

EXPERIMENTAL STUDIES ON UREA DEGRADATION IN SEAWATER

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ABSTRACT

The rate of urea degradation in seawater was studied under various conditions and the kinetics of urea degradation was evaluated. Urea decomposition experiments showed that the rates and quantity of ammonium oxidation were slower in the relatively polluted waters, resulting in the accumulation of ammonia. The urea decomposing bacteria varied between 7 and 40% among the total heterotrophic bacteria in Velsao bay, while these were from 15 to 22% near Mormugao. The rate for biological oxidation of urea was $18.6 \text{ n mole l}^{-1} \text{ day}^{-1}$ and for chemical oxidation it was $10.5 \text{ n mole l}^{-1} \text{ day}^{-1}$. The effects of different urea concentration and pH on the nitrogen transformation processes were also studied.

INTRODUCTION

Earlier studies in Velsao Bay along the coastline of Goa indicated that there was a continuous eutrophication problem associated with discharge of nitrogenous wastes from the fertiliser factory which had an adverse long term effect on fishery resources of this region (Verlencar and D'Silva, 1977).

Laboratory and field studies showed that though ammonia, urea, nitrate and nitrite-N are all utilized by phytoplankton for growth, ammonia seems to be the preferred form which may also inhibit the uptake of nitrate by phytoplankton when present in excess (Eppley, Rogers and McCarthy, 1969). Likewise, abnormally high concentrations of urea in a locality like the Velsao Bay (Goa), might prove to be harmful to the algae. Because of the potential impact of excess urea and its immediate degradation product on the phytoplankton productivity, an attempt has been made to study the rate of degradation of urea in seawater, the formation and accumulation of ammonia under various conditions and also the subsequent process of nitrification.

MATERIALS AND METHODS

Two stations were surveyed periodically (Fig. 1) and they were the effluent area of Velsao Bay (Station 1) and the relatively unpolluted open sea area, 3 km off Mormugao harbour (Station 2). First survey was carried out in Jan-June 1976, before the pipeline for the discharge of effluent was laid. Another survey was carried out in 1978. The concentration of urea, ammonia, nitrite and nitrate were estimated. Urea in seawater was estimated by the method of Newell, Morgan and Cundy (1967). Ammonia was determined by the indophenol blue method of Koroleff (1969). Nitrite and nitrate were estimated by standard procedures described by Strickland and

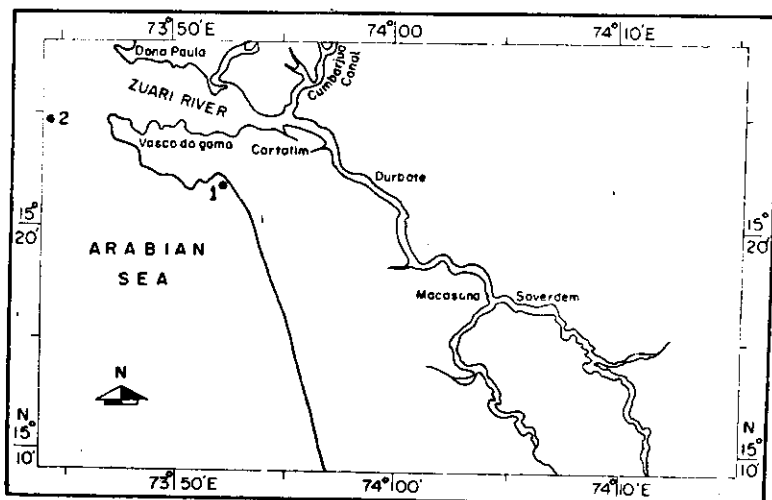


Fig. 1. Map showing the two stations sampled.

Parsons (1968). The number of total heterotrophic bacteria was estimated using ZoBell's 2216 E medium (Aaronson, 1970) and that of urea decomposing bacteria using the medium given by Remsen, Carpenter and Schroeder (1974).

Laboratory experiments were carried out to find the rate of urea degradation and ammonia oxidation by incubating the water samples filled in 300 ml dark BOD bottles, tightly sealed and kept in the dark at room temperature (25–30°C). Periodically one bottle was opened and the required chemical parameters were analysed. To find out the rate of nonbiological degradation process, the water sample was filtered in sterile condition through silver filters having 0.1 micron pore size and incubated in sterilized dark BOD bottles.

Similarly, incubation experiments were carried out to find out the effect of urea concentration on its degradation and subsequent other nitrogen transformation by adding 5, 40, 130 and 160 $\mu\text{g-at/l}$ of urea to the samples separately. The effect of pH on these processes was also estimated by altering the pH using either borate-borax buffer and bicarbonate-carbonate buffers that were prepared in artificial seawater according to Gomori (1955).

RESULTS AND DISCUSSION

Nitrogen transformation processes at the two stations

The work carried out at Station 1 and 2 in January–June, 1976, prior to laying the pipeline indicated that at these stations, ammonia varied between 4 to 10 $\mu\text{g-at/l}$, while the concentration of urea in the effluent area was found to vary widely between 12.5 $\mu\text{g-at/l}$ to 115.3 $\mu\text{g-at/l}$ and at the Station 2, it varied between 2 to 10.0 $\mu\text{g-at/l}$ only. Several authors reported varying urea concentration in different regions, but it never exceeded more than 5.0 $\mu\text{g-at/l}$. Newell (1967) and Remsen (1971) found values ranging from 0.5 to 5.0 $\mu\text{g-at/l}$ whereas McCarthy (1970) reported values from 0 to 0.67 $\mu\text{g-at/l}$.

The decomposition experiments showed variations in different nitrogen transformation processes. The decomposition of urea and the resultant ammonification were slower at Station 1 than at Station 2. The maximum production of ammonia was found to be after 40 days in the water from Station 2 and a maximum of only 10.0 $\mu\text{g-at/l}$ of ammonia was produced. On the other hand in the effluent water from Station 1, the rate of ammonia production was higher and within 17 days, a maximum between 22.0 to 44.4 $\mu\text{g-at/l}$ was found to be produced.

Ammonium oxidation by autotrophic bacteria was also found to vary between the two stations, despite a similarity between both waters in the lag phase for this process, *i.e.*, 4 to 6 days. The rate and quantity of ammonia oxidation were less at Station 1, resulting in the accumulation of ammonia. A maximum of 15 $\mu\text{g-at NH}_4\text{-N/l}$ was oxidised in 12 days in unpolluted waters, whereas it took 16–18 days for the same process in the waters from Station 1. In the same way, the nitrite oxidation was quite fast at Station 2 resulting a maximum nitrate production upto 22.2 $\mu\text{g-at/l}$ in 30 days. These variations between the two waters are thought to be due to the variability in the bacterial population, which is normally altered by the substrate concentration, pH, suspended particles and other physico-chemical factors. Ammonia oxidation and nitrite oxidation were found to be simultaneous with ammonia oxidation being the slower of the two processes.

As far as the nitrogen is concerned in the aquatic environment, the principal toxicity problem is form ammonia in the molecular form (NH_3) which can adversely affect the aquatic life in the receiving coastal waters. Increase in pH may cause elevation in toxicity, as the ammonium ion (NH_4^+) is progressively transformed into ammonia. Concentration of dissolved oxygen and carbon dioxide, increase in temperature and bicarbonate alkalinity are the factors that increase the ammonia toxicity at a given pH.

Distribution of urea decomposing bacteria

Water samples from 2 m depth and sediment samples were collected in sterilized tubes for four months (Feb–May, 1976) and tested for both total heterotrophic bacteria and urea decomposers. The results are given in the Table I.

Table I. Distribution of urea decomposing bacteria in relation to the total heterotrophic bacteria.

Station	Sample	Total heterotrophic bacteria				Urea decomposing bacteria			
		Feb	Mar	Apr	May	Feb	Mar	Apr	May
Velsao Bay (Station 1)	Water (No. $\times 10^4/\text{ml}$)	16	29	25	5	2.4	6.0	4.2	2.0
	Sediment (No. $\times 10^6/\text{g}$ dry wt)	37	29	20	47	1.2	1.7	3.9	1.7
Mormugao (Station 2)	Water (No. $\times 10^4/\text{ml}$)	25	32	27	10	0.4	1.4	2.7	1.3
	Sediment (No. $\times 10^6/\text{g}$ dry wt)	42	39	17	32	3.2	4.2	2.0	0.7

In the case of the total heterotrophic bacteria, there was not much difference in their numbers between Velsao Bay and Mormugao waters, though the numbers were less in Velsao Bay in most occasions. But the urea decomposers were very low in number in Mormugao waters. This may be due to the availability of the substrate, namely urea in sufficiently high concentration in Velsao Bay. It is observed that both the total heterotrophic bacteria and urea decomposers were not differing very much in distribution in both the stations. The urea decomposers varied between 7 to 40% among the total heterotrophic bacteria in Velsao waters, while 15 to 22% in Mormugao.

Rate of biological and non-biological degradation of urea

The formation and utilization of ammonia due to both biological and non-biological degradation are shown in Fig. 2. The process is comparatively slower in the samples collected at the mouth of the Zuari estuary (sample 1) than those in the sample 2, collected from Sanvordem in the Zuari estuary, nearly 32 km from the mouth. In sample 1, there was only production of ammonia though the process was slow. On

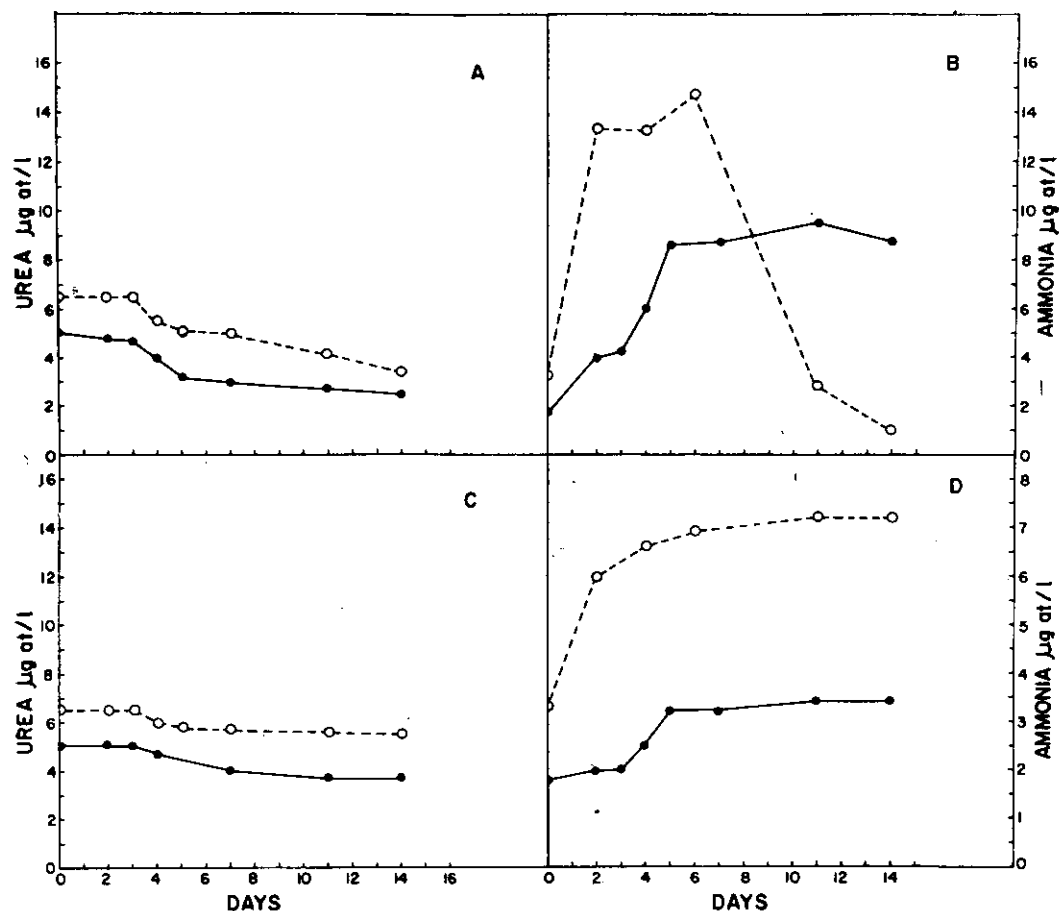


Fig. 2. Rate of biological and non-biological degradation process. A and B : Biological oxidation; C and D : Chemical oxidation (●—● Sample 1 and ○—○ sample 2)

the contrary, in the sample 2, the process was very fast even from the first day and the production was maximum, upto $14.75 \mu\text{g-at/l}$, from 2nd to 6th day of the experiments. Later the ammonia content was found to decrease sharply, probably due to the utilization of ammonia by heterotrophic bacteria that occurred more in Station 2 than at the mouth of the estuary (Fig. 2B).

During the non-biological (mostly chemical) degradation process, the production of ammonia in the sample 1 was very slow, more or less 30% of the total production due to both biological and non-biological degradation processes (Fig. 2D). But in the case of the sample 2, the production of ammonia was faster and more or less 50% of the total produced by both the processes. There was no decrease in the ammonia content because there was no heterotrophic bacterial utilization.

The results in the ammonia production showed significant difference in biological and non-biological oxidative capacities of the two samples. The variation in the urea content due to both microbial and non-biological degradation are shown in Fig. 2A & B. Though the initial concentration of urea in sample 2 was slightly higher than the sample 1 ($6.47 \mu\text{g at/l}$ in sample 2 and $5.07 \mu\text{g-at/l}$ in sample 1), the rate of degradation was more or less the same (2.56×10^2 n moles/l/day in sample 2 and 2.81×10^2 n mole/l/day in sample 1). The degradation process due to the non-biological degradation was very slow (Fig. 2C) and it was, however, higher in sample 1 (11.14×10^1 n moles/l/day) than the sample 2 (8.1×10^1 n moles/l/day).

While both urea degradation and ammonia production were essentially due to heterotrophic oxidative processes, the ammonia production was faster than the urea degradation. In other words, the ammonia produced was not entirely from urea but also from other dissolved organic nitrogen compounds, like amino acids etc.

The changes in the concentration of urea, ammonia and nitrite in both unfiltered and filtered seawater during 25 days of incubation are shown in Table II. These results are the average values of 4 experiments carried out at different periods during the year 1978-79. The results indicate the significance of biological degradation process and of the nitrifying bacteria. Though there was no lag period in urea degradation and ammonia formation, the rate of these two processes are comparatively slower in the first 8 days of incubation and between 9-18 days, the rates of urea degradation and ammonia formation were the highest (5625 n mole/l/day of urea removal and 925 n mole /l/day of ammonia addition). Later after 19 days, the ammonia

Table II. Rates of biological and chemical degradations of urea.

Day	Seawater unfiltered - change in n mole/l/day			Filtered seawater - change in n mole/l/day		
	Urea	Ammonia	Nitrite	Urea	Ammonia	Nitrite
1-4	-50	+770	0	-20	0	0
5-8	-375	+710	+40	-275	+315	0
9-18	-5625	+925	+2935	-500	+205	0
19-25	-75	+782	+800	0	+104	0

(Negative sign indicates the disappearance and the positive sign indicates the addition to the water)

was found to decrease and relatively increased rate of ammonium oxidation was found.

The lag period for nitrite production (resultant of ammonium oxidation) varied between 3-5 days. No nitrification was found in the filtered sea water samples. Urea decreased at a rate of 20 n moles/l/day in the first 4 days and in next 4 days the rate was found to be 275 n moles/l/day. This is due to hydrolysis of urea which is comparatively negligible. Similarly, very low rate of ammonia formation was found (315 n moles/l/day) in 5-8 days and later decreased still further.

These experiments show that along with the heterotrophic activity resulting biodegradation, autotrophic nitrification also is significantly required to avoid the accumulation of ammonia in the coastal waters. Nitrogen in the fixed forms of ammonia and nitrate ions is the major nutrient that cause the algal blooms and eventually resulting in eutrophication. So nitrogen removal from waste waters is the major task for the environmental scientists who recommend that nitrogen removal through nitrification followed by denitrification represents the most promising methods because it has the advantage of returning nitrogen to the atmosphere in its natural form. Nitrification is considered to be highly beneficial because of the oxygen source provided by nitrate which is the end product and thus accomplish a significant nitrogenous oxidation (NOD) in creating oxygen sag in the receiving coastal waters. Barth, Brenner and Lewis (1968) and Barth (1972) suggested a multiple stage, combined chemical-biological processes designed for nitrogen removal. It consisted of different stages of each biological systems such as nitrification, denitrification etc. and chemical supplements to enhance the overall efficiency.

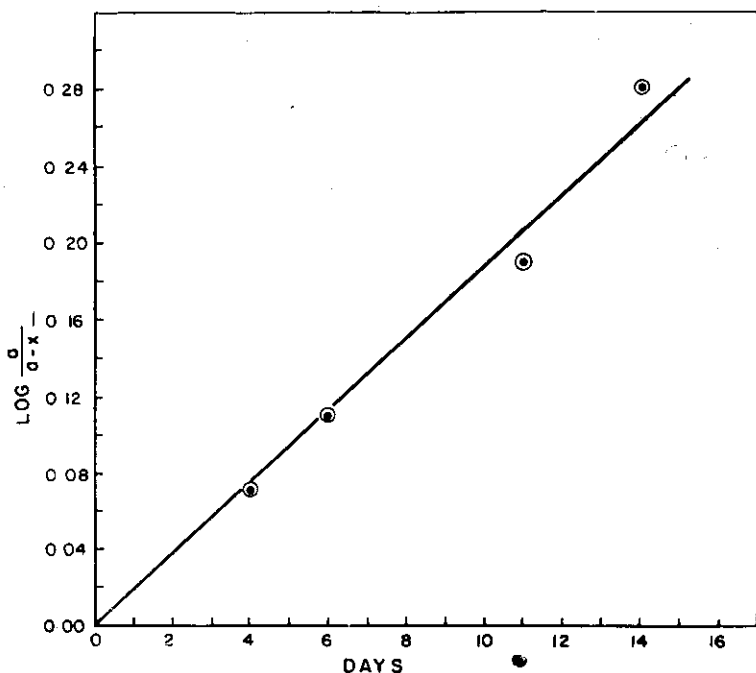


Fig. 3. Hydrolysis of urea due to biological oxidation.

Kinetic study on urea hydrolysis

Urea on hydrolysis gives ammonia and carbon dioxide as products which can be represented as



The rate of reaction can be expressed as

$$-\frac{d}{dt} [\text{NH}_2\text{CONH}_2] = K [\text{NH}_2\text{CONH}_2] [\text{H}_2\text{O}]$$

where $[\text{NH}_2\text{CONH}_2]$ is the concentration of urea and K is the rate constant of the hydrolysis. The hydrolysis of urea was found to be a first-order reaction for both biological and chemical oxidation processes (Figs. 3 and 4). The rate calculated for biological oxidation was 18.6 n mole/l/day and for the chemical oxidation it was only 10.5 n mole/l/day. The rate constants were also calculated and found to be $4.28 \times 10^{-2} \text{ day}^{-1}$ and $2.42 \times 10^{-2} \text{ day}^{-1}$ for the biological and chemical oxidations respectively. It was observed that the rate and the rate constant for the bacterial degradation was found to be more or less double as compared with the chemical hydrolysis where the hydrolysis was not catalysed by the biological agencies that usually give the activation energy needed to degrade the urea.

Since water, one of the reactant in this reaction is present in great excess, the concentration of water can be regarded as constant. Hence the rate equation of urea can be rewritten as

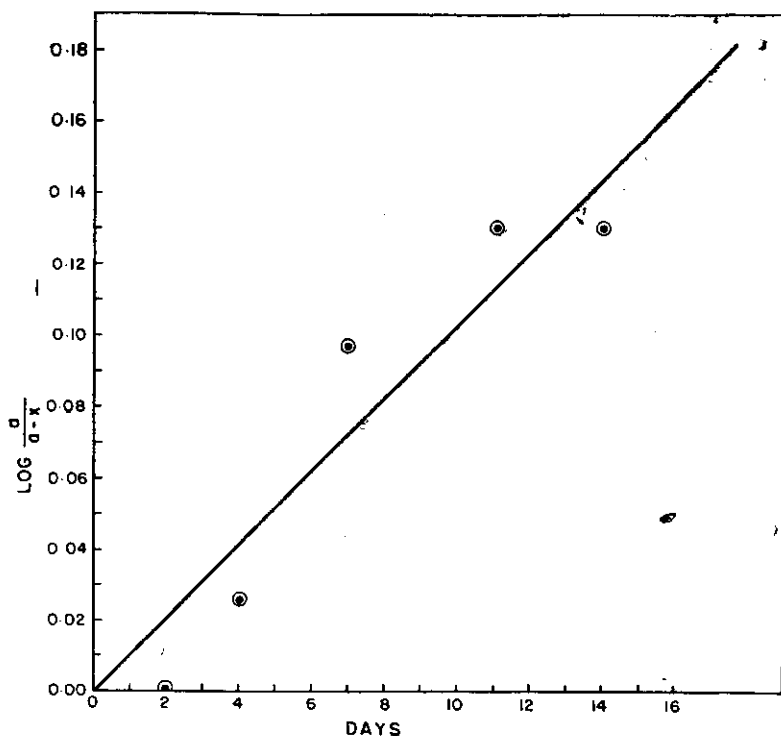


Fig. 4. Hydrolysis of urea due to chemical oxidation.

$$-\frac{d}{dt}[\text{NH}_2\text{CONH}_2] = K[\text{NH}_2\text{CONH}_2]$$

For this reason, urea hydrolysis can be considered as pseudo-first order reaction.

Effect of urea concentration on its degradation and subsequent nitrification

Experiments were conducted with 4 different initial concentrations of urea such as 5, 40, 130 and 160 $\mu\text{g-at/l}$ and variations in the lag phase, degradation of 50% urea, ammonia production and nitrite formation were observed. The results are shown in Fig. 5.

The lag phase in urea degradation was always 3 or 4 days in the lower urea concentrations, and at 160 $\mu\text{g-at/l}$ level, there was a lag phase of 6 days observed. At the concentrations of 5 $\mu\text{g-at/l}$, ammonia formation and nitrite formation were slow with longer lag phase and at higher concentrations, especially at 130 and 160 $\mu\text{g-at/l}$ levels, the lag phase was short and simultaneously ammonia and nitrite started to form within 3 days of the lag phase.

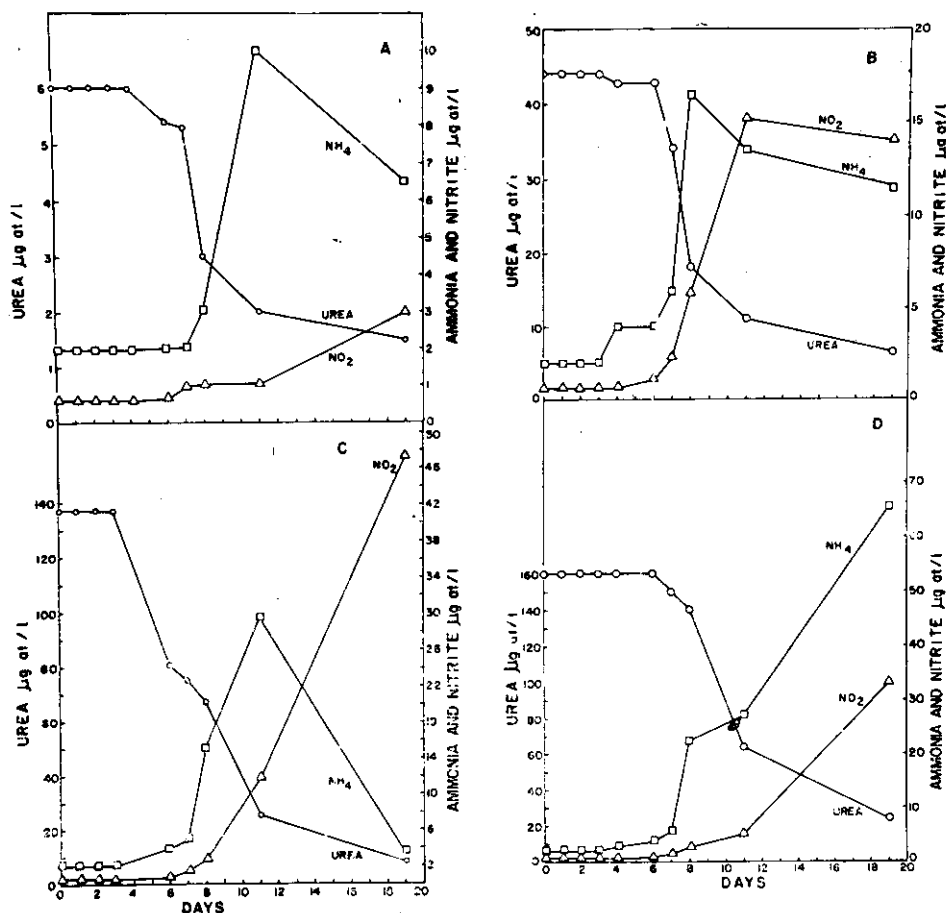


Fig. 5. Effect of different concentration of urea on its degradation and subsequent nitrification. (A) 5 $\mu\text{g-at/l}$; (B) 40 $\mu\text{g-at/l}$; (C) 130 $\mu\text{g-at/l}$ and (D) 160 $\mu\text{g-at/l}$.

At all concentration levels, except at 160 $\mu\text{g-at/l}$ the degradation of 50% urea was observed on 7th or 8th day. Only on 10th day, this occurred at 160 $\mu\text{g-at/l}$ level. Irrespective of the urea concentration, the ammonia production was found to start within 3 or 4 days and also at all on concentration levels, ammonia production and nitrite formation were simultaneous.

In the experiment of 5.0 $\mu\text{g-at/l}$ of urea level, ammonia started decreasing after reaching a maximum of 10.0 $\mu\text{g-at/l}$ on 11th day (Fig. 5A). In the higher urea concentration levels, ammonia did not reach relatively higher level. For example, ammonia began to decrease after reaching a maximum of 16.5 $\mu\text{g-at/l}$ on 8th day when 40 $\mu\text{g-at/l}$ of urea was used (Fig. 5B) and 29.5 $\mu\text{g-at/l}$ on 11th day when 130 $\mu\text{g-at/l}$ of urea was allowed to decompose (Fig. 5C). Only in the experiment in which 160 $\mu\text{g-at/l}$ of urea was used, it was observed that ammonia did not start decreasing even after 19 days and its concentration on 19th day was 65.5 $\mu\text{g-at/l}$ (Fig. 5D).

The above results showed that the concentration of decomposing urea did not affect the simultaneous production of ammonia and nitrate. Though there was no significant ammonia accumulation in the case of lower concentration of urea decomposed, ammonium oxidation was found to be slow at urea level higher than 130 $\mu\text{g-at/l}$ and consequently, there was a higher concentration of ammonia persistently. The overall rate of degradation was also found to be slower when higher concentration of urea was allowed to decompose. Table III shows the variation in the rate of urea decomposition and ammonia and nitrite production in relation to the amount of urea allowed to degrade in 10 days. The rates decreased only at maximum concentration of urea, but the rate of nitrite production started decreasing even after 40.0 $\mu\text{g-at/l}$ of urea level in the water sample. In spite of a short lag period in nitrification, there was no retardation due to the high concentration of urea or ammonia.

Effect of pH on urea degradation

The effect of pH (at 5.0, 6.0, 8.0 and 9.0) was observed on both urea decomposition and ammonia production during the early stages of degradation. There was no disappearance of urea in the first 12 hours at both 5.0 and 9.0 pH levels and in the intermediate pH levels, the decrease of urea began in 12 hours (Fig. 6). There was a gradual increase in the rate of degradation at lower pH levels (5.0–8.0) but beyond 8.0, it decreased. Among the four pH levels tested, the slowest rate was at 9.0 (Fig. 6).

Table III. Effect of various concentrations of urea on the degradation process.

Substrate concentration (urea) $\mu\text{g-at/l}$	Rate of urea decomposition n mole/l/day	Rate of ammonia production n mole/l/day	Rate of nitrite formation n mole/l/day
5.0	0.4×10^3	0.7×10^3	0.4×10^3
40.0	3.0×10^3	1.1×10^3	1.3×10^3
130.0	10.2×10^3	2.5×10^3	1.0×10^3
160.0	8.7×10^3	2.3×10^3	0.4×10^3

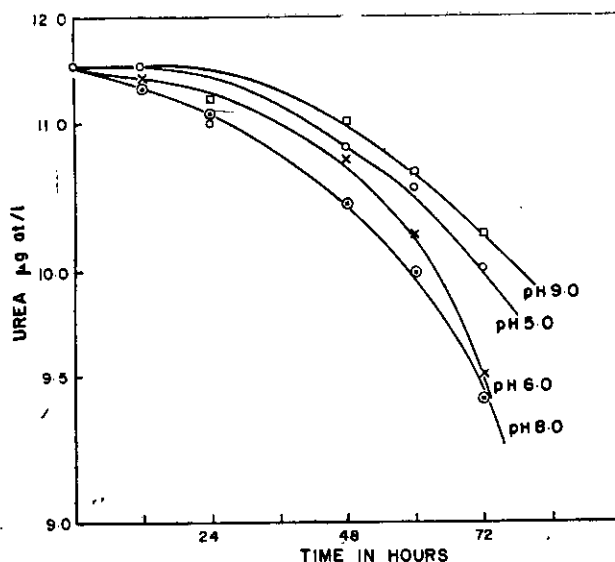


Fig. 6. Effect of pH on urea degradation.

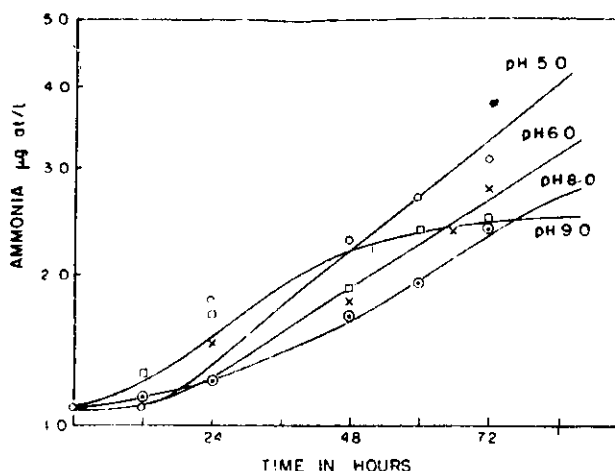


Fig. 7. Effect of pH on ammonia production.

With regard to the effect of pH on the ammonia production, the trend was different from that found in the degradation of urea. At pH 5.0 and 6.0, there was an increased rate of production of ammonia, especially after 24 hours (Fig. 7). A very high rate of ammonia production was found at pH 9.0 till 48 hours and afterwards it became very slow. At pH 8.0, there was a gradual increase in ammonia production but the rate was found to be slower among the four pH levels tested.

It was found that there was an increased rate of nitrification at pH 8.0 and 9.0 and the nitrite increased from 0.16 to 0.36 $\mu\text{g-at/l}$ and from 0.16 to 0.45 $\mu\text{g-at/l}$ respectively at 8.0 and 9.0 pH levels. Since there was a slow oxidation process of ammonia to nitrite at pH 5.0 and 6.0, there was an accumulation of ammonia in these experiments.

These experiments indicate that from 7.5 to 8.4, there was an increased rate of urea degradation and ammonium oxidation. As far as the Velsao Bay is concerned, it is desirable to have a high rate of urea degradation as well as a high rate of nitrifica-

tion so as to alleviate the toxic accumulation of ammonia in the environment. It was also found in the present experiments that there was a lag period in the oxidation of ammonia and this may be due to the time taken for acclimation of nitrifiers to the environmental pH. Although nitrifying bacteria initially prefer a pH environment of 7.0 to 8.0, it was found that they would acclimate to lower pH and approach the ammonia removal capacity attainable at the higher pH levels (EPA Manual, 1975).

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