



Investigation of the Role of Selected Fungal Strains in the Removal of Phosphate and Nitrate in Synthetic Wastewater

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Authors' contributions

This work was carried out in collaboration between all authors. Author TAAA carried out the laboratory analysis, performed the statistical analysis, carried out the analysis of results and proof read the first draft manuscript, author OBA designed the study, carried out the laboratory analysis, performed the statistical analysis and wrote the first draft of the manuscript and author BIA co-designed the study, managed the literature search and proof read the first draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigate the role of four fungi species in the removal of phosphate and nitrate in a low nutrient synthetic wastewater. Also investigated was the effect of initial inoculum size on the nutrient removal ability of the strains.

Materials and Methods: The fungal strains used for the study were *Aspergillus niger*, *Aspergillus flavus*, *Absidia spp* and *Fusarium spp*. Four different initial inoculum sizes of each of the respective isolates were used for the nutrient removal studies. After inoculation with the test strains, aliquot samples were taken from the media at time zero and every 24h, for the estimation of total phosphate, nitrate and pH in the medium, using standard methods.

Results: All the strains showed nitrate removal ability, irrespective of the initial inoculum size used for inoculation. After 96 h, the percent nitrate removed ranged from 25.25% to 77.52%, 26.12% to 39.80%, 8.88% to 44.23% and 29.50% to 87.34%, in the presence of *Aspergillus niger*, *Aspergillus flavus*, *Absidia spp* and *Fusarium spp*, respectively. None of the fungi exhibited phosphate removal ability, except *Aspergillus niger* which showed very

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slight potential for phosphate removal. Despite the observed differences in nitrate concentration removed by the different strains, these differences were not observed to differ significantly between the initial inoculum sizes used for investigation ($p \leq 0.05$). Similarly, the phosphate concentrations in the presence of the test strains did not differ significantly between the different initial inoculum sizes ($p \leq 0.05$). The pH values of the wastewater inoculated with the fungal strains increased with time of incubation. This trend was also observed irrespective of the initial inoculum size used.

Conclusion: The study was able to provide an insight into the phosphate and nitrate removal efficiency of the test strains under the experimental conditions.

Keywords: Phosphate; nitrate; fungi; nutrient removal; wastewater.

1. INTRODUCTION

Globally, the detrimental effects of pollution caused by the discharge of excess nutrients and heavy metals into receiving water bodies are sources of concern. The two major eutrophic nutrients are phosphorus and nitrogen [1], whose presence in excess amounts in water bodies leads to eutrophication, with ripple effects on aquatic and human life [2].

To safeguard the environment and avoid the negative impacts of eutrophication, there is need to reduce the concentrations of these nutrients to levels set by regulatory bodies before discharging into receiving water bodies. The two main technologies for nutrient removals from wastewater are chemical and biological. Due to the many disadvantages of chemical treatment processes, the biological nutrient removal process involving the use of microorganisms is advocated in recent years [3-5]. In addition, absorption with agricultural and industrial wastes, as well as modified wastes has been employed for both phosphate and nitrate removal from wastewaters [6-8].

Enhanced biological phosphorus and nitrogen removal through the use of microorganisms is indicated to be a current phenomenon, which may involve the uptake or mineralisation of the nutrients from wastewaters through various means [9,10]. A variety of mechanisms, such as assimilation, adsorption and biodegradation have been reported to be employed by microorganisms in nutrient removal [11]. It is indicated that some organisms nitrify ammonia to nitrate while others carry out denitrification of nitrates and nitrite to nitrogen. Some other organisms accumulate phosphorus and store it intracellularly [12].

Several bacteria, such as species of *Pseudomonas*, *Aeromonas*, *Bacillus*, as well as members of the Enterobacteriaceae have been implicated in nitrate and phosphate removal [13]. It is argued and proposed that fungi are better alternatives to bacteria in nutrient removal because they produce more valuable by-products and able to withstand inhibitory products during the process of nutrient removal. A number of fungi have been implicated in the bioremediation of polluted wastewater effluents [2,14-18]. This study was therefore aimed at investigating the role of four fungi species in the removal of phosphate and nitrate in low nutrient synthetic wastewater. The effect of initial inoculum size on the phosphate and nitrate removal ability of the strains was also investigated.

2. MATERIALS AND METHODS

The fungal strains used for this investigation were obtained from the Department of Microbiology Laboratory, Ekiti State University, Ado-Ekiti. Prior to use, the strains were

maintained on malt extract agar slants and stored at 4°C. The strains were *Aspergillus niger*, *Aspergillus flavus*, *Absidia spp* and *Fusarium spp*.

The composition of the synthetic wastewater used for the study comprised of 5 g/L sodium acetate; 0.5 g/L magnesium sulphate, 0.5 g/L potassium nitrate, 0.5 g/L potassium dihydrogen phosphate, 1 g/L meat extract, 1 g/L peptone and 0.5 g/L sodium chloride. The different components were dissolved in deionized water and dispensed in 200mL volume in 250 mL Erlenmeyer flasks. Before usage, the flasks containing the media were sterilized in an autoclave at 121°C at 1.05 kg/cm² for 15 min. After sterilization, the flasks were incubated for 24 h to ensure that there was no growth.

Before inoculation into the sterile medium, the fungi were plated on malt extract agar to ascertain their purity, after which they were suspended in normal saline and the mature spores were harvested and quantified as described by Aderiye et al. [19]. A known amount of the fungal suspension was inoculated into the flask containing the sterile synthetic wastewater.

For this investigation, four different initial inoculum sizes of each of the respective strains were used for inoculation. The different initial inoculum sizes were: 3.80 x 10² spores/mL, 7.50 x 10² spores/mL, 1.13 x 10³ spores/mL and 1.50 x 10³ spores/mL for the *Aspergillus niger*; 1.8 X 10³ spores/mL, 3.6 X 10³ spores/mL, 5.4 X 10³ spores/mL and 7.2 X 10³ spores/mL for the *Aspergillus flavus*; 5.80 x 10² spores/mL, 1.16 x 10³ spores/mL, 1.74 x 10³ spores/mL and 2.32 x 10³ spores/mL for the *Absidia spp*. and 1.60 x 10² spores/mL, 2.80 x 10² spores/mL, 4.40 x 10² spores/mL and 5.60 x 10² spores/mL for the *Fusarium spp*.

After inoculation, the flasks were incubated in a rotary shaker at a shaking speed of 120 rpm at 25°C. At 24 h intervals, aliquot samples were taken from each flask for the determination of the pH value, phosphate and nitrate concentrations of the initial and treated synthetic wastewater as described in standard methods [20].

The statistical analyses were carried out using the PAST: Paleontological statistics software package for education and data analysis, as described by Hammer et al. [21]. The test for comparison of means was carried out using the One-Way Analysis of Variance (ANOVA) while the test for relationship was carried out using the Pearson Correlation Index. All statistical analyses were done at a confidence interval of 95%.

All the reagents used were of analytical grades. All the experiments were carried out in triplicates and conducted twice.

3. RESULTS AND DISCUSSION

The variation in the nitrate levels of the wastewater in the presence of the *Aspergillus niger* is shown Fig. 1. With the respective initial inoculum sizes, significant nitrate reduction was observed in the wastewater. At initial inoculum sizes of 7.50 x 10² spores/mL, 1.13 x 10³ spores/mL and 1.50 x 10³ spores/mL, there were sharp decreases in nitrate concentration after 72 h incubation. From an initial nitrate concentration of 178.35 mg/L, the nitrate levels were observed to decrease after 72h incubation to 112.50 mg/L, 27.70 mg/L, 50.61 mg/L and 30.16 mg/L with initial inoculum sizes of 3.80 x 10² spores/mL, 7.50 x 10² spores/mL, 1.13 x 10³ spores/mL and 1.50 x 10³ spores/mL respectively (Fig. 1). Although the decreases in nitrate concentrations at the various initial inoculum sizes were observed to differ, these differences were however not observed to be significant (p≤ 0.05).

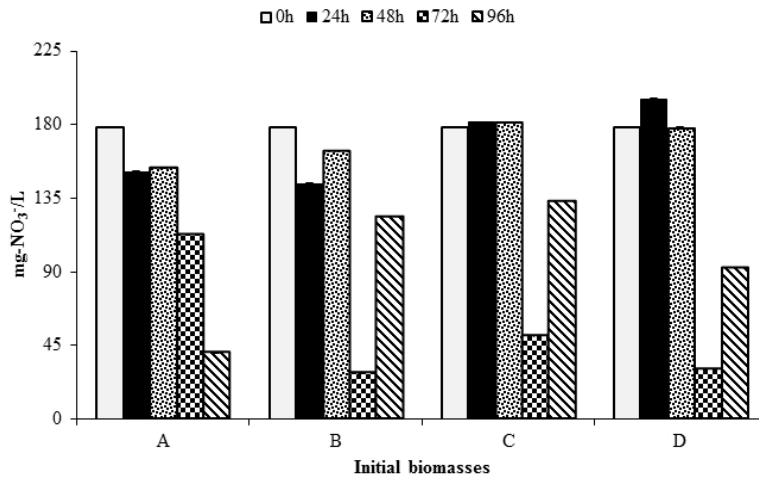


Fig. 1. Nitrate concentration of the wastewater inoculated with *Aspergillus niger* (A, B, C and D represent the inoculum sizes of 3.80×10^2 spores/mL, 7.50×10^2 spores/mL, 1.13×10^3 spores/mL and 1.50×10^3 spores/mL respectively)

In the presence of *Aspergillus flavus*, remarkable decreases in nitrate levels were observed after 72 h incubation. This trend was irrespective of the initial inoculum sizes. At the end of 96 h, nitrate concentration decreased from 178.35 mg/L at 0 h to 116.78 mg/L, 107.37 mg/L, 117.36 mg/L and 131.77 mg/L, at initial inoculum sizes of 1.80×10^3 spores/mL, 3.60×10^3 spores/mL, 5.40×10^3 spores/mL and 7.20×10^3 spores/mL respectively (Fig. 2). As was observed in the case of *Aspergillus niger*, although the decreases in nitrate levels in the presence of the different initial inoculum sizes were observed to differ, these differences were not observed to be significant ($p \leq 0.05$).

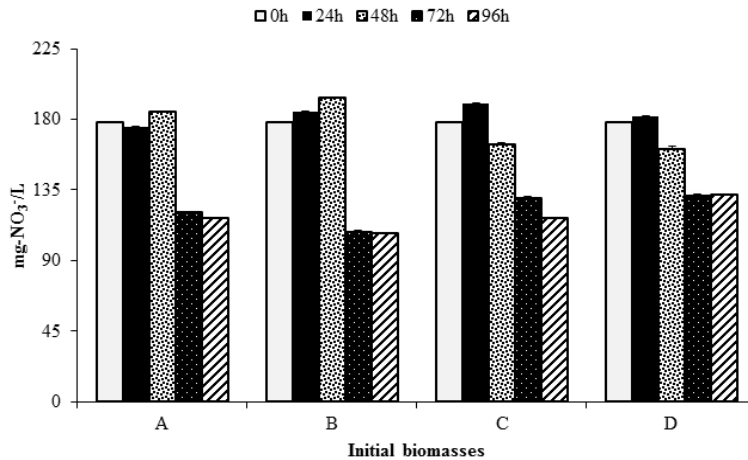


Fig. 2. Nitrate concentration of wastewater inoculated with *Aspergillus flavus* (A, B, C and D represent initial inoculum sizes of 1.80×10^3 spores/mL, 3.60×10^3 spores/mL, 5.40×10^3 spores/mL and 7.20×10^3 spores/mL respectively)

As shown in Fig. 3, with *Absidia spp*, no significant change in nitrate level was observed until after 72 h, where there a drastic decrease irrespective of the respective initial inoculum sizes. At the end of 72 h, with the least nitrate levels, the nitrate levels decreased from an initial concentration of 178.35 mg/L to concentrations of 32.11 mg/L, 37.05 mg/L, 25.89 mg/L and 36.53 mg/L, with the initial inoculum sizes of 5.80×10^2 spores/mL, 1.16×10^3 spores/mL, 1.74×10^3 spores/mL and 2.32×10^3 spores/mL respectively (Fig. 3). Despite the variation in the nitrate removal levels by the isolate at the various initial inoculum sizes, these variations were not observed to significantly different ($p \leq 0.05$).

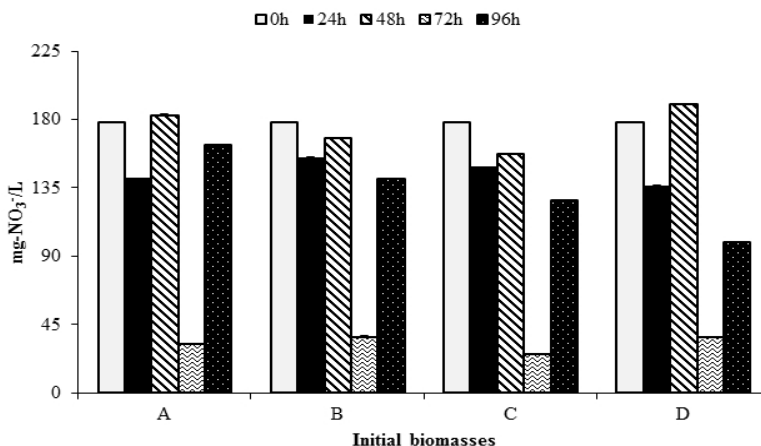


Fig. 3. Nitrate concentration of wastewater inoculated with *Absidia spp* (A, B, C and D represent initial inoculum sizes of 5.80×10^2 spores/mL, 1.16×10^3 spores/mL, 1.74×10^3 spores/mL and 2.32×10^3 spores/mL respectively)

With respect to nitrate removal in the presence of the *Fusarium spp*, remarkable removals were observed using initial inoculum sizes of 1.60×10^2 spores/mL, 2.80×10^2 spores/mL and 4.40×10^2 spores/mL. With initial inoculum size of 5.60×10^2 spores/mL, only a slight decrease in the nitrate concentration was observed after 96h. At the end of 72h incubation, where the least nitrate levels were observed with almost all the initial inoculum sizes, the nitrate levels were observed to decrease from an initial value of 178.35 mg/L to 68.81 mg/L, 28.42 mg/L, 22.58 mg/L and 139.16 mg/L, at initial inoculum sizes of 1.60×10^2 spores/mL, 2.80×10^2 spores/mL, 4.40×10^2 spores/mL and 5.60×10^2 spores/mL respectively (Fig. 4). At the end of the 96h, the nitrate level at an initial inoculum size of 5.60×10^2 spores/mL was observed to be significantly higher than when other initial inoculum sizes were considered ($p \leq 0.05$).

Generally, after 96 h incubation, there were decreases in the nitrate levels of the wastewaters treated with all the fungal strains irrespective of the initial inoculum sizes. In the presence of *Aspergillus niger*, a maximum removal level of 77.52% was observed at initial inoculum size of 3.80×10^2 spores/mL. With *Aspergillus flavus* treated wastewater, 39.80% reduction in the nitrate concentration was observed at initial inoculum size of 3.60×10^3 spores/mL. In the presence of the *Absidia spp* and *Fusarium spp.*, the highest nitrate removal of 44.23% and 87.34% at initial inoculum sizes of 2.32×10^3 spores/mL and 4.40×10^2 spores/mL, respectively was observed (Table 1).

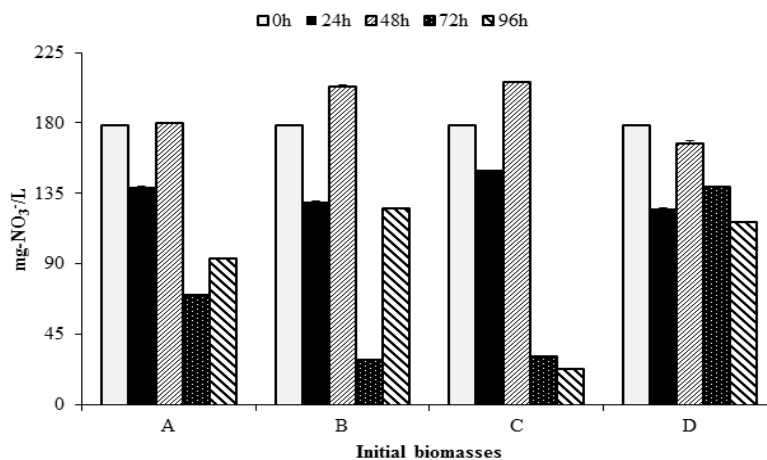


Fig. 4. Nitrate concentration of wastewater inoculated with *Fusarium spp* (A, B, C and D represent initial inoculum sizes of 1.60×10^2 spores/mL, 2.80×10^2 spores/mL, 4.40×10^2 spores/mL and 5.60×10^2 spores/mL, respectively)

Table 1. Percent Nitrate Removal by the fungal strains with different initial inoculum sizes

Initial inoculum sizes	Initial NO ₃ ⁻ (mg/L)	Final NO ₃ ⁻ (mg/L)	% NO ₃ ⁻ removed or released
<i>Aspergillus niger</i>			
3.80×10^2 spores/mL	178.35	40.09	77.52
7.50×10^2 spores/mL	178.35	123.79	30.59
1.13×10^3 spores/mL	178.35	133.32	25.25
1.50×10^3 spores/mL	178.35	92.58	48.09
<i>Aspergillus flavus</i>			
1.80×10^3 spores/mL	178.35	116.78	34.52
3.60×10^3 spores/mL	178.35	107.37	39.80
5.40×10^3 spores/mL	178.35	117.36	34.20
7.20×10^3 spores/mL	178.35	131.77	26.12
<i>Absidia spp.</i>			
5.80×10^2 spores/mL	178.35	162.91	8.66
1.16×10^3 spores/mL	178.35	140.52	21.21
1.74×10^3 spores/mL	178.35	126.71	28.95
2.32×10^3 spores/mL	178.35	99.46	44.23
<i>Fusarium spp.</i>			
1.60×10^2 spores/mL	178.35	93.75	47.43
2.80×10^2 spores/mL	178.35	125.73	29.50
4.40×10^2 spores/mL	178.35	22.58	87.34
5.60×10^2 spores/mL	178.35	116.97	34.42

Initial and final represent nitrate concentrations at time 0h and 96h respectively. All the values are average of triplicate analyses

In the case of phosphate removal in wastewater with *Aspergillus niger*, only very slight decreases in the concentrations were observed after 96h incubation. This trend was irrespective of the different initial inoculum sizes used for inoculation. After 96h, the

phosphate concentration decreased from an initial level of 155.34 mg/L to 141.90 mg/L, 142.89 mg/L, 153.41 mg/L and 129.50 mg/L, at initial inoculum sizes of 3.80×10^2 spores/mL, 7.50×10^2 spores/mL, 1.13×10^3 spores/mL and 1.50×10^3 spores/mL respectively (Fig. 5). Despite the differences in the phosphate concentrations at the end of incubation, these differences were not observed to be significantly different between the different initial inoculum sizes ($p \leq 0.05$).

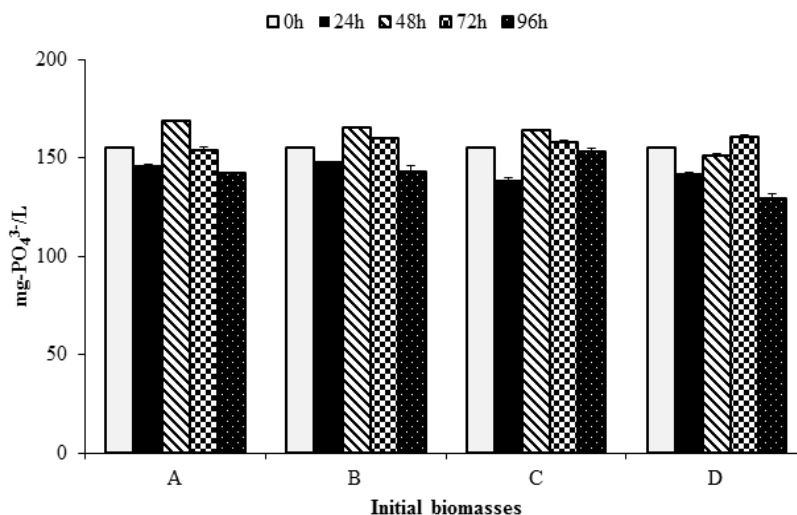


Fig. 5. Phosphate concentration of wastewater inoculated with *Aspergillus niger* (A, B, C and D represent inoculum sizes of 3.80×10^2 spores/mL, 7.50×10^2 spores/mL, 1.13×10^3 spores/mL and 1.50×10^3 spores/mL, respectively)

As shown in Fig. 6, with *Aspergillus flavus*, apart from the initial inoculum size of 3.60×10^3 spores/mL, where a slight decrease in phosphate concentration was observed, there were increases in concentrations at the end of 96 h. After 96 h, the phosphate concentration was observed to have changed from an initial level of 155.34 mg/L to 176.46 mg/L, 137.95 mg/L, 175.05 mg/L and 190.52 mg/L, when the initial inoculum sizes of 1.80×10^3 spores/mL, 3.60×10^3 spores/mL, 5.40×10^3 spores/mL and 7.20×10^3 spores/mL were used respectively (Fig. 6). The concentration of phosphate at an initial inoculum size of 3.60×10^3 spores/mL was observed to be significantly lower than concentrations with other initial inoculum sizes ($p \leq 0.05$).

In addition, with *Absidia spp.* treated wastewater, no remarkable decreases in the phosphate concentrations were observed throughout the period of incubation. This trend was irrespective of the initial inoculum sizes used for inoculation. After 96h, the phosphate levels showed an increase from 155.34 mg/L to 196.54 mg/L, 157.03 mg/L, 178.27 mg/L and 190.52 mg/L, with initial inoculum sizes of 5.80×10^2 spores/mL, 1.16×10^3 spores/mL, 1.74×10^3 spores/mL and 2.32×10^3 spores/mL respectively (Fig. 7). Although the differences were observed in the phosphate levels with different initial inoculum sizes, these differences were not observed to be significant ($p \leq 0.05$).

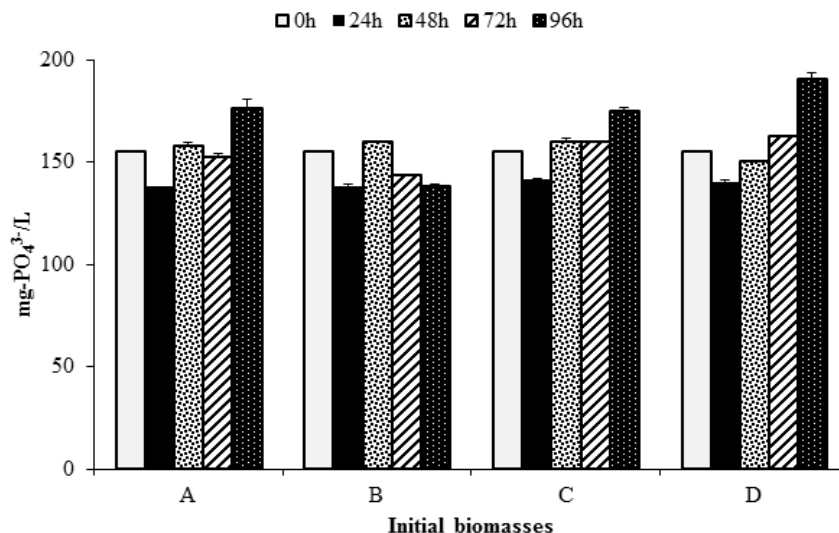


Fig. 6. Phosphate nitrate concentration of wastewater inoculated with *Aspergillus flavus* (A, B, C and D represent inoculum sizes of 1.80×10^3 spores/mL, 3.60×10^3 spores/mL, 5.40×10^3 spores/mL and 7.20×10^3 spores/mL respectively).

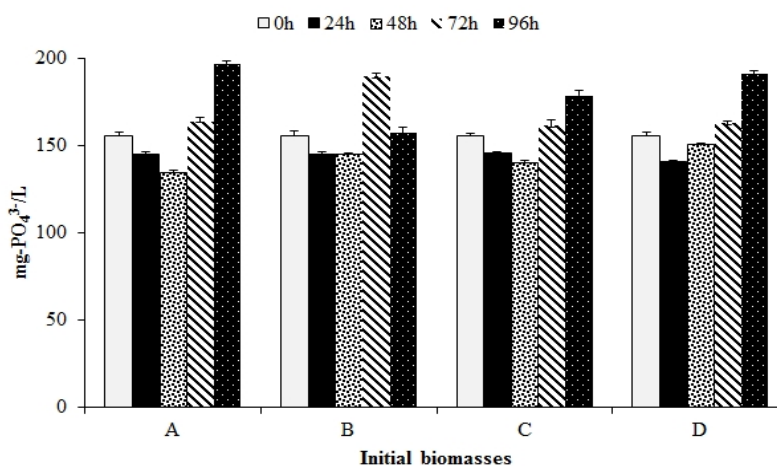


Fig. 7. Phosphate concentration of wastewater inoculated with *Absidia spp* (A, B, C and D represent inoculum sizes of 5.80×10^2 spores/mL, 1.16×10^3 spores/mL, 1.74×10^3 spores/mL and 2.32×10^3 spores/mL, respectively)

With respect to *Fusarium spp* no remarkable decreases in the phosphate concentration was observed throughout the period of incubation. This trend was irrespective of the initial inoculum size. After 96h, the phosphate concentrations at initial inoculum sizes of 1.60×10^2 spores/mL, 2.80×10^2 spores/mL, 4.40×10^2 spores/mL and 5.60×10^2 spores/mL were observed to increase from an initial concentration of 155.34 mg/L to 191.71 mg/L, 180.69 mg/L, 214.68 mg/L and 196.45 mg/L respectively (Fig. 8). Similarly, as was observed in the case of *Absidia spp*, the phosphate concentrations at different initial inoculum sizes were not observed to differ during incubation ($p \leq 0.05$).

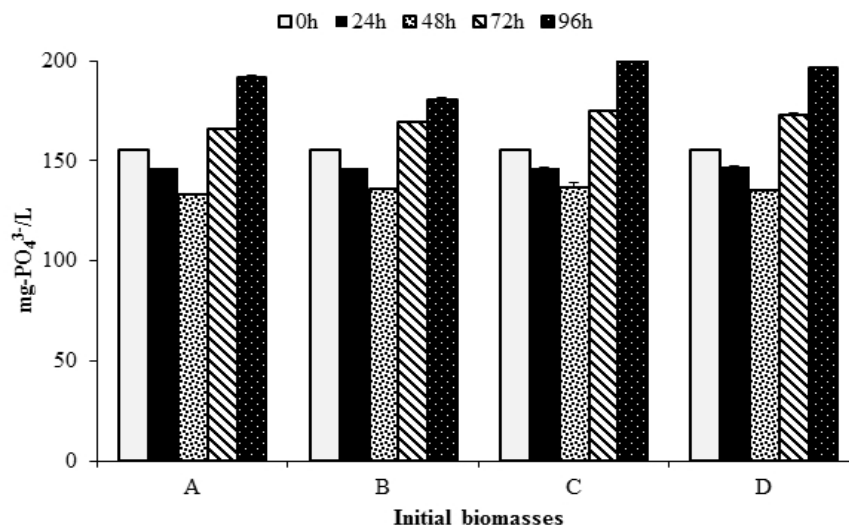


Fig. 8. Phosphate concentration of wastewater inoculated with *Fusarium spp* (A, B, C and D represent inoculum sizes of 1.60×10^2 spores/mL, 2.80×10^2 spores/mL, 4.40×10^2 spores/mL and 5.60×10^2 spores/mL, respectively)

There was slight phosphate removal in the presence of the *Aspergillus niger*. The other three fungal strains exhibited some phosphate releases after 96h irrespective of the initial inoculum sizes. The highest phosphate removal of 16.63% was observed in the presence of *Aspergillus niger* at an initial inoculum size of 1.50×10^3 spores/mL. With *Aspergillus flavus*, *Absidia spp* and *Fusarium spp*, the highest phosphate releases of 18.46%, 20.97% and 27.64% were observed when the initial sizes of the inocula were 7.20×10^3 spores/mL, 5.80×10^2 spores/mL and 5.60×10^2 spores/mL respectively (Table 2).

The pH value of the wastewater treated with each of the fungi was observed to increase after 96 h irrespective of the strains and the initial inoculum sizes used for inoculation. Generally, the percent pH value ranged from 10.61% to 14.49%, 7.81% to 18.06%, 7.81% to 18.06% and 4.84% to 11.94%, in the presence of the *Aspergillus niger*, *Aspergillus flavus*, *Absidia spp* and *Fusarium spp*, respectively (Table 3).

The present study revealed no relationship between the nutrient removal ability of the fungi and the initial inoculum sizes used implying that the increases in the population of the organisms did not translate into increases in nutrient removal. It was reported that excessive quantities of nutrients accumulate when microbial growth is arrested due to a lack of some nutrients [22]. This trend negates the findings of Akpor and Momba [23] and Momba and Cloete [24]. Akpor and Momba [23] reported a direct relationship between decreases in nutrient concentrations and increases in the cell densities of the protozoa studied. Earlier, Momba and Cloete [24] had reported an increase in phosphate uptake with increases in the growth *Pseudomonas fluorescens*, *Escherichia coli* and *Acinetobacter radioresistens* in mixed liquor.

Table 2. Percent phosphate removed or released by the fungi with different initial inoculum sizes

Initial inoculum sizes	Initial NO ₃ ⁻ (mg/L)	Final NO ₃ ⁻ (mg/L)	% NO ₃ ⁻ removed or released*
<i>Aspergillus niger</i>			
3.80 x 10 ² spores/mL	155.34	141.90	8.65
7.50 x 10 ² spores/mL	155.34	142.89	8.01
1.13 x 10 ³ spores/mL	155.34	153.41	1.24
1.50 x 10 ³ spores/mL	155.34	129.50	16.63
<i>Aspergillus flavus</i>			
1.80 x 10 ³ spores/mL	155.34	174.46	10.96*
3.60 x 10 ³ spores/mL	155.34	137.95	11.19
5.40 x 10 ³ spores/mL	155.34	175.05	11.26*
7.20 x 10 ³ spores/mL	155.34	190.51	18.46*
<i>Absidia spp.</i>			
5.80 x 10 ² spores/mL	155.34	196.55	20.97*
1.16 x 10 ³ spores/mL	155.34	157.96	1.66*
1.74 x 10 ³ spores/mL	155.34	178.27	12.86*
2.32 x 10 ³ spores/mL	155.34	165.86	6.34*
<i>Fusarium spp.</i>			
1.60 x 10 ² spores/mL	155.34	191.71	18.97*
2.80 x 10 ² spores/mL	155.34	180.69	14.03*
4.40 x 10 ² spores/mL	155.34	214.68	27.64*
5.60 x 10 ² spores/mL	155.34	194.45	20.11*

Values are average of triplicate analyses

*values represent percent increases

Table 3. pH variation during the nutrient removal study in presence of the test strains

Initial Inoculum size	Initial pH	Final pH	%increase in pH
<i>Aspergillus niger</i>			
3.80 x 10 ² spores/mL	5.9	6.6	10.61
7.50 x 10 ² spores/mL	5.9	6.6	10.61
1.13 x 10 ³ spores/mL	5.9	6.6	10.61
1.50 x 10 ³ spores/mL	5.9	6.9	14.49
<i>Aspergillus flavus</i>			
1.80 x 10 ³ spores/mL	5.9	6.4	7.81
3.60 x 10 ³ spores/mL	5.9	7.1	16.90
5.40 x 10 ³ spores/mL	5.9	7.2	18.06
7.20 x 10 ³ spores/mL	5.9	7.2	18.06
<i>Absidia spp.</i>			
5.80 x 10 ² spores/mL	5.9	6.4	7.81
1.16 x 10 ³ spores/mL	5.9	6.5	9.23
1.74 x 10 ³ spores/mL	5.9	6.6	10.61
2.32 x 10 ³ spores/mL	5.9	6.6	10.61
<i>Fusarium spp.</i>			
1.60 x 10 ² spores/mL	5.9	6.2	4.84
2.80 x 10 ² spores/mL	5.9	6.5	9.23
4.40 x 10 ² spores/mL	5.9	6.7	11.94
5.60 x 10 ² spores/mL	5.9	6.5	9.23

Initial and final represent nitrate concentrations at time 0h and 96h respectively.

All values are average of triplicate analyses

Although bacteria such as *Arthrobacter globiformis*, *Aerobacter aerogenes*, *Mycobacterium phlei*, *Streptomyces griseus*, *Thiosphaera* and *Pseudomonas spp* have been reported to be involved in nitrification and denitrification in wastewater treatment systems, some reports have indicated that the major organisms involved appear to be fungi, mostly *Aspergillus flavus*, *Penicillium* or *Cephalosporium* [25]. Several other fungi species have been reported in the removal of eutrophication agents and bioremediation of metal contaminated waste streams. There is also a growing interest in the use of fungi for the removal of nitrogen, phosphorus and metals from commercial and municipal waste [26].

All the strains used in this study showed nitrate removal ability while none of them showed any remarkable phosphate removal ability. Several species of fungi have been implicated in nitrate and heavy metal removals from wastewater. *Aspergillus niger* has been indicated in ammonium, nitrate and protein assimilation in a continuous fixed-slab reactor at 22°C [2]. The role of the *Aspergillus niger* in the removal of thallium, lead, zinc, cadmium, chromium, nickel and copper in wastewater systems have also been reported [16,27,28]. Among the four fungal species used in this investigation, only *Aspergillus niger* showed slight phosphate removal ability. In a study of twenty one fungal isolates that were screened for the elimination of compounds from raw wastewater under shaking flasks conditions, two strains (*Aspergillus niger* and *Trichoderma viride*) were found to be best in the elimination of total nitrogen, phosphate, ammonia, nitrate and chemical oxygen demand from raw wastewater [29]. The study indicated that *Aspergillus niger* removed over 86% and 95% of nitrogen and phosphorus from the wastewater.

The denitrification capability of *Fusarium spp.* in synthetic wastewater of defined quality has been investigated. Some workers have reported that *Fusarium spp* was capable of reducing nitrate and nitrite to form N₂O during denitrification [30,31]. In addition, other fungi species, such as *Aspergillus versicolor*, *Aspergillus niger*, *Aspergillus oryzae*, *Absidia fusca* and *Fusarium verticillioides* have been indicated to exhibit remediation ability in heavy metal and nutrient polluted wastewaters [2,17,28]. The use of *Fusarium spp* as a potential organism for magnesium and calcium removal from water in its immobilized form has been reported [18]. Guiraud et al. [32] have implicated *Absidia spp.* in the degradation of some xenobiotics.

4. CONCLUSION

This study aimed at investigating the role of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium spp.* and *Absidia spp.* in the removal of eutrophic nutrients from synthetic wastewater revealed the following:

- All the test fungistrains exhibited significant nitrate removal ability, irrespective of the initial inoculum sizes.
- Only *Aspergillus niger* showed slight phosphate removal ability after 96h of incubation. In the presence of *Aspergillus flavus*, *Fusarium spp.* and *Absidia spp.*, there was phosphate release into the wastewater rather than removal also irrespective of the initial inoculum sizes.
- There was no direct relationship between the nutrient uptake by the test fungal strains and initial inoculum sizes.

Although the study cannot be considered to be exhaustive, the present findings have given an insight into the nutrient removal ability of the test fungal strains under the experimental conditions investigated.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rocca CD, Belgiorno V, Meriç S. Overview of in-situ applicable nitrate removal processes. *Desalination*. 2007;204(1–3):46-62.
2. Hwang S, Lin C, Chen I, Chen J, Liu L, Dodds WK. Removal of multiple nitrogenous wastes by *Aspergillus niger* in a continuous fixed-slab reactor. *Bioresource Technology*. 2004;939(2):131-138.
3. Kassim TI. Possible use of microgreen algae to remove phosphate and nitrate from wastewater. *Proceedings of International Symposium on Environmental Pollution Control and Waste Management, 7-10 January 2002, Tunis (EPCOWM'2002)*. 2002;628-632.
4. Ayyasamy PM, Rajakumar S, Sathishkumar M, Swaminathan K, Shanthi K, Lakshmanaperumalsamy P, Lee S. Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. *Desalination*. 2009;242(1–3): 286-296.
5. Jalal, KCA, Zahangir MD, Matin WA, Kamaruzzaman BY, Akbar J, Toffazel H. Removal of nitrate and phosphate from municipal wastewater sludge by *Chlorella*, *Vulgaris*, *Spirulina platensis* and *Scenedesmus quadricauda*. *IJUM Engineering Journal*. 2011;12(4):125-132.
6. Rahman MA, Ahsan S, Kaneco S, Katsumata H, Suzuki T, Ohta K. Wastewater treatment with multilayer media of waste and natural indigenous materials. *Journal of Environmental Management*. 2005;74(2):107-110.
7. Krishnan KA, Haridas A. Removal of phosphate from aqueous solutions and sewage using natural and surface modified coir pith. *Journal of Hazardous Materials*. 2008;152(2):527-535.
8. Bhatnagar A, Sillanpaa M. A review of emerging adsorbents for nitrate removal from water. *Chemical Engineering Journal*. 2011;16(2):493-504.
9. McGrath JW, Quinn JP. Microbial phosphate removal and polyphosphate production from wastewaters. *Advances in Applied Microbiology*. 2003;52:75-100.
10. Seviour RJ, Mino T, Onuki M. The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiology Reviews*. 2003;27(1):99-127.
11. Wu Y, Li T, Yang L. Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: a review. *Bioresource Technology*. 2012;107:10-18.
12. Ekama GA. Biological nutrient removal: reference module in earth systems and environmental sciences, from treatise. *Water Science*. 2011;4:409-526.

13. Wang L, Huang L, Yun L, Tang F, Zhao J, Liu Y, Zeng X, Luo Q. Removal of nitrogen, phosphorus, and organic pollutants from water using seeding type immobilized microorganisms. *Biomedical and Environmental Sciences*. 2008;21(2):150-156.
14. Han R, Li H, Li Y, Zhang J, Xiao H, Shi J. Biosorption of copper and lead ions by waste beer yeast. *Journal of Hazardous Materials*. 2006;137(3):1569-1576.
15. Wang J, Chen C. Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. *Biotechnology Advances*. 2006; 24(5):427-451.
16. Ahluwalia SS, Goyal D. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology*. 2007;98(12):2243-2257.
17. Bairagi H, Khan MR, Ray L, Guha AK. Adsorption profile of lead on *Aspergillus versicolor*: a mechanistic probing. *Journal of Hazardous Materials*. 2011;186(1):756-764.
18. Mahmoud ME, Yakout AA, Abdel-Aal H, Osman MM. Immobilization of *Fusarium verticillioides* fungus on nana-silica (NSi-Fus): A novel and efficient biosorbent for water treatment and solid phase extraction of Mg(II) and Ca(II). *Bioresource Technology*. 2013;134:324-330.
19. Aderiye BI, Ogundana SK, Adesanya SA, Roberts M. Antifungal properties of yam (*Dioscorea alata* L). *Folia Microbiologica*. 1996;41(5):240 – 245.
20. APHA. Standard Methods for the Examination of Water and Wastewater, 22nd edition. APHA, Washington D.C; 2012.
21. Hammer O, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*. 2001;4(1):9. Available: http://palaeo-electronica.org/2001_1/past/issue1_01.htm
22. Fuhs GW, Chen M. Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. *Microbial Ecology*. 1975;2:119–138
23. Akpor OB, Momba MNB. Relationship of protozoan biomass to phosphate and nitrate removal from activated sludge mixed liquor. *Biotechnology Journal*. 2010;5:304-313.
24. Momba MNB, Cloete TE. Biomass relationship to growth and phosphate uptake of *Pseudomonas fluorescens*, *Escherichia coli* and *Acinetobacter radioresistens* in mixed liquor medium. *Journal of Industrial Microbiology*. 1996;16:364–369.
25. Vymazal J. Removal of nutrients in various types of constructed wetlands. *Science of the Total Environment*. 2007;380:48-65.
26. Price MS, Classen JJ, Payne GA. *Aspergillus niger* absorbs copper and zinc from swine wastewater. *Bioresource Technology*. 2001;77:41-49.
27. Peter AL, Viraraghavan T. Removal of thallium from aqueous solutions by modified *Aspergillus niger* biomass. *Bioresource Technology*. 2008;99(3):618-625.
28. Sepehr MN, Nasser S, Zarrabi M, Samarghandi MR, Amrane A. Removal of Cr (II) from tanning effluent by *Aspergillus niger* in airlift bioreactor. *Separation and Purification Technology*, 2012: 96: 256-262
29. Awad MF, Kraume M. Fungal diversity in activated sludge from membrane bioreactors in Berlin. *Canadian Journal of Microbiology*. 2011;57(8):693-698.
30. Shoun K, Tanimoto T. Denitrification by the fungus *Fusarium oxysporum* and involvement of cytochrome P450 in the respiratory nitrite reduction. *Journal of Biological Chemistry*. 1991;266:11078-11082.

31. Liu D, Zheng Y, Li P, Takaya N, Shoun H. Biological nitrogen removal from wastewater by denitrification of mix-culturing fungi and bacteria. *Acta Hydrobiologica Sinica*. 2006;30(6):671-675.
32. Guiraud P, Villemain D, Kadri M, Bordjiba O, Steiman R. Biodegradation capability of *Absidia fusca* Linnemann towards environmental pollutants. *Chemosphere*. 2003;52(4):663-671.

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