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Comparison of Fish Toxicity & Microtox Toxicity of Luminescent Bacteria Due to Bleach Plant Effluent Released from Agro & Wood Based Pulp and Paper Mills

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Abstract

With more public awareness about environmental issues mainly in developed countries when they found the Chlorophenolic compounds (AOX) in the fish stock receiving bleach plant effluent, draw much attention regarding the toxicity value of bleach plant effluent. In this paper, I have mainly described the fish toxicity and microtox toxicity value of bleach plant effluents and their relationship in agro & wood based mills. Toxicity studies were done on chlorination stage, Extraction stage, Hypo stage and combined bleach effluents of agro and wood based mills. Studies show that the E-stage effluents are having high fish mortality rate at lower % volume concentration & high % inhibition of Luminescent bacteria, indicating that it is more toxic than the other stage bleach effluent. Studies also shows that scenario of agro based small pulp and paper mills are not good and releases high toxic bleach effluent as compared to wood based large pulp and paper mills due to low scale of operation and use of high dose of bleach chemical due to economical reason.

Keywords: AOX; LC-50; Fish Toxicity; Microtox toxicity; % Inhibition; Bleach plant effluent

Introduction

In Indian pulp and paper mills, pulp mill section plays an important role for releasing high toxic chemicals in water streams. Most of the mills are employing conventional bleaching process (Chlorination stage, Extraction stage & Hypochlorite stage). The consumption of chlorine in these categories of mills is comparatively higher, consequently releasing high amount of chloro-organic compounds [1]. Adsorbable organic halogen (AOX), a measure of the sum of chlorinated organic compounds, averages about 4 to 5 kg per tonne of pulp in untreated effluents of mills using primarily elemental chlorine for bleaching [2]. The chemical stability of organochlorine compounds in bleach effluents was reflected in their resistance to microbial degradation [3]. The consequence of AOX compounds discharged in mill effluents were recognized by developed countries when high concentration of these compounds were detected in fish fat stocks receiving bleach plant effluent. These compounds also have tendency to accumulate in fish & were responsible for acute toxicity [4]. In Canadian pulp and paper Industry and senior manager have been legally charged for failing to meet the effluent toxicity regulation [5]. A case study of bioaccumulation of dioxins in a Canadian river system was described [6]. Chloro organic compounds become bound to natural matrices and also interfere with the bioavailability [7].

Fish exposed to chlorinated phenolics discharge by bleached pulp mill were reported to have demonstrated impaired function of liver, enzyme system, metabolic cycle as well as increase in the incidence of spinal deformities and reduced gonad development in both laboratories and field studies [8]. Some of the reported biological effects include adverse impact on growth rate, lever, primary production in natural mixed phytoplankton populations as well as mutagenic effects, disruption of regulatory hormones, reproductive and immune system disorders and abnormal fetal development [9]. The present study has been carried out with an objective to demonstrate toxicity of bleach plant mill effluent generated from agro based and wood based pulp mill.

Material and Method

Fish toxicity

Toxicity test is used to determine the potential harmful impact of wastewater on aquatic life. The conventional bleaching process followed by Indian pulp and paper industry, thus discharging high level of AOX which are toxic to the aquatic organism. Fish are particularly susceptible to the influence of toxic substance during the reproductive and early stages of development. Determination of the influence on early developmental stages is a more sensitive index of fish tolerance than obtained by determination of acute toxicity. The test fish species used should be selected on the basis of practical criteria, such as their ready availability throughout the year, ease of maintenance, convenience for testing, relative sensitivity to chemicals, and economic, biological or ecological factors which have any bearing. Easily available common carp fish were selected for toxicity test in the present study. Carp were imported from hatchery and allowed in the test pond for testing the toxicity. Toxicity inhibition response of bleach plant effluents on Luminescent bacteria (*Vibrio fisheri*) were also evaluated using Microtox Toxicity Analyzer which are widely used in developed countries to assess toxicity emission factor and also to establish a correlation between conventional LC_{50} & instrumental Microtox toxicity. The inhibition of light production of *Vibrio fisheri* luminescent bacteria indicates disturbance of the energy metabolism

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of this heterotrophic bacterium. Because this luminescent pathway is direct branch of electron transport chain the luminescent measurement assesses the metabolic status of this bacterium. Hence the change in bacterial luminescence when these bacteria are exposed to wastewater samples can be used as an indicator of potential toxicity. The sensitivity of the microtox method has been proven to be comparable to acute toxicity tests with fish and crustaceans in several comparative investigations [10]

Experimental work

The toxicity of bleach plant effluents collected from mills to Common Carp (Fish) were tested according to standard method (IS: 6582 – 1971 & ISO 7346-1 1996- Water quality - Determination of the acute lethal toxicity of substances to a freshwater fish - Part 1: Static method). The procedure is based upon direct exposure to fish to measure LC_{50} under controlled laboratory condition.

The fish (*Cyprinus carpio*) of weighing 5-6 gm and length 4-5 cm were purchased from a local fish hatchery. Prior to use, the fish were acclimated for 12 days to laboratory well water at a temperature 25± 3°C, at pH of 7.7, hardness of 210 mg/l. The same well water was used for effluent dilution purposes. Dissolved oxygen concentrations were maintained above 90% saturation with oil free compressed air. The fish were fed a commercial diet at a daily rate of 1% body weight. Feeding were stopped 48h before the beginning of each exposure.

Exposures lasting 96h were conducted in glass aquarium of 20 Lit. Capacity at a temperature of 25±3°C. 10-20 fish of approximately the same weight and length were placed in aquaria containing effluents. The fish were not fed during experiments but all other parameters were monitored daily like measurements of pH, dissolved oxygen and temperature are carried out daily. The fish were inspected after the first 2 to 4 hours and at least at 24-hour intervals. Fish were considered dead if touching of the caudal peduncle produces no reaction, and no breathing movements are visible. Dead fish were removed when observed and mortalities were recorded

Results and Discussion

Toxicity of bleach plant effluents (C-stage, E-stage, H-stage & combined) collected from a wood based mill were determined by using bioassay technique in lab scale aquarium using fish and results obtained are shown in table 1. Toxicity of bleach plant effluents (C-stage, E-stage, H-stage & combined) collected from an agro based mill were determined by using bioassay technique in lab scale aquarium using fish and results obtained are shown in table 2.

Acute toxicity is the discernible adverse effect induced in an organism within a short time (days) of exposure to a substance. In the present test, acute toxicity was expressed as the median lethal concentration (LC50), which is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure which must be stated. LC – 50 values of the fish toxicity of Bleach Plant effluents collected from wood based and agro based mills are given in the table 3 & 4.

The toxicity of the effluents were also tested according to the standardized luminescence bacteria test procedure (DIN 38412 Teil 34 1991- standardized procedure established by the DIN-Arbeitskreis "Leuchtbakterientest" (Working Group of the German Institute for Standardization for the luminescent bacteria test)) by *ToxAlert* 10 instrument. Comparison of Microtox Toxicity with other toxicity tests has pointed out the satisfactory sensitivity of microtox for analyzing the toxicity of pulp and paper mill effluents [11,12]. The luminescence inhibition tests were accomplished by combining bleach effluents collected from wood and agro based mills with the luminescence bacteria *Vibrio fischeri* NRRL B-11177. The luminescence inhibition (I%) of bleach effluents (C-stage, E-stage, H-stage & combined) collected from wood based mills and agro based mills were measured. The results obtained are summarized in table 4. In the table 5 and 6 shows the relation between the fish toxicity & microtox toxicity of luminescent bacteria (% inhibition) of bleach plant effluents. Higher the % inhibition and lower the LC-50 was having the high toxic effluent

Effluent Concentration %	%Fish Mortality after 96hr (Sample-1)	%Fish Mortality after 96hr (Sample-2)	%Fish Mortality after 96hr (Sample-3)
C-Stage Effluent			
0	0	0	0
30	30	20	30
50	70	40	60
70	80	70	90
E-Stage Effluent			
0	0	0	0
30	40	30	50
50	80	70	70
70	90	70	90
H-Stage Effluent			
0	0	0	0
30	20	25	30
50	40	35	40
70	60	55	70
Combined Bleach Effluent			
0	0	0	0
30	20	30	20
50	50	65	45
70	70	75	70

Table 1: Fish Toxicity from bleach effluent of wood based mill at DO 7.3 to 7.7 mg/l and temperature of 25±2°C.

Effluent Concentration %	%Fish Mortality after 96hr (Sample-1)	%Fish Mortality after 96hr (Sample-2)	%Fish Mortality after 96hr (Sample-3)
C-Stage Effluent			
0	0	0	0
30	40	35	45
50	70	65	70
70	90	75	85
E-Stage Effluent			
0	0	0	0
30	45	50	50
50	75	70	80
70	90	85	100
H-Stage Effluent			
0	0	0	0
30	30	35	30
50	55	55	65
70	65	70	70
Combined Bleach Effluent			
0	0	0	0
30	40	30	30
50	60	70	60
70	85	80	80

Table 2: Fish Toxicity from bleach effluent of agro based mill at DO 7.3 to 7.7 mg/l and temperature of 25±2°C.

	C-Stage	E-Stage	H-Stage	Combined Effluent
WOOD BASED MILL				
Sample- 1	40	35	60	50
Sample- 2	50	40	65	42
Sample- 3	44	30	57	55
AGRO BASED MILL				
Sample- 1	37	34	47	40
Sample- 2	40	33	45	40
Sample- 3	35	30	43	44

Table 3: Fish Toxicity (Lc_{50} Value) of Bleach Plant Effluents.

	C-Stage	E-Stage	H-Stage	Combined Effluent
WOOD BASED MILL				
Sample-1	70	85	50	65
Sample-2	75	90	55	65
Sample-3	65	85	50	70
AGRO BASED MILL				
Sample-1	80	95	52	68
Sample-2	75	85	65	75
Sample-3	70	95	60	70

Table 4: Microtox Toxicity of Luminescent Bacteria (% Inhibition).

		Sample-1	Sample-2	Sample-3
C-Stage	% Inhibition	70	75	65
	LC-50	40	50	44
E-Stage	% Inhibition	85	90	85
	LC-50	35	40	30
H-Stage	% Inhibition	50	55	50
	LC-50	60	65	57
Combined Bleach Effluent	% Inhibition	65	65	70
	LC-50	50	42	55

Table 5: Fish Toxicity (Lc_{50} Value) & Microtox Toxicity of Luminescent Bacteria (% Inhibition) of Bleach Plant Effluents of Wood BASED MILL.

		Sample-1	Sample-2	Sample-3
C-Stage	% Inhibition	80	75	70
	LC-50	37	40	35
E-Stage	% Inhibition	95	85	95
	LC-50	34	33	30
H-Stage	% Inhibition	52	65	60
	LC-50	47	45	43
Combined Bleach Effluent	% Inhibition	68	75	77
	LC-50	40	40	44

Table 6: Fish Toxicity (Lc_{50} Value) & Microtox Toxicity of Luminescent Bacteria (% Inhibition) of Bleach Plant Effluents of Agro Based Mill.

and lower the % inhibition and higher the LC-50 was having less toxic effluents.

Fish toxicity test (LC_{50})

Toxicity inhibition response of bleach plant effluents on fish mortality were evaluated of wood based and agro based mill. From the results it was found that C-stage effluents collected from wood based mill were found toxic to the fish between 40-50% volume concentration & 35-37 % in agro based mill. E-stage effluents collected from wood based mill were found toxic to the fish between 30-40% volume concentration & 30-34 % in agro based mill. The result shows that E-stage effluents were more toxic than other stage effluent. H-stage effluents collected from wood based mill were found toxic to the fish between 57-65% volume concentration & 43-47 % in agro based mill. Combined Bleach Plant effluents collected from wood based mill were found toxic to the fish between 50-50% volume concentration & 40-44 % in agro based mill. LC_{50} of E-stage bleach plant effluents were very less (30-40) of wood and agro based mills & this indicate that E-stage bleach plant effluents were highly toxic than the other stage effluent and due to this E-stage effluent needs to be treated before being discharged to the aquatic environment. R. Nagarathnamma and Pratima Bajpai [13] was studied and reported that initial 50% lethal concentration LC-50 (96 h) of extraction-stage effluent was in the range of 50% by volume. H- stage effluent is very less toxic and all effluent recycle in the plant itself. Gergov M et al. [14] also reported that toxicity of Extraction stage filtrate was rather high as compared to other stage effluent and it was even higher during bleaching with a higher chlorine dioxide concentration.

Microtox toxicity test (% Inhibition)

Toxicity inhibition response of bleach plant effluents on Luminescent bacteria were evaluated using Microtox Toxicity Analyzer. Inhibition of luminescent bacteria in C- stage effluents of wood based mill were observed between 65- 70 % and 70-80 % in agro based mill. Inhibition of luminescent bacteria in E- stage effluents of wood based mill were observed between 85-90 %and 85-95 % in agro based mill. Inhibition of luminescent bacteria in H- stage effluents of wood based mill were observed between 50-55 % and 52-65 % in agro based mill. Maximum inhibition (>85%) were observed in E-stage bleach plant effluents of wood and agro based mills. This indicates that

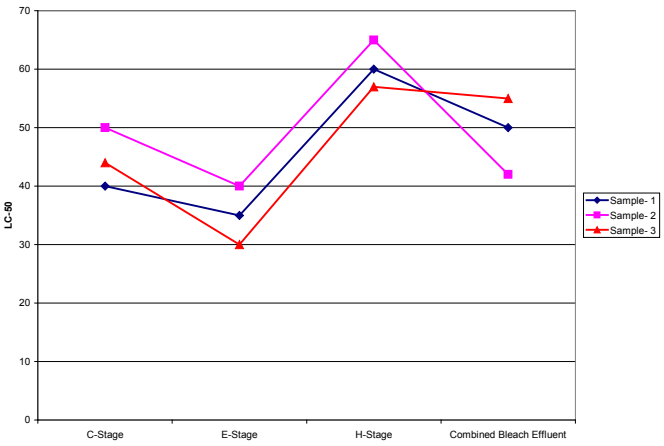

Figure 1: LC-50 Value of Bleach Plant Effluents of Wood Based Mill.

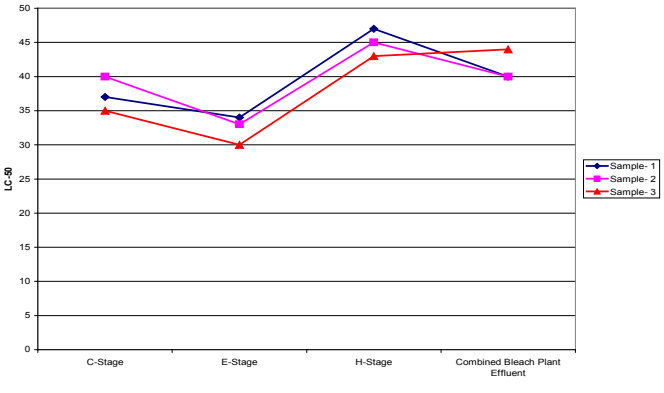

Figure 2: LC-50 Value of Bleach Plant Effluents of Agro Based Mill.

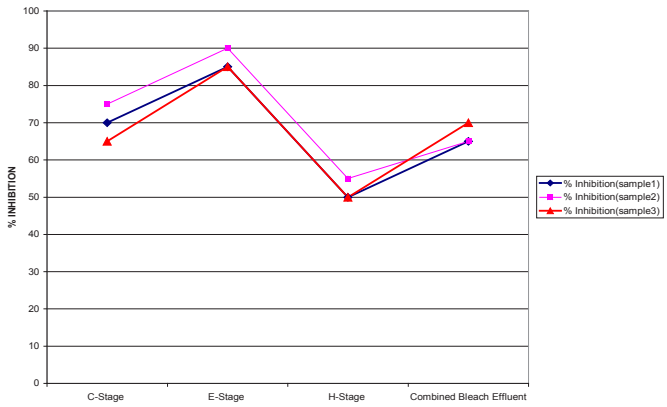

Figure 3: % Inhibition of Luminescent Bacteria Bleach Effluents of wood based mill.

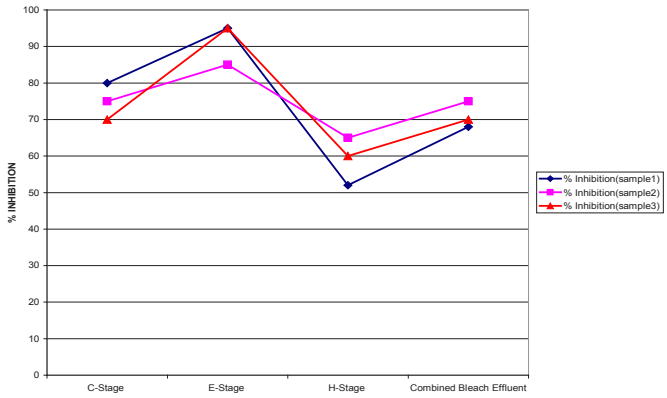

Figure 4: % Inhibition of Luminescent Bacteria Bleach Effluents of agro based mill.

E-stage bleach plant effluents were highly toxic and needs to be treated before being discharged to the aquatic environment. The toxicity results shows that E-stage effluents were more toxic than C & H stage effluent and more emphasis were given to treat and reduce the AOX value in E-stage effluent. Wagner [15] also studied & reported that water pollution problem and toxicity in the pulp and paper industries originate largely from Bleach Plant Effluent.

Conclusion

The bleach plant effluent is of wide environmental concerns in pulp and paper mills in India because it contains high amount of AOX which are very toxic to aquatic environment. The E – stage effluent is found to have high toxicity value as compared to other bleach stage effluent. Therefore it is advisable to segregate the high polluted stream of bleach plant followed by any treatment application. A three way approach is suggested for treatment or reducing AOX in paper mill effluent which is as follows:

- in house control measures
- End of pipe(EoP) treatment
- Integrated Approach (combination of In-house & end of pipe treatment)

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