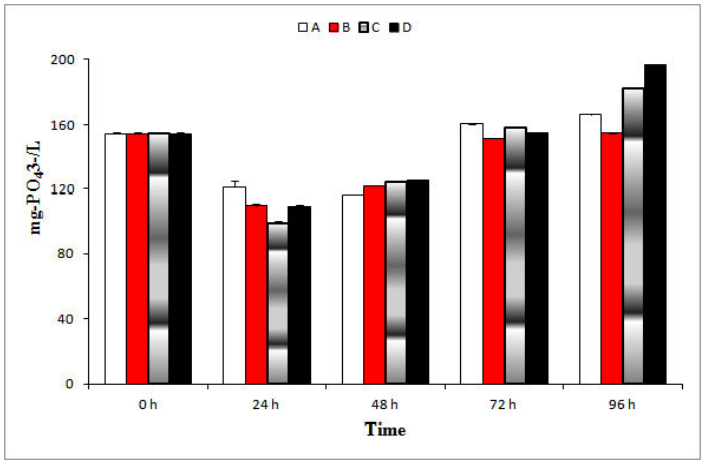

Fig. 3: Variation in phosphate concentration in presence of the different initial biomasses of the *Lysinibacillus* sp. (A, B, C and D represent initial biomass concentrations at1.75 x 10^8 cfu/mL, 3.49 x 10^8 cfu/mL, 5.24 x 10^8 cfu/mL and 6.98 x 10^8 cfu/mL, respectively)

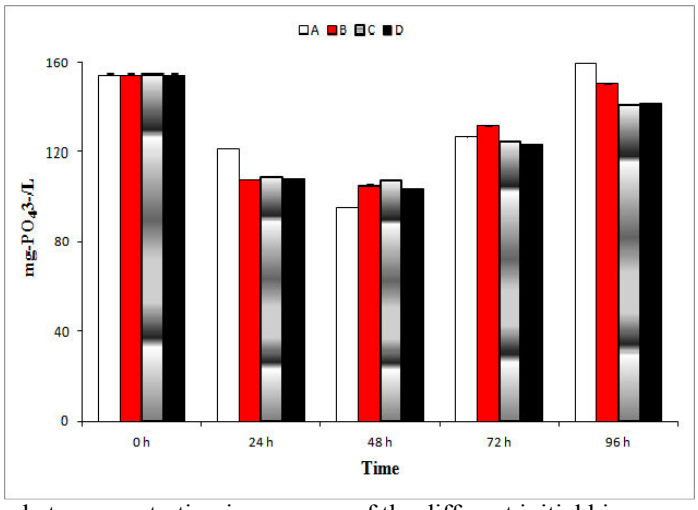

Fig. 4: Variation in phosphate concentration in presence of the different initial biomasses of the *Staphylococcus* sp. (A, B, C and D represent initial biomass concentrations at7.1 x 10^8 cfu/mL, 1.42 x 10^9 cfu/mL, 2.13 x 10^9 cfu/mL and 2.84 x 10^9 cfu/mL, respectively)

Generally, the results revealed that after the 96 h incubation period, phosphate removal rates that ranged from 8.23 % to 14.05 % and from 2.72 % to 8.61 % were observed in the presence of the *Klebsiella* sp. and

Staphylococcus sp., respectively. In the presence of the *Pseudomonas* sp. and *Lysinibacillus* sp., phosphate increases that ranged from 2.62 % to 17.70 % and from 0.06 % to 27.39 %, respectively were observed after the 96 h incubation period (Table 1).

Table 1: Change in phosphate concentration in the wastewater after 96 h incubation in the presence of the different isolates at the different initial biomass concentrations

	Initial PO_4^{3-} (mg/L)	Final PO_4^{3-}/L (mg/L)	% change
***Klebsiella* sp.**			
A	154.40	141.70	8.22
B	154.40	137.70	10.82
C	154.40	158.11	-2.40
D	154.40	132.71	14.05
***Pseudomonas* sp.**			
A	154.40	144.67	6.30
B	154.40	168.73	-9.28
C	154.40	158.45	-2.62
D	154.40	181.73	-17.70
***Lysinibacillus* sp.**			
A	154.40	165.57	-7.23
B	154.40	154.50	-0.06
C	154.40	182.02	-17.89
D	154.40	196.70	-27.39
***Staphylococcus* sp.**			
A	154.40	159.74	-3.46
B	154.40	150.20	2.72
C	154.40	141.11	8.61
D	154.40	141.70	8.22

All values are in percentages. Positive and Negative values represent percentage decreases and increases, respectively. A, B, C and D represent the different initial biomasses of the test bacterial species used for inoculation

3.2 Nitrate removal in presence of the test bacterial species

As revealed in figure 5, nitrate concentration in the presence of the *Klebsiella* sp, significant decreases in concentrations were observed with time. This trend was irrespective of the initial biomass used. At the expiration of the 96 h incubation period, the concentrations of nitrate at initial biomasses of 2.91 x 10^8 cfu/mL, 5.82 x 10^8 cfu/mL, 8.73 x 10^8 cfu/mL and 1.16 x 10^9 cfu/mL were found 223.11mg/L 52.10mg/L at 20.83mg/L 17.32mg/L and 70.59 mg/L, respectively (Fig. 5). Although significant nitrate removal was observed at the different initial biomasses, no differences were observed between nitrate concentrations at the different biomasses ($p \leq 0.05$).

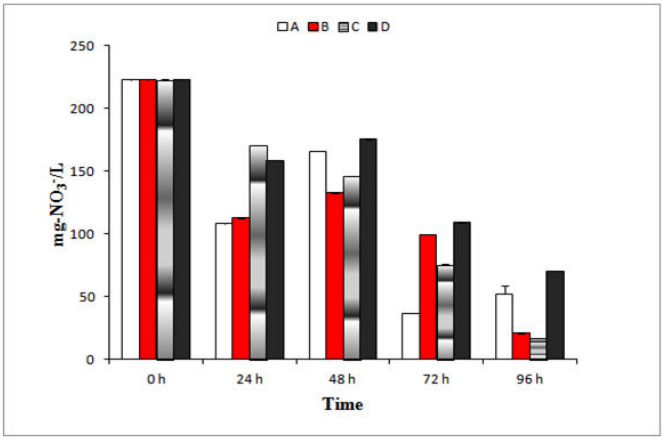

Fig. 5: Variation in nitrate concentration in presence of the different initial biomasses of the *Klebsiella* sp. (A, B, C and D represent initial biomass concentrations at 2.91 x 10^8 cfu/mL, 5.82 x 10^8 cfu/mL, 8.73 x 10^8 cfu/mL and 1.16 x 10^9 cfu/mL, respectively)

The trend in nitrate concentration in the presence of *Pseudomonas* sp. at the various biomass concentrations revealed a gradual decrease in concentration within the first 24 h of incubation after which there were sharp decreases with time after 48 h incubation, which was maintained throughout the 96 h incubation period. After the 96 h incubation period, nitrate concentration was observed to decrease from 223.11 mg/L at 0 h to 8.30mg, 10.51mg/L, 11.16 mg/L, 11.68 mg/L, at initial biomasses 6.31 x 10^8 cfu/mL, 1.26 x 10^9 cfu/mL, 1.89 x 10^9 cfu/mL and 2.52 x 10^9 cfu/mL, respectively, (Fig. 6). At the end of incubation, the decreases in nitrate concentration in the presence of the *Pseudomonas* sp. were observed to be significant. However there were no significant differences between decreases at the different initial biomasses ($p \leq 0.05$).

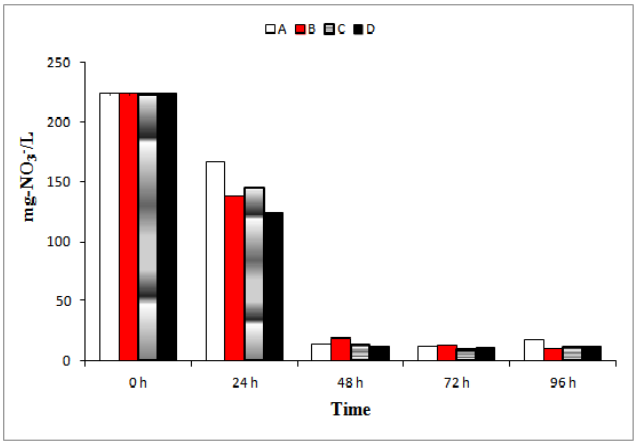

Fig. 6: Variation in nitrate concentration in presence of the different initial biomasses of the *Pseudomonas* sp. (A, B, C and D represent initial biomass concentrations at6.31 x 10^8 cfu/mL, 1.26 x 10^9 cfu/mL, 1.89 x 10^9 cfu/mL and 2.52 x 10^9 cfu/mL, respectively)

As shown in figure 7, throughout the period of incubation, nitrate concentration in presence of the *Lysinibacillus* sp. was not observed to show any significant decrease in concentration. After the 96 h incubation period, the concentration of nitrate was observed to be 215.98 mg/L, 205.79 mg/L, 197.47 mg/L and 198.14 mg/L at initial biomasses 1.75 x 10^8 cfu/mL, 3.49 x 10^8 cfu/mL, 5.24 x 10^8 cfu/mL and 6.98 x 10^8 cfu/mL, respectively (Fig. 7). As was observed in the case of phosphate, throughout the period of incubation, no significant differences were observed between the nitrate levels at the different initial biomasses ($p \leq 0.05$).

In the presence of the *Staphylococcus* sp., there was only a slight reduction in nitrate concentration within the first 24 h of incubation, after which there was a drastic reduction with time, a trend that continued till the end of the incubation period. This observation was irrespective of the initial biomass used for inoculation. Nitrate concentration in the wastewater after the 96 h incubation period was found to decrease from 223.11 mg/L at 0 h to 21.54 mg/L, 29.52 mg/L, 15.96 mg/L and 12.65 mg/L, at initial biomasses of 7.1 x 10^8 cfu/mL, 1.42 x 10^9 cfu/mL, 2.13 x 10^9 cfu/mL and 2.84 x 10^9 cfu/mL, respectively (Fig. 8). Although significant decrease in nitrate concentration was observed at the different initial biomasses, these differences were not observed to differ between the different initial biomasses used for inoculation ($p \leq 0.05$).

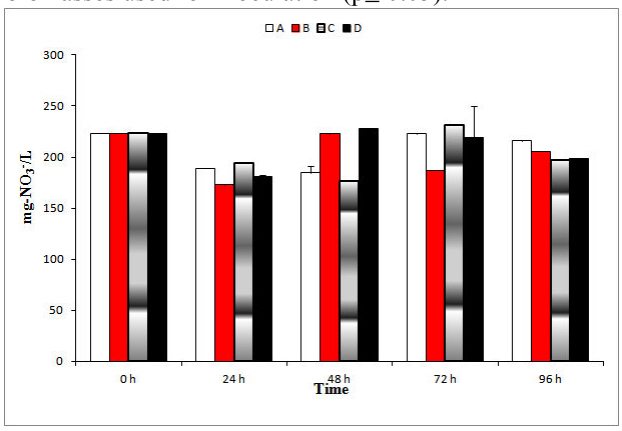

Fig. 7: Variation in nitrate concentration in presence of the different initial biomasses of the *Lysinibacillus* sp. (A, B, C and D represent initial biomass concentrations at1.75 x 10^8 cfu/mL, 3.49 x 10^8 cfu/mL, 5.24 x 10^8 cfu/mL and 6.98 x 10^8 cfu/mL, respectively)

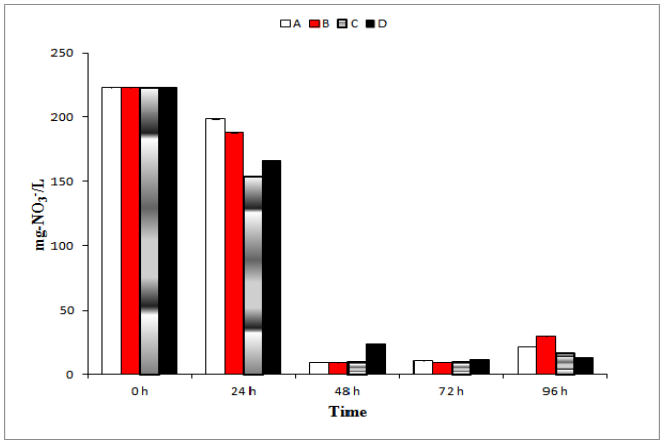

Fig. 8: Variation in phosphate concentration in presence of the different initial biomasses of the *Staphylococcus* sp. (A, B, C and D represent initial biomass concentrations at7.1 x 10^8 cfu/mL, 1.42 x 10^9 cfu/mL, 2.13 x 10^9 cfu/mL and 2.84 x 10^9 cfu/mL, respectively)

After the 96 incubation period, nitrate concentration in the wastewater in the presence of the test isolates showed removal rates that ranged from 68.36% to 92.24 %, from 91.80 % to 95.29 %, from 3.20 % to 11.49 % and from 86.77 % to 94.33 %, at the different initial biomasses of the *Klebsiella* sp., *Pseudomonas* sp., *Lysinibacillus* sp. and *Staphylococcus* sp., respectively (Table 2).

Table 2: Change in nitrate concentration in the wastewater after 96 h incubation in the presence of the different isolates at the different initial biomass concentrations

	Initial NO_3^- (mg/L)	**Final NO_3^-/L (mg/L)**	**% change**
Klebsiella sp.			
A	223.11	52.10	76.65
B	223.11	20.83	90.67
C	223.11	17.32	92.24
D	223.11	70.59	68.36
Pseudomonas sp.			
A	223.11	18.30	91.80
B	223.11	10.51	95.29
C	223.11	11.16	95.00
D	223.11	11.68	94.77
Lysinibacillus sp.			
A	223.11	215.98	3.20
B	223.11	205.79	7.76
C	223.11	197.49	11.49
D	223.11	198.14	11.20
Staphylococcus sp.			
A	223.11	21.54	90.35
B	223.11	29.52	86.77
C	223.11	15.96	92.85
D	223.11	12.65	94.33

All values represent percentage decreases. A, B, C and D represent the different initial biomasses of the test bacterial species used for inoculation

3.3 pH and growth rates in presence of the test bacterial species

As shown in Table 3, in the presence of the test isolates, pH of the wastewater was observed to increase gradually with incubation time. Despite the observed increases with time, the values were still the near neutral ranges. This trend was irrespective of the test isolates or the initial biomasses used for inoculation. At the end of the 96 h incubation period, from the original value of 5.9 at 0 h, pH values at the different initial biomasses were observed to range from 7.1 to 7.3, 7.0 to 7.8, 7.3 to 76.5 and 6.8 to 7.5, in the presence of the *Klebsiella* sp., *Pseudomonas* sp., *Lysinibacillus* sp. and *Staphylococcus* sp., respectively (Table 3).

Table 3: pH variations during the nutrient removal studies in presence of the test isolates at the different initial biomasses used for inoculation

Time	A	B	C	D
***Klebsiella* sp.**				
0h	5.9	5.9	5.9	5.9
24h	6.3	6.4	6.4	6.4
48h	6.5	6.9	6.9	6.9
72h	6.9	7.4	7.4	7.3
96h	7.1	7.3	7.3	7.3
***Pseudomonas* sp.**				
	A	**B**	**C**	**D**
0h	5.9	5.9	5.9	5.9
24h	6.4	6.3	6.3	6.4)
48h	7.0	7.0	7.0	7.0
72h	7.7	7.5	7.5	7.6
96h	7.0	7.6	7.6	7.8
***Lysinibacillus* sp.**				
	A	**B**	**C**	**D**
0h	5.9	5.9	5.9	5.9
24h	6.4	6.3	6.3	6.4
48h	6.8	6.7	6.7	7.0
72h	7.2	7.1	7.1	7.4
96h	7.3	7.2	7.2	7.5
***Staphylococcus* sp.**				
	A	**B**	**C**	**D**
0h	5.9	5.9	5.9	5.9
24h	6.2	6.2	6.2	6.4
48h	6.4	6.4	6.4	7.0
72h	6.6	6.5	6.5	7.4
96h	6.8	6.6	6.6	7.5

All values are average of triplicate analysis. A, B, C and D represent the different initial biomasses of the test bacterial species used for inoculation

With respect to growth rates of the isolates during the nutrient removal studies, there were steady increases with time. This trend was irrespective of the test isolates or the initial biomass used for inoculation. At the end of the 96 h incubation, growth rates of the isolates at the respective initial biomasses were observed to range from 1.97 d^{-1} to 1.98^{-1}, 1.96 to 2.00 d^{-1}, 1.97 to 2.08 d^{-1} and from 1.98 d^{-1} to 2.15 d^{-1}, for the *Klebsiella* sp., *Pseudomonas* sp., *Lysinibacillus* sp. and *Staphylococcus* sp., respectively (Table 4).

Table 3: Growth rate (d^{-1}) of the different isolates during the nutrient removal studies at the different initial biomass concentrations

	0h	24h	48h	72h	96h
***Klebsiella* sp.**					
A	0	1.54	1.55	1.96	1.97
B	0	1.54	1.95	1.97	1.99
C	0	1.94	1.95	1.97	1.98
D	0	1.94	1.96	1.97	1.98
***Pseudomonas* sp.**					
A	0	1.94	1.96	1.99	2.00
B	0	1.54	1.96	1.98	1.99
C	0	1.94	1.95	1.96	1.96
D	0	1.95	1.96	1.99	2.00
***Lysinibacillus* sp.**					
A	0	1.68	2.04	2.08	2.08
B	0	1.94	1.95	1.96	1.97
C	0	1.97	1.96	1.97	1.97
D	0	1.94	1.96	1.97	1.98
***Staphylococcus* sp.**					
A	0	1.94	1.95	1.97	1.98
B	0	1.75	1.84	1.94	1.99
C	0	1.84	1.93	2.01	2.15
D	0	1.85	1.94	2.00	2.08

All values are average of triplicate analysis. A, B, C and D represent the different initial biomasses of the test bacterial species used for inoculation

4. Discussion

The present study revealed slight phosphate removal in the presence of *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus* sp. In the presence of the *Lysinibacillus* sp. phosphate releases were observed at the various initial biomasses used for inoculation. Some species of *Klebsiella* sp. and *Pseudomonas* sp. have been implicated in phosphate removal by previous investigators (Momba and Cloete, 1996; Krishnaswamy *et al*., 2011; Diep and Cuc, 2013).

The trend in pH in this investigation showed slight increases with time, although all values were within the near neutral ranges. Increases in pH during nutrient studies have been reported by some investigators (Chevalier *et al*., 2000; Akpor *et al*., 2007). According to Akpor and co-workers (2007), during nutrient removal in activated sludge mixed liquor using protozoan isolates, at the end of the incubation period, pH was observed to increase from an average of 7.5 to 8. The findings of this study negated the observation of some other investigators. In the study by Krishnaswamy *et al*., (2011), a phosphate removal of 92.5% was obtained with a pH change of the culture medium from 7.2 to 5.9. They postulated that the reduction in pH may be due to the production of various organic acids by the phosphate reducers in the culture medium.

It is reported that acidic pH is favourable for phosphate removal, which is based on the suggestion that acidic pH seems to be favourable to acid phosphatase, which aids in the removal of phosphate (Bouquet *et al*., 1987; Krishnaswamy *et al*., 2011). Mulan and co-workers (2002) have also reported an enhanced phosphate uptake and polyphosphate accumulation in *Burkholderia cepacia* under acidic conditions. It is reported that during nutrient removal, the initial pH at neutral and acidic conditions is favourable for the optimum removal of phosphate by the individual strain of *Pseudomonas* sp. and consortium (Krishnaswamy *et al*., 2011). Some workers have also indicated similar observations of phosphate utilizing microorganisms producing various organics and consequently fall in pH of the medium (Kundu, 1984; Satar and Gaur 1984). None of the isolates used in this study showed significantly phosphate removal ability during the experimental conditions. The majority of the isolates used for this investigation showed significant nitrate removal efficiency. It is however indicated that during denitrification process, alkalinity is produced (Bitton, 1999).

The present study revealed significant nitrate removal in the presence of *Pseudomonas, Klebsiella* and *Staphylococcus*. According to Diep and Cuc, (2013), *Pseudomonas stutzeri* is said to have excellent denitrifying ability under aerobic and anaerobic conditions. All the isolates used in study were investigated under aerobic condition. In the study by Vymazal, (2007), diverse bacteria groups have been observed to be involved in nitrogen mineralisation, such as nitrification and denitrification. Although denitrifying bacteria are said to include organotrophs, lithotrophs, phototrophs and diazotrophs, the majority of them are said to be chemotrophs.

In aquatic environments, the most important denitrifying bacteria are implicated to be *Pseudomonas, Aeromonas and Vibrio*. Other bacteria, such as *Arthrobacter globiformis, Aerobacter aerogenes, Mycobacterium phlei, Streptomyces griseus, Thiosphaera* and *Corynebacterium* have also been implicated for nitrogen mineralisation (Vymazal , 2007; Diep and Cuc, 2013).

.

5. Conclusion

This study revealed that three of the isolates (*Pseudomonas, Klebsiella* and *Staphylococcus*) have significant nitrate removal ability under the experimental conditions investigated. In addition, although there were only slight decreases in phosphate concentration in the wastewater in presence of the *Pseudomonas* sp., *Klebsiella* sp and *Staphylococcus* sp. These observations were irrespective of the respective initial biomasses used for inoculation. No remarkable phosphate or nitrate removal ability was observed in the presence of the *Lysinibacillus* sp. This study also observed consistent but slight increases in pH during nutrient removal studies. This trend was also irrespective of the initial biomass used for inoculation. Finally, the study did not observe any relationship between nutrient removal and the initial biomasses used for inoculation.

Acknowledgements

The authors wish to thank the management of Landmark University, Omu Aran, Kwara State, Nigeria for providing the resources and facilities to carry out this investigation. The contribution of the Microbiology Department of the Ekiti State University, Ado Ekiti, Nigeria in the provision of the isolates used for the study is also appreciated.

References

Akpor, O.B., Momba, M.N.B. & Okonkwo J. (2007). Phosphorus and nitrate removal by selected wastewater protozoa isolates. *Pakistan Journal of Biological Sciences*, 10(22), 4008-4014

APHA (2012), *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association, Washington DC

Bitton G. (1994). *Wastewater Microbiology*. John Wiley & Sons, Inc., Publication; USA

Chevalier, R., Proulx, D., Lessard, P., Vincent, W.F.& De La Noue J. (2000). Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. *Journal of Applied Phycology*, 12, 105-112

DebRoy, S., Das, S., Ghosh, S. & Banerjee, S. (2012). Isolation of nitrate and phosphate removing bacteria from various environmental sites. *Online Journal of Biological Sciences*, 12(2), 62-71

Diep, C.N. & Cuc, N.T. (2013), Heterotrophic nitrogen removal bacteria in sedimentaryand water of striped catfish ponds in the Mekong Delta,Vietnam. *American Journal of Life Sciences*, 1(1), 6-13

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

Gray, F.N. (2002). *Water Technology: An Introduction for Environmental Scientists and Engineers*. Butterworth-Heinemann, Oxford pp 35-80

Hammer, O., Harper, D.A.T. & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Jalal, K.C.A., Zahangir Alam, M.D., Matin, W.A., Kamaruzzaman, B.Y., Akbar, J. & Toffazel, H. (2011). Removal of Nitrate and Phosphate from Municipal Wastewater Sludge by *Chlorella vulgaris*, *Spirulina platensis* and *Scenedesmus quadricauda. IIUM Engineering Journal*, 12(4), 125-132

Krishnaswamy, U., Muthuchamy, M. & Perumalsamy, L. (2011). Biological removal of phosphate from synthetic wastewater using bacterial consortium. *Iranian Journal of Biotechnology*, 9(1), 37-49

Kundu, B.S. & Gaur, A.C. (1984). Rice response to inoculation with N-fixing and p-solubilizing microorganism. *Plant Soil,* 79, 227-234

Mbwele, L. (2006). Microbial phosphorus removal in wastewater stabilization pond. *A Licentiate Thesis from the School of Biotechnology*: A Royal Institute of Technology, Albanova, Stockholm, Sweden

Momba, M.N.B. & Cloete, T.E. (1996). Biomass relationship togrowth and phosphate uptake of *Pseudomonas fluorescens*,*Escherichia coli* and *Acinetobacter radioresistens* in mixed liquor medium. *Journal of Industrial Microbiology,* 16, 364–369

Mullan, A., Quinn, J.P. & McGrath J.W. (2002), Enhanced phosphate uptake and polyphosphate accumulation in *Burkholderia cepacia* grown under low-pH conditions. *Microbial Ecology*. 44, 69-77

Ramothokang, T.R., Simelane, S.C. & Bux, F. (2006). Biological nitrogen and phosphorus removal by filamentous bacteria in pure culture. *Water Institute of South Africa (WISA) Biennial Conference*, Durban, South Africa

Satar, M.A. & Gaur, A.C. (1984). Solubilization of insoluble and fixed phosphates in crop rhizobium by phosphomicroorganisms. Proc. Int. Symposium on soil test crop responses correlation studies. *International Congress Meeting of Soil Science*, Feb. 7-10, Dhaka, pp. 237-251

USGS. (1996), National Water-Quality Assessment (NWQA) Program. *US Geological Survey Fact Sheet*, FS218-96

Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. Science of the Total Environment, 380: 48–65

Ward, M.H., deKok, T.M., Levallois, P., Brender, J., Gulis, G.., Nolan, B.T. & VanDerslice, J. (2005). Workgroup report: drinking-water nitrate and health-recent findings and research needs. Environmental Health Perspectives, 113(11), 1607-14