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Effects of Abattoir Wastes on Ammonium and Nitrite Consumptions in a Tropical Fresh Water Ecosystem

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ABSTRACT

Despite the attendant environmental consequences, direct discharge of untreated waste water from industries into water bodies is common in many developing countries. This study evaluates the impact of abattoir wastes on microbial ammonium and nitrite consumptions in a tropical fresh water ecosystem. Samples were collected from points (source point, upstream and downstream) about 300 m apart. Autotrophic and heterotrophic ammonium and nitrite consumptions were estimated by incorporation of allyl thiourea and sodium chlorate, inhibitors of autotrophic ammonium and nitrite oxidations, respectively. Source point sample was the most turbid, richer in nutrients and microbial populations than the upstream and downstream samples. Ammonium and nitrite consumption patterns were active at the upstream and downstream stations. Autotrophic ammonium consumption was completely repressed while autotrophic and heterotrophic nitrite consumptions were very active at the source point. Correlations between ammonium and nitrite consumptions at the various sampling points were negative ($r = -0.96$) for autotrophs at the source point, and positive ($0.96<r<1.0$) for autotrophs and heterotrophs at the other stations. Nitrite consumption rates were greater for heterotrophs than autotrophs. The highest rate of autotrophic ammonium consumption was 14.254 μg NH_4^+-N h^{-1}, observed upstream while the highest rate of heterotrophic nitrite consumption was 122.917 μg NO_2^- -N h^{-1}, observed at the source point. The adverse effect of abattoir wastes on nitrification in the tropical fresh water ecosystem was through repression of autotrophic ammonium oxidation. This could change composition of available nutrients and is capable of causing shifts in microbial community structure and altering aquatic nitrogen cycle.

Key words: Allyl thiourea, ammonium, autotrophs, heterotrophs, nitrite, sodium chlorate

INTRODUCTION

Nitrogen plays a critical role in the productivity of every ecosystem, hence nature ensures accurate balance through its recycling between organic and inorganic forms by using microorganisms in mineralisation, nitrification and denitrification processes. In aquatic ecosystem, improper management of nitrogen can contribute to water quality degradation, therefore identification of nitrogen sources is crucial for management response and regulation of water quality issues (Showers *et al*., 2006). Nitrification, the oxidation of ammonium to nitrite and then to nitrate is a fundamental process in aquatic environments brought about by both autotrophic and heterotrophic microorganisms. Unlike autotrophic nitrification which provides energy for autotrophs, heterotrophic nitrification does not provide energy for heterotrophs (Lin *et al*., 2007).

Autotrophic nitrification is mediated by 2 groups of obligatory autolithotrophic bacteria that use ammonium and nitrite as their sole energy sources and carbon dioxide as their main carbon source. These are Ammonia-Oxidising Bacteria (AOB) and Nitrite-Oxidising Bacteria (NOB) (Robertson and Groffman, 2007). Research has shown that all strains of AOB isolated from terrestrial and freshwater environments belonged to a single evolutionary group within the β-subclass of the class Proteobacteria. A number of studies suggested that Nitrosospira-like organisms dominate among AOB populations in freshwater environments (Kowalchuk and Stephen, 2001). Also cultivation independent molecular methods and immunological techniques have revealed that uncultured bacteria related to the genus Nitrospira and not Nitrobacter are the dominant NOB in many aquatic ecosystems (Daims *et al*., 2001; Spieck *et al*., 2006). Even though nitrifiers appear to be well adapted to a wide range of environmental conditions, they are subject to numerous environmental and physiological control variables such as temperature, Dissolved Oxygen (DO), NH_4-N availability and pH (Strauss *et al*., 2002). Autotrophic nitrifiers often compete with phytoplanktons (Ogbebo and Ochs, 2008), algae (Rier and Stevenson, 2002) and heterotrophs (Horz *et al*., 2004) for dissolved inorganic nutrients.

Apart from being more abundant, naturally occurring heterotrophic bacteria grow faster than nitrifying bacteria and thus should out-compete nitrifying bacteria for available ammonium in N-limited environments (Strauss and Lamberti, 2000). In river water, maintenance of a balanced supply of nitrogen is necessary since excess nitrate could lead to eutrophication and insufficient supply of assimilable nitrogen to microorganisms which are the primary decomposers could drastically affect the ecosystem.

In many developing countries where environmental regulations are either lacking or not properly enforced, coastal regions are replete with industrial and agricultural activities so that wastes from these activities are channeled directly into the rivers. In Nigeria, notable among these activities are slaughtering of animals (Omole and Longe, 2008) and palm oil milling (Olaleye and Adedeji, 2005). Such rivers also serve as sources of domestic water supply for coastal communities. Abattoir wastes have the potential for generating large quantities of solid wastes and waste water with high BOD (GTZ/TBW, 2001). Channeling untreated municipal waste water directly into receiving water bodies could impair the legitimate uses of aquatic ecosystems and lead to a change in microbial community structure (Montuelle *et al*., 2001) due to toxicity to aquatic organisms (Moosavi *et al*., 2005). These are not in sync with the principle of sustainable development.

Microbial communities quickly respond to changes in many environmental parameters, hence the dynamics of their population and metabolism are relevant indicators of the ecological status of many ecosystems. Nitrification has been used as a bioindicator in stream systems influenced by mining activities (Niyogi *et al*., 2003). In view of the role biological nitrification plays in eutrophication and in removal of nitrogen through the two-step nitrification/denitrification process (Mpenyana *et al*., 2008), the factors controlling nitrification in fresh waters are integral to the health of the ecosystem. This study evaluates the effects of abattoir wastes on ammonium and nitrite oxidation in a tropical fresh water ecosystem.

MATERIALS AND METHODS

Study area: Otamiri River flows through various communities in Imo State, Nigeria and serves as a source of drinking water for these communities. In Egbu community, the river is a discharge point for wastes from an abattoir located about 300 m away. Three sampling stations namely upstream, downstream and source point which were about 300 m apart and located at N 05°

28.329´, E 07°03.113´; N 05°28.386´, E 07°02.991´ and N 05°28.422´, E 07°02.803´, respectively were chosen.

Sample collection: River water samples were collected from the three sampling stations at a depth of about 10-20 cm below the water surface using three separate 5 L plastic containers. Samples were transported to the laboratory for analysis within one hour of collection. Portions of the samples were used for microbiological and physicochemical analyses while the other was used for monitoring ammonium and nitrite oxidation over a 24 h period.

Microbiological analysis: Microbial counts were estimated by plating out 0.1 mL of the 10^{-1} to 10^{-3} dilutions of the various samples in normal saline on appropriate agar plates. Total heterotrophic bacterial count was done in nutrient agar while ammonium and nitrite oxidizing bacterial counts were done in modified Schmidt and Belser medium (Schmidt and Belser, 1994). The medium composition for the ammonia oxidizing bacteria was (g L^{-1}): NH_4Cl-0.136, $MgSO_4.7H_2O$-0.04, KH_2PO_4-0.204, EDTA-0.005, Trace metal solution-1 mL, agar-15 g. For nitrite oxidizing bacteria the medium was composed of (g L^{-1}): $NaNO_2$-0.006, $MgSO_4.7H_2O$-0.0098, K_2HPO_4-0.0556, NaCl-0.1168, $CaCl_2$-0.0294, EDTA-0.001, Trace metal solution-1 mL, agar -15 g. The trace metal solution used was composed of (g L^{-1}) H_3BO_4-0.1976, $ZnSO_4.7H_2O$-0.1724, $FeSO_4.7H_2O$-3.8924, $CuSO_4.5H_2O$-0.250. Ketoconazole at 50 μg mL^{-1} was added to the medium for the nitrifiers and the pH adjusted to 7.5. The inoculated plates were incubated at room temperature 28(±2) °C for 1-2 days for the heterotrophs and 7-9 days for the nitrifiers.

Physicochemical analysis: The physicochemical analysis of the water samples were carried out in the geological laboratory of Federal University of Technology, Owerri, by standard methods for water analysis as adopted by HACH (1992) using a portable HACH DR-EL/5 Laboratory Kit. Total carbon was determined by the wet combustion method as modified by Nelson and Sommers (1982) and total nitrogen was by the semi-micro Kjeldhal method (Bremner and Mulvany, 1982).

Estimation of ammonium and nitrite consumption: Extents and rates of ammonium and nitrite consumptions were measured using inhibitors of chemoautotrophic nitrifying bacteria during short-term 24 h incubation experiments following the procedures by Bianchi *et al.* (1994). Some 2.5 L portions of river water from each site were put in screw capped containers and supplemented with basic food solution consisting of (g L^{-1}) NaCl-0.73, $CaCl_2$-0.1825, $MgSO_4.7H_2O$-0.0625, KH_2PO_4-0.0675, $FeSO_4.7H_2O$-0.0000025, $ZnSO_4$-0.0000025 (Juliastuti *et al.*, 2003). Ammonium was added in form of NH_4Cl to a final concentration of 50 μM while nitrite was added as $NaNO_2$ to a final concentration of 0.36 mM (Cebron *et al.*, 2003). The pH of each sample was adjusted to 7.5 with NaOH. Each sample was dispensed in 250 mL amounts into three triplicate bottles labeled A, Na and W. The bottles were given different treatments which enabled the estimations of ammonium and nitrite consumptions by autotrophic and heterotrophic microorganisms as follows:

- A-Allyl Thiourea (ATU), an inhibitor of the oxidation of NH_4^+ to NO_2^- by autotrophic bacteria was added to a final concentration of 10 mg L^{-1} (Hall, 1984)
  This treatment estimated ammonium consumed by heterotrophs ($NH_{4\ hetero}$).

- Na-$NaClO_3$, an inhibitor of the oxidation of NO_2^- to NO_3^- by autotrophic bacteria was added to a final concentration of 10 mM (Belser and Mays, 1980)

This treatment estimated nitrite consumed by heterotrophs ($NO_{2\ hetero}$).

- W-This contained no inhibitor. Both autotrophs and heterotrophs are free for ammonium and nitrite consumption

This treatment estimated:

- Ammonium consumed by both autotrophs and heterotrophs ($NH_{4\ hetero\ +\ auto}$)
- Nitrite consumed by both heterotrophs and autotrophs ($NO_{2\ hetero\ +\ auto}$)

Therefore, autotrophic ammonium ($NH_{4\ auto}$) and nitrite consumptions ($NO_{2\ auto}$) were estimated as follows:

- $(NH_{4\ auto}) = (NH_{4\ hetero\ +\ auto}) - (NH_{4\ hetero}) = (W\text{-}A)$
- $(NO_{2\ auto}) = (NO_{2\ hetero\ +\ auto}) - (NO_{2\ hetero}) = (W\text{-}Na)$

The extents of ammonium and nitrite consumptions were calculated from the decrease in ammonium and nitrite concentration during the incubation. Rates of consumption were calculated by dividing the ammonium or nitrite consumed with time of estimation (h).

Incubation conditions and analysis: The samples were incubated in the dark, at room temperature 28 (±2)°C. Ten milliliter aliquots of each sample were collected at 0, 6, 12 and 24 h and held at 0-5°C prior to analysis. One milliliter aliquots of each sample collected at different time intervals were used for the determination of NH_4^+-N and NO_2^--N. Ammonium-nitrogen was determined by the modified Keeney and Nelson (1982) method while nitrite-nitrogen was determined by the Greiss method as modified by Promega (2005). Ammonium and nitrite consumed were estimated from reductions in ammonium and nitrite concentrations relative to the initial concentrations.

RESULTS

Physicochemical analysis of the river water (Table 1) samples showed that sample from the Source Point (SP) was the most turbid (10 FTU) and had higher conductivity (82 μmhos cm^{-1}), total dissolved solids (49.2 mg L^{-1}), nitrates, bicarbonates, total suspended solids and C:N ratio than the Upstream (UP) and Downstream (DN) stations. Microbiologically, SP sample also had the highest BOD (0.75 mg L^{-1}) and was richer in the various microbial populations. At the various stations, the populations of the total heterotrophic bacteria were significantly higher than those of the nitrifiers with the SP having the highest count of 16.6×10^3 cfu mL^{-1}.

The patterns of cumulative ammonium consumptions showed active autotrophic and heterotrophic ammonium consumptions at the upstream and downstream stations throughout the incubation period (Fig. 1). After 24 h, the autotrophs have consumed 203.95 μg NH_4^+ and 124.78 μg NH_4^+ at the upstream and downstream stations, respectively. On the contrary, at the source point, autotrophic ammonium consumption was completely repressed *ab initio*.

Figure 2 showed the patterns of cumulative nitrite consumption at the three stations. In all the stations, nitrite consumptions by both groups of organisms were active throughout the incubation

Table 1: Physicochemical and microbiological properties of the river samples at the three stations

Properties	Sampling stations		
	Sourcepoint (SP)	Downstream (DN)	Upstream (UP)
i) Physicochemical			
pH@ 28±2°C	6.42	6.34	6.75
Conductivity (µmhos cm^{-1})	82.00	56.00	58.00
Total dissolved solid (mg L^{-1})	49.20	33.60	34.80
Turbidity (F.T.U)	10.00	6.00	4.00
Iron (mg L^{-1})	0.06	0.01	ND*
Calcium (mg L^{-1})	26.00	9.60	6.00
Magnesium (mg L^{-1})	17.01	2.43	3.16
Potassium (mg L^{-1})	1.56	1.02	1.02
Sulphate (mg L^{-1})	4.90	8.20	1.80
Nitrate (mg L^{-1})	1.02	0.133	0.098
Chloride (mg L^{-1})	0.75	0.50	0.50
Bicarbonate (mg L^{-1})	86.93	28.98	33.55
Total Hardness (mg L^{-1})	135.00	34.00	28.00
Manganese (mg L^{-1})	0.033	0.02	ND
Total suspended solids (mg L^{-1})	75.00	50.00	25.00
Chemical oxygen demand (mg L^{-1})	75.00	30.00	22.00
Total organic Carbon (%)	0.0112	0.0052	0.0048
Total Nitrogen (%)	0.0045	0.0032	0.0067
C: N ratio	2.91	1.74	0.83
ii) Microbiological			
Biological oxygen demand (mg L^{-1})	0.75	0.30	0.22
Total heterotrophic count ($\times 10^3$ cfu mL^{-1})	16.60	0.70	0.60
Ammonium oxidizing bacterial count ($\times 10^3$ cfu mL^{-1})	1.04	0.02	0.03
Nitrite oxidizing bacterial count ($\times 10^3$ cfu mL^{-1})	0.67	0.05	0.07

ND*: Not determined

Table 2: Rates of ammonium and nitrite consumption by different microbial communities

Sampling points/Time	Consumption rates			
	Autotrophs		Heterotrophs	
	NH_4^+ (µg h^{-1})	NO_2^+ (µg h^{-1})	NH_4^+ (µg h^{-1})	NO_2^+ (µg h^{-1})
Upstream				
0-6 h	14.254	16.667	10.088	37.500
6-12 h	10.965	16.667	13.377	37.500
12-24 h	4.386	11.007	9.978	31.250
Downstream				
0-6 h	6.579	7.666	15.57	30.417
6-12 h	6.542	15.643	22.167	37.917
12-24 h	3.838	11.543	11.623	34.583
Sourcepoint				
0-6 h	-6.579	29.749	13.175	45.833
6-12 h	-8.772	38.693	26.333	52.366
12-24 h	-3.289	0.000	16.456	122.917

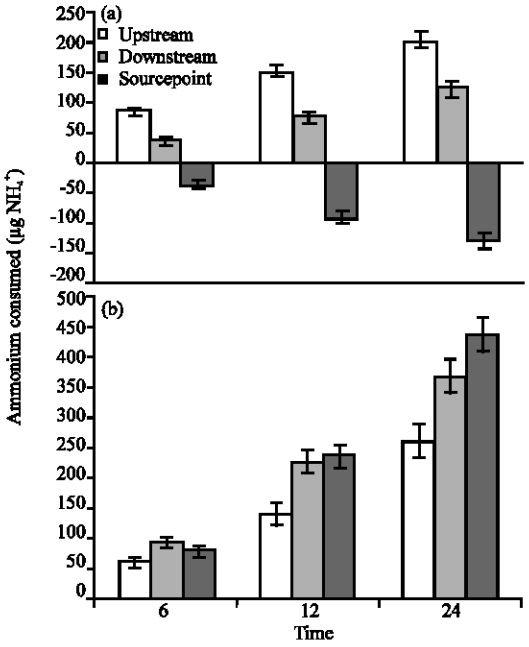

Fig. 1(a-b): Patterns of cumulative (a) autotrophic and (b) heterotrophic ammonium consumption at different sites (<0% = inhibition)

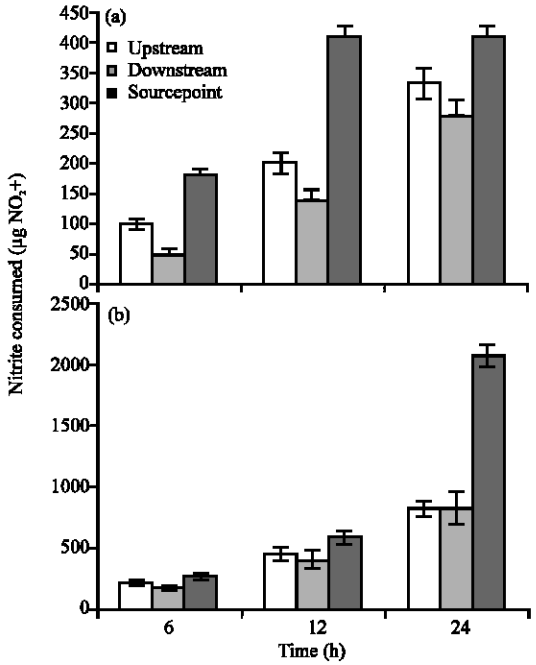

Fig. 2(a-b): Patterns of cumulative (a) autotrophic and (b) heterotrophic nitrite consumption at different sites

period. At 24 h, the autotrophs have consumed 332.09 and 278.37 µg NO_2^+ at the upstream and downstream stations, respectively. At the source point, autotrophic nitrite consumption was active

in the first 12 h, consuming 410.66 μg NO_2^+. At 24 h, no additional nitrite was detected in the medium. Hence, the cumulative nitrite consumption at 24 h was same as that at 12 h.

The rates of ammonium and nitrite consumption at the different stations by the autotrophic and heterotrophic microbial communities are shown in Table 2. The highest rate of autotrophic ammonium consumption was 14.254 μg NH_4^+-Nh^{-1} observed upstream between 0-6 h, however, this steadily declined with time. The rate of autotrophic nitrite consumption upstream was constant (16.667 μg NO_2^--Nh^{-1}) in the first 12 h and slightly declined thereafter. In all the stations, the rates of nitrite consumption by heterotrophs were greater than those by autotrophs. At the SP, the rate of heterotrophic nitrite consumption increased with time and the highest rate of 122.917 μg NO_2^--Nh^{-1} was observed between the 12-24 h.

Estimation of the relationships between ammonium and nitrite consumptions by autotrophs and heterotrophs at the various sampling points using the Pearson's product moments correlation analysis showed that apart from the source point where a strong negative correlation (r = -0.96) existed between both parameters among the autotrophs, the relationships were strongly positive (0.96< r > 1.0) among autotrophs and heterotrophs in the other stations.

DISCUSSION

The physicochemical and microbiological conditions at the source point station which is a direct recipient of abattoir waste, compared to other stations indicate that the waste adversely affected Otamiri River. High turbidity could reduce light penetration into the water and this may inhibit primary productivity (Sandrin *et al*., 2009). Studies by Shah *et al*. (2008) indicated that water transparency is a major physical factor affecting the distribution and seasonal variation of phytoplanktons which represents the biological wealth of a water body and a vital link in the food chain (Hossain *et al*., 2007). In a similar study, Adesalu (2010) attributed poor phytoplankton population in River Oli partly to high turbidity. Increased organic matter can alter energy relationships in the stream and this is capable of disrupting biotic community structure and function (Wakelin *et al*., 2008; Spanhoff *et al*., 2007). This is evident in the higher populations of the various microbial groups in the source point station. Omole and Longe (2008) also reported a high population of fecal coliforms at the source point of a river impacted by abattoir effluent. Increased microbial population could lead to a disruption in microbial community structure that can create shifts in ecosystem-level carbon, energy and nutrient flows (Schimel *et al*., 2007). The greater population of heterotrophs relative to the nitrifiers is in line with the higher growth rate of heterotrophs relative to the nitrifiers (Rittmann and McCarty, 2001) and is consistent with the observations of Fernandes *et al*. (2010) in shrimp pond with elevated organic carbon availability.

Repressed autotrophic ammonium consumption at the source point indicates that the conditions imposed by the abattoir waste at the source point adversely impacted the process. Part of the reason for this could be the high organic carbon content of the waste which imposed nitrogen limitation leading to higher C:N ratio at this point compared to other points. With the high C:N ratio, the faster growing, more abundant heterotrophic bacteria would have out-competed the autotrophic nitrifying bacteria for available NH_4^+. These observations are consistent with those of Strauss and Lamberti (2000) who reported that addition of organic carbon to aquatic environment significantly decreased nitrification rates but increased total microbial population. Another possible reason for the repressed nitrification is the microbial predilection for reduced nitrogen in form of NH_4^+ for cellular incorporation since reduced nutrients are incorporated directly into the Krebs cycle

intermediate for amino acid synthesis. Thus the NH_4^+ must have been immobilised rather than nitrified. The impact was however relieved downstream where even though autotrophic ammonium consumption was observed, it was not as vibrant as the ammonium consumption observed at the unimpacted upstream.

Positive correlations between ammonium and nitrite consumptions among the autotrophs and heterotrophs in the upstream and downstream stations imply normal nitrification process. Thus at the source point, normal nitrification by autotrophs was impeded probably due to conditions imposed by the abattoir waste. Repressed autotrophic ammonium oxidation signifies an impairment of a critical process in the biological removal of nitrogen and has serious implications. It leads to a reduction in the formation of nitrite and nitrate which are substrates for denitrification, the second process in biological nitrogen removal and hence a reduction in the self purifying ability of the river. This can create an imbalance in the nitrogen cycle. Secondly, it could provide assimilable nitrogen for microorganisms and some phytoplanktons. Research has shown that diatoms prefer nitrates (Yool *et al.*, 2007) while phytoplanktons such as dinoflagellates prefer ammonium as a growth nutrient (Lima *et al.*, 2010). Increased ammonium will therefore enhance the growth of dinoflagellates relative to diatoms, thus causing a shift in the phytoplankton community composition. Furthermore it could lead to the accumulation of potentially toxic ammonium salts which could harm aquatic lives (Wee *et al.*, 2007). Ammonium salts have also been reported to have microbicidal effects (Xiao *et al.*, 2008) and so can affect the ammonium oxidizers themselves. This has far reaching implications because ammonium oxidizers possess the Ammonia Mono-Oxygenase (AMO) enzyme complex which has a broad substrate range that permits the co-oxidation of numerous recalcitrant aliphatic, aromatic and halogenated molecules that could cause environmental problems (Robertson and Groffman, 2007). The change in microbial community structure due to shifts in nutrient composition and reduction in substrates for denitrification could alter the aquatic nitrogen cycle and lead to eutrophication of the river. These adverse effects of the abattoir wastes are however, relieved downstream.

Although the upstream station was not impacted, a steady decline in rate of autotrophic ammonium consumption with time was observed there. This could be due to substrate limitation imposed by a progressive reduction in the concentration of the ammonium substrate with time. This however does not seem to affect nitrite whose rate of consumption by autotrophs was relatively constant in the first twelve hours. It thus appears that the overall extent of autotrophic nitrification will be affected more by the concentration of ammonium than the concentration of nitrite. This is consistent with the views expressed by Prosser and Embley (2002) that ammonium oxidation is the rate limiting step in overall nitrification. The fact that nitrite consumption rates by heterotrophs were greater than those by autotrophs suggested that the heterotrophs made more demands on nitrite than the autotrophs. This is consistent with the higher population and faster growth rate of heterotrophic bacteria relative to nitrifying bacteria (Strauss and Lamberti, 2000).

CONCLUSIONS

The abattoir waste adversely impacted nitrification in the fresh water ecosystem through its effect on autotrophic ammonium oxidation. Ammonium oxidation by heterotrophs and nitrite oxidation by both autotrophs and heterotrophs were not affected. Repression of nitrification by autotrophic microbial population acclaimed to be the most prolific nitrifiers could disrupt the nitrogen balance and have far reaching effects on the aquatic ecosystem.

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