

THE REACTION OF ORGANIC NITROGEN COMPOUNDS WITH CHLORINE AND CHLORINE DIOXIDE

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ABSTRACT

When chlorine and chlorine dioxide are separately interacted with the nitrogenous compounds, namely, adenine, cytosine, thymine, and uracil, and in the concentration range of 0.001 to 0.28 mm/l, chlorine exhibited a higher selectivity for cytosine and thymine, when the concentrations of nitrogenous compounds were held constant. Under similar conditions, there were no reactions with chlorine dioxide. These findings herald inroads into an understanding of chlorine-DNA dynamics and the resulting mutagenic effect, and the associated health impacts of disinfecting municipal water and wastewater by chlorination.

Chlorination is the most widely used method for disinfecting municipal water and wastewater. In an aqueous environment, free chlorine in the form of unionized HOCl is a very potent biocidal agent [1]. However, it further dissociates into H^+ and OCl^- . At pH levels higher than 7.5, OCl^- dominates, and at lower pH values HOCl is the dominant species. Aqueous chlorine impairs a wide variety of subcellular components and disrupts metabolic function [2]. Nitrogen is a ubiquitous component of surface waters [1-13].

Williams contends that domestic sewage, treated or untreated, and food industries are the main contributors of organic nitrogen to surface water [14]. It is estimated that total nitrogen loading in domestic sewage ranges from 0.5 to 2.2 grams per capita per day, of which about 40 percent is organic nitrogen.

As early as 1957 [15], α -amino-nitrogen compounds were found in fresh water, later amino acids were also detected in Lake Mendota. Ram identified samples [16], and at times the concentration was up to 860 $\mu\text{g/l}$. He estimated that the concentration of heterocyclic compounds varied from 0.2 to 18.8 percent of the total organic nitrogen. However, the total organic nitrogen concentration varied from 0.34 to 21.7 mg/l as nitrogen.

In the past, much work has been conducted on the formation of chloramines in water; however, investigation on the interaction of organic nitrogen and aqueous chlorine has been rather limited.

Recent investigations of Rook [17] and Bellar [18] have implicated the chlorination because it reacts with certain precursors in water to produce halogenated compounds, some of which may be carcinogens or mutagens. Because of health implications caused by such compounds, much attention has been given to alternative disinfection by chlorine dioxide (ClO_2).

How chlorine or chlorine dioxide affects disinfection at the cellular level is not well understood at this time. Knox *et al.* claim that deactivation of the mercapta group of the protein molecule explains HOCl 's action as a disinfectant [12]. On the other hand, Olivieri contends f_2 type viruses take exception; rather, the disruption of transformation-ribonucleic acid (RNA) has been found to be the principal disinfection mechanism for f_2 viruses [13].

In this research, the selected organic nitrogen compounds were four heterocyclic amines: adenine, cytosine, thymine, and uracil. They widely occur in the cells of plants and animals. A heterocyclic amine is a vital part of the nucleotide. Each monomer unit of the nucleotide consists of a pentose sugar, a phosphate, and a heterocyclic amine. Heterocyclic amines are also referred to as nucleic acid bases.

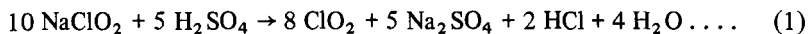
Nucleotides undergo hydrolysis to form nucleic acids. When the pentose sugar is "ribose," the resulting nucleic acid is called RNA. And when the pentose sugar is "deoxyribose," it is referred to as deoxyribonucleic acid (DNA). DNA constitutes the actual chemical of the genes.

This study evaluated the chemical interaction of organic nitrogen with chlorine and chlorine dioxide. The concentration of organic nitrogen ranged from 0.0375 to 0.28 millimoles per liter.

METHODOLOGY

1. All glassware was washed with acid cleansing solution followed by Super "Q" water. Experiments were conducted in one liter beakers in batch form at 25°C .
2. Stock solutions of chlorine were prepared by the dissolution of chlorine gas in water. The final pH was adjusted to 4.2 with 1N NaOH. The lower pH precludes the loss of chlorine by volatilization by converting some of the free chlorine to hypochlorous acid.

3. Chlorine dioxide was generated in the laboratory according to *Standard Methods* [19]. Chlorine dioxide was generated by reacting acid (10% H_2SO_4) with activated sodium chlorite solution. The reaction proceeded according to the following equation:



This reaction usually provides chlorine-free ClO_2 , however, trace amounts of chlorine were found, which were neutralized by glycine [19].

A predetermined concentration of disinfectant was added to the beaker containing nucleic acid solution. The final volume in all cases was 500 ml. The system was gently mixed by magnetic stirrer to provide homogeneity. Samples of 20 ml were withdrawn from the system at various time intervals, and the samples were immediately tested for residual chlorine or chlorine dioxide concentration, using DPD-FAS method [19].

RESULTS AND DISCUSSION

Figure 1 represents a plot of residual free chlorine with respect to time for various thymine-chlorine concentrations. In these experiments, the organic nitrogen concentration was held constant while varying the chlorine concentration.

The reaction proceeded rapidly when the ratio was 1.0 to 1.0. The thymine to chlorine in general exhibited multiphasic characteristics, as evidenced by the varied rate of depletion. Similar curves were plotted for each organic-nitrogen compound.

Figure 2, a plot of free residual chlorine with respect to time, is typical. In these experiments, however, the concentration of disinfectant was held constant and the concentration of organic nitrogen varied. For example, the reaction between uracil and chlorine was slow within the first ten minutes of reaction, that is, for chlorine-uracil concentrations of 7.5:1.0 and 7.5:2.5. Thereafter, the reaction rate increased, then tapered off after twenty-five minutes.

The objective of these plots was to obtain the rate of reaction for each nitrogen compound under investigation. The kinetics were governed by the following equation:



where

A = organic nitrogen concentration

B = disinfectant's concentration

Products = chlorinated organic nitrogen compound and other by-products.

From equation (2) the rate of the reaction is as follows:

$$r = k (NA)^\alpha (Cl)^\beta \tag{3}$$

where

- r = rate of reaction
- NA = organic nitrogen concentration
- Cl = concentration of disinfectant (Cl or ClO₂)
- k = reaction constant
- α = order of reaction with respect to NA
- β = order of reaction with respect to chlorine or chlorine dioxide.

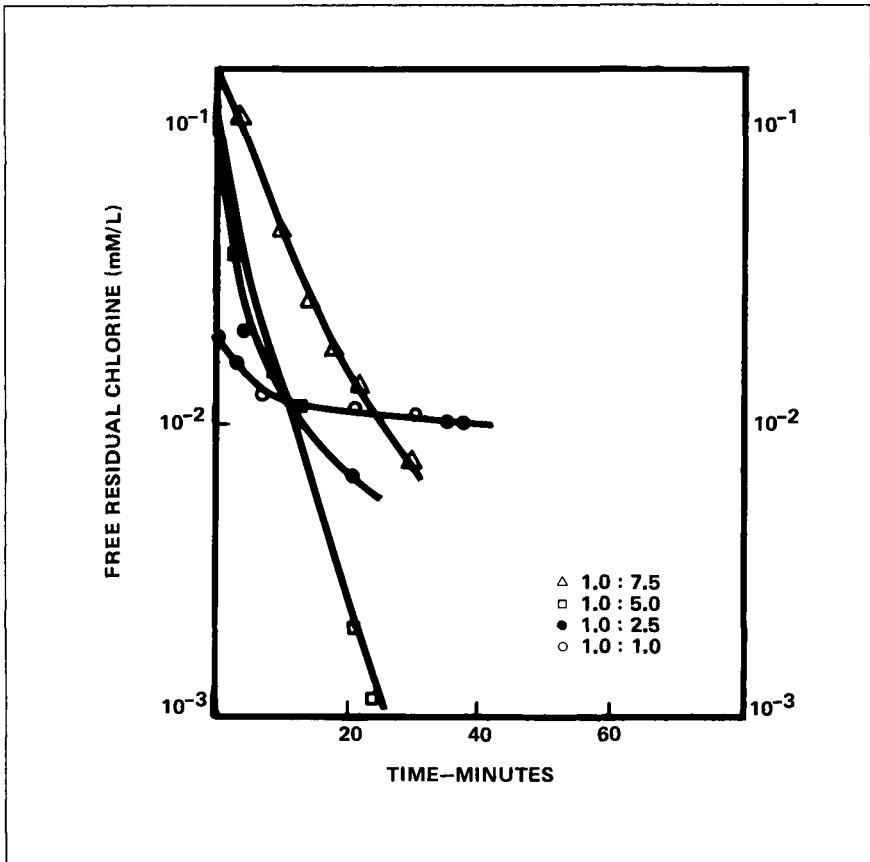


Figure 1. Free residual chlorine in nucleic acid-chlorine system.

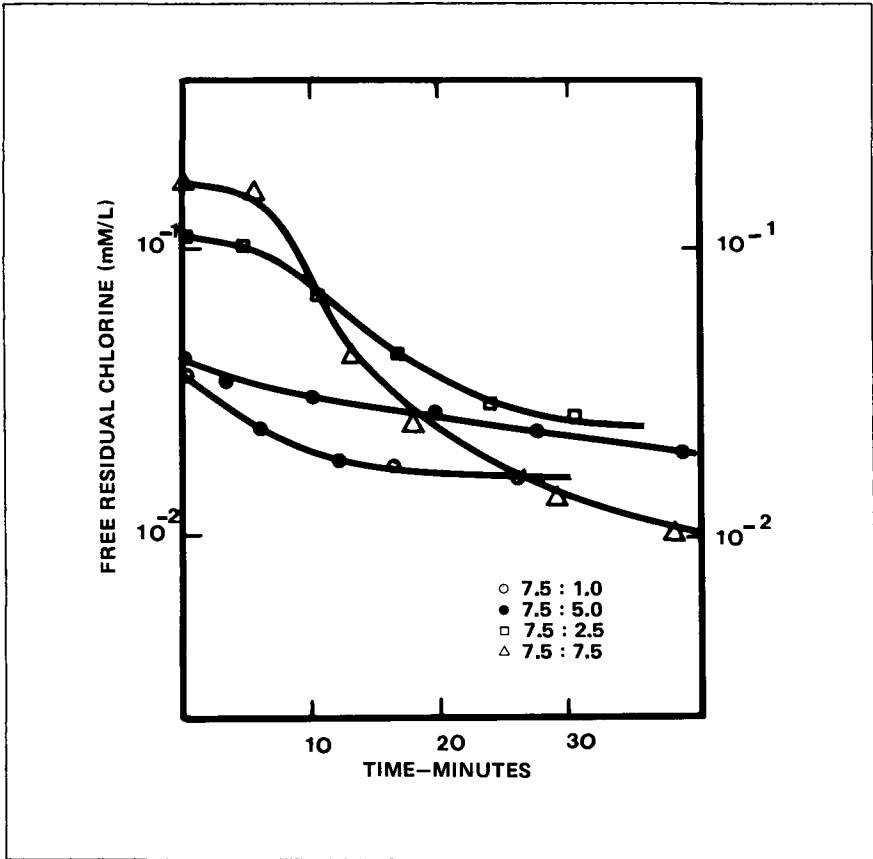


Figure 2. Free residual chlorine in nucleic acid-chlorine system.

Equation (3), when differentiated, becomes

$$-\log \text{rate} = \log [K] + \alpha \log [\text{NA}] + \beta \log [\text{disinfectant}] \dots \quad (4)$$

Figure 3 represents plots between initial rate of reaction and initial concentration of the organic nitrogen compound. The slopes of the curves are represented by α .

The initial rates were obtained from graphical plots of disinfectant's residual concentration with respect to time. Typical graphs are shown in Figures 1 and 2. Zero slopes were obtained for cytosine and thymine, as shown in Figure 3, thus indicating the nonreactivity of these two compounds with chlorine in the concentration range between 0.001 to 0.28 mm/l. Adenine and uracil reaction orders were 1.08 to 0.89 min^{-1} , respectively.

Table 1.

Organic Nitrogen Compound	α -Order ^a (min ⁻¹)	β -Order ^b (min ⁻¹)
Adenine	1.08	0.63
Uracil	0.89	0.91
Cytosine	0.00	1.57
Thymine	0.00	0.49

^a Rate expression when chlorine concentration was held constant.

^b Rate expression when organo-nitrogen compound was held constant.

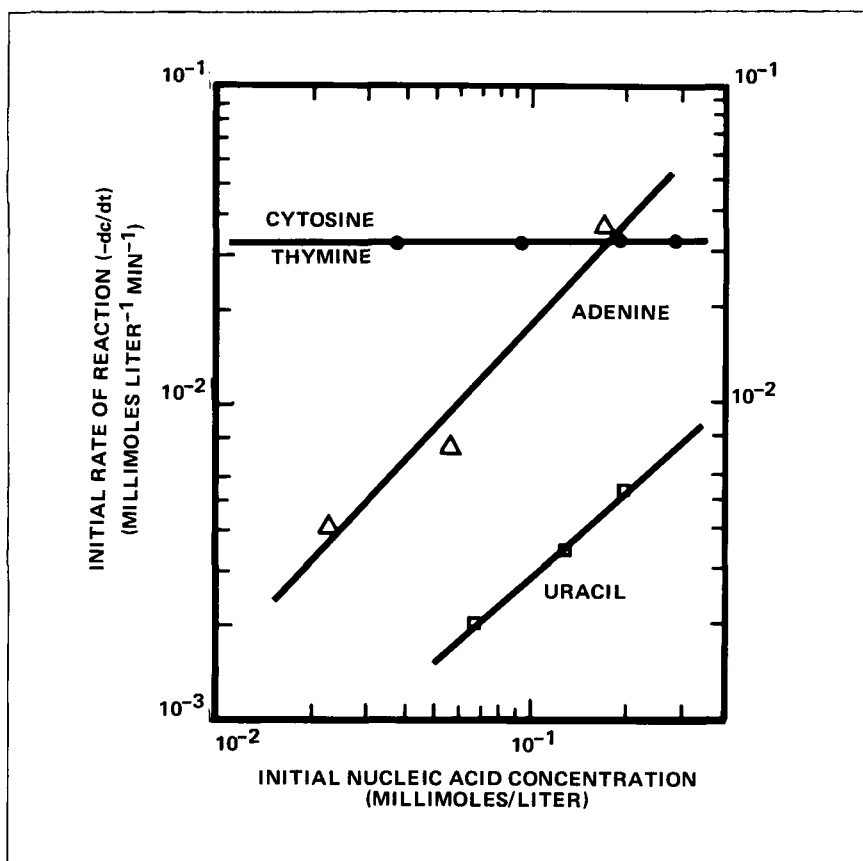
Figure 3. Reaction order curve in α for nucleic acid-chlorine system.

Table 1 shows the numerical value of β for various organic nitrogen compounds, varying from 0.49 min^{-1} (thymine) to 1.57 min^{-1} (cytosine).

The increasing reaction orders with respect to β have shown that thymine < adenine < uracil < cytosine with corresponding values of 0.49, 0.63, 0.91, and 1.57, respectively. On the other hand, adenine and uracil show α values of 1.08 and 0.89, respectively, with cytosine and thymine showing zero order.

$$-r = dC_A/dt = -dC_B/dt = k' C_A C_B . \quad (4)$$

Parallel studies were also conducted with chlorine dioxide. Here, too, equation (4) governed. The amount of C_A or C_B , that is, disinfectant or organic nitrogen, respectively, reacting at any time, t , should be equal. This is given in the relationship shown in equation (5).

$$C_A = C_B = (C_{A0}) (X_A) = (C_{B0}) (X_B) , \quad (5)$$

where

- C_A = millimolar concentration of disinfectant at anytime, t .
- C_B = millimolar concentration of organic-nitrogen compound at anytime, t .
- C_{A0} = initial concentration of disinfectant (chlorine or chlorine dioxide).
- C_{B0} = initial concentration of organic nitrogen compound.
- X_B = mole fraction of organic nitrogen compound, at anytime, t .
- X_A = mole fraction of (Cl or ClO_2) at anytime, t .
- t = time (min)
- k = reaction rate .

The rate equation becomes

$$-r_a = (C_{A0}) (dX_A)/(dt) = k'(C_{A0}) (C_{B0} - C_{A0} X_A) . \quad (6)$$

By separation of terms, integration, breakdown into partial fractions, and rearrangement, equation (5) becomes

$$1/C_A - C_{A0} = (C_{A0}) (X_A/(1 - X_A)) = kt \quad (7)$$

Figure 5 is a plot of equation (7) to test for bimolecularity between organic nitrogen compound and disinfectant. The resulting plot should, theoretically, exhibit linearity. However, it is evident that cytosine and thymine were non-linear, with thymine showing a major departure from linearity. On the other hand, adenine and uracil tend towards linearity, approximating a pseudo-second order reaction.

In Figure 5, the slope of the graph expresses the rate of reaction as dictated by equation (7). If equation (7) was to be satisfied, then the reaction would be described as a classical second order reaction.

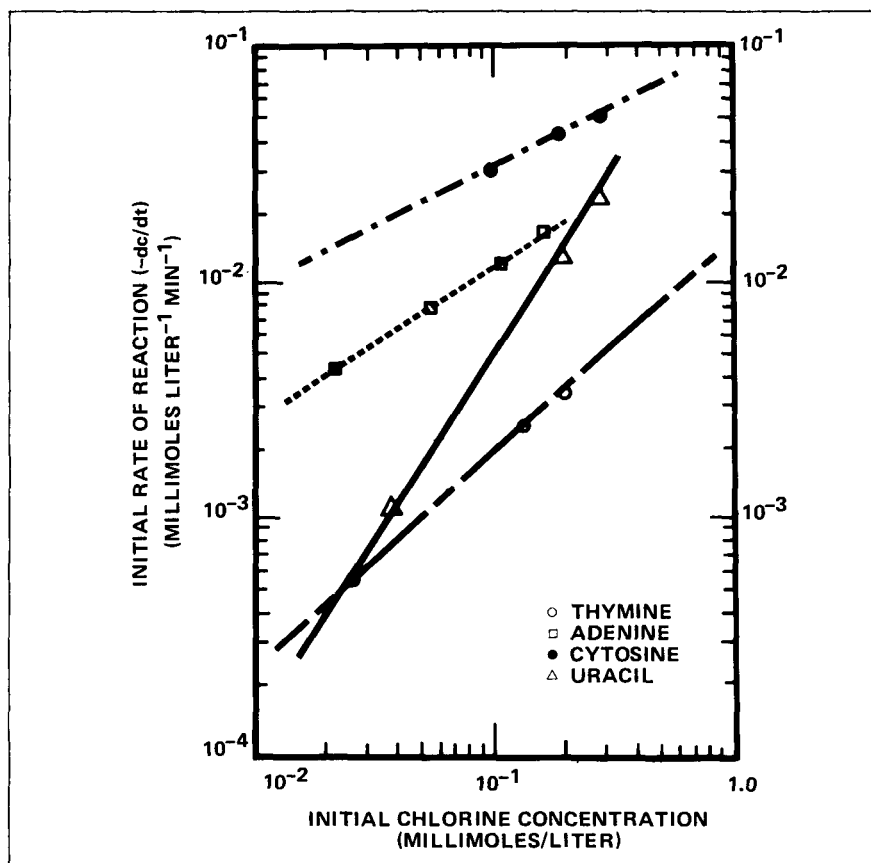


Figure 4. Reaction order curve in β for nucleic acid-chlorine system.

The N-organo compounds, cytosine, adenine, thymine, and uracil, did not show any interaction with chlorine-dioxide. Losses in chlorine-dioxide, as determined by measurement of residual chlorine-dioxide, were perhaps associated with agitation in the reaction vessel. These losses have also been evidenced in the chlorine dioxide control.

In summary, chlorine reacted with organic nitrogen compounds investigated, namely, thymine, cytosine, adenine, and uracil. These are nucleic acid bases (heterocyclic amines). These bases are part of the backbone of deoxyribonucleic acid (DNA), the molecule which is encoded with genetic information and through which genetic transcription occurs. The magnitude of reaction for thymine, cytosine, adenine, and uracil showed corresponding values of 0.49, 1.57, 1.71, and 1.80.

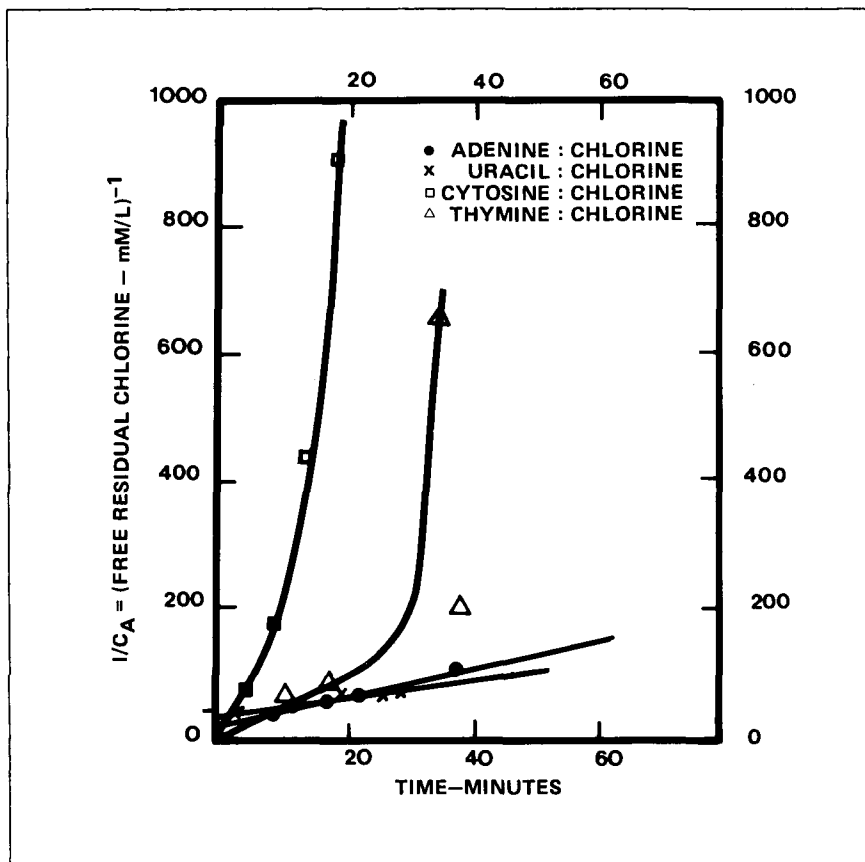


Figure 5. Test of bimolecular mechanism for equimolar system of nucleic acids and chlorine.

Therefore, it is recommended that nucleic acid bases should be further investigated in order to determine:

1. How hydrogen bonding is affected by chlorinated nucleic acid bases;
2. If a methodology can be established to determine the possible miscoding of DNA, through genetic transcription, due to the influence of chlorinated bases; and
3. The eventual effect of chlorinated bases upon embryological development.

Notably, chlorine-dioxide did not show any interaction with the aforementioned nucleic acid bases at concentrations between 0.001 and 0.28 mm L^{-1} . This signifies that, at these concentrations, chlorine-dioxide does not appear to be mutagenic and should therefore be considered as an alternative disinfectant.

To this end, further studies should be conducted in which other concentrations of chlorine dioxide are considered.

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