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Research Paper

ACUTE TOXICITY OF THIOCYANATE USING *DANIO RERIO* AND MICROBIAL DEGRADATION OF THIOCYANATE

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Thiocyanate is used in a variety of industrial processes such as photofinishing, herbicide and insecticide production, dyeing, coking, metal separation, thiourea manufacturing, fertilizer, and electroplating. Due to wide industrial use, it is frequently found in waste streams and soil, which releases into the aquatic environment. Thiocyanate is a stable, non-hydrolysable, non-volatile compound and persists into environment even in the presence of acidic conditions. Therefore, thiocyanate removal from industrial wastewater system and other contaminated sites is in well demand. In the present study, acute toxicity of thiocyanate to zebra fish (*Danio rerio*) and biodegradation of thiocyanate in synthetic solution and in coke oven wastewater of Steel Plant were carried out using different bacterial media. The effects of pH, phenol additive and hydraulic loading rate on biodegradation of thiocyanate were also studied. The results showed that the permissible concentration range of thiocyanate for industrial effluent was 405 to 465 ppm. By comparing the biodegradation of thiocyanate concentration with the addition of two different bacterial media i) *Pseudomonas* and ii) *Thiobacillus*, the bacterial medium, *thiobacillus* was proved to be more effective for the biodegradation of thiocyanate, since the number of days taken for degradation were 5 days.

Keywords: Acute toxicity, *Danio rerio*, biodegradation, thiocyanate, *thiobacillus*, *pseudomonas*

INTRODUCTION

Man in his attempt to lead a comfortable life extracts all the resources from the mother earth. In this process of utilization of useful materials, he lets into environment many hazardous substances, unaware of their effect on his own

life. The rate of discharge of these materials into ecosystem is more than that of their degradation by natural process, resulting in the accumulation of such substances in the environment. A variety of organic and inorganic compounds that are toxic to the normal biological activity, and their

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persistence and accumulation in the food chain are hazardous to all human activity (Lanza and Bertazzoli, 2002). Of the many toxic pollutants, phenols, cyanides, thiocyanates, formaldehyde, heavy metals such as mercury, cadmium, zinc, copper, chromium are more hazardous (Young, 2001 and Sharma, 2003). Thiocyanate is a toxic anion which arises from a diverse range of natural and industrial sources. In both animals and plants it occurs as the non-functional detoxification product from cyanide ingestion, or as a defense mechanism against microbial infection (Westly, 1981, Wood, 1975).

Industrial sources of thiocyanate-containing effluents are wide spread, but one of the major sources of such wastes is the carbonization of coal to produce coke. Cyanide reacts readily with sulfur to produce thiocyanate, and any industry with cyanide in its waste is a potential source of thiocyanate contamination. Such industries include steel manufacturing, metal mining and electroplating (Jane Stratford, 1994).

Although thiocyanate is less toxic than cyanide, thiocyanate containing wastes are produced in vast quantities. Thiocyanate must either be removed totally from effluents or have its concentration greatly reduced in wastes prior to their disposal as it can inhibit the degradation of other pollutants present in the waste and has a detrimental effect on aquatic flora and fauna (Jane Stratford, 1994). A huge quantity of pollutants in the form of domestic and industrial effluent is discharged directly or indirectly into the water bodies, which has severe impact on its biotic and abiotic environment (Turk, 1984). The standard quality of waste effluent has traditionally been based on the control of global parameters such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) or Total

Suspended Solids (TSS) according to Council Directive (1991) concerning wastewater treatment. The detection of these parameters alone is not sufficient, as the wastewater generated from small scale industries may contain large amount of chemicals, many of which may be present in such a low concentration that these may be beyond detection limit and for many of them even the analytical techniques are inadequate.

Secondly, the physico-chemical analysis is not only quite complicated, expensive and time consuming process, but also lacks the information about the additive, antagonistic or synergistic effects of various chemicals on biotic community in aquatic ecosystem (Villegas, 1999). Therefore, effective tools for the evaluation of negative effects on living organisms are needed. The use of biological assays can provide a direct and appropriate measure of toxicity to complement the physico-chemical measures of quality of wastewater (Hernando, 2005). The toxicity test is one of such parameters, which covers all above shortcomings and can be used as summary parameters (easier, cheaper, effective and less time consuming).

The measure of toxicity is an integral view of the sum of all interacting components in the sample. The purpose of regulatory toxicity testing is to produce baseline data for environmental hazard and risk assessment of chemicals, to be used in regulating the discharge of wastewater treatment systems. Bioassay has been extensively used to document toxicity of surface water and evaluate the potential toxicity of discharges into these waters. Numerous studies have been made to understand the toxic effects of waste effluent on fish, but relatively little attention has been paid to their adverse effects on plankton.

The present work on toxic effect of waste effluent to *Danio rerio* (Zebra fish), which is highly sensitive to toxic substances, has short generation time, multiplies very rapidly, easily acclimatizes in laboratory condition, cultured in a small space and can be measured in a relatively short period (APHA, 1998). The use of *Danio rerio* (Zebra fish) in toxicology is accepted in several countries to monitor wastewater treatment systems, to establish quality criteria to determine permissible concentrations of pollutants, limits of impurity in water from natural effluent, and to determine the efficacy of a good sanitation method (Villegas, 1999).

The acceptance of bioassay toxicity test as an effective analytical tool requires guarantees of standardization and validation of the experimental procedure to evaluate its sensitivity, accuracy or precision. In this sense, the main objectives of this work were to assess the utility and validity of toxicity tests and to study the acute toxicity of thiocyanate to *Danio rerio* (Zebra fish).

The objective of the present study is to study the acute toxicity of thiocyanate to zebra fish (*Danio rerio*), the biodegradation of thiocyanate in synthetic solution and in coke oven waste water using bacterial media, *Pseudomonas* and *Thiobacillus*. Experimental parameters affecting the biodegradation of thiocyanate in coke oven waste water such as pH, phenol additive and hydraulic loading rate were also studied.

MATERIALS AND METHODS

Acute Toxicity of Thiocyanate

Test-Fish Species

Danio rerio (zebra fish) having a size of 30 ± 5 mm corresponding to approximately 0.2 to 0.3 g

mass were collected from Vishaka Aquariums, Visakhapatnam. The fish were kept at a temperature of 25 °C approximately in aerated chlorine free portable water of roughly having similar characteristics of dilution water. The population density of the fish was not exceeded 1g/L.

The daily illumination was in the range of 12 to 16 h. The stock was kept on a normal diet. The fish were free of manifest diseases or visible malformations. The minimum acclimatization period was 10 days prior to test under conditions of water quality and illumination similar to those used in the test. Mortality was not exceeded 1% per week. For each test, fish was selected from the same stock tank, the population of which is under conditions of water quality and illumination similar to those applied in the test. Feeding of the fish was stopped from 24 h prior to the start of the test.

Preparation of Dilution water

The water used in the test was collected from the de-ionized water plant from the site (Steel plant). This water had its conductivity range of 3 to 5 $\mu\text{S}/\text{cm}$. Dilution water was prepared by using 25ml each of the following four stock solutions in 1L of de-ionized water. Stock solution of calcium chloride, magnesium sulphate, sodium bicarbonate and potassium bicarbonate of desired concentration have been prepared by dissolving appropriate amount of calcium chloride dehydrate, magnesium sulphate hepta hydrate, sodium bicarbonate and potassium chloride in distilled water. All chemicals used in this study were of analytical grade. The characteristics of dilution water were given in Table 1. After preparing dilution water it was aerated for 24 h prior to the test.

Table 1: Characteristics of Dilution Water

S.No.	Parameter	Concentration
1.	pH	7.7
2.	Dissolved Oxygen(DO), ppm	7.8
3.	Hardness(as CaCO ₃), ppm	230
4.	Calcium (Ca), ppm	107
5.	Magnesium (Mg), ppm	25

Preparation of Test Solution

Since thiocyanate is not available in its direct form, potassium thiocyanate is converted to thiocyanate by using simple chemistry. After calculating the thiocyanate concentration in ppm, it was taken in a 3 L capacity borosilicate glass beaker and dilution water was added to make a 2 L test solution. After thorough mixing of this solution, its temperature, pH and DO were noted.

Experimental Procedure

Zebra fish were exposed to different concentrations of dilution series (1, 10, 100, and 1000). Five numbers of zebra fishes per 2 L of test solution (synthetic water + dilution water) were taken in 3000 ml glass beakers for four consecutive concentrations (1, 10, 100, and 1000). One control set containing only dilution water was also run simultaneously. Observation was made for death of zebra fish for which zero to 100 percent mortality should be attained after 24 h to 48 h. The samples were analyzed as per the standard techniques established by APHA 1998 (American Public Health Association – 20th Edition) using more sophisticated instruments like UV – spectrophotometer (chemito – 2700) for chemical analysis, Multi 340i WTW (Merck make) meter for pH measurement and Orion meter with DO electrode for dissolved oxygen.

After exposing the zebra fish to standard dilution series, the experiments were run to find the exact range of thiocyanate where zero to 100% mortality was attained.

Biodegradation of thiocyanate using bacterial medium, thiobacillus

Raw influent characteristic of coke oven plant are tabulated in Table 2. The bacteria which behave like all other living beings, need favorable conditions like pH, constant level of toxicants, avoidance of shock load, continuous and constant air flow and mainly continues feed of nutrients. With these conditions the bacteria will be active and toxicant reduction will be more efficient.

Table 2: Raw Influent Characteristics

S.No.	Characteristics	Value
1.	pH	7
2.	Free ammonia, mg/L	100
3.	Fixed ammonia, mg/L	500
4.	Phenol, mg/L	400
5.	Thiocyanate (SCN), mg/L	400
6.	Cyanides (CN), mg/L	20
7.	Oil and grease, mg/L	500
8.	COD, mg/L	2500

Preparation of Bacterial Medium, Thiobacillus

Distilled water was taken in a 5 L glass jar and it was heated for 3 h to remove the free chlorine. After obtaining negative test (Silver nitrate test) for chlorine, this water was used to prepare the thiocyanate bacterial media (Jones and Carrington, 1972). To this water, KH₂-PO₄-3.5 g/L, KOH-1.4 g/L, solution A-10 ml and solution B – 10 ml were added. Final pH of the solution was maintained between 7.0-7.2 by adding sufficient quantities of HCL and NaOH. Solution A was prepared by dissolving MgSO₄-1g, MnSO₄-

0.2g, FeCl_3 -0.035g in 1 L distilled water. Solution B was prepared by dissolving CaCl_2 -0.5g, MgCl_2 -1g, MnCl_2 -1g in one liter distilled water. Sodium Succinate (0.1g/L) was added as growth factor. Bacterial culture-10 mg/L taken from the process bacterial tank was added to the contents of the bottle.

Description of Bench Scale Experimental set-up

5 L glass jar of three numbers were used as reservoir or equalization tank, reactor or aeration tank and sludge settling tank. The schematic diagram of the experimental set up is shown in Figure 1. The influent was placed in the reservoir and pumped continuously into aeration tank by a suction pump. The flow rates were adjusted by adjusting the airflow to the pump. The flow rate was regulated in such a way that a retention time of 4 h was maintained, in the reactor. The settled sludge from the reactor was sent to the sludge settling tank by suction pump. Part of the settled sludge was re-circulated from the settler to the reactor by means of suction pump. Air flow was

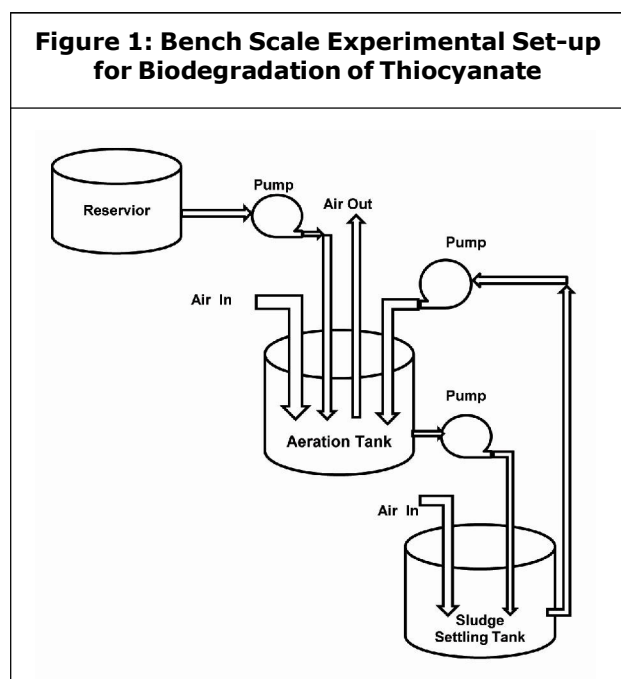
maintained in the reactor by means of a pump. Air was provided for agitation as well as oxygen requirement in reactor and in sludge settler. The glass vessels were provided with vents. The temperature of the reactor content was measured daily twice with mercury thermometer. An electric sodium vapour lamp was placed nearer the reactor to heat up the solution in the reactor. A part of sludge (30%) was sent into the aeration tank on an intermittent basis. Air was taken from the plant wet air circuit.

Experiments with Synthetic Solution With the Addition of Bacterial Medium

(Thiobacillus): After adding thiocyanate media and 1200 mg/L - KCN to distilled water (5 L), it was fed into the equalization tank and pumped continuously into the aeration tank. For every 24 h thiocyanate concentration was estimated from the reactor content.

Experiments with Coke Oven Wastewater Using Bacterial Medium, Pseudomonas

A few experiments were conducted for the biodegradation of thiocyanate of coke oven wastewater using a bacterial medium, pseudomonas available in bacterial tank of MBC plant. This culture was maintained by its addition of nutrient level by ortho-phosphoric acid (not less than \neq 5mg/L). P_1 water (wastewater after 1st stage aeration) from MBC plant after phenol degradation was taken for the study purpose. To this waste water pseudomonas bacterial medium described above was added, and placed in the equalization tank and pumped continuously by a suction pump into bioreactor (aeration tank). After 24 h the liquid from the bioreactor was led into the sludge settler for settling the biomass. For every 24 h thio-cyanate concentration in the reactor was estimated. In this study, it was planned to conduct some experiments utilizing



the bacterial medium, pseudomonas and comparing the results with similar experiment conducted by addition of bacterial medium, thiobacillus on a bench scale. The samples were taken daily from this tank and were analyzed for thiocyanate concentration.

Experiments with coke oven wastewater with bacterial media, thiobacillus:

P₁ water (wastewater after 1st stage aeration) from MBC plant after phenol degradation was taken for this study purpose. To this wastewater, thiocyanate media as described earlier was added, and placed in the equalization tank and pumped continuously by a suction pump into bioreactor (aeration tank). After 24 h the liquid from the bioreactor was led into the sludge settler for settling the biomass. For every 24 h thiocyanate concentration in the reactor was estimated. When the concentration of thiocyanate reached zero, thiocyanate media was again added to the contents of the reservoir, and it was operated in a continuous mode. The concentrations of sulphate, ammonium and thiocyanate were determined during this period.

Effect of pH on Biodegradation of thiocyanate

Ludberg and Nicks, 1969 had described that the strains of bacteria most effectively oxidize thiocyanate function best at a lesser pH. The optimum pH for these bacteria is about 7. The experiments were carried out to determine the optimum pH for this bacteria. As described earlier, process P₁ water (wastewater after 1st stage aeration) was taken for the study purpose. Water was taken in the equalization tank and ortho-phosphoric acid was added to the contents of the equalization tank to decrease the pH to the required value. After adjusting the pH to the desired level it was fed continuously into aeration vessel. For every 24 h, thiocyanate, sulphate and ammonium concentrations were measured.

Effect of phenol additive on Biodegradation of thiocyanate

P₁ water (waste water after 1st stage aeration) was fed to the equalization tank. To this, different dosages of phenol was added and operated in a continuous mode. The bacterial culture that was developed in the bench scale model was added to the aeration tank. For every 24 hrs, samples were collected and concentrations of thiocyanate, ammonium were measured. The pH of the aeration tank was maintained at 5.

Effect of hydraulic loading rate on Biodegradation of thiocyanate

Generally the hydraulic loading rate is an important variable in the wastewater treatment from the point of view of shock loads. Hence it was studied as a variable in the present study.

As the plant authorities desired that these studies were to be carried out in plant scale model, an aeration tank (aeration tank no 24) was used for this study. The aero tank was completely drained and cleaned free of sludge and other dirt before commencement of the experiment. The aero tank was sterilized (steamed thoroughly for 48 h). The culture that was developed in bench scale model was transferred to the aero tank. Treated wastewater after first stage (after phenol removal) was taken into aeration tank. Nutrient was added to the aeration tank at a required levels (>5mg/L). When the required MLSS (Mixed Liquid Suspended Solids) concentration of 350 mg/L in the aero tanks has attained, the aeration tank was taken for study purpose. In the studies of hydraulic loading rate the experiments were conducted at pH – 6.8 of the influent wastewater. The flow rates were varied from 5 to 30 cum/h and the air flow rates were adjusted according to the requirement.

RESULTS AND DISCUSSION

Aquatic ecosystems may be polluted with a variety of contaminants from many industrial, agricultural, or domestic activities and naturally occurring processes that release contaminants. "There was no such thing as a single chemical exposure" (Yang *et al.*, 1998). The main objective of an individual chemical toxicity test is to define the concentration at which a test material is capable of producing some selected response, usually deleterious, in a population under controlled conditions of exposure. The appropriate way to do this is by use of the "quantal response" (*i.e.* by having only two experimental alternatives dead or alive, all or none) from which the relation between concentration and percentage effect can be defined (Catchpole and Stanford, 1977). In simple application, acute toxicity tests are time-dependent. That is, the length of exposure is pre determined; usually 0 to 48 h (Catchpole and Stanford, 1977).

The potential use of *Danio rerio* (Zebra Fish) as a test organism for the evaluation of thiocyanate chemical toxicity in synthetic solution and its degradation by using thiobacillus and pseudomonas was investigated in the present study.

Acute Toxicity of Thiocyanate Using Zebra Fish

The objective is to determine the possible range

of chemical concentration that causes mortality to the test organism over a constant period of time (48 h). Many experimental runs were made to determine the toxicity of thiocyanate. The experiments were conducted for different concentrations of thiocyanate ranging from 280 to 410 ppm (280,340,375,410) and the mortality was tested along with pH and DO for a contact time of 0 to 48 h. The results obtained are tabulated in Table 3.

From Table 3, it is observed that the mortality rate (0 to 100 %) was irregular for the concentration of thiocyanate ranging from 0 to 410 ppm for the contact time of 0 to 48 h. It was also observed that the pH and DO were decreased with an increase in thiocyanate concentration from 0 to 410 ppm and also decreased with an increase in time of contact from 0 to 48 h. Since the mortality rate was irregular, the experiment was repeated with different concentrations of thiocyanate ranging from 340 – 410 ppm (340, 375, 410, and 445). The results obtained are tabulated in Table 4.

From Table 4, it is observed that the mortality rate (0 to 100 %) was not attained for the concentration of thiocyanate ranging from 340 to 445 ppm. In this case also the pH and DO were decreased with an increase in thiocyanate concentration and time of contact. Since the

Table 3: Acute Toxicity of Thiocyanate to Zebra Fish for 0 to 410 ppm

Sample	0 h			24 h			48 h		
	pH	DO	Mortality	pH	DO	Mortality	pH	DO	Mortality
Blank	7.51	8.14	-	7.42	7.13	0/5	7.31	7.31	0/5
280 ppm	7.41	8.12	-	7.3	7.04	0/5	7.24	7.02	0/5
340 ppm	7.3	8.10	-	7.25	6.98	0/5	7.16	6.80	3/5
375 ppm	7.23	8.06	-	7.1	6.86	0/5	7.02	6.75	2/5
410 ppm	7.16	8.04	-	7.06	6.81	4/5	6.93	6.73	4/5

Table 4: Acute Toxicity of Thiocyanate to Zebra Fish for 340 to 410 ppm

Sample	0 h			24 h			48 h		
	pH	DO	Mortality	pH	DO	Mortality	pH	DO	Mortality
Blank	7.80	8.30	-	7.72	8.15	0/5	7.56	8.14	0/5
340 ppm	7.68	8.23	-	7.53	6.97	1/5	7.41	6.77	0/5
375 ppm	7.52	8.16	-	7.42	6.83	1/5	7.32	6.62	0/5
410 ppm	7.41	8.04	-	7.39	6.76	2/5	7.21	6.56	4/5
445 ppm	7.32	7.09	-	7.21	6.68	2/5	7.12	6.53	4/5

mortality rate (0 to 100 %) was not attained, the experiment was repeated with different concentrations of thiocyanate ranging from 410 – 605 ppm (410, 475, 540 and 605). The results obtained are tabulated in Table 5.

From Table 5, it is observed that the mortality rate (0 to 100 %) was attained for the concentration of the thiocyanate ranging from 410 to

605 ppm. In this case also the pH and DO were decreased with an increase in thiocyanate concentration and time of contact. Though the mortality rate (0 to 100 %) was attained, for the correct range of the thiocyanate concentration the experiment was repeated with different concentrations of thiocyanate ranging from 405 – 465 ppm (405, 425, 445, and 465). The results obtained are tabulated in Table 6. From Table 6, it

Table 5: Acute Toxicity of Thiocyanate to Zebra Fish for 410-605 ppm

Sample	0 h			24 h			48 h		
	pH	DO	Mortality	pH	DO	Mortality	pH	DO	Mortality
Blank	8.189	7.8	-	7.96	7.48	0/5	7.85	7.33	0/5
410 ppm	7.738	7.63	-	7.55	6.48	0/5	7.49	6.52	2/5
475 ppm	7.535	7.54	-	7.46	6.39	2/5	7.32	6.28	5/5
540 ppm	7.473	7.47	-	7.30	6.23	5/5	7.23	6.12	5/5
605 ppm	7.386	7.33	-	7.23	5.81	5/5	7.12	5.31	5/5

Table 6: Acute Toxicity of Thiocyanate to Zebra Fish for 405-465 ppm

Sample	0 h			24 h			48 h		
	pH	DO	Mortality	pH	DO	Mortality	pH	DO	Mortality
Blank	8.3	8.0	-	7.78	7.6	0/5	7.71	7.56	0/5
405ppm	8.189	7.8	-	7.43	6.40	0/5	7.21	5.97	0/5
425ppm	8.173	7.77	-	7.31	6.07	0/5	7.11	5.71	2/5
445ppm	8.150	7.69	-	7.251	5.71	0/5	6.94	5.65	3/5
465ppm	8.142	7.52	-	7.15	5.56	2/5	6.83	5.53	5/5

is observed that the mortality rate 0 to 100 % was attained for thiocyanate concentration ranging from 405 to 465 ppm, where a fish has not sustained its life. Hence the permissible concentration of thiocyanate suggested for industrial effluent is upto 405 ppm.

Biodegradation of Thiocyanate

The studies on biodegradation of thiocyanate were carried out in synthetic solution and in coke oven wastewater with different bacterial media Thiobacillus and Pseudomonas. The effects of pH, phenol additive and hydraulic loading rate on biodegradation of thiocyanate in coke oven wastewater were also studied.

Biodegradation of Thiocyanate in Synthetic Solution with the Addition of Bacterial Medium (Thiobacillus)

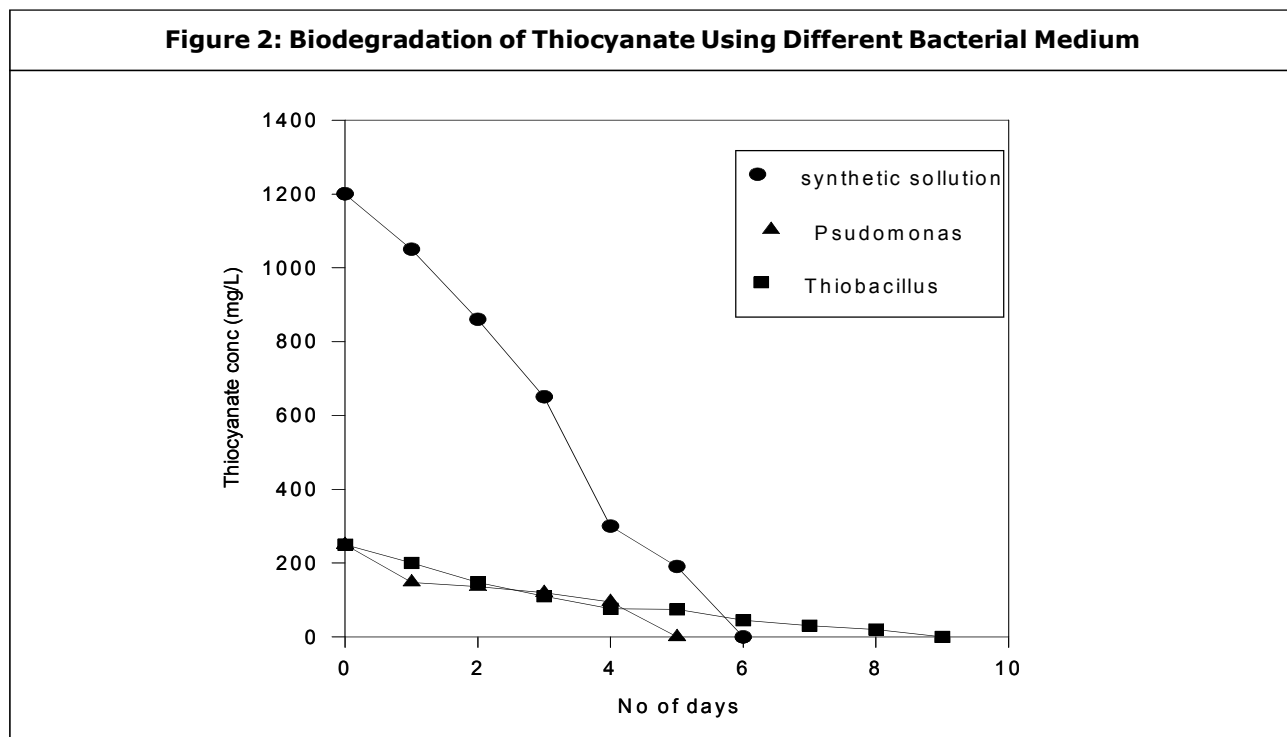
The experiment was conducted at constant temperature (25 °C) for finding out the thiocyanate concentration in synthetic solution for different number of days. The concentration of thiocyanate in synthetic solution was decreased with an

increase in number of days from 1 to 5 and further decreased to below detectable limit during 6th day as shown in Figure 2. This is due to the addition of bacterial medium, Thiobacillus. During this process, the bacterial medium, Thiobacillus takes the thiocyanate as its food and the oxidation process takes place to release Sulphate, Total Suspended Solids (TSS) and Ammonia. Biodegradation of thiocyanate was occurred during 6th day to below detectable limit from an initial concentration of 1200 mg/L.

Biodegradation of Thiocyanate in Coke Oven Wastewater

The concentration of thiocyanate was measured in coke oven wastewater for different number of days using two different bacterial media.

- (i) Bacterial culture, pseudomonas from MBC plant of Visakhapatnam Steel Plant.
- (ii) Thiobacillus cultured in the laboratory for this experiment.
- (i) In case of bacterial medium, pseudomonas,



the thiocyanate concentration was degraded from 250 mg/L to below detectable limit with an increase in number of days from 0 to 9 as shown in Figure 2. Due to oxidation reaction between thiocyanate and bacterial medium, pseudomonas, Ammonia was formed and the concentration of Ammonia was also measured. Biodegradation of thiocyanate was occurred to below detectable limit by adding the bacterial medium, pseudomonas, during 9 days.

- ii) In case of bacterial medium, thiobacillus (which was cultured in the laboratory), the thiocyanate concentration was degraded from 250 mg/L to below detectable limit with an increase in number of days from 0 to 5 as shown Figure 2. During this process, the bacterial medium, thiobacillus takes the thiocyanate as its food and the oxidation process takes place to release Ammonia and Sulphate. In this case, biodegradation of thiocyanate was occurred during 5 days.

Biodegradation of thiocyanate was also studied by Paruchuri and Sivaraman (1990) using same bacteria, thiobacillus for the coal carbonization wastewater and the number of days taken for biodegradation were 6 days.

By comparing the biodegradation of thiocyanate concentration with the addition of two different bacterial media i) Pseudomonas and ii) Thiobacillus, the bacterial medium, thiobacillus was proved to be more effective for the biodegradation of thiocyanate, since the number of days taken for degradation were 5 days.

Effect of pH on Biodegradation of Thiocyanate

The hydrogen ion concentration is an important quality parameter for both natural and wastewater. The concentration range suitable for the existence

of most biological life is quite narrow and critical. When the pH of wastewater is high, it is difficult to treat by biological system, and some bacteria will be active only when the desired level of pH is maintained. Thus pH plays a very important role in biological treatment system. The concentration of thiocyanate was measured in coke oven wastewater using the bacterial medium, thiobacillus for different number of days by varying pH of the solution from 6.5 to 7.5. The biodegradation of thiocyanate to below detectable limit was occurred for the pH values of 6.5 to 6.9 during two days. For the pH values of 7.0 to 7.2, the biodegradation of thiocyanate to below detectable limit was occurred during 3 days. For pH values of 7.3 to 7.5, the biodegradation of thiocyanate to below detectable limit was occurred during 4 days. The results were shown in Figures 3, 4 and 5.

A cross plot was drawn between pH and % reduction of thiocyanate as shown in Figure 6. The % reduction of thiocyanate was increased from 92 to 98% with an increase in pH of the solution from 6.5 to 6.9 and it was decreased to 60.96% with an increase in pH from 6.9 to 7.5. At lower pH values, the strains of the bacteria were effectively oxidize the thiocyanate. The optimum pH for the biodegradation of thiocyanate using bacterial medium, thiobacillus was 6.8. Ludberg and Nicks 1969 had observed 7.0 as the optimum pH for this bacterium.

Effect of Phenol additive on biodegradation of thiocyanate

In order to discharge a satisfactory quality of effluent, when dealing with waste water containing more than one toxic substance, it is necessary to understand the effect of the toxicants on the biodegradation of each other, so that the system can be properly designed.

Figure 3: Vatiation of % Reduction of Thiocyanate with No. of Days

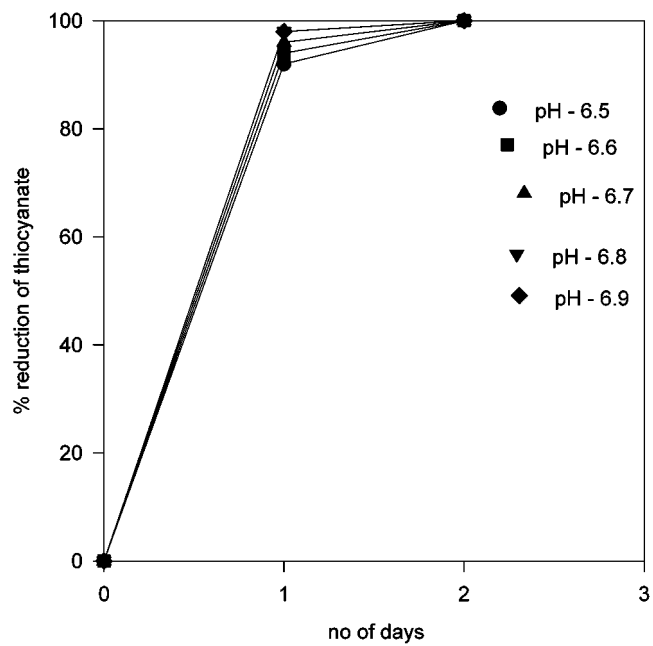
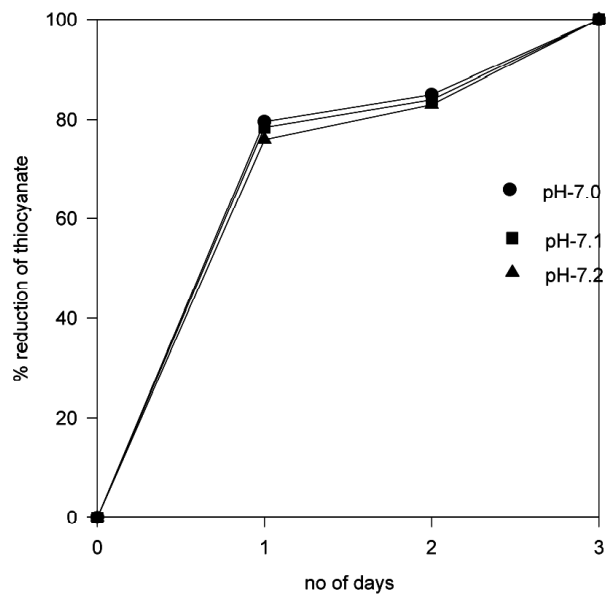


Figure 4: Vatiation of % Reduction of Thiocyanate with No. of Days



The concentration of thiocyanate was measured for different number of days by adding phenol content (0 to 400 mg/L) to coke oven wastewater using bacterial medium, thiobacillus. The results were shown in Figure.7.

The thiocyanate concentration was reached below detectable limit during 2 days for zero phenol added concentration, 3 days for phenol added concentration of 50 and 100 mg/L, 5 days for phenol added concentration of 200mg/L, 6

Figure 5: Variation of % Reduction of Thiocyanate with No. of Days

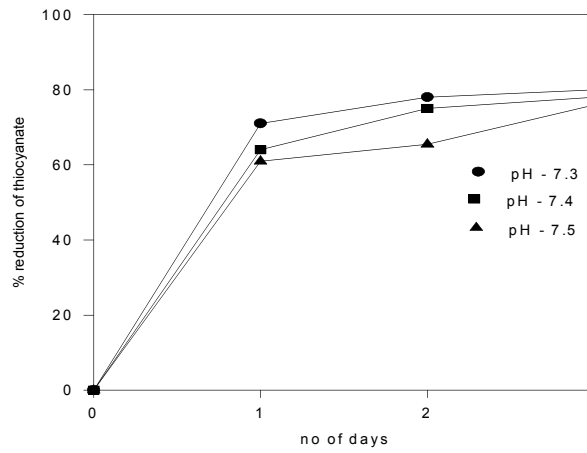


Figure 6: Cross Plot for Variation of % Reduction of Thiocyanate with pH

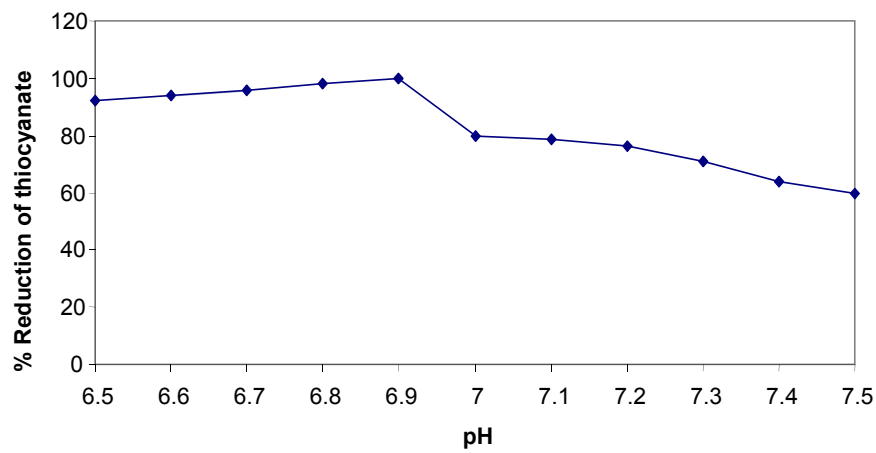
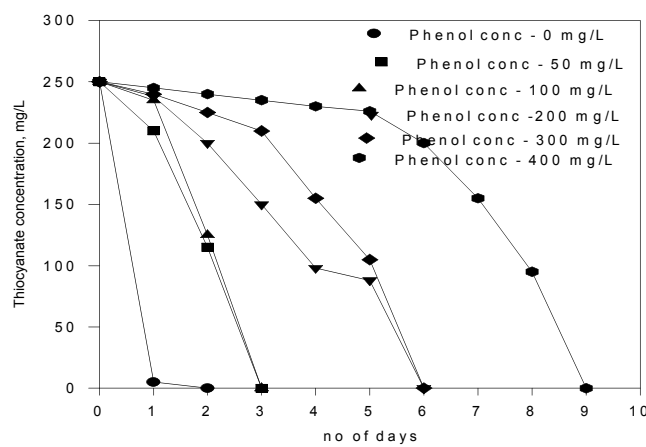


Figure 7: Biodegradation of Thiocyanate with Varying Phenol Concentration



days for phenol added concentration of 300 mg/L and 9 days for phenol added concentration of 400 mg/L. The number of days for biodegradation of thiocyanate was increased with an increase in phenol added concentration from 0 to 400 mg/L. This is because of the toxic effect of phenol on bacterial medium, thereby the concentration of bacterial medium was decreased and the oxidation reaction was not carried out in a proper way.

Effect of Hydraulic Loading Rate on Biodegradation of Thiocyanate

Generally the hydraulic loading rate is an important variable in the wastewater treatment process from the point of view of shock loads. Hence it was studied as a variable in the present study.

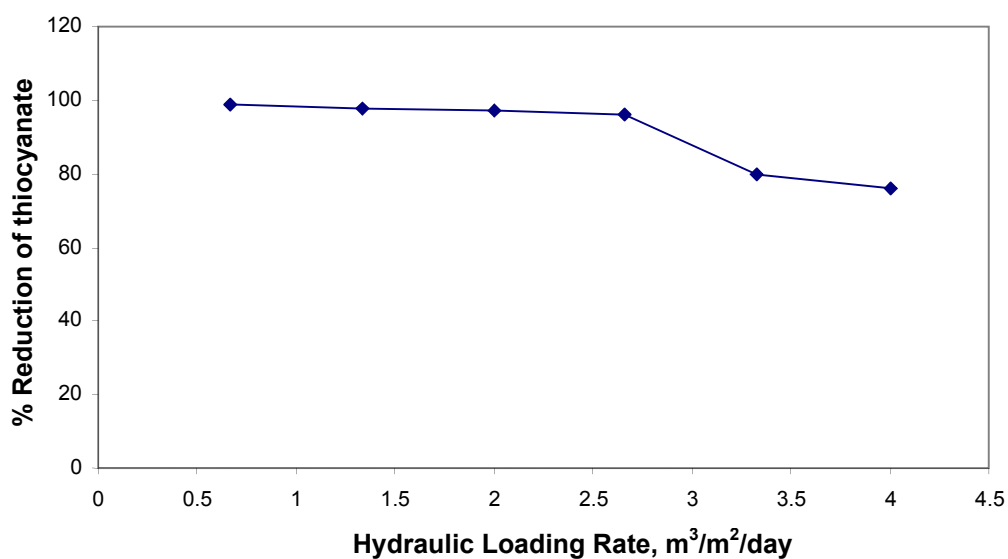
The effect of hydraulic loading rate on biodegradation of thiocyanate was studied by varying the flow rate of influent (coke oven wastewater) and the % reduction of thiocyanate was calculated and shown in Figure 8. The % reduction of thiocyanate was decreased from 99 to 75.83% with an increase in the flow rate of

influent from 5 to 30 m³/hr and also with an increase in hydraulic loading rate from 0.67 to 4 m³/m²/day. This is because of the increase in the hydraulic loading rate, as more food in the form of thiocyanate is available to the bacterial medium thiobacillus.

CONCLUSION

The present study shows that the mortality rate, 0 to 100% was attained for thiocyanate concentration ranging from 405 to 465 ppm, hence the permissible concentration of thiocyanate suggested for industrial effluent is up to 405. It was observed that the concentration of thiocyanate in synthetic solution was decreased with an increase in number of days from 1 to 5 and further decreased to below detectable limit during 6th day by adding the bacterial medium thiobacillus. Biodegradation of thiocyanate in coke oven wastewater was occurred to below detectable limit by adding the bacterial medium, Pseudomonas during 9 days and thiobacillus during 5 days. The biodegradation of thiocyanate

Figure 8: Variation of % Reduction of Thiocyanate with Hydraulic Loading Rate at pH – 6.8.



in coke oven wastewater to below detectable limit was occurred for the pH of 6.5 to 6.9 during two days. For the pH of 7.0 to 7.2, the biodegradation of thiocyanate to below detectable limit was occurred during 3 days. For the pH of 7.3 to 7.5 the biodegradation of thiocyanate to below detectable limit was occurred during 4 days. The % reduction of thiocyanate was increased from 92 to 98% with an increase in pH of the solution from 6.5 to 6.9 and it was decreased to 60.96% with an increase in pH from 6.9 to 7.5. The optimum pH for the biodegradation of thiocyanate in coke oven wastewater using bacterial medium thiobacillus was 6.8. The number of days for biodegradation of thiocyanate in coke oven wastewater was increased with an increase in phenol added concentration from 0 to 400 mg / L. The % reduction of thiocyanate in coke oven waste water was decreased from 99 to 75.83% with an increase in the flow rate of influent from 5 to 30 m³/hr and also with an increase in hydraulic loading rate from 0.67 to 4 m³/m²/day.

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