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Potential of Magnetite Nanoparticles on Dairy Effluent Nitrate and Phosphate Bioremediation

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ABSTRACT

Despite nanotechnology got a high public awareness due to its implementation in various human activities, the effect of nanoparticles on bacterial performance in wastewater treatment is still unclear. This study examines the role of magnetite nanoparticles (Fe_3O_4 NPs) on the dairy effluent nitrate and phosphate bioremediation using diverse inoculum sources. Two inoculum sources (wastewater and sludge) were got from the dairy wastewater treatment plant unit. A culture was prepared to check the role and efficacy of Fe_3O_4 NPs. After five days of incubation, the culture of dairy diverse inoculum sources was verified to be effective in effluent treatment. By applying sludge as an inoculum source, the reduction efficacy was enhanced up to 48.54% and 46.13% for nitrate and phosphate, respectively compared to control. In the case of applying wastewater as an inoculum source, the reduction was enhanced up to 64.12% and 36.85% for nitrate and phosphate, respectively compared to control. Furthermore, the bacterial abundance showed a significant variance between control and another sample (high abundance than control). Results revealed that Fe_3O_4 NPs can improve the microbial growth of diverse inoculum sources which are efficient in the bioremediation of dairy effluent. Overall, the concentration (40 ppm) of Fe_3O_4 NPs was obtained the optimal abundance for inoculum sources and the reduction of nitrate and phosphate as well.

KeyWords:
Activated sludge, Bacterial wastewater treatment, Nitrate, Phosphate, Magnetite nanoparticles.

1. INTRODUCTION

Population growth, cumulative urbanization migration, and progress over the years have affected the request for freshwater resources. It had been estimated by the World Health Organization (WHO) [1] that 50% of the worldwide population would be living in water-stressed areas. The industrial invention, mining, exhaustive agriculture, in addition to urban utilization have led to a rise in water usage, which ultimately has significantly impacted the water quality obtainable around the world [2].

Healthy water had become a challenge for wholly suitably operative countries considering its great importance in human health [3]. Water pollution is a reason for millions of deaths each year around the world, mainly in developed countries, in this regard some eco-friendly nanoparticles might be utilized as a real water disinfectant to deliver pollution-free water which is harmless for human usage [4].

Nanotechnology is the greatest radical technological modernization in this century to offer a scientific output intended for human life. A major part of research in nanotechnology chiefly contracts with the nanoparticle's synthesis with diverse chemical structures, dimensions, and organization [5-7].

Magnetite nanoparticles signify a novel generation of ecological remediation methods that would deliver effective cost solutions for some of the greatest challenging ecological cleanup difficulties [8]. They are metallic oxide nanoparticles owning magnetic possessions as well as greater biocompatibility [9]. Fe_3O_4 NPs have been broadly utilized because of their biocompatibility as well as their superb optoelectronic besides magnetic possessions, they simply removed from aqueous solution through using an exterior magnetic field

[10]. El-kassas et al. [8] explore an experimental study to explain the bioremediation of industrial wastewater using Fe_3O_4 NPs [8].

In summary, this study examines the effect of magnetite nanoparticles in microbial activation for dairy effluent nitrate and phosphate bioremediation. Specifically, the Fe_3O_4 nanoparticles concentrations and their influence on the isolated bacterial growth and the reduction of nitrate and phosphate bioremediation were investigated and discussed in this study.

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2. MATERIALS AND METHODS

2.1. Inoculum sample

Fresh dairy activated sludge and wastewater inoculum samples had been obtained from a unit of dairy wastewater treatment in Altayb Dairy Products Factory found in Jumasa, Egypt. The samples were kept at 4 °C to prevent any alterations in inoculum properties, then carried to the laboratory, for further use.

2.2. Magnetite nanoparticles (Fe_3O_4 NPs)

2.2.1 Preparation of Fe_3O_4 NPs

Hydrothermal technique was used to synthesis a colloidal solution of Fe_3O_4 NPs capped with ascorbic acid. 0.0017 M of $FeCl_3$.$6H_2O$ was used as a precursor salt that was dissolved in 25 mL H_2O with continuous stirring. 10 mL 0.6 M of Na_2CO_3 was added to the prepared solution gradually; after 10 min, 0.12 g ascorbic acid was added to solution with continues stirring. The solution color was turned into black that confirmed the Fe_3O_4 NPs creation. To improve the size distribution stirred for another 15 minutes, then transported and wrapped in a 40 mL Teflon sealed autoclave. Autoclaved at 160 °C for 3 h then cooled in the open air. The final product was separated from the solution via centrifugation. Using three cycles of centrifugation/ washing/centrifugation process in deionized water than in alcohol was done beforehand drying in oven for 12 hours at 60 °C [11-13].

2.3. Experimental design

2.3.1. Influence of some environmental factors on the microbial growth and nitrate and phosphate bioremediation

100 mL of different inoculum source solutions were inoculated separately in a reactor containing 300 mL of culture media that composed of: 2.5 g L^{-1} d-glucose anhydrate; 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, and 0.18 g L^{-1} KNO_3 dissolved in distilled water [14]. To examine the microbial growth and nitrate and phosphate reduction under different environmental conditions, three pH values 6, 7, 8, and three temperature degrees 15, 25, 35 ±2 ˚C were determined. The pH adjustment before sterilization was done using HCl to obtain pH 6 and NaOH to obtain pH 8. The aliquot samples were used to determine nitrate and phosphate concentrations (ppm) using ion chromatography (Thermo Scientific, Dionex ICS-1100) [15]. The microbial growth was measured using Jenway model 6800 spectrophotometer at wavelength of 450 nm [16,17]. Tests had been carried out in triplicate and the averages and change % than control had been recorded.

2.3.2. Influence of different concentrations of Fe_3O_4 NPs on the microbial growth and nitrate and phosphate bioremediation

To investigate the influence of different concentrations of magnetite nanoparticles on microbial growth and nitrate and phosphate bioremediation, magnetite NPs concentrations in the samples were adapted as follows: 10, 20, 30, 40, and 50 ppm, and the free nanoparticle sample was used as a control. The samples were incubated at 35 °C and pH 7. The microbial growth and nitrate and phosphate reduction were measured.

3. RESULTS AND DISCUSSION

3.1. Fe_3O_4 NPs characterization

3.1.1. X-ray diffraction analysis (XRD)

The Fe_3O_4 NPs capped with ascorbic acid XRD pattern is presented in Fig. 1. When applying ascorbic acid as capping agents well-definite peaks were achieved. The diffraction patterns for the prepared nanoparticles demonstrated peaks at 27.34°, 31.69°,45.45°, 53.87° and 66.22° corresponding to [220], [311], [400], [422], [511], and [440] planes of cubic **Fe_3O_4** lattice, respectively. The study results are agreed with many studies results [18-20].

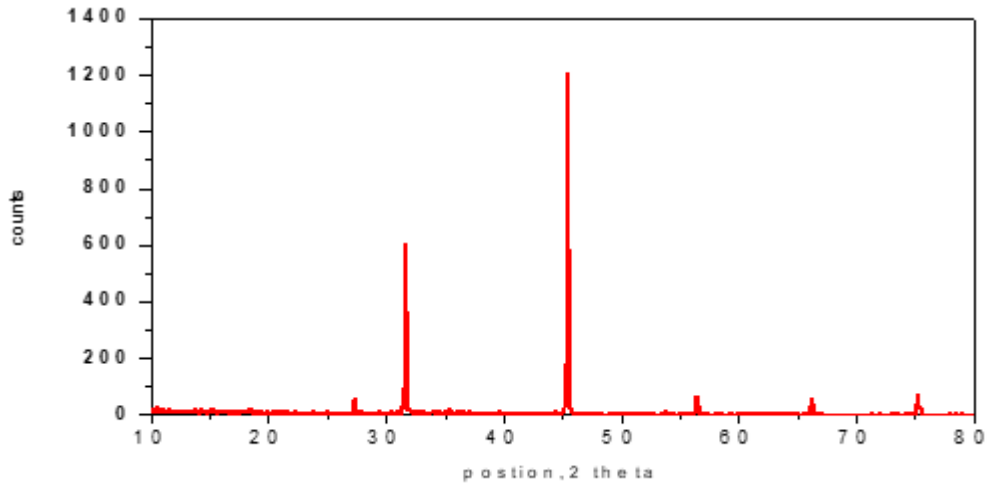

Fig. 1. X-ray diffraction pattern of the prepared Fe_3O_4 NPs.

3.1.2. Transmission electron microscope (TEM)

The TEM photomicrograph of the prepared Fe_3O_4 NPs is presented in Fig. 2. Supporting that, the prepared nanoparticles diverse from sphere-shaped to egg-shaped with fairly identical shape and size, that is compatible with [21,22]. With an average size of around 5nm.

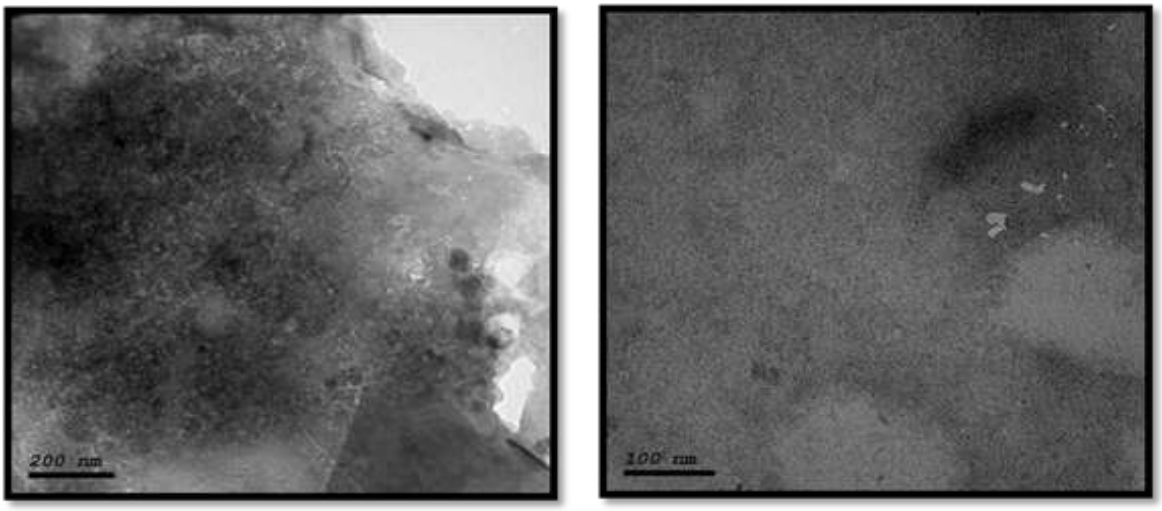

Fig. 2. Transmission electron microscope photomicrograph of the prepared Fe_3O_4 NPs.

3.1.3. Zeta potential

Zeta potential value of prepared Fe_3O_4 NPs capped with ascorbic acid is -28.9 mV and presented in Fig. 3. Nanoparticles with zeta potential values higher than +25 mV or less than -25 mV usually have more degrees of colloidal stability, because of the repulsive forces that avoid the agglomeration of NPs [3.23]. The obtained result of Fe_3O_4 NPs showed that the nanoparticles have appropriate dispersion ability in a hydrous medium.

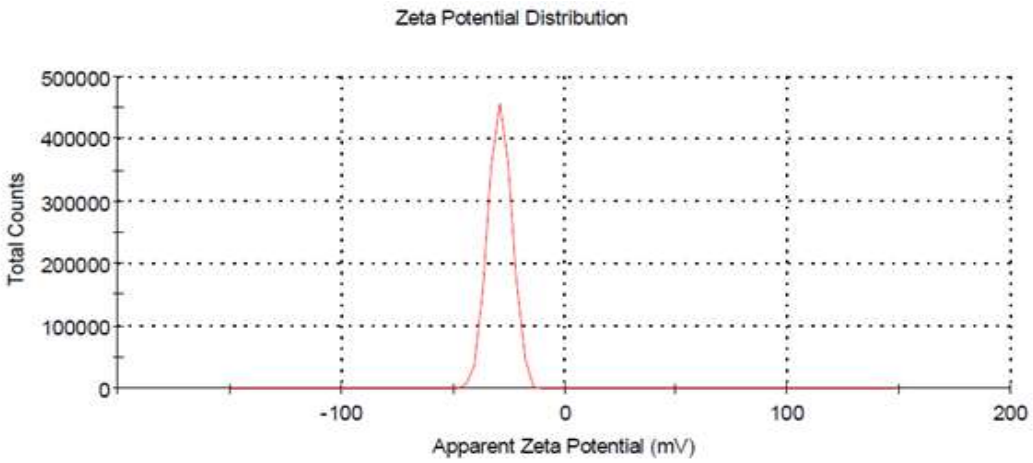

Fig. 3. Zeta potential of the prepared Fe_3O_4 NPs having peak at-28.9 mV.

3.2. Microbial wastewater treatment

Biological treatment employs a range of microbes having different metabolic ways to degrade the inorganic and organic contaminants in a polluted matrix and, henceforth, is observed as eco-friendly, effectively cost technique for treatment and management of wastewater with the modest structural arrangement, wider implementation, easily functioning, and a little sludge generation [24-29].

Biological wastewater treatment methods are intended to eliminate nutrients, generally dissolved phosphorus as well as nitrogen [29]. The microorganisms could achieve decomposition under both aerobic as well as anaerobic circumstances [30].

Utmost removal of the nitrogen from wastewater is done by microorganism communities through manipulating an ammox besides nitrification-denitrification apparatuses within crops water management systems (CWMS) [31,32]. Thus, the elimination of phosphorus via mineralization besides immobilization is likewise partly influenced by microbial activities [33,34].

Some microbes provide an approach aimed at contaminants-removal such as nitrogen, phosphorus, and carbon from wastewater whereas generating biomass that might find a use for the manufacture of high-value chemicals or biogas through anaerobic digestion [35]. Wang et al. [36] informed a reduction in nitrogen is (83% N as NH_4+) besides phosphorus reduction is (90% P as PO_4^{3-}) in urban wastewater via microbial organisms [37].

Nutrient reduction in wastewater using the chemical methods were reached about 80-98 % for nitrogen as well as 85-99 % for phosphorus [38,39]. While when comparing these approaches with the biological treatment we were satisfying the needed elimination efficiency without any harmful environmental impact [40-43].

Bioremediation of nitrogen is the main procedure for ammonium elimination in the wastewater treatment process. The traditional biological nitrogen removal comprises nitrification and denitrification [43-46]. According to Silkina et al. [47], the microbial consortia had greater rates of nitrogen and phosphorous reduction, as a result, the diverse species might utilize diverse reduction mechanisms.

3.3. Influence of some environmental factors on the microbial growth and nitrate and phosphate bioremediation

The growth of wastewater and sludge inoculum under different environmental factors as using three pH values (6,7, and 8) were presented in Fig. 4, while the effect of the three temperature degrees (15, 25, 35) ±2 ℃ was presented in Fig. 5.

An enhancing effect of pH and temperature on microbial growth was obtained at pH 7 and temperature 35℃ ±2 for both wastewater inoculum and sludge inoculum. While the other pH values and temperature degrees' effects were lesser than pH 7 and temperature 35℃ ±2 enhancing effect. The significant variations ($P < 0.05$) in the absorbance pattern of microbial growth media at wavelength 450 nm among different environmental factors was illustrated in Tables 1 and 2.

Table 1. GLM test for variation in absorbance (450 nm) among different PH values using different inoculum sources**.**

Growth media	Source	df	SS	AS	AM	F-value	P-value
Different inoculum sources	Columns	1	528323	528323	528323	344.51	0.000
	Rows	2	51989	51989	25994	16.95	0.000
	Error	14	21469	21469	1534	-	-
	Total	17	601782	-	-	-	-

General Linear Model (GLM),
Degree of freedom (df),
Sequential sums of squares(SS),
Adjusted sums of squares (AS),
Adjusted mean squares (AM)

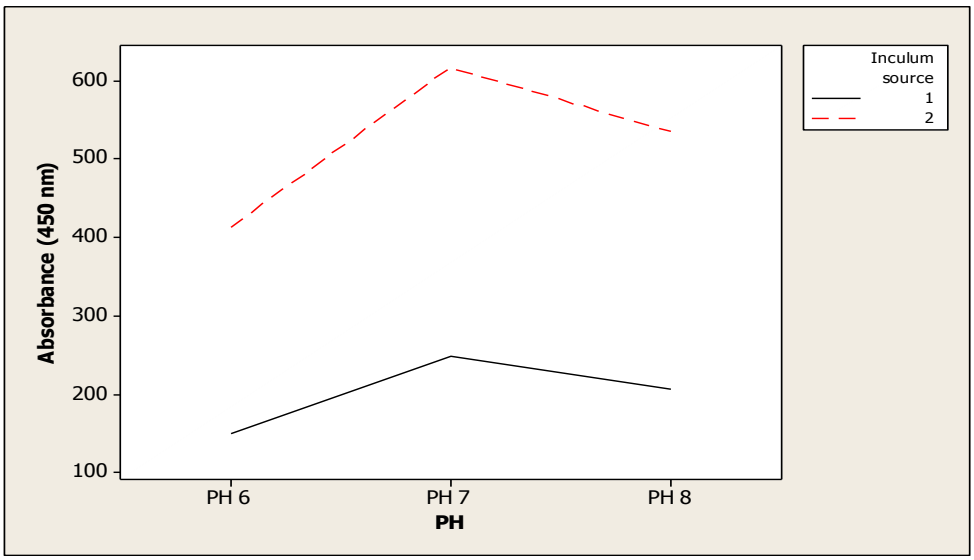

Fig. 4. x̄ of microbial growth media absorbance at wavelength (450 nm) using different inoculum sources and different pH values, at temperature 35.0°C, after 5.0 days of incubation.

Table 2. GLM test for variation in absorbance (450 nm) among different temperature degrees using different inoculum sources

Growth media	Source	df	SS	AS	AM	F-value	P-value
Different inoculum sources	Columns	1	4261254	4261254	4261254	302.26	0.000
	Rows	2	219227	219227	109614	7.78	0.005
	Error	14	197369	197369	14098	-	-
	Total	17	4677850	-	-	-	-

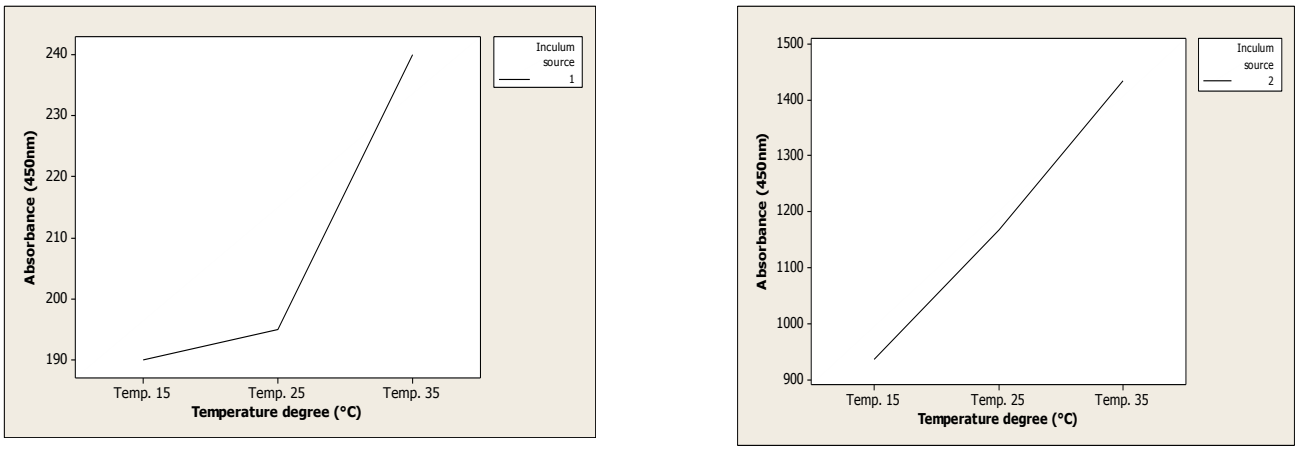

Fig. 5. x̄ of microbial growth media absorbance at wavelength (450 nm) using different inoculum sources and different temperature degrees (°C), at pH 7, after 5.0 days of incubation.

The nitrate and phosphate bioremediation using wastewater and sludge inoculum under different environmental factors are presented in Figs. 6, 7, 8 and 9, respectively. The significant variations ($P < 0.05$) in nitrate and phosphate bioremediation among different environmental factors was illustrated in Tables 3 and 4.

Table 3. GLM test for variation in nitrate concentration (ppm) reduction (upper section of figure) and phosphate concentration (ppm) reduction (lower section of figure) among different PH values and using different inoculum sources.

Growth media	Source	df	SS	AS	AM	F-value	P-value
	Columns	1	47342	47342	47342	149.47	0.000
	Rows	5	8449	8449	4224	13.34	0.001
	Error	29	4434	4434	317	-	-
	Total	35	60224	-	-	-	-
Different inoculum sources	Source	df	SS	AS	AM	F-value	P-value
	Columns	1	231.72	231.72	231.72	411.52	0.000
	Rows	2	13.56	13.56	6.78	12.04	0.000
	Error	14	7.88	7.88	0.56	-	-
	Total	17	253.16	-	-	-	-

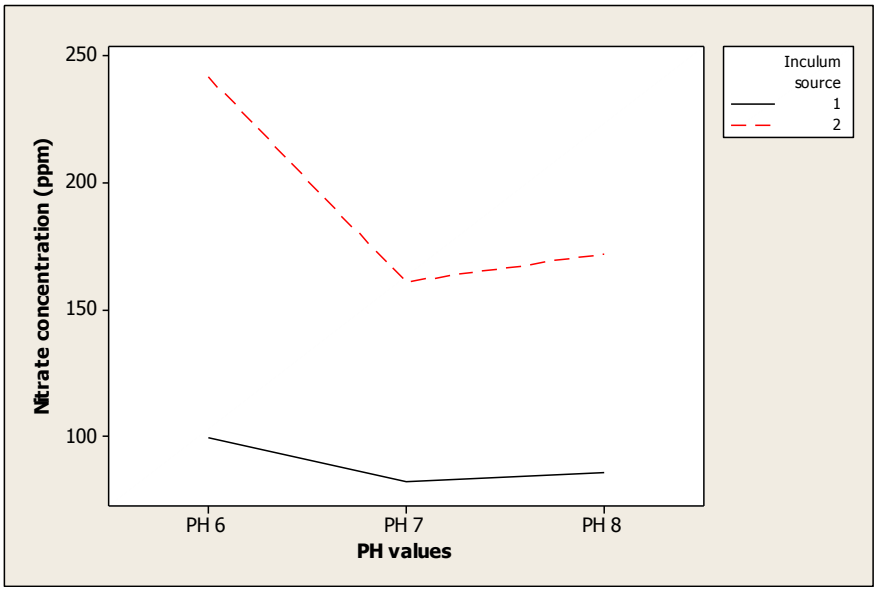

Fig. 6. Nitrate concentration (ppm) reduction, with different pH values.

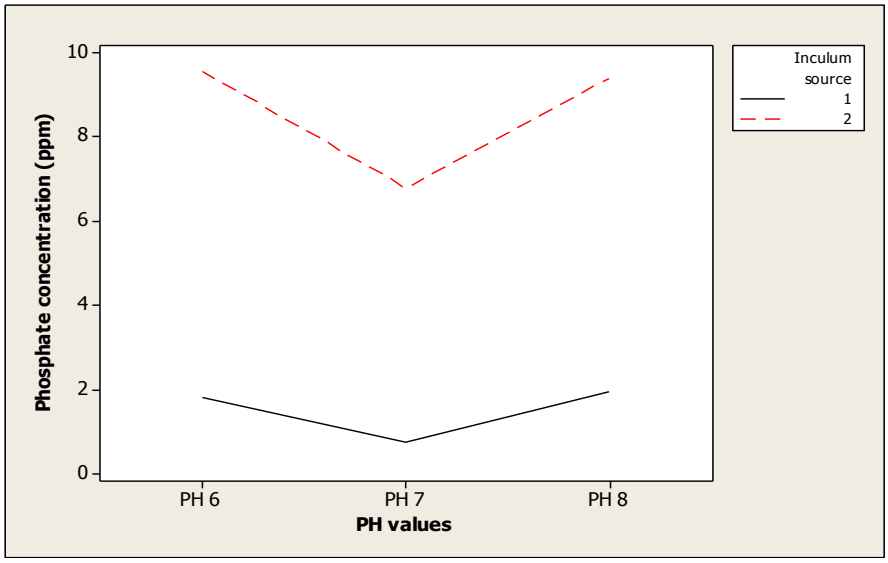

Fig. 7. Phosphate concentration (ppm) reduction, with different pH values.

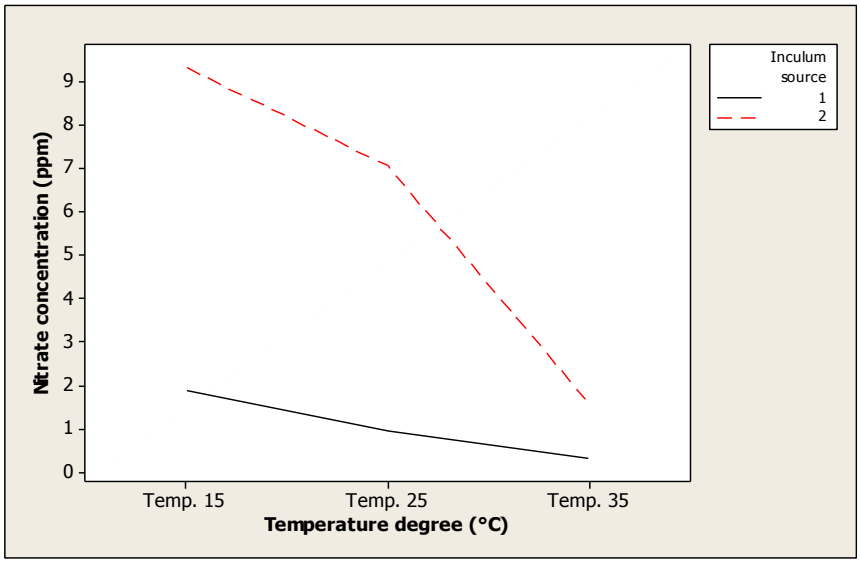

Fig. 8. Nitrate concentration (ppm) reduction, using different temperature degrees (°C).

Table 4. GLM test for variation in nitrate concentration (ppm) reduction (upper section of figure) and phosphate concentration (ppm) reduction (lower section of figure) among different temperature degrees (°C) and using different inoculum sources.

Growth media	Source	df	SS	AS	AM	F-value	P-value
	Columns	1	110.61	110.61	110.61	41.22	0.000
	Rows	2	66.74	66.74	33.37	12.44	0.001
	Error	14	37.57	37.57	2.68	-	-
	Total	17	214.92	-	-	-	-
Different inoculum sources	Source	df	SS	AS	AM	F-value	P-value
	Columns	1	57.14	57.14	57.14	110.45	0.000
	Rows	2	82.06	82.06	41.03	79.31	0.000
	Error	14	7.243	7.243	0.517	-	-
	Total	17	146.44	-	-	-	-

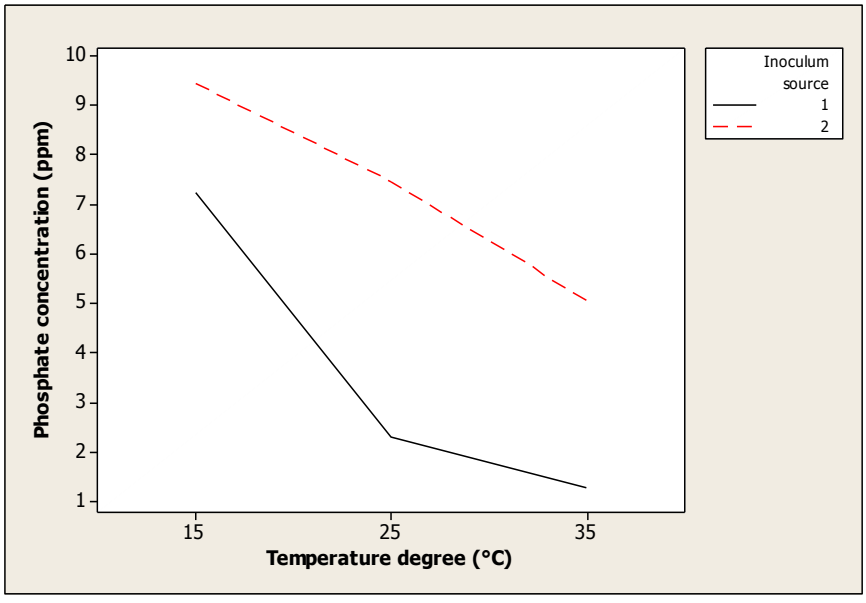

Fig. 9. Phosphate concentration (ppm) reduction, using different temperature degrees (°C).

Comparing the results of microbial growth increasing with the result of nitrate and phosphate concentrations (ppm) reduction, we can conclude that the highest microbial growth were (208.67 for wastewater inoculum and 617.00 for sludge inoculum) which coincide with the highest nitrate reduction (83.20 ppm and 160.90 ppm) and highest phosphate reduction (0.73 ppm and 6.79 ppm), respectively using pH 7 in the nutrient media

(wastewater and sludge inoculums separately), while the lowest values of nitrate reduction (99.37 ppm and 242.01 ppm) and the lowest phosphate reduction (1.79 ppm and 9.57 ppm) were recorded at pH 6 using wastewater as inoculum and sludge as inoculum respectively.

The study results were agreed with many other studies reported that the best pH value for nitrate and phosphate bioremediation depending on microbial activation is pH 7 [48-52]. Esfandiari et al. [3] reported that magnetite nanoparticles are more stable at pH 7, which made them appropriate for the water treatment application.

Also, the greatest microbial growth was 230 for wastewater inoculum and 1433.67 for sludge inoculum, which coincides with the highest values of nitrate reduction (0.31 ppm and 1.60 ppm), and the highest values of phosphate reduction (1.26 ppm and 5.06 ppm) at temperature 35 ℃ in the nutrient media (wastewater and sludge as inoculums separately). While the lowest values of nitrate reduction (1.87 ppm and 9.33 ppm) and the lowest values of phosphate reduction (7.23 ppm and 9.46 ppm) were recorded at 15 °C using wastewater and sludge as inoculums respectively.

The study results, in agreement with other many studies results, indicate that the nitrate and phosphate reduction decreases at diverse temperatures, and is more related to the bacteria sensitivity to the procedure optimum temperature [53,54]. According to Sibiya and Muzenda [55], the optimum temperature for nitrate and phosphate bioremediation is 35°C.

3.4. Influence of different concentrations of prepared Fe_3O_4 NPs on the microbial growth

The effect of different concentrations of Fe_3O_4 NPs ranged from 10 to 50 ppm on microbial growth, are presented in Fig. 10. An enhancing effect of Fe_3O_4 NPs on the microbial growth was detected when using Fe_3O_4 NPs with a concentration (40 ppm) for wastewater and sludge inoculum. However, a high-dose inhibition for microbial growth was observed with Fe_3O_4 NPs concentrations higher than 40 ppm. Significant variations ($P < 0.05$) in the absorbance pattern of microbial growth media at wavelength 450 nm among different inoculum sources using different concentrations of Fe_3O_4 NPs (Table 5).

Table 5. GLM test for variation in absorbance (450 nm) among different concentrations of the prepared Fe_3O_4 NPs capped with ascorbic acid using different inoculum sources.

Growth media	Source	df	SS	AS	AM	F-value	P-value
Different inoculum sources+ different concentrations of Fe_3O_4 NPs capped with ascorbic acid	Columns	1	2120907	2120907	2120907	1422.97	0.000
	Rows	5	113857	113857	22771	15.28	0.000
	Error	29	43224	43224	1490	-	-
	Total	35	2277988	-	-	-	-

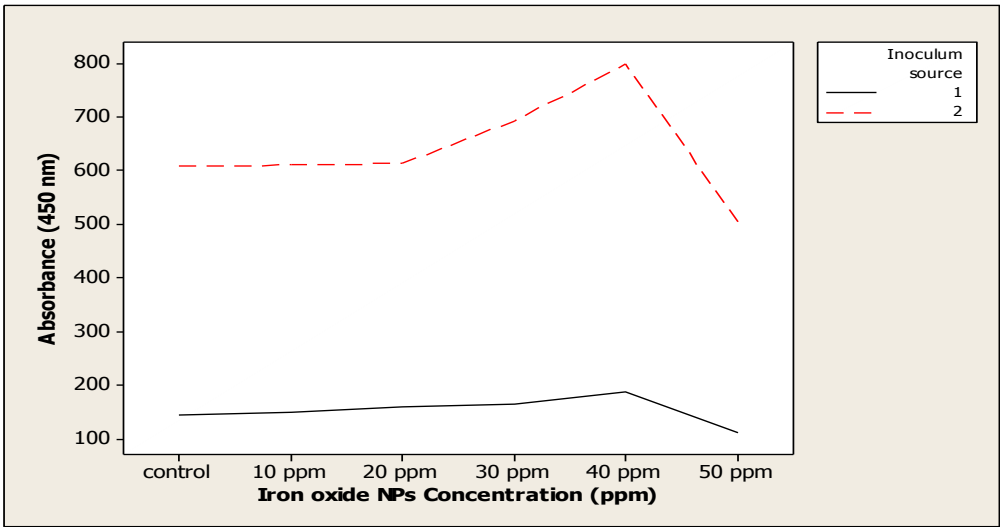

Fig. 10. Microbial growth media absorbance, using different concentrations of the prepared Fe_3O_4 NPs.

The study results, in agreement with those of [56-59], have approved and evaluated the enhancing effect of Fe_3O_4 nanoparticles on the microbial growth and activated sludge performance. Magnetite nanoparticles have commonly been used to enhance microbial activity as some bacteria might obtain energy aimed at their growth from ferrous (Fe^{2+}) oxidation to ferric (Fe^{3+}) [60]. Furthermore, Fe_3O_4 NPs have likewise known enzymes activators similar isocitratelyase which consumed by the microbial cells throughout microbes growth on the hydrophobic substrate, in addition to throughout acetyl-CoA incorporation into C4 complexes or throughout the bio-surfactant synthesis [61,62].

Many studies [63-66] were reported that heavy metals were significant for bionics besides might be required by the body in moderately little concentrations. For example, the vital heavy metals (Mn, Fe, Co, Ni, Mo, Cu, and Zn) were micronutrients otherwise trace elements for microbial organisms [67]. Moreover, magnetite nanoparticles with chemical inertness, biological compatibility, and less toxicity display an amazing possibility together with biotechnology and many environmental applications [9,22,68].

3.5. Influence of Fe_3O_4 NPs concentrations on dairy effluent nitrate and phosphate bioremediation

The effect of different microbial inoculum sources on nitrate and phosphate reduction (ppm) was examined using different concentrations of Fe_3O_4 NPs (ranged from 10 to 40 ppm) and presented in Figs. 11 and 12. The highest microbial growth was 188 for wastewater inoculum and 798.67 for sludge inoculum, which coincides with the highest nitrate reduction (64.12% and 48.54%) and the highest phosphate reduction (36.85% and 46.13%) using 40 ppm of Fe_3O_4 NPs in the nutrient media (wastewater and sludge inoculums separately) compared with the control sample after five days of incubation at pH 7 and temperature 35 °C.

The nitrate and phosphate concentration (ppm) reduction were linked linearly with the increasing concentration of Fe_3O_4 NPs from 10 to 40 ppm for wastewater and sludge inoculums. A higher concentration of Fe_3O_4 NPs showed some lower bioremediation efficacy. Significant variations ($P < 0.001$) in nitrate and phosphate (ppm) reductions among different inoculum sources using different Fe_3O_4 NPs concentrations (Table 6).

Table 6. GLM test for variation in nitrate concentration (ppm) reduction (upper section of figure) and phosphate concentration (ppm) reduction (lower section of figure) among different concentrations of the prepared Fe_3O_4 NPs using different inoculum sources.

Growth media	Source	df	SS	AS	AM	F-value	P-value
Different inoculum sources+ different concentrations of Fe_3O_4 NPs capped with ascorbic acid	Columns	1	4408.07	4408.07	4408.07	430.33	0.000
	Rows	5	581.09	581.09	116.22	11.35	0.000
	Error	29	297.06	297.06	10.24	-	-
	Total	35	5286.23	-	-	-	-
	Source	df	SS	AS	AM	F-value	P-value
	Columns	1	6347225	6347225	6347225	321.17	0.000
	Rows	5	565615	565615	113123	5.72	0.001
	Error	29	573121	573121	19763	-	-
	Total	35	7485961	-	-	-	-

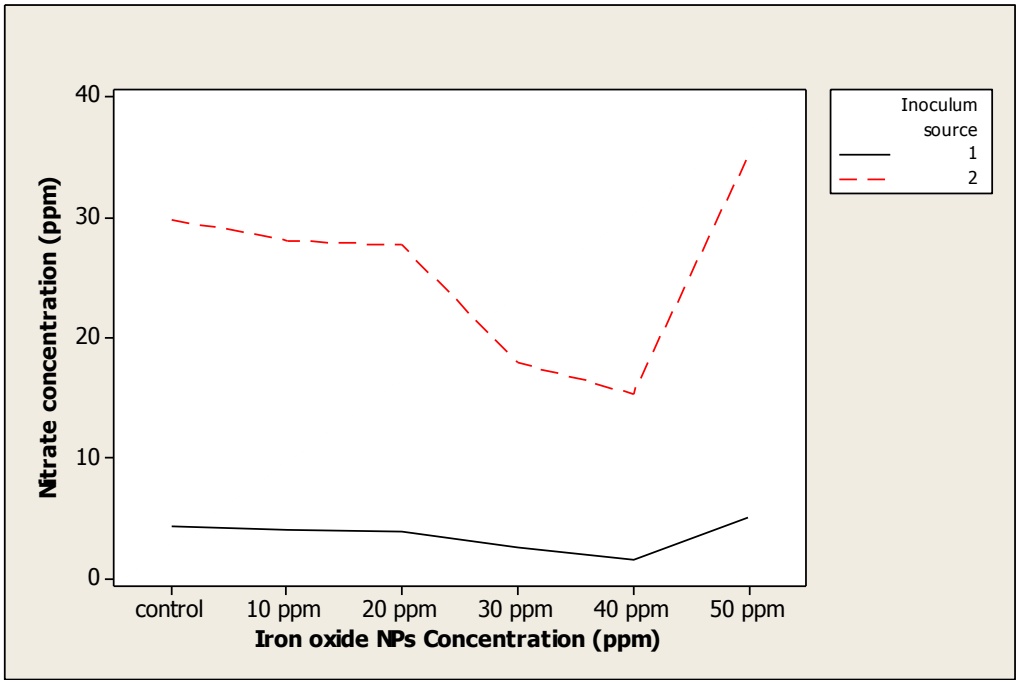

Fig. 11. Nitrate concentration (ppm) reduction pattern, using different concentrations of the prepared Fe_3O_4 NPs.

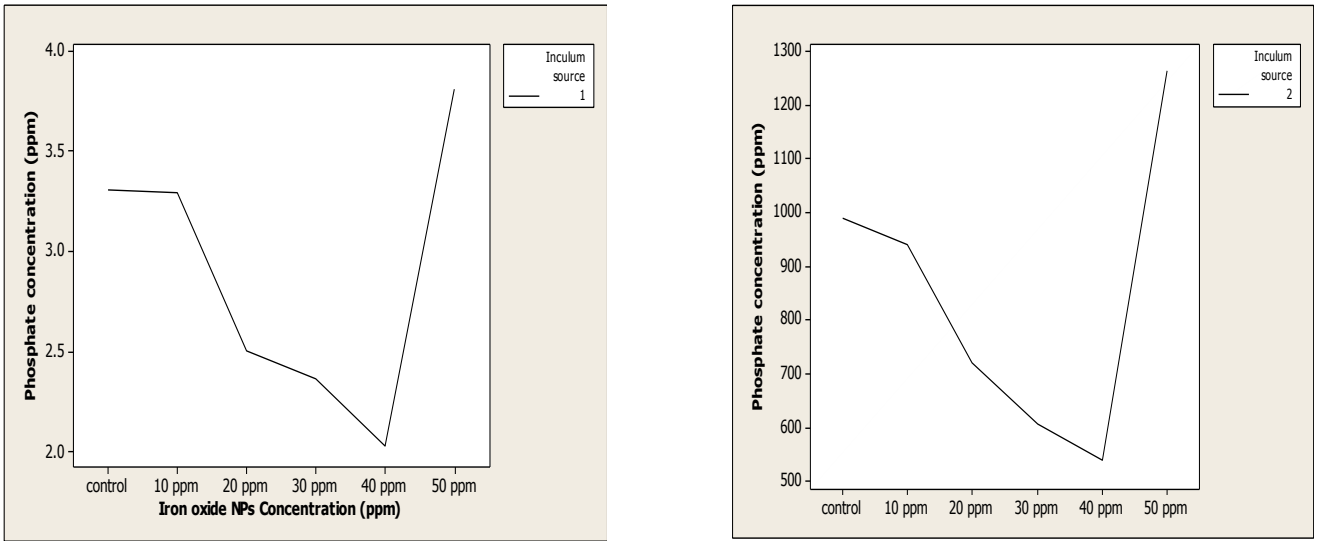

Fig. 12. Phosphate concentration (ppm) reduction pattern, using different concentrations of the prepared Fe_3O_4 NPs (left figure using wastewater as inoculum source while the right figure using sludge as inoculum source).

The study results agree with some previous studies that the magnetite nanoparticles could be used for the decomposition of contaminants involved in the wastewater effluent, generally for the industrial sewage treatment. Thus, they could be used for the reduction of nitrates, phosphates and heavy metals in the water [69,70].

4. CONCLUSION

The bacterial growth and the enhancing of nitrate and phosphate bioremediation have a linear relationship with the increase of Fe_3O_4 NPs concentration from 10 up to 40 ppm in the following order: 40 ppm > 30 ppm > 20 ppm > 10 ppm at pH 7 and temperature 35℃ ±2.

The nitrate reduction efficacy using wastewater as inoculum source was enhanced up to 64.12% compared to control, and for phosphate the reduction was enhanced up to 36.85% compared to control. When using activated sludge as an inoculum source the reduction of nitrate was enhanced up to 48.54% compared to control, and the phosphate reduction was enhanced up to 46.13% compared to control.

It is concluded that using diverse inoculum sources along with 40 ppm of the prepared Fe_3O_4 NPs capped with ascorbic acid is an effective technique for dairy effluent nitrate and phosphate bioremediation.

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SUPPLEMENTARY INFORMATION

Annex 1. Average (± SD) of microbial growth media absorbance at (450 nm), using different inoculum sources and different pH values and temperature degrees (°C) (highest and lowest values are underlined)

Environmental parameter	Values	Average ± SD of growth media absorbance at (450 nm) using wastewater as inoculum	Average ± SD of growth media absorbance at (450 nm) using sludge as inoculum
pH	6.00	148.66 ± 18.03	412.33± 10.02
	7.00	208.67 ± 10.69	617.00 ± 40.95
	8.00	182.06 ± 11.43	535.00 ± 13.23
Temperature	15.0°C	190 ± 2.64	934.33 ± 46.80
	25.0°C	195 ± 3.00	1166.33 ±24.09
	35.0°C	230 ± 16.64	1433.67 ±128.44

Annex 2. Average (± SD) of nitrate and phosphate concentration (ppm) reduction, using different inoculum sources and different PH values (highest and lowest values are underlined)

Growth media	PH Values	Average ± SD nitrate reduction using wastewater inoculum (450 nm)	Average ± SD nitrate reduction using sludge inoculum (450 nm)
Different inoculum sources	6.00	99.37 ± 5.00	242.01 ± 13.37
	7.00	83.20 ± 3.22	160.90 ± 7.88
	8.00	85.59 ± 7.49	171.96 ± 7.51
	PH Values	**Average ± SD phosphate reduction using wastewater inoculum (450 nm)**	**Average ± SD phosphate reduction using sludge inoculum (450 nm)**
	6.00	1.79 ± 0.07	9.57 ± 0.10
	7.00	0.73 ± 0.06	6.79 ± 1.04
	8.00	1.68 ± 0.50	9.40 ± 1.045

Annex 3. Average (± SD) of nitrate and phosphate reduction, using different inoculum sources and different temperature degrees (°C) (highest and lowest values are underlined)

Growth media	Temperature degrees (°C)	Average ± SD nitrate reduction using wastewater inoculum (450 nm)	Average ± SD nitrate reduction using sludge inoculum (450 nm)
Different inoculum sources	15.00	<u>1.87 ± 0.47</u>	<u>9.33 ± 1.44</u>
	25.00	0.94 ± 0.12	7.06 ± 0.74
	35.00	<u>0.31 ± 0.20</u>	<u>1.60 ± 0.17</u>
	Temperature degrees (°C)	**Average ± SD phosphate reduction using wastewater inoculum (450 nm)**	**Average ± SD phosphate reduction using sludge inoculum (450 nm)**
	15.00	<u>7.23 ± 0.49</u>	<u>9.46 ± 0.56</u>
	25.00	2.80 ± 0.36	7.46 ± 0.33
	35.00	<u>1.26 ± 0.40</u>	<u>5.06 ± 0.61</u>

Annex 4. Average (± SD) of microbial growth media absorbance at (450 nm), using different inoculum sources and change % of absorbance than control, using different concentrations of iron oxide nanoparticles (highest and lowest values are underlined)

Growth media	Nanoparticles concentration (ppm)	Average ± SD absorbance using wastewater inoculum (450 nm)	Change % of absorbance	Average ± SD absorbance using sludge inoculum (450 nm)	Change % of absorbance
Different inoculum sources + Fe3O4 NPs capped with ascorbic acid	0.00	145.33 ± 11.06	0.00	607.67 ± 0.58	0.00
	10.00	<u>149.00 ± 3.61</u>	2.53	<u>610.67 ± 1.53</u>	0.49
	20.00	158.33 ± 5.03	8.95	613.33 ± 2.52	0.93
	30.00	163.33 ± 4.073	12.38	693.00 ± 11.72	14.10
	40.00	<u>188.00 ± 2.65</u>	29.36	<u>798.67 ± 2.52</u>	31.43
	50.00	111.67 ± 6.03	-23.16	504.67 ± 7.37	-16.95

Annex 5. Average (± SD) of nitrate and phosphate concentration (ppm) reduction, using different inoculum sources and change % of reduction than control, using different concentrations of iron oxide nanoparticles (highest and lowest values are underlined)

Growth media	Nanoparticles concentration (ppm)	Average ± SD nitrate reduction using wastewater inoculum (450 nm)	Change % of reduction	Average ± SD nitrate reduction using sludge inoculum (450 nm)	Change % of reduction
Different inoculum sources + Fe3O4 NPs capped with ascorbic acid	0.00	4.32 ± 0.05	0.00	29.83 ± 0.06	0.00
	10.00	4.02 ± 0.03	-6.94	28.13 ± 0.02	-5.69
	20.00	3.80 ± 0.23	-12.03	27.71 ± 0.35	-7.10
	30.00	2.51 ± 0.44	-41.89	17.96 ± 0.12	-39.79
	40.00	1.55 ± 0.31	-64.12	15.35 ± 0.87	-48.54
	50.00	5.10 ± 0.01	18.05	35.11 ± 1.11	17.70
	Nanoparticles concentration (ppm)	**Average ± SD phosphate reduction using wastewater inoculum (450 nm)**	**Change % of reduction**	**Average ± SD phosphate reduction using sludge inoculum (450 nm)**	**Change % of reduction**
	0.00	3.31 ± 0.11	0.00	989.42 ± 21.60	0.00
	10.00	3.29 ± 0.07	-0.30	940.71 ± 11.34	-4.92
	20.00	2.50 ± 0.32	-24.47	721.14 ± 37.72	-27.11
	30.00	2.36 ± 0.28	-28.07	606.25 ± 48.42	-38.72
	40.00	2.09 ± 0.74	-36.85	533.88 ± 29.31	-46.13
	50.00	3.81 ± 0.57	15.11	1264.63 ± 36.18	27.82