Bioremediation of Nitrates from Selected Water Bodies within the Tarkwa Nsuaem Municipality

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Abstract

Nitrate-containing compounds in the soil are generally soluble and readily migrate into surface and ground waters. Though they act as nutrients in soils, streams and rivers, excess concentration causes eutrophication, while oxidation of nitrites (NO_2^-) to nitrates (NO_3^-) in fresh water causes oxygen depletion in the water. Some water quality studies in the Tarkwa Nsuaem Municipality have reported high concentrations of nitrate, exceeding the WHO guideline value of 50 mg/L. This paper therefore set out to identify the potential sources of the nitrates and nitrites in the Tarkwa Municipality, and to stimulate a denitrifying environment using the bacteria, *Thiobacillus denitrificans*. This was achieved through field visits and water sampling from Bediabewu River and around an explosive manufacturing plant, followed by incubation with *T. denitrificans.* The sources of nitrates were identified as handling, manufacturing and use of explosive materials for mining activities, as ammonium nitrate is the major raw material in the explosives. The residue from explosive manufacturing plants flowed from active sumps with an average concentration of 22180 mg/L into nearby water bodies like the Bediabewu River, which had an average concentration of 380 mg/L. The bacteria required an adaptation period of 7 to 8 days, with denitrification activity observed on the 8th and 9th days of incubation. A batch treatment of standard and field samples resulted in about 90% denitrification within 8-9 days. The bacteria were active at a temperature range of 27 ^oC – 30 ^oC and pH values above 5. The results demonstrate the ability of *T. denitrificans* to denitrify nitrates in the environment. With further investigations, the bacteria can be adapted, in combination with phytoremediation, to treat excess process water from the manufacturing plants and through the discharge routes into the nearby water bodies.

Keywords: Nitrate Wastewater, *Thiobacillus denitrificans*, Denitrification, Tarkwa Municipality

1 Introduction

Nitrogen is one of the most abundant elements in the environment, constituting about 78% of air. It is found in the cells of all living things and it is a major component of proteins. Inorganic nitrogen may exist in the free-state as a gas (N_2), or as a nitrate (NO_3^-), nitrite (NO_2^-), or ammonia (NH_3^+) (Galloway *et al.*, 2004).

Nitrate (NO_3^-) and nitrite (NO_2^-) are inorganic ions that are part of the nitrogen cycle. Microbial action in soil or water decomposes wastes containing organic nitrogen into ammonia, which is then oxidised to nitrite and nitrate. Because nitrite is easily oxidised to nitrate, nitrate is the compound predominantly found in groundwater and surface waters. Nitrate-containing compounds in the soil are generally soluble and readily migrate into groundwater (Pitt *et al*., 1999).

Nitrogen-containing compounds act as nutrients in streams and rivers and, when in excess, causes eutrophication. Oxidation of nitrites (NO_2^-) to nitrate (NO_3^-) in fresh water causes oxygen depletion. Thus, aquatic organisms depending on the supply of oxygen in the streams and rivers may die. It is therefore important to remove these contaminants or at least minimise their concentrations and hence their danger to health. The major routes of entry of nitrates into water bodies are municipal and industrial wastewater, septic tanks, animal wastes, fertilisers and from the use of explosives in the mining industry. Bacteria species such as Nitrobacter in aerobic conditions

convert nitrites (NO_2^-) to nitrates (NO_3^-) (Sliekers *et al.,* 2002).

Water quality studies done in the Tarkwa Nsuaem municipality and bore holes and wells found high levels of nitrates and nitrites within the Akoon Green Compound and Brenu Akyim and the Bediabewu River in the Tarkwa municipality. According to Quansah and Amankwah (2010), high concentrations of nitrate, exceeding the WHO guideline value of 50 mg/l were detected in both the Akoon Green compound borehole (61 mg/l) and the Brenu Akyim well (59 mg/l).

The possible cause of this pollution can be attributed to active manufacturing of explosives and blasting activities of various mines, "Galamsey" operators and use of fertilisers in the municipality (Quansah and Amankwah, 2010). Nitrate can be reduced or removed from water by three main methods. Demineralistion by distillation or reverse osmosis, Ion exchange and Blending.

Demineralisation removes nitrate and all other minerals from the water. Distillation is one of the oldest, most effective type of demineralisation. The nitrate and other minerals remain concentrated in the boiling tank. Another method to demineralise water is reverse osmosis. It reduces but does not remove all nitrates. Water is put under pressure in a reverse osmosis system and forced through a membrane that removes minerals and nitrate. One-half to two-thirds of the water remains behind the membrane as rejected water. The quantity of treated water to reject water is associated with the pressure applied. A lower water pressure connotes a greater volume of reject water. Greater-yield systems use water pressures above 150 psi (Adams *et al*., 2007).

Ion exchange presents another substance that exchanges places with the nitrate. Most often, nitrate is replaced by chloride. The ion exchange unit is a tank filled with special resin beads that may be charged with chloride. As water containing nitrate flows through the tank, the resin takes up nitrate in exchange for chloride. All the chloride will be exchanged for nitrate in time. The resin can then be recharged by back washing with a brine solution (sodium chloride) and reused and, hence, can be used for treating large volumes of water (Sallenave *et al*., 2017).

There are, however, some downsides to ion exchange systems. First, in addition to exchanging nitrate, the resin beads will also take up sulphate in exchange for chloride (Swistock *et al.,* 2014). Therefore, if sulphates are present in the water supply, the capacity of the resin to take up nitrate reduces. Secondly, the resin may also make the water corrosive and for this reason, the water must go through a neutralising system after going through the ion exchange unit. Finally, backwash brines, containing high levels of nitrate, must be disposed of properly so they do not re-contaminate the groundwater supply.

The final way to reduce nitrates is to dilute the nitrate-contaminated water by mixing it with water from an alternate source that has low nitrate concentrations. Blending the two sets of water from different sources produces water that is low in nitrate concentration. Blended water is not completely safe but can and is mostly used for other applications. However, boiling and filtration have zero impact on reducing the nitrate content (Sallenave *et al*., 2017).

Hence, there is the need for a better, efficient and effective way of removing nitrates from water using microbial systems. This would have little to no after-effect and complications as compared with the existing methods of removing nitrates from water. Bioremediation is a waste management technique that involves the use of biological agents to remove or neutralise pollutants from contaminated sites, water bodies and other environments (Shah, 2014). The elimination of a wide range of pollutants and wastes from the environment requires increasing understanding of the relative importance of microbes in our environment and, hence, will certainly accelerate the development of bioremediation technologies and biotransformation processes.

Denitrification is the conversion of nitrate (NO_3^-) to nitrogen gas (N_2). Heterotrophic bacteria (capable of utilising only organic materials as a source of food) utilise the nitrate as an oxygen source under anoxic conditions to break down organic substances (Smil, 2000). Most denitrifiers produce nitrous oxide (N_2O) instead of di-nitrogen (N_2) under aerobic conditions. Examples of denitrifying bacteria include *Thiobacillus denitrificans* and *Paracoccus denitrificans* (Takaya *et al*., 2003).

Thiobacillus denitrificans belongs to an important group of autotrophic bacteria occurring in nature linking the biogeochemical cycles of nitrogen and sulphur. It has a biosafety level of one and, hence, non-pathogenic (Beller *et al.*, 2006). *T. denitrificans* is a facultative anaerobe and uses a simple denitrification process to convert nitrogen oxides back to gaseous nitrogen as shown in Equation 1. The nitrate dissociation leads to the release of energy which is utilised by the microbes for normal metabolism.

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \quad (1)$$

With the aim to mitigate the levels of nitrates in the environs of Tarkwa Nsuaem using *T denitrificans*, this paper therefore set out to identify the potential sources of the nitrates and nitrites in the Tarkwa Municipality, and to stimulate a denitrifying environment using the bacteria, *Thiobacillus denitrificans.*

2 Resources and Methods Used

Two series of batch experiments (A - D) were prepared to verify the course and extent of denitrification by *Thiobacillus denitrificans.* The experiments were carried out under different conditions in an MRC LOM-150 Orbital Shaker Incubator at a set temperature to avoid contamination and provide a controlled environment for the set-ups.

2.1 Materials

The sulphidic ore with a sulphur content of 2% used in this experiment was obtained from a mine in West Africa sub-region while the precipitated sulphur was obtained from University of Mines and Technology, Minerals Laboratory. Measuring equipment used for the research was obtained from the University of Mines and Technology, Minerals Laboratory except for the Nitrate Ion meter that was bought from Bante Instruments in China. The major equipment included: Ohaus PA214 Laboratory Scale Balance, ADWA AD 132 pH/mV Meter, Schott Handy Lab pH 11 Meter, MRC Steriliser Autoclave Model 9, MRC LOM-150 Orbital Shaker Incubator and Bante 321 Nitrate Ion Meter.

The bacteria were received as freeze-dried cells which was rehydrated and grown in appropriate medium for use during this research.

2.2 Culture Preparation and Growth of Bacteria

The vial containing the freeze-dried bacterial cells was opened and diluted with 10 ml of 295 S8 medium. Approximately, 2 ml of the aliquot was contacted with 250 ml of 295 S8 broth medium in tubes and incubated at 30 ^oC for 7 days. After 7 days, 0.2 ml of the cloudy broth, indicating growth was used to inoculate the bacteria on Agar plates. Growth was observed on the Agar plates after 7-14 days of inoculation as orange colonies of the microorganism. Figure 1 shows sterile Agar plates with inoculations from the microbial cultures.

Fig. 1 Sterile Plates with Bacteria Culture

2.3 Simulation of Nitrate Environment

A series of batch experiments were prepared to verify the activity of the Thiobacillus denitrificans in an artificial environment. This experiment was carried out in Erlenmeyer flasks and was subjected to similar experimental condition (temperature and environment). The Erlenmeyer flasks were labelled A to D, where A and D contained distilled water, B contained distilled water and sulphidic ore while C contained distilled water and elemental sulphur. All the flasks (A – D) contained nitrate and *T. denitrificans* with the exception of bottle D which contained no *T. denitrificans*. Each bottle containing 200 ml of nitrate solution was inoculated with 2 ml of the bacteria culture with 10 g of sulphidic ore added to flask B while 10 g of precipitated sulphur was added to flask C. The experiment was kept in the MRC Orbital Shaker Incubator for 21 days with daily checks of both pH and nitrate level.

2.4 Water Sampling and Laboratory Testing

Random water samples were taken at four different sampling points (Sampling Point A, Sampling Point B, Bediabewu River and from the Maxam Plant as the Source) and combined to form a single sample. Figure 2 shows an Aerial view showing the Bediabewu River located at the AngloGold Iduapriem Mine about 60 meters from the Maxam Plant premises located at the following coordinates 5.247123, -2.046455. Red markers show river sample collection points, yellow shows sampling point A and orange for sampling point B. Where A and B are designated sampling points with water sources from the plant sumps which ends up finally in the Bediabewu River.

Fig. 2 Aerial view showing the Bediabewu River and the Maxam Plant. (Source: Google maps, 2018)

The samples were first diluted before denitrification using a dilution of 1 ml of the samples to 99 ml deionised water. Three out of the four samples were selected and subjected to denitrification process using *T. denitrificans* culture. The denitrification test on the field samples was monitored for 14 days with daily monitoring of pH and nitrate concentration levels. This was done to assess the level of denitrification as a function of pH and drop in the nitrate concentration. Table 1 depicts the experimental conditions to which the laboratory simulations were subjected while Table 2 shows the alphanumeric coding used to represent the various water sample sources and the microbial conditions under which the observation and analysis was done for the field samples.

Table 1 Experimental Conditions of the Laboratory Simulated Samples

	Bacteria Only	Bacteria and Sulphidic Ore	Bacteria and Precipitated Sulphur	Control
Source Sample	A1	B1	C1	D1
Sampling Point A	A2	B2	C2	D2
Sampling Point B	A3	B3	C3	D3

Table 2 Experimental Conditions of the Set-up for the Field Samples

Parameter	Condition
Initial Concentration of Nitrates	650 mg/L
Temperature	27 ^{o}C
Set-up A	Bacteria Only
Set-up B	Bacteria with Sulphidic Ore
Set-up C	Bacteria with Precipitated Sulphur
Set-up D	Blank (Control)

3 Results and Discussions

3.1 Determination of Optimum Remedial Time

The optimum remedial period of the *Thiobacillus denitrificans* was determined, and Fig. 3 shows the microbial activity on the artificial nitrate solution samples used for the laboratory simulations.

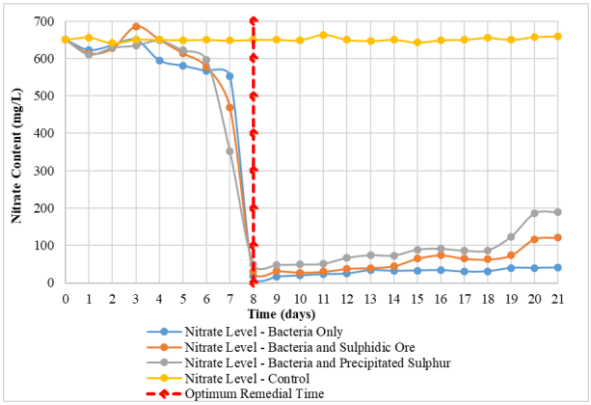

Fig. 3 Changes in Nitrate Level with Time under Artificial Conditions

Growth and activity of the microbes in the medium as shown in Fig. 3 follows the model with four different phases which has been summarised in Table 3.

Table 3 Microbial Activity for Artificial Nitrate Solution Simulations

Growth Phase	Period (Days)
Lag	0 – 6
Exponential	6 – 8
Stationary	8 – 14
Death	14 – 21

The results from the laboratory simulations indicate the ideal conditions observed during the bioremediation process.

3.2 Activity of Microorganisms

The microorganisms required an adaptation period of 0 – 3 days as observed in Fig. 3 before significant decrease in the nitrate content. The effective period of 8 – 9 days is required for an extensive remediation to occur in the medium. From the Laboratory simulations, the set-up with Bacteria only, Bacteria and Sulphidic Ore and Bacteria and Precipitated Sulphur had an effective reduction potential of 97.38%, 95.07% and 92.62% respectively. Autotrophic denitrification using *Thiobacillus denitrificans* is influenced by several agents and factors. The factors involve the type of electron donor (sulphide, elemental sulphur or thiosulphate), temperature and pH.

Nitrate removal in autotrophic denitrification is accompanied by the production of hydrogen ions causing a drop in pH using elemental sulphur as shown by Equation 2 (Blazková *et al*., 2017):

$$5S + 6NO^{3-} + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+ \qquad (2)$$

The precipitated sulphur however could not dissolve in the medium. This can only dissolve in organic medium such as tetrachloromethane hence the introduction of soil samples with a known concentration of sulphur. The microbes were however able to work in all set-ups since the microbe culture used had some amount of sulphate present during the medium preparation.

The sulphur serves as the energy source and nitrate as a terminal electron acceptor under anaerobic conditions. Also, high nitrate-nitrogen concentrations above 660 mg/L reduced the rate of sulphur-driven autotrophic denitrification. The high level of water hardness and the presence of sulphide in the effluent serve as limiting factors. This is caused by the precipitation crust formed by calcium sulphide on the sulphur surface during denitrification using sulphur and limestone as a pH modifier (Wang *et al*., 2017).

The temperature of the set-ups was maintained between 27 ºC – 30 ºC within the MRC Orbital Shaker Incubator. This facilitated the remediation process. Figure 4 shows the pH trend observed during the laboratory simulations and has been discussed below.

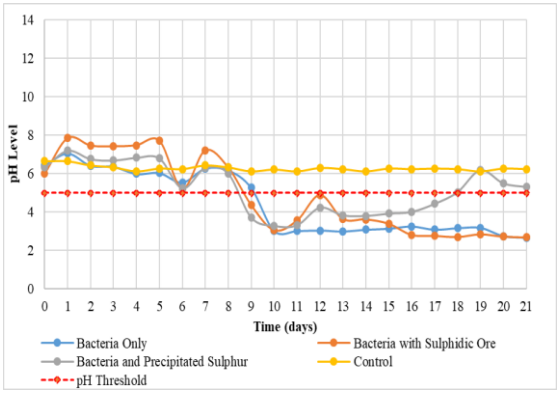

Fig. 4 Combined pH for Laboratory Simulation

During the denitrification by *Thiobacillus denitrificans*, sulphur is oxidised to sulphuric acid, which acidifies the environment. Denitrification processes are decreased when pH drops below 5. Therefore, it is necessary to add limestone (as a pH modifier) to sustain the reaction (Blazková and Palarcik, 2014). The limestone reacts with sulphuric acid to form calcium sulphate and carbon dioxide. The calcium sulphate however is a limiting factor for the denitrifying activity (Koenig and Liu, 2004) when the electron donor is sulphur. Carbon dioxide however can be utilised as the carbon energy source by *Thiobacillus denitrificans* (Liu and Koenig, 2002; Moon *et al*., 2004). Nevertheless, results from the study show some denitrification activity in pH values below 5 that can be attributed to carbon as the energy source. The growth of the denitrifying microbes is limited in low pH medium and hence the observed trend in most of the set-ups (Wang *et al.*, 2009). No significant changes in the Control were observed since the set-up only contained deionised water without microbes.

3.3 Tests on Field Samples

Samples collected from the field were analysed and results presented in Table 4 indicate significant levels of nitrate in the various sampling points. This indicates that the contaminations are high at the manufacturing plant facility, and smaller amounts of the nitrate eventually end up at the designated sampling points.

Table 4 Summary of Results from Testing of Field Samples

Sample ID	Nitrate Content (mg/L) Dilution – 1:100 ml	Nitrate Content (mg/L) Actual Value	pH (Average)
Plant Source	221.8	22180	6.12
Sampling Point A	18.8	1880	7.45
Sampling Point B	4.8	480	7.03
Bediabewu River	3.8	380	8.85

The high value of nitrate (22180 mg/L) was recorded at the explosive manufacturing plant site during the operational cycle whilst manufacturing was on-going, accounting for the high reading. The significant drop in the concentrations at sampling points A and B may be due to dilution from rain water and other processed water from the plant (Johnson, 1969), phytoremediation and solar disinfection (Carreiral, 1995; Susarla, 2002; Richard *et al*., 2007). The nitrate contamination identified in the Bediabewu River can be traced to the explosive manufacturing site and some of the active heaps within the immediate vicinity. In cases of severe rainfall, sumps containing significant levels of nitrates at the explosive manufacturing site overflow into the nearby water system, leading to nitrate contamination in the nearby water bodies.

3.3.1 Bioremediation Test on Field Samples

Results from the nitrate remediation carried out on the plant source samples are shown in Fig. 5. Tests using samples from the plant source followed the artificial nitrate solution simulations; however, there were a few variations due to the presence of other microbes and contaminants in the samples that could not be isolated.

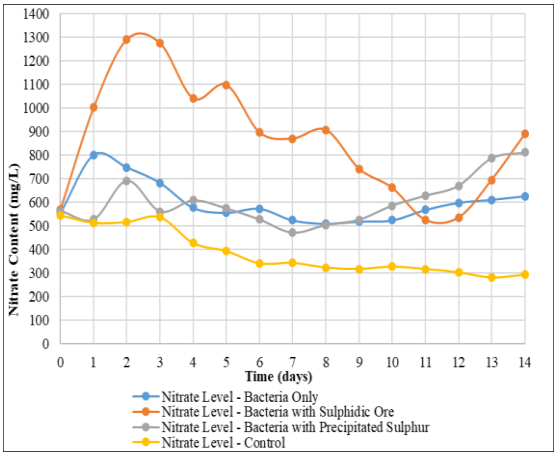

Fig. 5 Plant Source Combined Nitrate Reduction

From Fig. 5, it is observed that though there is reduction in the nitrate content it did not fall below the accepted WHO guideline value of 50 mg/L. This can be attributed to the high nitrate content and the presence of other compounds in the field samples collected. Also, the pH of the set-up followed the expected path as the initial laboratory simulations discussed from Fig. 4. The presence of calcium ions greatly retards the action of the microbes and hence the low level of remediation as compared to the artificial nitrate solution simulation which contained only the nitrates.

The use of the sulphidic ore also introduced some foreign ions which were not accounted for into the system as the sulphur content could not be isolated. This was to mimic the prevailing soil conditions where the water samples are located as well as the Bediabewu River. The increase in the nitrate level of the sulphidic ore step up can be attributed to the adaptation of the microbes under the new conditions. Fig. 6 indicates the trend of pH during the period of testing using the microbes on the plant source samples. The results show that there was some microbial disinfection at pH values below 5.

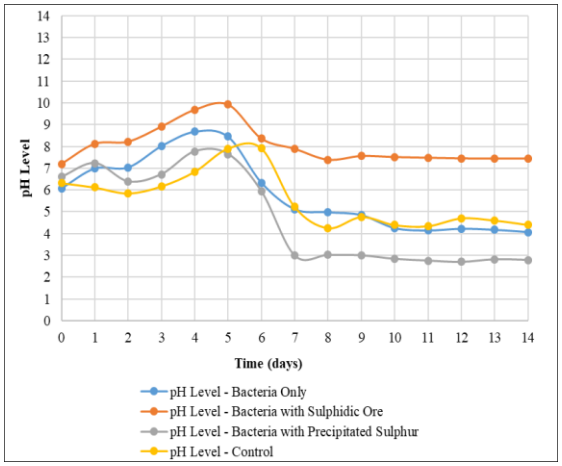

Fig. 6 Plant Source - Combined pH Readings from Set-ups

The remediation dropped after day 7 which had pH values lower than 5 due to the formation of some sulphuric acid. This has also been observed by Blazková and Palarcik (2014). For the Control set-up, there was some form of degradation of the nitrate level due to the presence of microbes naturally found in the environment where the samples were collected.

3.3.2 Sampling Point A

Figure 7 shows the graphical representation of the nitrate degradation in relation to the microbial activity and the field sample from Point A.

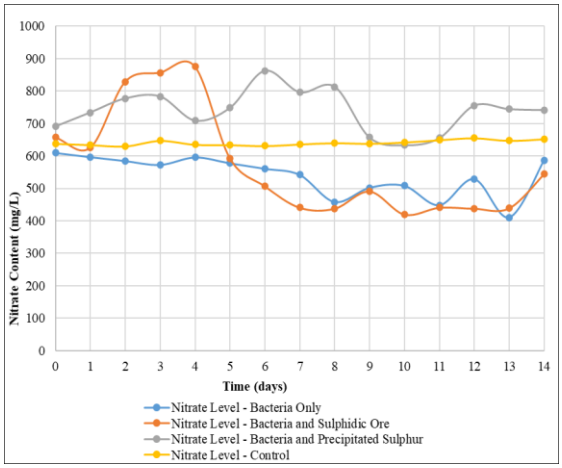

Fig. 7 Sampling Point A - Combined Nitrate Reduction

Figure 8 shows the graphical representation of the pH and period of microbial activity of the field sample from Point A. This also followed the observed trend as shown from the Plant Source sample analysis with the microbes, where there was gradual reduction in microbial activity because of low pH values.

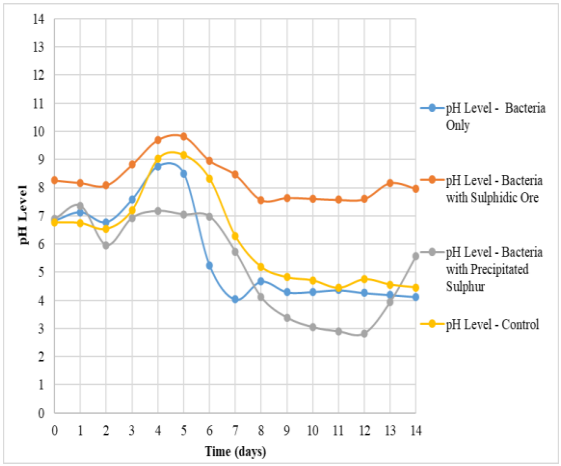

Fig. 8 Changes in pH Level of Water Samples with Time - Sampling Point A

The results from Fig. 8 indicated a phenomenon corresponding to the growth of microbes at a constant temperature of the set-ups with no pH control. The remediation trend as observed in Fig. 7 was different from the artificial nitrate solution tests with the laboratory samples where no significant remediation was observed. This can be attributed to water hardness, presence of biogenic elements, other compounds and the formation of calcium sulphide. The remediation also declined after day 7 which had pH value lower than 5 due to the formation of sulphuric acid as reported also by a research done by Blazková and Palarcik in 2014. Degradation of nitrate in the Control sample was observed due to the presence of microbes naturally found in the environment where the samples were collected.

3.3.3 Sampling Point B

Figure 9 shows the graphical representation of the nitrate degradation in relation to the microbial activity. This indicates the phenomenon corresponding to the growth of microbes at a constant temperature of the set-ups with no pH control.

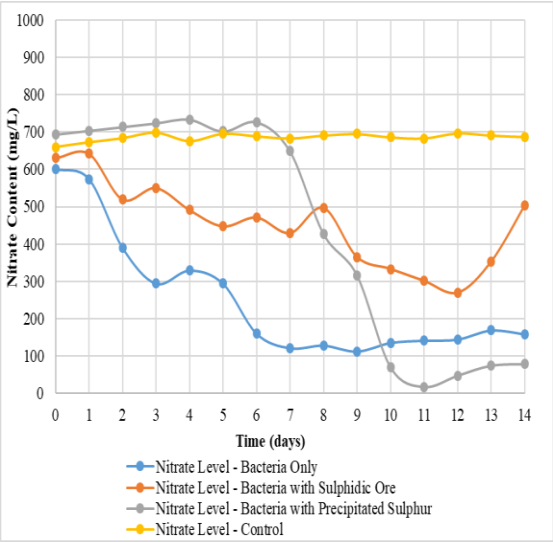

Fig. 9 Changes in Nitrate content with time - (Sampling Point B)

However, the remediation trend was similar to Sampling Point A. Significant remediation was observed from the set-ups after day 7 which had pH values lower than the initial, between pH 7 and 8. This can be attributed to the exponential growth of the microbes leading to reduction in nitrate concentrations and generation of sulphuric acid. The graphical representation of the pH and period of microbial activity is presented in Fig. 10.

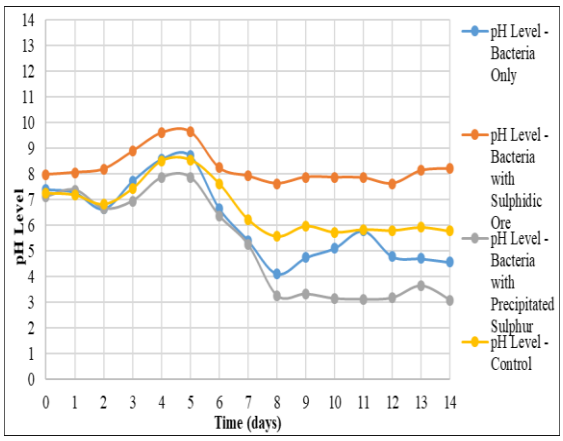

Fig. 10 Changes in pH Level of Water Samples with Time - Sampling Point B

There was no significant degradation of the nitrate level in the Control set-up which deviated from the other samples from the field.

4 Conclusions

The following conclusions drawn from the discussions of the results obtained from this research indicates that the residue from explosive manufacturing plants flows from active sumps with a concentration of 22180 mg/L to nearby water bodies like the Bediabewu River which had a concentration of 380 mg/L. This, however, is still above the allowable WHO standard of 50 mg/L. Denitrification investigations conducted using the artificial nitrate solution samples and the field samples showed that, *Thiobacillus denitrificans* can denitrify the nitrates in the environment. The bacteria required an adaptation period of 7 to 8 days with denitrification activity of up to 90% observed on the 8th and 9th day in both the artificial simulated nitrate solution and test work on the actual field samples. The bacteria were active at a temperature range of 27 ºC – 30 ºC and pH values above 5. The results obtained clearly indicates the potential use of *Thiobacillus denitrificans* in denitrification of contaminated water bodies. Further investigations involving domestication of the bacteria with phytoremediation are required to treat excess process water from the manufacturing plants and through the discharge routes into the nearby water bodies.

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