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PII: S0043-1354(00)00393-6

Wat. Res. Vol. 35, No. 5, pp. 1304–1310, 2001

Printed in Great Britain
0043-1354/01/$ - see front matter

DETERMINATION OF N-CHLORAMINES IN AS-SAMRA CHLORINATED WASTEWATER AND THEIR EFFECT ON THE DISINFECTION PROCESS

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(First received 23 September 1999; accepted in revised form 18 July 2000)

Abstract—Chlorine is the most widely used disinfectant of wastewater due to its capacity to inactivate most pathogenic microorganisms quickly. However, chlorine reacts with natural organic compounds present in wastewater to produce some undesirable organic byproducts. One such class of compounds is the nitrogenous compounds. The reaction between chlorine and compounds containing a nitrogen atom with one or more hydrogen atoms attached to it will form chloramines which have lower disinfection efficiency. Eighty percent of the wastewater generated in Jordan is treated at the Khirbet As-Samra wastewater treatment plant for eventual use in agriculture. In this study efficiency of chlorination was studied by collecting samples from the effluent of the treatment plant. The yield concentration of N-chloramines upon chlorination was determined. Efficiency of disinfection process as a function of contact time, concentration of chlorine dosage, concentration of nitrogenous compound and pH were studied. In this study, it has been found that at a chlorine dosage of 15 mg/L and contact time of 15 min, the percentage total coliform kill in As-samra wastewater sample was 100%. After addition of histidine, glycine and phenylalanine (15 mg/L in each case) to the wastewater sample, the percentage of total coliform kill dropped to 58, 78 and 79% respectively. When chlorine dosage was increased to 24 mg/L the percentage total coliform kill reached 96, 99 and 100% in wastewater samples treated with 5 mg/L histidine, glycine and phenylalanine, respectively. The percentage total coliform kill dropped to zero in wastewater samples treated with histidine, glycine and phenylalanine at a concentration of 30 mg/L each. This work supports the theory that amino acids and ammonia preferentially react with chlorine to form N-chloramine which significantly reduces the disinfection efficiency of the chlorination process.

Key words—chlorination, disinfection, glycine, histidine, N-chloramines, phenylalanine, wastewater

INTRODUCTION

Among the major problems facing developing countries are those related to shortage of surface and underground water. This problem can be partially solved by identifying new water sources. Treated effluents can be used for irrigation under controlled conditions to ensure minimum health risk as a result of pathogenic and toxic pollution.

Chlorination is the most important method of disinfection. It is able to effect a proper kill of pathogenic organisms commonly found in sewage effluents. For this purpose, chlorine is added to water either as chlorine gas (Cl_2) or hypochlorite solution (NaOCl).

$$Cl_2 + H_2O \rightleftarrows HOCl + H^+ + Cl^- \quad (1)$$

$$OCl^- + H_2O \rightleftarrows HOCl + OH^- \quad (2)$$

A mixture of HOCl and OCl^- is referred to as free available chlorine. Hypochlorous acid is known to be the most effective disinfecting agent (White, 1972).

The addition of chlorine to wastewater containing ammonia and organic nitrogen compounds may result in the formation of N-chloramines. There are two distinct classes of N-chloramines:

Inorganic N-chloramines: are formed by the reaction of chlorine with free ammonia naturally occurring in wastewater. These are monochloramine (NH_2Cl), dichloramine ($NHCl_2$) and tri-chloramine (NCl_3).

Organic N-chloramines: are formed by the reaction of chlorine with amino acid and proteins naturally occurring in wastewater (Ward *et al.*, 1984a, b; Ayoote and Gray, 1986; Fleischaker and Randtke, 1983; Scully *et al.*, 1988).

This study was carried out to determine N-chloramines that result upon chlorination in As-Samra wastewater stabilization pond system (WSP).

Samples were collected from As-Samra wastewater effluent. Concentration of N-chloramines and per-

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centage kill of bacteria were measured. Efficiency of disinfection process as a function of contact time, concentration of chlorine dosage, concentration of nitrogenous compound and pH were investigated.

As-Samra wastewater stabilization pond system (WSP)

As-Samra WSP is one of the largest WSPs in the world, located near AL-Hashimiya village, north of Zarqa city, about 60 km northeast of Amman. The pond system consists of three parallel trains and a chlorination pond at the outlet of the trains, occupying a total area of 200 h. Each train consists of two anaerobic ponds, four facultative ponds and four maturation ponds operating in series. The effluent from the maturation ponds of all three trains is collected in a channel, where chlorine is added to the effluent before it is introduced into the chlorination pond.

Table 1 shows the average chemical and biological characteristics of influent and effluent of As-Samra.

METHODOLOGY

Chlorine working solution (1000 mg/L as Cl_2) was prepared by dilution of 5% sodium hypochlorite and was standardized by iodometric titration (Standard Methods, 1995). Working solutions of nitrogenous compounds (ammonium chloride, histidine, glycine and phenylalanine) of (600 mg/L as N) were prepared by dissolving calculated amount of selected nitrogenous compound in one liter deionized water. Standard chloramine solutions were prepared by adding standard aqueous chlorine solution (200 mg/L) to the standard nitrogenous compound solution (600 mg/L) while mixing rapidly.

The most probable number method was used for measuring the bacterial count (Tortora *et al*., 1992; Ayres and Mara, 1996). N,N-diethyl-p-phenylene diamine (DPD) spectrophotometric method was used for measuring free chlorine (Standard Methods, 1995). Total residual chlorine was measured by rapid spectrophotometric method (Yoon and Jensen, 1995). The HPLC (UV detector at wavelength 254 nm) was used to measure the concentration of inorganic monochloramine (McCormick *et al*., 1993; Conyers and Scully, 1993). Analytical conditions were as follows:

C_{18} ultrasphere column (5 μm packing, 4.6 mm × 7.5 cm, Waters Co., Milford, MA), mobile phase A = 90% water (containing 1% acetic acid adjusted to pH 4), 10% acetonitrile, mobile phase B = 90% acetonitrile, 10% water (containing 1% acetic acid adjusted to pH 4). Five minutes of isocratic elution with 85% A/15% B was followed by a linear gradient to 55% A/45% B over 20 min and a flow rate of 1.0 ml/min.

The plan for all experiments was to vary one of the following parameters: concentration of nitrogenous compound, concentration of chlorine dosage, contact time and pH. For pH control, three solutions with pH values 6.8, 7.5 and 8.0 were prepared by addition of phosphate buffer.

Four different wastewater samples were investigated for N-chloramine formation during chlorination process:

1. effluent of As-Samra;
2. effluent of As-Samra treated with histidine;

Table 1. Average chemical and biological characteristics of the influent and effluent of As- Samra wastewater treatment plant (Jordan Water Authority, 1995)

Parameter	Symbol	Unit	Influent	Effluent
Total suspended solid	TSS	mg/L	433 ± 24	125 ± 24
Biochemical oxygen demand	BOD	mg O_2/L	530 ± 10	83 ± 10
Chemical oxygen demand	COD	mg O_2/L	1112 ± 13	294 ± 13
Sulfate	SO_4	mg/L	73.0 ± 0.13	31 ± 0.13
Ammonium	NH_4	mg/L	75.6 ± 0.1	76.5 ± 0.1
Nitrate	NO_3	mg/L	—	0.45 ± 0.01
Detergent	MBAS	mg/L	24.7 ± 0.3	13.3 ± 0.3
Boron	B	mg/L	0.43 ± 0.06	0.65 ± 0.06
Totol dissolved solid	TDS	mg/L	1092 ± 21	1243 ± 21
Electrical conductivity	EC	us/cm	1947 ± 1	2129 ± 1
Dissolved oxygen	DO	mg O_2/L	—	4.3 ± 0.1
pH	pH	—	6.8 ± 0.01	8.2 ± 0.01
Sodium	Na	mg/L	188 ± 3	221 ± 3
Chloride	Cl	mg/L	269 ± 10	329 ± 10
Calcium	Ca	mg/L	—	102 ± 3
Magnesium	Mg	mg/L	—	26 ± 2
Bicarbonate	HCO_3	mg/L	—	838 ± 5
Total coliform	—	MPN[a]/100 ml	1.7×10^8	9.7×10^3
Feacal coliform	—	MPN[a]/100 ml	4.0×10^7	6.1×10^2
Intestinal nematodes	—	T.Eggs/L	1	0
Chlorophyll (a)	—	μg/L	—	126.1
Algae	—	cell/ml	—	2.5×10^4

[a] MPN: Most probable number of organism present in 100 mL of sample at 95% confidence limit. It is the unit used in multiple tube fermentation technique for measuring coliform count.

3. effluent of As-Samra treated with glycine;
4. effluent of As-Samra treated with phenylalanine.

The samples were treated with 5, 15 and 30 mg/L of amino acids to increase the nitrogen content in As-Samra effluent wastewater that already contains 30 mg/L organic nitrogen. Thus, the percentage increase of organic nitrogen was 17, 50 and 100%, respectively.

RESULTS AND DISCUSSION

Chemical studies

Correlation between chlorine dosage and formation of N-chloramine (Break point phenomena). The break point chlorination scheme explains the changes in chlorine residuals as a function of $Cl_2:N$ ratio at neutral pH. Break point for As-Samra wastewater was determined by chlorinating As-Samra wastewater sample to 100, 150, 200, 250, 300 and 350 mg/L Cl_2. The concentration of inorganic monochloramine was measured by HPLC-direct injection method after a contact time of 15 min.

The break point curve in Fig. 1 appeared normal for a wastewater containing a high concentration of ammonia and exhibited a monochloramine maximum at 150 mg/L chlorine dosage (96.33 mg/L monochloramine) and a break point at 350 mg/L chlorine dosage (0.00 mg/L monochloramine).

The chlorine dosage applied in As-Samra was 15 mg/L, this chlorine dosage is located before break point. This means that the form of chlorine residual will be as inorganic monochloramine (NH_2Cl) which has a lower disinfection efficiency than free chlorine and its stability in water is low. Thus, bacteria will reproduce and the chlorination process will be unreliable.

Effect of amino acids on formation and stability of NH_2Cl. The presence of selected nitrogenous organic compounds (as amino acids) can reduce the disinfection efficiency of free chlorine (HOCl) and inorganic mono-chloramine (NH_2Cl) by interfering with the formation and stability of the disinfectant.

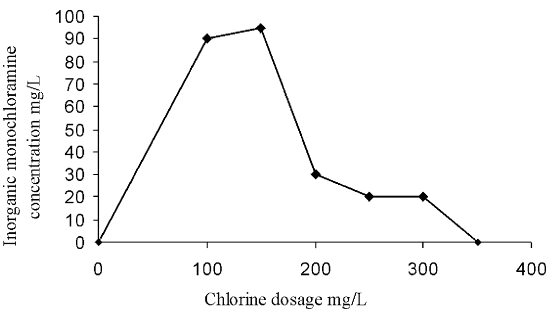

Fig. 1. Break point graph for wastewater sample (chlorine dosage vs. concentration of NH_2Cl).

Effect of histidine. Inorganic monochloramine (NH_2Cl) was measured by the HPLC-direct injection method. As shown in Fig. 2 the sample to which 30 mg/L histidine was added showed a sharp decrease in inorganic monochloramine and free chlorine over all chlorine dosages. For example, at 100, 150 and 300 mg/L chlorine dosage the concentration of inorganic chloramine was reduced by 95.18, 66.67 and 100% respectively, in a sample to which histidine was added.

From chemical studies, the reduction of inorganic monochloramine in the presence of histidine is due to the fact that chlorine binds faster to histidine than to ammonia, forming N-chloraminoacid compound and subsequently decomposes to more simple compounds. A general scheme for chlorination of aminoacids is proposed in Fig. 3 (Conyers and Scully, 1993).

The scheme indicates that amino acid consumes chlorine and this will decrease the disinfection efficiency.

Effect of glycine. Inorganic monochloramine (NH_2Cl) was measured by the HPLC-direct injection method. As shown in Fig. 4, the sample to which 30 mg/L glycine was added showed a sharp decrease in inorganic monochloramine and free chlorine over all chlorine dosages. For example, at 100, 150 and 300 mg/L chlorine dosages the concentration of inorganic chloramine was reduced by 49.22, 68.88 and 8.13%, respectively, in a sample to which glycine was added. From chemical and biological studies the effect of glycine is similar to the histidine effect.

Effect of phenylalanine. The third amino acid used in this study was phenylalanine. To test the effect of treating wastewater with phenylalanine, an experiment was conducted in which phenylalanine was supplemented into the test As-Samra wastewater sample at a level of 30 mg/L. The behavior of

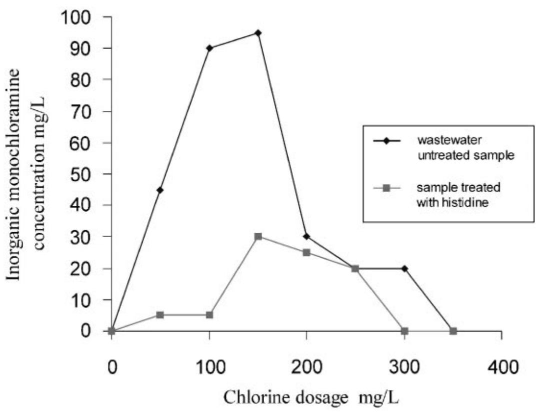

Fig. 2. Break point graph for wastewater sample treated with 30 mg/L histidine (chlorine dosage vs. concentration of NH_2Cl).

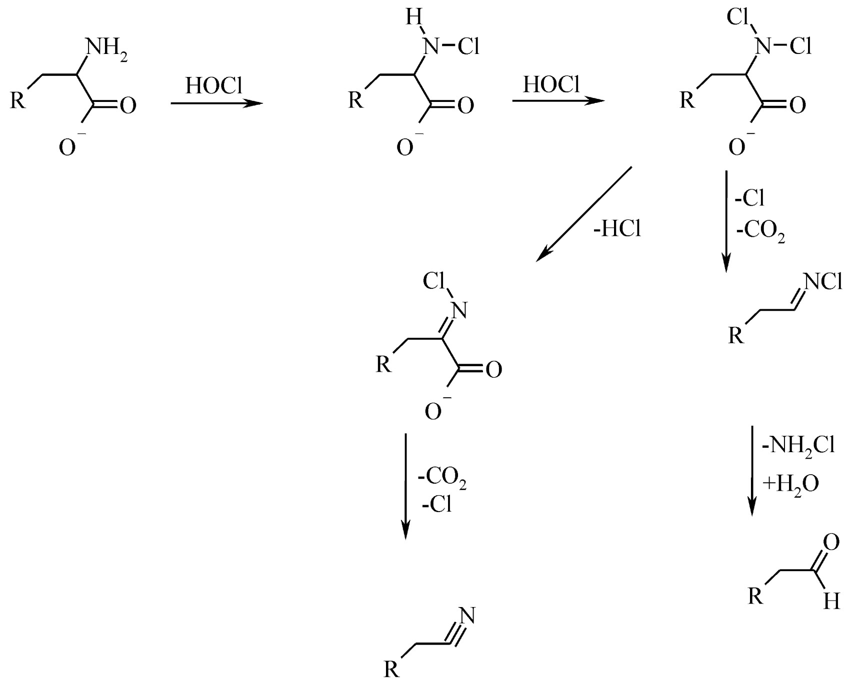

Fig. 3. A general scheme for chlorination of aminoacids.

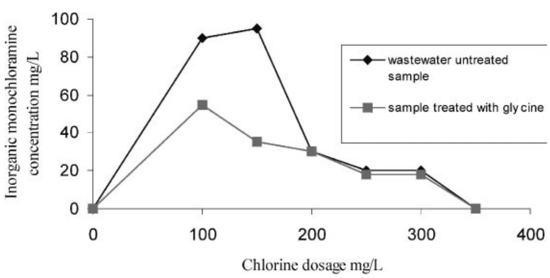

Fig. 4. Break point graph for wastewater sample treated with 30 mg/L glycine (chlorine dosage vs. concentration of NH_2Cl).

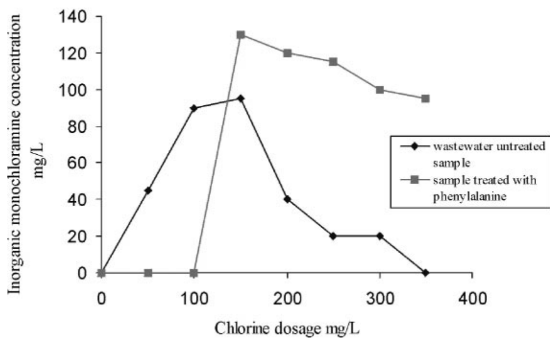

Fig. 5. Break point graph for wastewater sample treated with 30 mg/L phenylalanine (chlorine dosage vs. concentration of NH_2Cl).

phenylalanine was different from that of histidine and glycine. The plots of inorganic monochloramine (NH_2Cl) conc. vs chlorine dosage in the presence of phenylalanine showed a break point curve different from that for As-Samra wastewater sample. As seen in Fig. 5, when chlorine dosage was 100 mg/L the concentration of inorganic monochloramine formed in As-Samra wastewater was 88.88 mg/L, but was zero in the sample treated with phenylalanine. A new peak at retention time 1.5 min appeared, but as the chlorine dosage was increased to 150 mg/L this peak began to decrease until it reached zero at 350 mg/L chlorine dosage and the peak of inorganic monochloramine at retention time 0.883 min began to appear.

From the above, it can be concluded that the chlorine preferred to react with phenylalanine more than ammonia to form N-chlorphenylalanine which is more stable than N-chlorglycine and N-chlorhistidine. Thus in the chromatograms it was clear that by increasing chlorine dosage N-chlorphenylalanine decomposed very fast to form inorganic monochloramine.

Effect of time on monochloramine concentration. As shown in Fig. 6, the decrease of inorganic monochloramine in As-Samra wastewater sample as a function of time is very low. After 15 min contact time the concentration of inorganic monochloramine was 88.84 and after 60 min contact time it was 80.19 mg/L; so the reduction was only 9.74%. In the sample treated with 30 mg/L histidine the reduction in monochloramine concentration reached 100% and it was 64.34% in the sample treated with 30 mg/L glycine.

This can be explained by chlorine transfer from inorganic monochloramine to histidine or glycine. Thus, their presence in water consumes chlorine residual with time. The opposite effect was, however, found in the sample treated with 30 mg/L phenylalanine. The concentration of inorganic monochloramine increased from 0 mg/L at 15 min contact time to 108.85 mg/L at 60 min contact time. This increase is caused by decomposition of N-chlorphenylalanine with time to give inorganic monochloramine.

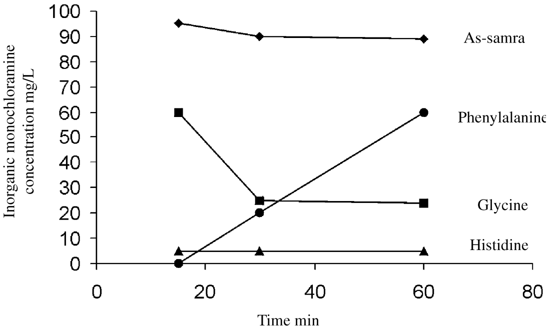

Fig. 6. The concentration of inorganic monochloramine as a function of time.

Biological studies

Correlation between concentration of chlorine dosage and percentage total coliform kill

Effect of histidine, glycine, and phenylalanine. The biological studies clearly indicated decreased disinfection efficiency when wastewater was treated with histidine, glycine and phenylalanine (Fig. 7). The results show that:

(a) The percentage total coliform kill was decreased from 100% in As-Samra wastewater sample to 58, 78 and 79% in samples treated with 5 mg/L histidine, glycine and phenylalanine, respectively. The contact time was 15 min and the chlorine dosage was 15 mg/L at pH 6.8.

(b) When chlorine dosage was decreased to 9 mg/L, the percentage total coliform kill in As-Samra wastewater was reduced to 58% and to zero in samples treated with 5 mg/L histidine, glycine or phenylalanine.

(c) The presence of histidine, glycine, and phenylalanine decreased the concentration of free chlorine and of inorganic monochloramine.

(d) The percentage total coliform kill reached 100% at a chlorine dosage of 21 mg/L in samples treated with 5 mg/L phenylalanine at contact time 15 min and pH 6.8. In samples treated with histidine and glycine the highest percentage total

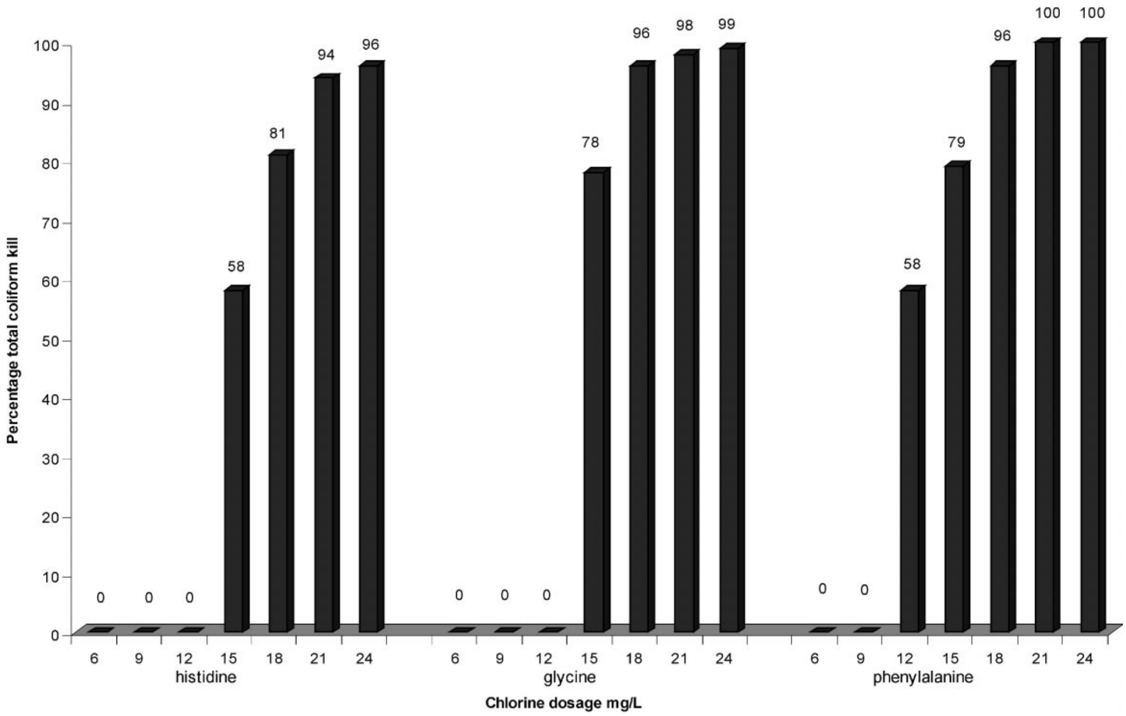

Fig. 7. Percentage total coliform kill as a function of chlorine concentration (mg/L), for wastewater sample treated with 5 mg/L histidine, glycine and phenylalanine (initial conditions: contact time 15 min, pH 6.8).

coliform kill was 96, 99%, respectively, at a chlorine dosage of 24 mg/L.

Effect of nitrogenous compound concentration on the percentage of bacterial kill. The $Cl_2:N$ ratio affects the rate of inactivation of bacteria. As shown in Fig. 10, there is a general trend between concentration of nitrogenous compounds and the percentage of bacterial kill. It was found that as the concentration of nitrogenous compounds increases, the percentage total coliform kill decreases.

As shown in Fig. 8, when the concentration of ammonium chloride was increased from 5 to 30 mg/L the percentage total coliform kill was decreased from 100 to 81% .The same trend was noticed when the concentration of histidine, glycine and phenylalanine increased from 5 to 30 mg/L. The percentage total coliform kill decreased from 58 to 0% in the case of histidine, from 78% to 0% in the case of glycine and from 78 to 0% in the case of phenylalanine.

From the above it can be seen that there is a strong correlation between increasing the concentration of nitrogenous compounds in wastewater and the decrease in disinfection efficiency.

Time effect. As indicated earlier, there is a correlation between the concentration of nitrogenous compounds and disinfection efficiency. It was found that as the concentration of nitrogenous compounds increases the disinfection efficiency decreases.

This problem can be solved by increasing the contact time, because different forms of chlorine residual as inorganic monochloramine and N-chloraminoacids have lower disinfection efficiency than free chlorine.

From Fig. 9 it can be concluded that histidine and glycine are in need of longer contact time to get 100% total coliform kill than phenylalanine and ammonium chloride. Also, it can be seen that as the concentration of amino acids increases the contact time needed for 100% total coliform kill increased, which implies that nitrogenous compounds may adversely impact the efficiency of disinfectant.

pH effect: The pH range 6.8–8.0 was chosen for this study because the pH of As-Samra influent is about 6.8 and the pH of effluent is about 8.0. From Table 2 it can be seen that the total coliform killing was not affected in the pH range 6.8–8.0 for all samples.

CONCLUSIONS

1. Evidence was presented that selected nitrogenous organic compounds can significantly reduce the disinfection efficiency of chlorine. Amino acids and ammonia preferentially react with chlorine to form inorganic N-chloramine and N-chloraminoacids.
2. This study demonstrated that inorganic N-chloramine and N-chloraminoacids are considerably less effective than free chlorine for inactivating bacteria.
3. The rate of inactivation of bacteria has been found to be greatly dependent on concentration of chlorine, concentration of nitrogenous compound, and contact time.
4. Break-point determination must be practiced during the chlorination process because if the chlorine dosage is more than break-point dosage the available chlorine will be present as free

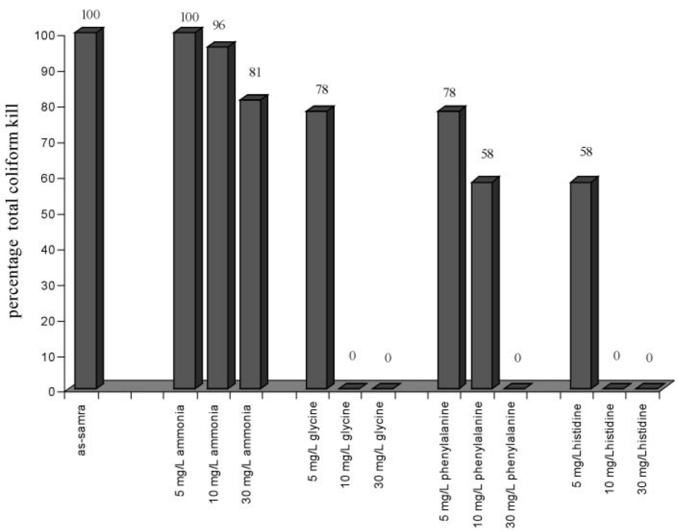

Fig. 8. Percentage total coliform kill as a function of nitrogenous compound concentration (mg/L) (initial conditions: chlorine dosage of 15 mg/L contact time 15 min, pH 6.8).

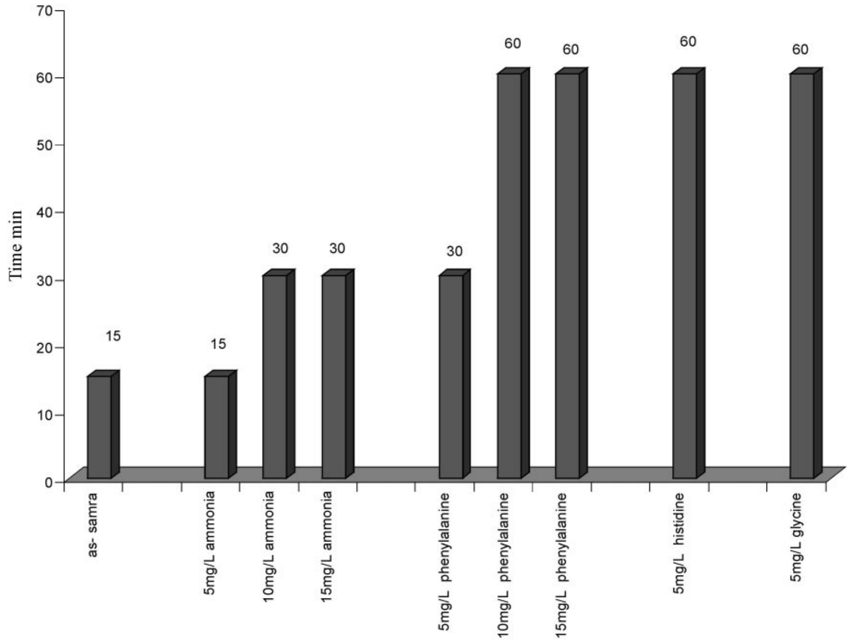

Fig. 9. Minimum time needed for 100% total coliform kill as a function of nitrogenous compound concentrations (mg/L) (chlorine dosage 15 mg/L, pH 6.8).

Table 2. pH effect on percentage total coliform kill (initial conditions: chlorine dosage 15 mg/L, contact time 30 min, concentration of nitrogenous compound 30 mg/L)

Sample	Percentage total coliform kill at different pH values		
	6.8	7.5	8.0
As-Samra sample	100	100	100
Ammonium chloride	100	100	99
Glycine	0	0	0
Phenylalanine	89	89	89
Histidine	86	86	86

chlorine and disinfection efficiency is very good but if chlorine dosage is less than break point dosage the available chlorine will be present as N-chloramine with lower disinfection properties.

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