International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 2, Feb 2016 IF- 2.818 ISSN: (2349-4077)
© Associated Asia Research Foundation (AARF)
Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

INFLUENCE OF SOME ENVIRONMENTAL ATTRIBUTES ON NITRIFICATION POTENTIAL OF CRUDE OIL CONTAMINATED SEDIMENT OF QUA IBOE RIVER ESTUARY MANGROVE ECOSYSTEM AMENDED WITH NPK FERTILIZER

[1]Richard C. John, [1]Emem S. Ntino and [2]Donald I. Ikpe
1. Department of Microbiology, University of Uyo
2. Department of Science & technology, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, AkS

ABSTRACT

The aim of this study was to evaluate the influence of temperature, pH, organic carbon, salinity and ammonium concentration on the nitrification potential and nitrification rate of crude oil contaminated sediment of Qua Iboe River estuary mangrove ecosystem treated with NPK fertilizer. Nitrification potential rate (NPR) and Nitrification rate (NR) of the mangrove sediment were determined by amending the sediment with sodium chlorate to inhibit the oxidation of NO_2-N to NO_3-N. The Oil-contaminated and the remediated sediments were examined. The physicochemical parameters of the remediated sediment such as temperature, pH, salinity as well as total organic carbon and total nitrogen content showed distinct variations with time. The NPR of the oil-contaminated sediment were very low compared to the remediated sediments that had 96.4, 70.5 and 62.6 nmol N/g Dry weight sediment (DWS)/ h at the depth of 0-3, 3–6 and 6–9cm respectively. Nitrification rate (NR) obtained for the remediated sediment was relatively high in the temperatures range of 25-35^oC with optimum at 28.6^oC, pHs range of 7.5-8.0 with the optimum at pH 8.0, Organic carbon range of 0–1 mgCL^{-1} with optimum at 0 mgCL^{-1}, Salinity range of 10 to 15ppt with the optimum salinity at 15ppt for nitrification. The relationship between ammonium concentration and NR was highly significant (R^2= 0.98). It shows a maximum of NR rate (Vmax) 29.8 nmol N/g DWS/h at NH_4-N concentration of 2,800μM and the half saturation constant (ks) 700 μM NH_4-N. These results imply that environmental determinants affect NR significantly. It also indicates that treatment of crude oil contaminated mangrove sediment with nutrient supplements increased nitrification potential rate, a reliable indicator of nitrifying bacteria population. To our knowledge, this is the first report on nitrification of sediment of Qua Iboe

River estuary mangrove ecosystem in the Niger Delta of Nigeria and Its optimum temperature, pH, organic carbon, salinity and ammonium concentration as individual parameter.

Keywords: Influence; some environmental attributes; nitrification potential; crude oil contaminated; Sediment; NPK fertilizer.

INTRODUCTION

The Qua Iboe River estuary mangrove ecosystem is a mesotidal estuary and like other estuaries located in the Niger Delta, Nigeria, it supports productive processes as it provides an active habitat for biotic components of aqua-terrestrial ecosystem. Decomposition of fallen mangrove leaves by means of crabs, fungi and bacteria ensures availability of nutrients called fine particles organic matter (FPOM) which is the source of energy for many estuarine organisms. The unique mangrove ecosystem of the Niger Delta of Nigeria with estuaries and tidal mudflat areas provides nutrients supply (i.e nitrate, nitrate, ammonium, organic nitrogen and phosphorus) which are responsible for its productivity.

The Niger Delta terrestrial and aquatic systems are generally the main recipients of crude oil spillages; sometimes resulting in large-scale contamination of these environments. Oil spills in the last two decades have given rise to increased scientific knowledge of the behavior of hydrocarbons and have led to the development of new intervention methods (Ladousee and Tramier, 1991). Of the many remediation methods currently in use, bioremediation is viewed as one of the most promising technologies. The approach that has been exploited most, consists of stimulation of the soil indigenous microflora by adding an electron acceptor and/or nutriments, in particular nitrogen in the form of ammonium salts (Allen-King *et al*. 1994; Walworth and Reynolds, 1995; Walworth *et al*., 1977). This nitrogen source is exploited mainly by microbial biomass for growth and production of degradative enzymes.

Nitrification is the microbiological oxidation of ammonia to nitrite and subsequently to nitrate, that involves ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Ammonia oxidation is often the rate limiting step of nitrification in a wide variety of environments, and therefore, critical to nitrogen removal and global N cycling (Kowalchuk and Stephen, 2001; Choi and Hu, 2008). The rate of nitrification is significantly affected by the nature of nitrifying bacteria and a variety of environmental factors, such as substrate concentration (Kim *et al*., 2008; Miranda *et al*., 2008), dissolved oxygen (DO) (Kemp and Dodds, 2002), temperature, pH, salinity (Jones and Hood,

1980, Kim *et al*., 2008; Miranda *et al*., 2008), organic carbon (C) availability and CN ratio (Strauss and Dodds, 1997; Strauss *et al*., 2002). Most of these literatures reported the effect of environment factors in fresh water and water treatment systems. In addition, the individual effect of various environmental factors on nitrification of marine sediment is little known. In this study, we investigated the single effects of temperature, pH, salinity and substrate concentration on the NR of the Qua Iboe mangrove sediment.

MATERIALS AND METHODS

Study area

The study area of this investigation is the coastal zone of Nigeria in West Africa, which lies between the River Niger Delta ($7^{o}30^{1}$E) and Rio del Ray ($8^{o}30^{1}$E) in the Cameroun Republic (Tahal consultants, 1979; Tuegels *et al*., 1992. Within this coastal stretch, mangrove occurs in the estuaries of Imo River, Qua Iboe River and Cross River. The three estuaries connected to each other by means of interriverine creeks constitute a homogenous ecological unit. In this study only the mangrove swamp ecosystem of Qua Iboe River estuary was investigated. Crude oil spillage (Usari-Idoho QIT Mobil oil spill) occurred on the 4th December, 2009 at the vicinity of Qua Iboe estuary. This study was conducted between June and September after the May 1, 2010 Usari-Idoho-QIT Mobil oil spill short term post impact assessment study.

Experimental design

Three sampling stations were established in the ecosystem. These include Iwo-Okpom, Mkpanak and Upenekang. At each station, sediment from five sampling points were separately and systematically mapped out and samples were collected from the intertidal zone (epipellic sediment, 0-15cm depth) at 10m apart giving a total of fifteen samples. The samples were thoroughly mixed and the composite sample was stockpiled. Precisely, 10kg of the composite sediment sample was transferred into four 2x2ft porous-bottomed wooden boxes to serve as control. 100kg of 20:10:10 NPK fertilizer was applied to 10kg of the same composite sample was transferred into another four 2x2ft boxes to serve as treated sediment. Both the treated and the control sediment boxes were exposed to atmospheric condition undisturbed for one week period which was enough for conditioning of biological activities in the sediment. Using destructive approach, samples were collected for physicochemical analysis of

the soil characteristics, NPR experiment and monthly sampling for NR experiment and other environmental parameters.

Sediment physicochemical parameter

The soil sample was placed into a polypropylene centrifuge tube and tightly capped. After centrifugation at 4,000 g for 15 min, the supernatant was collected, filtered through 0.45 µm-pore size cellulose ester filter (Advantec, Toyo Roshi Kaisha, Tokyo, Japan), and frozen immediately until analysis (Lerat *et al*., 1990; Nissenbaum *et al*., 1990; Magni and Montani, 2006). NH_4-N, NO_2-N, NO_2+NO_3-N, PO_4, Total Nitrogen (TN) and Total Phosphate (TP) were analyzed by an automated water analyzer (Water auto-analyzer, swAAT, BLTEC, Tokyo, Japan). NH_4-N concentration was determined by the method of the alkali phenolhypochlorite reaction detected photometrically at 630 nm. NO_2-N concentration was analyzed by diazotizing with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form a highly colored azo dye and detected photometrically at 550 nm. NO_3-N was determined by the same method for NO_2-N after NO_3 was reduced by the cadmium reduction process. PO_4 was determined by the ascorbic acid method at 800 nm. TN and TP concentrations were measured by peroxodisulfate oxidation (Ebina *et al*., 1980).

Density of ammonium-oxidizing bacteria (AOB) in sediment samples

Density of ammonium-oxidizing bacteria was determined by the Most Probable Number (MPN) method in 1.5 mL sterile microtubes. The microtubes were filled with 900 mL sterile medium for ammonia-oxidizing bacteria as described by Cote and Gherna (1994). The medium was composed with $(NH_4)_2SO_4$, 1.32g/L; KH_2PO_4, 20 mg/L; $MgSO_47H_2O$, 0.1 g/L; $FeCl_2$ $6H_2O$, 0.014 g/L; $CaCl_22H_2O$, 0.18 g/L; $Na_2MO_42H_2O$, 100 mg/L; EDTA, 1.0 mg/L, phenol red, 0.002 g/L, dissolved in 1000ml of distilled water. The medium was adjusted to pH 8 with Na_2CO_3. One gram of sediment was suspended with 9 mL sterile water and 100 mL of the suspension was used to inoculate the microtube in triplicates, and serially tenfold diluted. Incubation was carried out at 28^oC for 20 days. The tubes which exhibited the color change from red to yellow due to acid production were tested by adding three drops of a nitrite color reagent (sulfanilamide, 10 g/L; *n*-(1-naphtyl)-ethylenediamine 2HCl, 0.50 g/L; concentrated HCl, 100 mL/L). Tubes that exhibited a red color after addition of the reagent were scored positive for nitrite. The bacterial density was calculated by the MPN formula in Visual Basic program (Koch, 1994).

Nitrification potential rate (NPR)

NPR was determined by the method described previously (Bianchi *et al*., 1997; Welsh and Castadelli, 2004; Dollhopf *et al*., 2005. The samples were homogenized and slurried (20.0 g of wet sediment/200mL of sterile water) and placed into 300 mL Erlenmeyer. Duplicate flasks from each depth were amended with ammonium ($(NH_4)_2SO_4$; 500 mM) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and sodium chlorate ($KClO_3$; 10 mM) (Wako Pure Chemical Industries Ltd.). Control flasks contained ammonium, sodium chlorate, and allylthiourea (ATU; 20 mg/L) (Sigma-Aldrich, St. Louis MO, USA) (Belser and Mays, 1980; Dollhopf *et al*., 2005). Flasks were capped with aluminum foil and incubated in the dark at 28ºC with constant stirring with a magnetic stirrer at 100 rpm for 72 h. Samples were collected at intervals over the incubation time. NO_2-N was determined in interstitial waters of the slurry samples after filtering through 0.45 µm cellulose ester membrane filter (Advantec, Toyo Roshi Kaisha, Japan). Since sodium chlorate is a specific inhibitor of nitrification, which blocks the oxidation of NO_2-N to NO_3-N (Belser and Mays,1980) , in the presence of 10mM chlorate, NPR can be determined as the linear accumulation of nitrite with time (Welsh and Castadelli, 2004; Dollhopf *et al*., 2005). The linear regression analyses were done after the NO_2 concentrations in all treatments were corrected by subtracting its concentration in the ATU control flash.

Effects of temperature, pH, Organic carbon and salinity on Nitrification Rate (NR): The sample used in these experiments was mud obtained from cores with 0-3 cm of sediment depth. The experiments were carried out by the method as used in the NPR experiment without emendation of $(NH_4)_2SO_4$. Incubation was carried out at 28^{o}C except for temperature experiment, which were incubated at 20, 25, 30, 35 and 40^{o}C. For pH experiment the artificial seawater was buffered with 0.1mM tris-HCl buffer (Kanto Chemical Co. Inc., Tokyo, Japan) and the pHs were adjusted at 7, 7.5, 8, 8.5 and 9. Organic carbon was added in form of D-glucose at concentration of 1, 5, 10, 15 and 20 $mgCL^{-1}$, Salinity experiment was conducted by adjustment the salinity of artificial seawater at 5, 10, 15, 20, 25 and 30ppt. pH and salinity were measured by a pH meter (ION meter IM-20E, TOA electronics Ltd., Tokyo, Japan) and a hand refractometer (ATC-S/Mill-E, ATAGO Co. Ltd, Tokyo, Japan), respectively.

Effect of ammonium concentrations on nitrification rate (NR)

The experiment used the same method as described above with NH_4-N addition at various concentrations. Since the mud sample contained NH_4-N, the regression analyses were carried out

based on the actual concentration of NH_4-N in each treatment determined at the initial time of the experiment (0 h) rather than the concentrations of NH_4-N added.

Determination of Total Hydrocarbons content in sediment (THC)

Standard procedures were adopted for the analysis of THC levels in sediment (Law and Klungsoyr, 2000; Radojevic and Bashkin, 1999; APHA, 1998). Precisely 50g of extracted sediment samples were acidified with 5 ml of 0.1M H_2SO_4 followed by the addition of 25ml of diethyl ether. The mixture was then vigorously shaken. Organic layer was separated, filtered and re-extracted with 25 ml diethyl ether followed by filtration. This procedure was repeated foe each sample which was stored in a dessicator placed inside a fume cupboard, and evaporated under hood. The resulting residue was dissolved in ether and chromatographed through a silica gel column (15–40μm). The filtrate was evaporated to dryness while the residue was weighed to determine the hydrocarbon using the formula:

$$\text{THC } (mg1^{-1}) = \frac{R}{V} \times 100$$ Where R= residue, V= Volume

The THC levels in the sediment were reported in $mgkg^{-1}$ dry weight.

RESULTS AND DISCUSSIONS

Table 1 shows mean values and standard deviation of pH, total phosphorous (mg/kg), total organic carbon (%,) total organic nitrogen (%,) Salinity (ppt), total hydrocarbon content (mg/kg), and Mineral nitrogen (NO_3-N, NO_2-N, NH_4-N, PO_4) in sediment samples studied. The sediments of Qua Iboe estuary mangrove ecosystem showed slight acidic to neutral pH range of 6.9 and 7.82.The soil pH decreased to 6.78 during remediation treatment but increased gradually after 4 weeks to 8.54. Tisdale and Nelson (1975) made a similar observation and reported that decreased in pH during remediation treatment may have resulted from the production of acids radicals through the process of nitrification of the applied fertilizer. There was no significant different in the effect of remediation treatment on the soil pH value at 5% probability levels. The mean total organic carbon (TOC) levels in sediment analyzed ranged from 9.3 to 11.5%. The level of TOC dropped between 9.1 to 10.5 during remediation treatment .The relatively high concentrations of TOC in epipellic sediments studied reflect “high” organic matter flux to epipellic sediments of Qua Iboe estuary mangrove ecosystem which can be

related to the decomposition of mangrove litter and hydrolysis of tannin in mangrove plants releasing various kinds of organic matter and acids (Liao, 1990).

In this study, the relationship between organic carbon content and remediation period showed a correlation (r= - 0.194) which suggests that the amount of carbon reduced with time. The total organic nitrogen (TON) ranged from 0.42% to 0.54% but increased in the remediated soil (0.45–0.59) probably due to the application of the nitrogenous fertilizer. The correlation analysis (Table 2), between the total nitrogen content and remediation period, showed a negative relationship (r = -0.368) at a 5% probability level. There was a marked decrease in the percentage of the THC in the remediated soil compared to the control. After 16 weeks of remediation, 43.86%, 60.33%, 69.30% and 82.46% reduction was recorded at 4, 8, 12 and 16 weeks respectively. The results indicate that the applied fertilizer increased the degradation of the hydrocarbons (Fig. 1).

A hypothesis that would account for such behavior is that the bacterial population was readily utilizing the available nitrogen for hydrocarbon degradation: hence the available nitrogen was

Table 1: Some physicochemical characteristic and ammonium oxidizing bacteria density of sediment of Qua Iboe estuary mangrove ecosystem

	Control Sediments		Treated Sediments	
	June, 2009 – September, 2009		June, 2009 – September, 2009	
Parameters	Range	Average	Range	Average
Temp ^{o}C	27.4 – 28.6	28.1 ± 0.5	27.2 – 28.8	27.9 ± 0.8
pH	6.94 – 7.82	7.38 ± 0.3	6.76 – 8.54	7.65 ± 0.4
Total Phos. (TP) mg/kg	5.36 – 5.75	5.87 ± 0.61	4.24 – 5.18	4.71 ± 0.48
Total Organic C. %	9.3 – 11.5	10.4 ± 0.02	9.1 – 10.5	9.8 ± 0.2
Total Organic N. %	0.42 - -0.54	0.48 ± 0.08	0.45 – 0.59	0.52 ± 0.7
C/N		21.7		18.8
NO_3-N (µg/L)	Undetectable – 0.1	0.1 ± 0.5	Undetectable – 0.2	0.1 ± 0.5
NO_2-N (µg/L)	0.4 – 0.8	0.6 ±0.2	1.4 – 1.8	1.6 ± 0.3
NH_4-N (µg/L)	12.4 – 46.8	29.6 ± 0.21	18.6 – 92.2	55.4 ± 1.2
PO_4 (µg/L)	6.9 - 24.3	15.6 ± 0.11	12.8 – 28.6	20.7 ± 0.42
Salinity (ppt)	15 – 32ppt	28ppt	18 – 36ppt	30ppt
THC mg/kg	2.2 - 2.28	2.24 ± 1.5	0.4 – 1.28	0.84 ± 0.8
Density of ammonium-oxidizing bacteria	$8.95x10^4$–$1.86x10^5$	1.38×10^5	$5.84x10^4$ – $1.96x10^6$	1.01×10^6

Data reported are means, ± SD, n=12. Measurements are indicated

Table 2: The relationships between time elapsed and environmental factors after remediation at 5% probability level

Correlation regression factor	Correlation co-efficient, r	Significance equation
Time and Temp.	+0.088	Y = 16.8 + 0.6x
Time and Ph	+0.025	Y = 6.55 + 0.08x
Time and TP	-0.432	Y = 0.048 – 0.05x
Time and Organic C	-0.194	Y = 0.293 – 0.24x
Time and TN	-0.368	Y = 0.042 – 0.02
Time and Sailinty	+0.324	Y = 2.38 + 0.3x
Time and THC	-0.286	Y = 35.7 – 113x

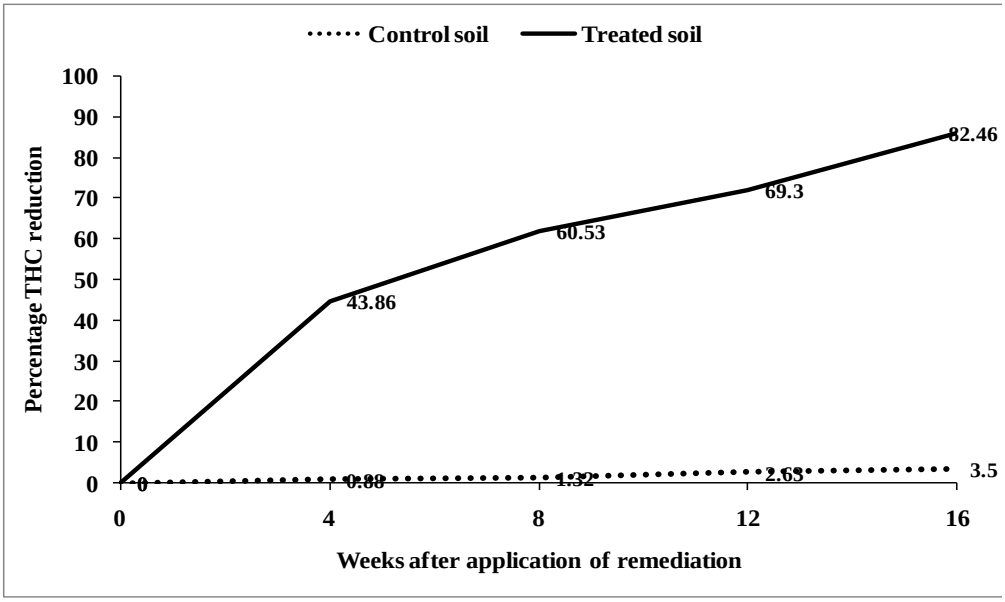


Figure 1: Rate of Hydrocarbon loss

diminishing with time. Brady and Weil, (1999) made a similar observation and concluded that, during degradation, nitrogen may be lost to the atmosphere when nitrate ions are converted to gaseous forms of nitrogen by a series of widely-occurring biochemical reduction reactions, brought about by denitrifying bacteria, such as *Pseudomonas, Bacillus* and *Micrococcus,* especially when localized micro-sites of low oxygen exist well within the soil aggregates. Correlation analysis between the total nitrogen content and remediation period showed a negative relationship (r = -0.368) at a 5% probability level. The palaeoenvironmental significance of the C/N ratio and its usefulness as an organic matter identifies has been emphasized by Mayers (1994). The C/N ratios have been used to distinguish between organic matter inputs in estuaries, since autochthonous marine organisms rich in

protein material have C/N values (4 -10) much lower than terrestrial plants (>20) (Kawamura and Ishiwatari, 1981; Meyer, 1994 and 1997). In this work,the C/N ratios of control sediments was 21.7 indicating an input of different mixtures of land and aquatic organic matter.

The concentrations of NO_3-N and NO_2-N in sediment were low in the control with average values of 0.1±0.5µg/L and 0.6±0.2 µg/L respectively. Similar trend was established in the treated sediment but there was slight increased in NO_3-N with an average value of (1.6±0.3) (Table 1): NH_4-N that was the dominant fraction of dissolved inorganic nitrogen in the sediment ranged from 12.4–46.8 and 18.6–92.2 µg/L with an average concentration of 29.6±0.21 and 55.4±1.2 in the control and treated sediment respectively. High NH_4-N levels in the treated soil could be indication of enhance ammonification (Deenik, 2006). The average concentration of phosphate was 15.6±0.11 and 20.7±0.42 in the control and treated sediment respectively. The density of AOB ranged from $8.95x10^4$–$1.86x10^5$ and $5.84x10^4$–$1.96x10^6$ cells/g of wet sediment in the control and treated sediment respectively. The later can be compared to the bacterial density determined by ammonia monooxygenase (a*MOA*) gene copy numbers in salt marsh sediments, which ranges from $5.6x10^4$ to $1.3x10^6$ cells/g of wet sediment (Dollhopf *et al*, 2005).

NPR, a reliable indicator of nitrifying bacteria population (Jenkins and Kemp, 1984) indicates the NR in the presence of ammonia and oxygen concentrations. NPR can be used to estimate actual in situ NR, when the depth of oxygen penetration into the sediment is known (Henriksen *et al*., 1981). In this study, ammonia was amended by adding $(NH_4)_2SO_4$, and oxygen was supplied by continues stirring. NPRs of sediment of Qua Iboe estuary mangrove ecosystem varied in the different sediment depths of 0-3, 3-6 and 6-9 cm. It amount to 96.4, 70.5 and 62.6 nmol/g dry weight sediment (DWS/h), respectively (Fig. 2). The lower layers of sediment at the depth of 3–6 and 6–9 cm showed high NPR, 70.5 and 62.6 nmol N/g DWS/h respectively. NO_2-N as the product of nitrification at all range of sediment depths was linearly accumulation indicating growth of ammonium oxidizing bacteria during 72h incubation. The NO_2 accumulation and incubation time also showed a high correlation (R^2) ranging from 0.91 to 0.97. The accumulation of nitrogen measured as NO_2 amounted to 5.6±1.2, 4.8±1.5 and 4.6±0.6 nmol N/g DWS during 72h incubation for the depth 0-3, 3–6 and 6–9 cm, respectively. Relatively high NPRs in the sediment suggest the high population of nitrifying bacteria. The nitrification that occurs even in the sediment depth of 6-9 cm indicates the presence of the bacteria in the sample.

There are at least three reasons to figure out nitrification in the lower depth sediment, which is usually unoxygenated well. Firstly, nitrifying bacteria are able to tolerate and actively grow under a very low oxygen condition (Caffrey *et al*., 2003). Secondly, there is perturbation that allow the oxygen penetrate to the deeper sediment The bioturbation in the sediment is likely contributed mainly by the population of burrowing organisms such as mud skipper (Japanese: *mutsugoro*) (*Boleophthalmus pectinirostris*), worm and several species of crabs. Bartlett *et al*. (2008) also proved that this anoxic nitrification is a microbially mediated reaction, and its significance and persistence depend on the frequency and magnitude of sediment perturbation. Thirdly, physical disturbance due to the high tidal range and the exposure to the air of this tidal flat allow penetration of oxygen to the deeper sediment.

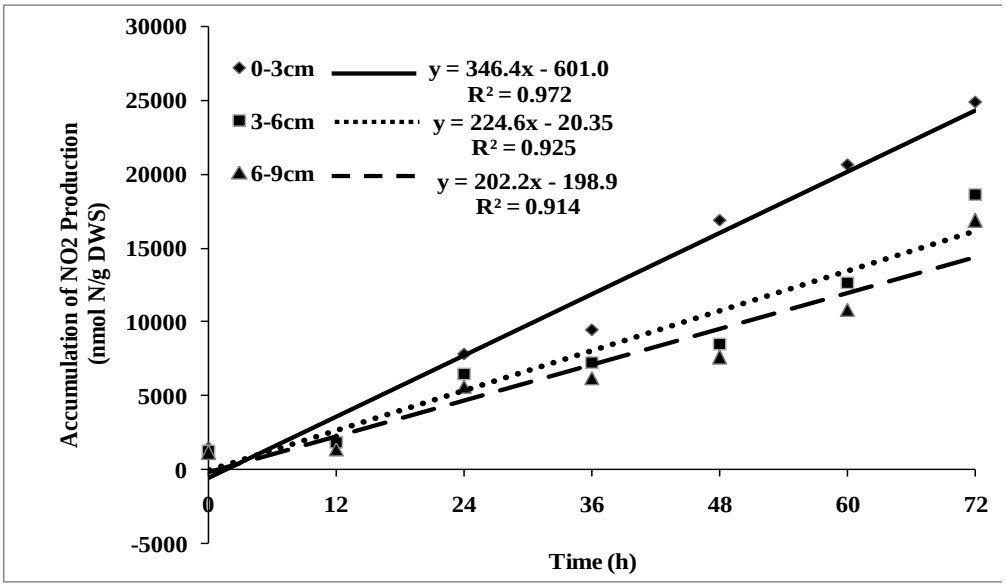


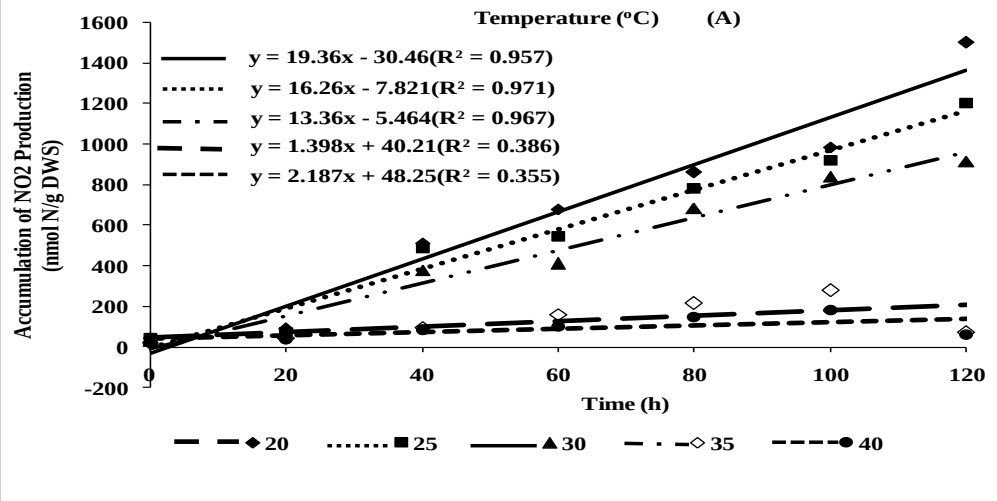
Figure 2: Nitrification potential rate (NPR) of sediment at various core depths determined by the accumulation of $N0_2$-N production at 72h incubation

Henriksen *et al*. (1981) also found nitrifying bacteria in anoxic sandy and muddy sediments. Diverse community of ammonia-oxidizing bacteria population including *Nitrosomonas* and *Nitrospira* in anoxic marine sediment have reported by Freitag and Prosser (2003). Nitrification potential rate is strongly correlated and enhanced by macrofaunal burrow abundance and activity (Dollhopf *et al*., 2005). The rate of nitrification is significantly affected by the nature of nitrifying bacteria and a variety of environmental factors, such as substrate concentration (Kim *et al*., 2008; Miranda *et al*., 2008), dissolved oxygen (DO) (Kemp and Dodds, 2002), temperature, pH, salinity (Jones and Hood, 1980, Kim *et al*., 2008; Miranda *et al*., 2008), organic carbon (C) availability and CN ratio (Strauss and Dodds, 1997; Strauss *et al*., 2002). Most of these literatures reported the effect of environment factors

in fresh water and water treatment systems. In addition, the individual effect of various environmental factors on nitrification of hydrocarbon contaminated estuary mangrove sediment is not known.

In this study, we investigated the single effects of temperature, pH, Organic carbon, salinity and substrate concentration on the NR of the Qua Iboe estuary mangrove sediment. Production of NO_2-N was significantly affected by temperature. Relatively high NO_2-N productions were observed at the temperature range of 25 - 35ºC. The highest NO_2-N production occurred at 28.6ºC which amounted to 1368.2 nmol N/g DWS for 120 h incubation (Fig 3a). The NO_2-N production was very low with 12.5 to 65.2 nmol N/g DWS at 20ºC and 40ºC. As indicated by the NO_2-N production, the NRs were also clearly affected by temperature. The highest NR, 14.8 nmol N/g DWS/h equivalent to 168.8 mmol N/m^2/h, was also obtained at 28.6ºC. Incubation at 20, 35 and 40ºC gave lower NRs, 6.9, 10.2 and 6.8 nmol N/g DWS/h, respectively (Fig. 3b). However, NRs of the sediment were very low at temperatures of 20ºC and at 40ºC. The rate increased drastically by increasing temperature from 20 ºC to 28.6ºC, but decreased by increasing temperature above 29.3ºC (Fig. 3). Kim *et al*. (2008) reported similar finding, in which ammonia oxidation rate increases significantly with the increase in temperature from 10 to 30ºC. However, a higher optimum temperature, 40ºC was reported for an estuarine isolate of nitrifying bacterium, *Nitrosomonas* (Jones and Hood, 1980).

The activation energy of ammonia oxidation at the temperature ranges of 10-20ºC (87.1 kJ/mol) is significantly higher than at 20-30ºC (38.6 kJ/mol) (Kim *et al*., 2008).

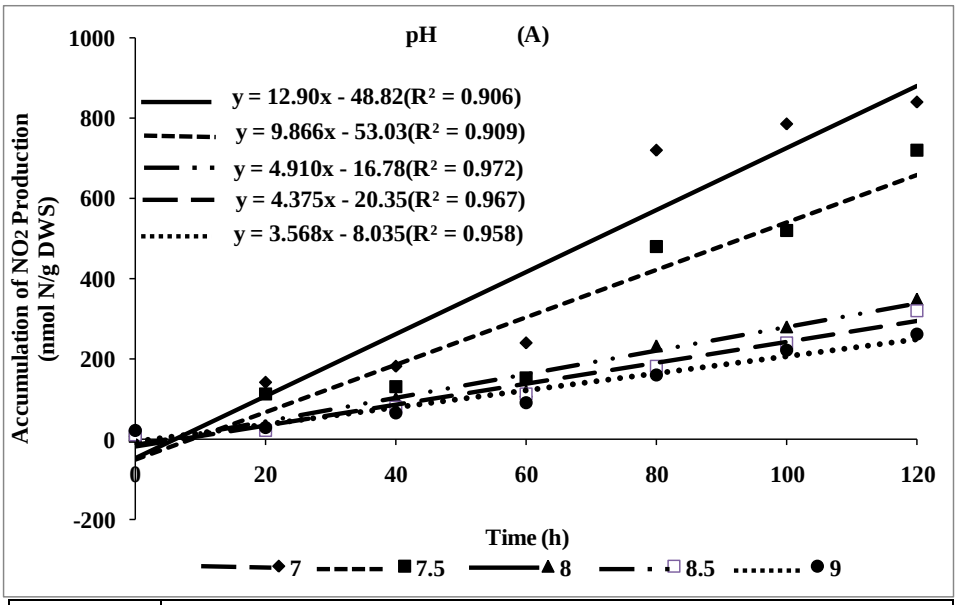


NO_2-N Production	Temperature (ºC) (B)					
	20	25	28.6	30	35	40
NR nmol	6.9	11.4	14.8	12.5	10.2	1.18
NO_2-N	12.5	1128.6	1368.2	1164.6	1112.8	65.2

Figure 3: Nitrification rate (NR) of sediment at various temperatures determined by the accumulation of NO_2 production at 120h incubation (A). Relationship between temperature and NR (B).

pH is one of the most major factors in nitrification both in freshwater and marine systems (Strauss *et al*., 2002; Miranda *et al*., 2008). In this study, the accumulation of NO_2-N productions during 120h (Fig. 4a) incubation at pH 7, 7.5, 8, 8.5 and 9.0 and their linear regression indicated that the NRs were greatly affected by pH. This was noticeable in pH range of 7.0 to 9.0. High NRs were found at 7.5 and 8.0 which reached 6.2 and 8.6 nmol N/g DWS/h, respectively (Fig. 4b). A lower NR was obtained at pH 9 and the highest at 8.0. The positive correlation between pH and NR was clearly detected in the pH range of 7 to 8 but a negative correlation occurred above pH 8.0. This positive correlation is related to the increase in available NH_3 as a true substrate of oxidation (Suzuki *et al*., 1974). Emerson *et al*. (1975) stated that the relative NH_3 concentration increases by nearly a full order of magnitude by increasing each pH unit. The negative correlation between pH and nitrification above the optimum pH is likely caused by the negative effect of increasing pH on enzyme activity (Strauss *et al*., 2002). Effect of pH as a single factor on nitrification of a marine and estuarine system has not been studied. Miranda *et al*. (2008) could not determine clearly the effect of pH on NR of marine sediment, but detect a tendency, though weak, that nitrification increases by increasing pH. The authors also suggested the positive relationship between pH and nitrification without defining the range of pH clearly. The optimum pH for nitrification varies depends on the nature of the system. In the freshwater sediment, Strauss *et al*. (2002) have determined that the maximum NR occurs at pH 7.5 over a pH range of 5.9 - 8.7. Antoniou *et al*. (1990) determined the maximum nitrification occurs at pH approximately 7.8 in the wastewater treatment sludge.

Organic carbon is an important regulator of nitrification rates and is of key importance in understanding N dynamics in freshwater ecosystem. In natural environment, We hypothesized that when environmental C:N ratios are high, heterotrophic bacteria are subject to N limitation and will out-compete nitrifying bacteria for available NH_4^+, thereby reducing nitrification rate. In our experiment, the accumulation of NO_2-N productions during 120h organic carbon amendment (using glucose as carbon source) at concentrations 1, 5, 10 and 20 $mgCL^{-1}$ and their linear regression indicated that the NRs were greatly affected by organic carbon (Fig. 5a).

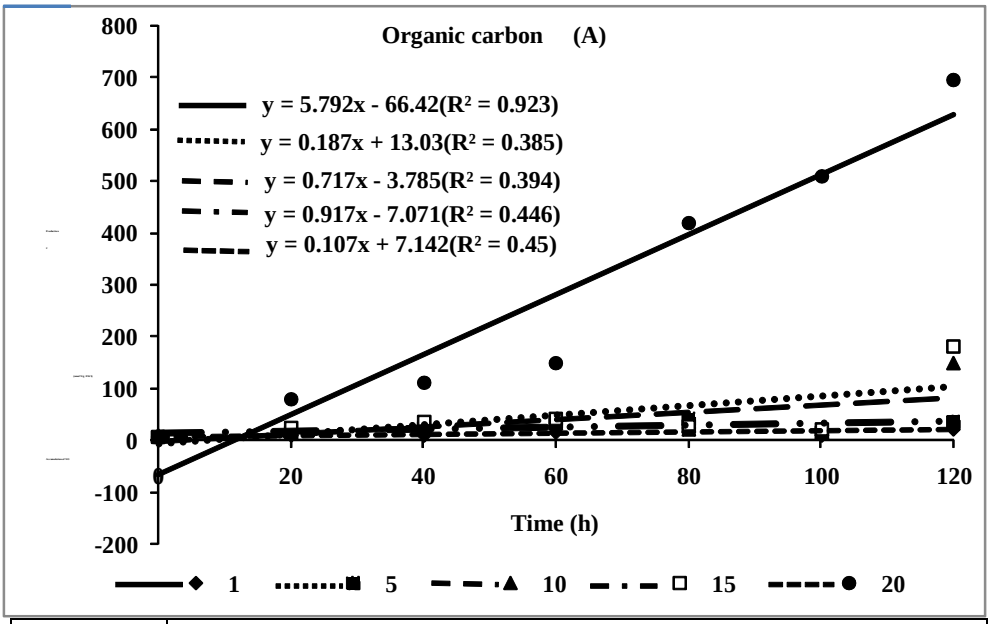


NO_2-N Production	pH (B)					
	7	7.5	7.8	8.0	8.5	9.0
NR nmol	3.4	6.2	7.6	8.6	5.4	1.3
NO_2-N	106.2	175.8	188.5	195.9	120.6	98.2

Figure 4: Nitrification rate (NR) of sediment at various pHs determined by the accumulation of NO_2 production at 120h incubation (A). Relationship between pH and NR (B).

High NR was found between no organic carbon amendments and 1mgCL^{-1} while lower NR was obtained at organic carbon amendment greater than 1mgCL^{-1}(Fig. 5b). Nitrification in the mangrove sediment was completely inhibited at amendment rate of 20mgCL^{-1} with or without addition of NH_4^+. Therefore, organic carbon additions significantly decreased nitrification rates but increased total microbial activity. Salinity of sediment samples in this study had a typical characteristic of estuarine salinity with a wide fluctuation from 18-30 ppt. Although the nature of nitrifying bacteria in this estuarine are adaptable to this fluctuation, the NR is greatly affected by salinity. High NRs of sediment of the Qua Iboe estuary mangrove ecosystem were found at intermediate salinity and drastically decreased at low and high salinities. Previous studies on nitrifying bacteria indicated that high NR occurs at intermediate salinities. Jones and Hood (1980) and Helder and de Vries (1983) found high NR of the bacteria at 5-10 ppt and 10-25 ppt, respectively. A maximum NR is determined at 10 ppt (MacFarlane and Herbert, 1984). NRs of marine and estuarine samples are also higher at low or intermediate salinities (Somville, 1984; Berounsky and Nixon, 1993; Bianchi *et al*., 1994; Magalhâs *et al*., 2005; Miranda *et al*., 2008). In this study, the NRs varied between 1.12 to 14.5nmol N/g DWS/h at

the salinity range of 5 – 30ppt (Fig. 6a). NRs increased drastically in the salinity range to 10 to 15ppt but it decreased sharply in the range above 15ppt (Fig. 6b). The optimum salinity was obtained at 15 ppt with NR 14.5 nmol N/g DWS/h. Somville (1984) reported that the optimum salinity for NR of estuarine samples is affected by the *in situ* water salinity. In contrast, Rysgaard *et al*. (1999) indicated that the optimum salinity for NR is not influenced by the *in situ* salinity with the highest NR at 0 ppt. The finding reported by the later authors is supported by Santoro and Enrich-Prast (2009), who reported that NPRs of sediment from saline shallow coastal lagoons have negative correlation to the increase of salinity ranging from 0 to 30 ppt.

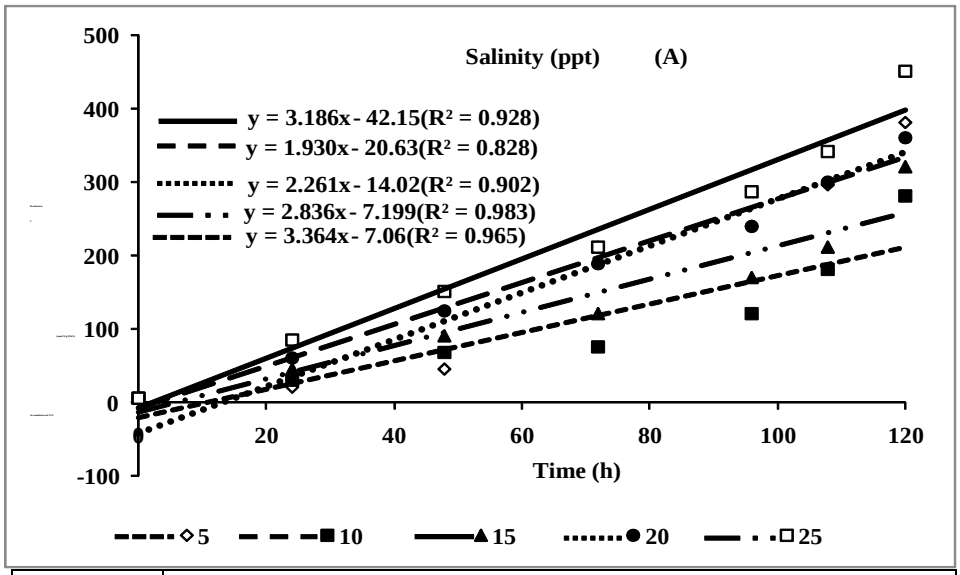


NO_2–N Production	Organic carbon ($mgCL^{-1}$) (B)					
	0	1	5	10	15	20
NR nmol	9.8	6.5	1.2	0.5	0.2	Nil
NO_2-N	128.4	115.2	27.8	12.6	10.5	Nil

Figure 5: Nitrification rate (NR) of sediment at various organic carbons determined by the accumulation of NO_2 production at 120h incubation (A). Relationship between organic carbons and NR (B).

Since nitrification is an enzymatic reaction, its reaction rate is directly affected by the availability of the substrate. Bianchi *et al*. (1999) found that 74% of the variability in nitrification in the estuarine area of the Rhone River can be explained by a single variable, NH_4-N availability. Kim *et al*. (2008) could not determine the effect of free ammonia (NH_3-N) concentration in the range of 5.6-90.1 mg/L on the specific substrate utilization rate as well as the relative nitrite accumulation.

In this study, we used much higher ammonium concentration to know the kinetic of ammonium oxidation and to know the possibility of construction of Mecheleis Menten curve since the reaction is an enzymatic reaction. To obtain an asymptotic curve, the ammonium concentration was increased to some levels, which were much higher than its field concentration. *Vmax* and *Ks* were found at high concentrations of ammonium over its actual concentration in the field. This finding will be relevant to predict the rate of nitrification at various NH_4 concentrations. In this study, Ammonium concentrations up to approximately 1,500µM increased the accumulation of NO_2-N production exponentially. The accumulation rate was lower at NH_4-N concentration around 1,500 to 2,800µM. The production was relatively constant at the NH_4-N concentration above 2,800µM (Fig. 7a). This similar pattern was also found in the NR by the increase of this concentration. The highest NR, 36.8 nmol N/g DWS/h, was found when the ammonium concentration was 3088µM. Relationship between ammonium concentration and NR resulted a hyperbolic Michaelis-Menten type curve ($R^2 = 0.99$) (Fig. 7) with 34.8 nmol N/g DWS/h of the maximum NR rate (*V*max) at NH_4 concentration 3,000µM. The half saturation constant (*Ks*) of the nitrification was determined at NH_4 concentration 700 µM. It is the first study determining *V*max and *Ks* of estuarine sediment sample of Qua Iboe estuary mangrove ecosystem.

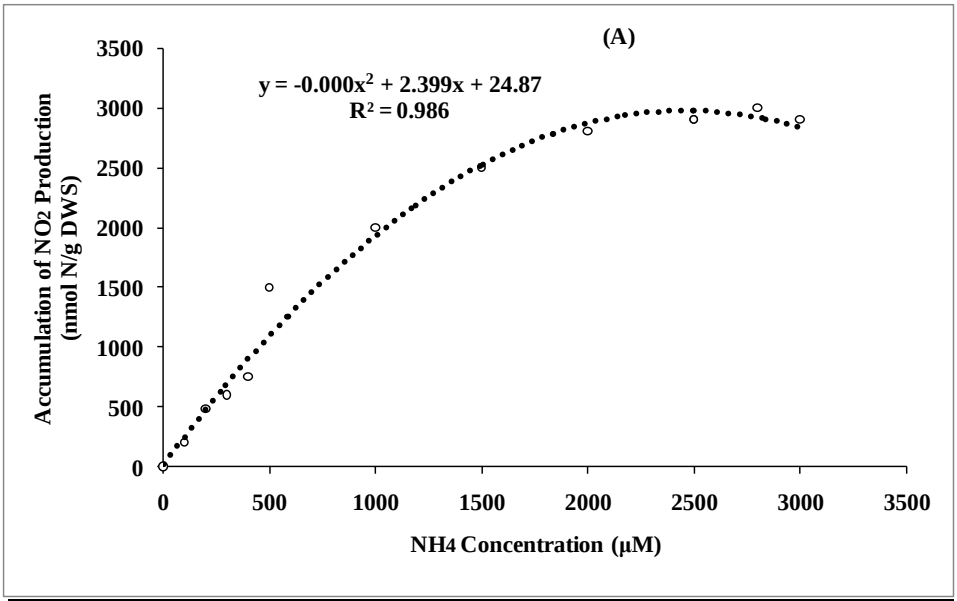


NO_2-N Production	Salinity (ppt) (B)					
	5	10	15	20	25	30
NR nmol	11.6	10.2	14.5	3.8	3.1	1.12
NO_2-N	76.4	138.2	196.2	64.2	58.6	34.8

Figure 6 : Nitrification rate (NR) of sediment at various salinity determined by the accumulation of NO_2 production at 120h incubation (A). Relationship between salinites and NR (B).

Cébron *et al*. (2005) previously reported NR of nitrifier denitrification of mixed nitrifying bacteria populations from Seine river water. They estimated the maximum N_2O production rate (*V*max) to be 8 to 9 µg N-N_2O–/mg C biomass/h with *Ks* of nitrifier-denitrification 1.5 to 3 mg N-NH_4/L for ammonium, and 1 to 4 mg N-NO_2/L for nitrite. The ammonium concentration and NR had a positive correlation in the concentrations range of 20 to 3,000 µM (Fig. 7b).

However, above the later concentration, the correlation became negative suggesting the nitrification inhibition by excessive ammonia concentration. Inhibition of nitrification by excessive concentration of ammonium in a marine system is little studied. The inhibition effect of an excessive free ammonia concentration on ammonia oxidation has been reported by Anthonisen *et al*. (1976). The ammonia concentration that inhibits its oxidation is greatly affected by degree ofadaptation of nitrifying bacteria to high ammonia concentration. Kim *et al*. (2008) described that the nitrifying bacteria and enzyme involve in the nitrification process are more resistant to high concentration of ammonia when they are adapted to the high ammonium concentration in advance, while the significant inhibition of nitrification often occurred in a system with low ammonium concentration. For example, the nitrification inhibition occurs at NH_3 concentration ranging from 0.1 to 150 mg/L (Anthonisen *et al*., 1976), activity of ammonia oxidizing bacteria, *Nitrosomonas* are significantly inhibited at free ammonia over 100 mg/L (Neufeld *et al*., 1980), NRs of high ammonium concentration adapted-nitrifiers are not inhibited by ammonium concentration ranging from 500 to 3000 mg N/L (Mahne *et al*., 1996), and *Nitrosomonas europaea* prevailing at an extreme substrate is not inhibited by substrate concentration up to 500 mmol/L (Hunik *et al*.,1992).



NO_2-N Production	Ammonium concentration (µM) (B)					
	500	1000	1500	2000	2500	3000
NR nmol	5	15	20	23.6	25.8	34.8
NO_2-N	35	80	106.5	1128	1325	1683

Figure 7: Nitrification rate (NR) of sediment at various concentration og NH_4-N determined by the accumulation of NO_2 production at 120h incubation (A). Relationship between NH_4-N and NR (B).

CONCLUSIONS

All the above literatures reported the inhibition of nitrification in freshwater and wastewater with high ammonia concentration. In this study, we found that nitrification inhibition occurred at relatively low ammonium concentration comparing to the inhibition reported in the above references. The relative low inhibition concentration of ammonia in this study is likely caused by the nitrifying bacteria in the Qua Iboe mangrove sediment are never adapted and exposed to the extreme concentration of ammonium. The nature of sediment also affects the inhibition concentration of NH_4-N. Magalhâs *et al*. (2005) found that nitrification of sandy sediment is inhibited by NH_4-N addition at 200 µM, but the same concentration does not inhibit nitrification of rocky biofilm.

In summary, the NPR of Qua Iboe estuary mangrove sediment is lower in the deeper sediment, but it was still noticeable even at 6-9 cm sediment depth suggesting the important role of perturbation and possible occurrence of anoxic nitrification. In this study, individual effect of temperature, pH, organic carbon, salinity and NH_4-N concentration on NR of Qua Iboe estuary mangrove sediment were

succeeded to be evaluated by controlling other factors in laboratory experiment. The results indicated that these environmental parameters affected NR significantly. High NR occurred at 25 to 35^{o}C, pH 7.5-8.0, organic carbon at 0-1mgCL^{-1}and salinity around 15ppt. The effect of ammonium concentration on NR exhibited typical kinetics of enzymatic reaction with Michaelis-Menten curve. This report represents the first study on nitrification of the Qua Iboe estuary mangrove sediment and the single effect of several environment parameters, which significantly improves the understanding of nitrification kinetic in this area.

Acknowledgment

We thank Dr. Moses Ekpeyong of the department of Computer Science, Faculty of Science, University of Uyo, Akwa Ibom State, Nigeria, for his valuable correction on the MPN program in Visual Basic.

REFERENCES

Allen-King RM, Barker JF, Gillham RW, Jensen BK (1994) Substrate- and nutrient-toluene biotransformation in sandy soil. *Environ. Toxicol. Chem.*.13:69

Anthonisen AC., Loehr RC, Prakasam TBS, Srinath EG (1976) Inhibition of nitrification by ammonia and nitrous acid. *Wat. Pollut. Control Fed.,* 48: 835-852

Antoniou P, Hamilton J, Koopman B, Jain R, Holloway B, Lyberatos G, Svoronos SA (1990) Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. *Water Res.,* 24: 97-101

APHA (1998) Standard methods for the examination of water and waste water (20th ed.) Washington, DC: American Public Health Association, APHA-AWWA-WPEF.

Bartlett R, Mortimer RJG, Morris K (2008) Anoxic nitrification: Evidence from Humber Estuary sediments. *UK Chem. Geol*., 250: 29-39

Belser LW, Mays EL (1980) Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Appl. Environ. Microbiol*., 39: 505-510

Berounsky VM, Nixon SW (1993) Rates of nitrification along an estuarine gradient in Narragansett Bay. *Estuaries Coasts*, 16: 718-730

Bianchi M, Bonin P, Feliatra F (1994) Bacterial nitrification and denitrification rates in Rhône River plume (Northwestern Mediterranean Sea). *Mar. Ecol. Prog. Ser*., 103: 197-202

Bianchi M, Feliatra F, Tréguer P, Vincendeau MA, Morvan J (1997) Nitrification rates, ammonium and nitrate distribution in upper layers of the water column and in sediments of the Indian sector of the Southern Ocean. *Deep Sea Res. II*, 44: 1017-1032

Bianchi M, Feliatra LD, Lefevre D (1999) Regulation of nitrification in the land-ocean contact area of the Rhone River plume (NW Mediterranean). *Aquat. Microb. Ecol.*, 18: 301-312

Brady NC, Weil RR (1999) The nature and properties of soils. 12th ed, Prentice Hall publishers, London. Pp 1-9, 453-536, 739-740

Caffrey JM, Harrington N, Solem I, Ward BB (2003) Biogeochemical processes in a small California estuary. 2. Nitrification activity, community structure and role in nitrogen budgets. *Mar. Ecol. Prog. Ser.*, 248: 27-40

Cote RJ, Ghena RL (1994) Nutrition and Media. In: Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R.Krieg, (Eds.), Methods for General and Molecular Bacteriology. *Am. Soc. Microbiol*., pp: 155-277

Cébron A, Garnier J, Billen G (2005) Nitrous oxide production and nitrification kinetics by natural bacterial communities of the lower Seine River (France). *Aquat. Microb. Ecol*., 41: 25-38

Cho OK, Hu ZQ (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ. Sci. Technol.*, 42: 4583-4588

Deenik J (2006) Nitogen mineralization potential in important agricultural soils of Hawaii: *Soil and crop management.* 16:1-5

Dollhopf SL, Hyun JH, Smith AC, Adams HJ, Brien SO, Kostka JE (2005) Quantification of ammonia-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. *Appl. Environ. Microbiol.*, 71: 240-246

Ebina J, Tsutsui T, Shirai T (1980) Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. *Water Res*., 17: 1721-1726

Emerson K, Russo RC, Lund RE,Thurston RV (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can*., 32: 2379-2383

Freitag TE, Prosser JI (2003) Community structure of ammonia oxidizing bacteria within anoxic marine sediments. *Appl. Environ. Microbiol*., 69: 1359-1371

Helder W, De Vries RTP (1983) Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard estuary). *Neth. J. Sea Res*., 17: 1-18

Henriksen K, Hansen JI, Blackburn TH (1981) Rates of nitrification, distribution of nitrifying bacteria, and nitrate fluxes in different types of sediment from Danish waters. *Mar. Biol.,* 61: 299-304

Hunik JH, Meijer HJG, Tramper J (1992) Kinetics of *Nitrosomonas europaea* at extreme substrate, product and salt concentrations. *Appl. Microbiol. Biotechnol*., 37: 802-807

Jenkins MC, Kemp WM (1984) The coupling of nitrification and denitrification in two estuarine sediments. *Limnol.Oceanogr*., 29(3): 609-619

Jones RD, Hood MA (1980) Effects of temperature, pH, salinity and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. *Microb. Ecol*., 6: 339-347

Kawamura K, Ishiwatari R (1981) Polyunsaturated fatty acids in a lacusterine sediment as a possible indicator of paleoclimate. *Geochimica et cosmochimica Acta*, 45: 149-155

Kemp MJ, Dodds WK (2002) The influence of variable ammonium, nitrate, and oxygen concentrations on uptake, nitrification, and denitrification rates. *Limnol. Oceanogr.,* 47: 1380-1393

Kim JH, Guo X, Park HS (2008) Comparison study of the effects of temperature and free ammonia concentration on nitrification and nitrite accumulation. *Process Biochem.,* 43:154-160

Kim JK, Kraemer GP, Yarish C (2008) Physiological activity of *Porphyra* in relation to eulittoral zonation. *J. Exp. Mar. Biol. Ecol*., 365: 75-85

Koch AL (1994) Growth Measurement. In: Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R. Krieg, (Eds.) Methods for general and molecular bacteriology. *Am. Soc. Microbiol*., pp: 248-277

Kowalchuk GA, Stephen JR (2001) Ammoniaoxidizing bacteria: A model for molecular microbial ecology. *Ann. Rev. Microbiol*., 55: 485-529

Ladousse A, Tramier B (1991) Results of 12years of research in Spilled oil bioremediation. Inipol EA 22.*Proceedings of the 1991 oil spill conference American petroleum Institute.* Washington D.C.

Law RJ, Klungsoyr RJ (2000) The analysis of polycyclic aromatic hydrocarbon in marine samples. *Int. J. Environ. Pollut.*, 13 (1-6) 262-283

Lerat Y, Lasserre P, le Corre P (1990) Seasonal changes in pore water concentrations of nutrients and their diffusive fluxes at the sediment-water interface. *J. Exp. Mar. Biol. Ecol.,* 135:135-160

Liao JF (1990) The chemical properties of the mangrove solonchak in the northeast part of Hainan Island. Acta Scientiarum Naturalium Universitatis sunyatseni. 9 (4): 67-72 supp

MacFarlane G, Herbert RA (1984) Effect of oxygen tension, salinity, temperature and organic matter concentration on the growth and nitrifying activity of an estuarine strain of *Nitrosomonas*. *FEMS Microbiol. Lett.,* 23: 107-111

Magalhâes CM, Joye SB, Moreira RM, Wiebe WJ, Bordalo AA (2005) Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. *Water Res.,* 39: 1783-1794

Magni P, Montani S (2006) Seasonal patterns of pore-water nutrients, benthic chlorophyll *a* and sedimentary AVS in a macrobenthos-rich tidal flat. *Hydrobiologia.,* 571: 297-311

Mahne I, Prin A…i… and F. Megušar, 1996. Nitrification/denitrification in nitrogen high-strength liquid wastes. *Water Res.,* 30: 2107-2111

Meyers PA (1994) Preservation of elemental and Isotopic source identification of sedimentary organic matter. *Chemical Geology* 144:289-302

Meyers PA (1997) Organic geochemical proxies of paleoceanographic,paleolimnologic and paleoclimatic processes. *Organic Geochemistry*. 27:213-250

Miranda J, Balachandran KK, Ramesh R, Wafar M (2008) Nitrification in Kochi backwaters. *Estuar. Coast Shelf Sci.,* 78: 291-300

Neufeld RD, Hill AJ, D.O. Adekoya (1980) Phenol and free ammonia inhibition to *Nitrosomonas* activity. *Water Res.,* 14: 1695-1703

Nissenbaum A, Stiller M, Nishri A (1990) Nutrients in pore waters from Dead Sea sediments. *Hydrobiologia,* 197: 83-89

Radojevic M, Bashin VN (1999) *Practical environmental analysis.* Royal society of chemists. 465pp

Rysgaard S,Thastum P, Dalsgaard T, Christensen PB, Sloth NP (1999) Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries*, 22: 21-30

Santoro AL, Enrich-Prast A (2009) Salinity control of nitrification in saline shallow coastal lagoons. *Acta Limnol. Bras.,* 21: 263-267

Somville M (1984) Use of nitrifying activity measurements for describing the effect of salinity on nitrification in the Scheldt Estuary. *App. Environ. Microbiol.,* 47: 424-426

Strauss EA, Dodds WK (1997) Influence of protozoa and nutrient availability on nitrification

rates in subsurface sediments. *Microb. Ecol*., 34: 155-165

Strauss, E.A., N.L. Mitchell and G.A. Lamberti, 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. *Can. J. Fish. Aquat. Sci.*, 59: 554-563.

Suzuki I, Dular U, Kwok SC (1974) Ammonia or ammonium ion as substrate for oxidation by *Nitrosomonas* cells and extracts. *J. Bacteriol*., 120: 556-558

Tahal consultants (1999) (Nigeria) Ltd. Qua Iboe River Basin pre-feasibility study. Cross River Basin Development Authority, Nigeria, Vol. 2, Annex 11: A 1-14

Teugels GC, Reid FM, King RP (1992) Fishes of the Cross River State (Cameroun-Nigeria): taxonomy, zoogeography, ecology and conservation. *Annals Science Zoologiques* 1: 216-248

Tisdale S, Nelson W (1995) *Soil fertility and fertilizer,* 3rd ed; Macmillan publisher Co., Inc. New York, USA.

Walworth JL, Reynolds CM (1995) Bioremediation of a petroleum-ontaminated cryic soil: effects of phosphorus, nitrogen, and temperature. *J. Soil Contam.* 4:299–310

Walworth JL, Woolard CR, Braddock F, Reynolds CM (1997) Enhancement and inhibition of soil petroleum bioremediation through the use of fertilizer nitrogen: an approach to determining optimum levels. *J. Soil Contam*. 6**:**465–480

Welsh DT, Castadelli AG (2004) Bacterial nitrification activity directly associated with isolated benthic marine animals. *Mar. Biol*., 144: 1029-1037