Acute Toxicity of Distillery Spent Wash on Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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ABSTRACT

Acute toxicity tests using distillery spent wash, treated by anaerobic digestion process, on Nile tilapia (*Oreochromis niloticus*) fingerlings were conducted under laboratory conditions for 96 h using static bioassay method. In the definitive test, seven groups with three replications of experimental fish (body weight 5.6±0.45 g/fish) were exposed to seven concentrations of distillery spent wash (0, 12, 14, 16, 18, 20 and 22 ml/L). No mortality was observed during the 96 h in groups of control fish (0 ml/L) and fish exposed to 12 ml/L. The 100% mortality rate was achieved only in fish group exposed to 22 ml/L of distillery spent wash. The median lethal concentration (LC_{50}) of distillery spent wash to fish for 24, 48, 72 and 96 h were 18.547, 18.024, 17.965 and 17.965 ml/L, respectively. In addition, health of fish exposed to over 12 ml/L (14, 16, 18, 20 and 22 ml/L of distillery spent wash) were affected. The behavioral changes of fish in these groups displayed rapid opercular movement and frequent gulping of air at the surface of the water. This abnormal behavior of fish increased after 24 h. Fish swimming activity decreased and later fish settled to the bottom of the aquaria, and their opercula moved slower until they eventually stopped when mortality occurred. Gill coloration was pale and the gills were clogged with sediment from distillery spent wash. Tilapia mortality was directly related to undesirable properties of distillery spent wash. Therefore, distillery spent wash should be suitably treated before disposal to the ecosystem or utilization in aquaculture.

Keywords: Distillery spent wash, Nile tilapia, Acute toxicity, Physiological observations

INTRODUCTION

Distillery spent wash, a.k.a. distillery effluent, is the unwanted residue liquid generated from alcohol production which consists of three steps including material preparation, fermentation and distillation. Distillery spent wash contains high contents of inorganic substances, such as nitrogen (N), potassium (K^+), phosphate (PO_4^{3-}), calcium (Ca^{2+}) and sulphate (SO_4^{2-}), and has high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and BOD/COD ratio. Further, it has a high concentration of organic matter, which is fermented mash with acidic character (Pant and Adholeya, 2007; Mohana

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et al., 2009). As a waste product from agriculture, it has been reported that distillery spent wash has several applications, namely in rice paddy fields, wheat, maize, cucumber, chili, onion and tomato, to increase crop yields as it has an abundance of desired nutrients. Distillery spent wash are mineral sources for plant, in terms of potassium (K), sulphur (S), nitrogen (N) and phosphorus (P), as well as micronutrients for plant such as calcium, sulphate, copper (Cu) and zinc (Zn), which are essential for plant (Phanapavudhikul, 1999; Ramana *et al*., 2002a,b; Orhue *et al*., 2005; Mohana *et al*., 2009; Das *et al*., 2010).

The disposal of untreated distillery spent wash into rivers and other reservoirs poses an extremely environmental pollution hazard as it is acidic and has a high temperature resulting in water quality levels which are hazardous and directly affecting microorganisms and aquatic animals (Inmuong, 1998; Satyanarayan, 2011; Botelho *et al*., 2012). Besides, a high percentage of fermented mash in distillery spent wash could reduce sunlight penetration in rivers which is one of the essential factors in photosynthesis to produce dissolved oxygen for aquatic animals. High COD, total nitrogen and total phosphate levels in distillery spent wash could lead to eutrophication in natural water bodies (Kumar *et al*., 1997; Saxena and Chauhan, 2003).

Previously published studies on the toxicity of distillery spent wash on fish reported that untreated and improperly treated distillery spent wash significantly cause adverse impacts on aquatic animals and on the behavioral changes in common carp (*Cyprinus carpio*), spotted snakehead fish (*Channa punctatus*), Indian giant gourami (*Colisa fasciatus*) and guppy (*Lebistes reticulatus*) associated with exposing distillery spent wash were observed (Kumar *et al*., 1995; Kumar and Gopal, 2001; Ramakritinan *et al*., 2005; Patil and Ghole, 2010; Shukla and Shukla, 2012a,b: Saroja *et al*., 2013; Mahesh, 2015). The LC_{50} values for 96 h period of distillery spent wash were observed at varying concentrations of distillery spent wash in these studies because the characteristics of distillery spent wash vary depending on the raw materials used and ethanol production process, and treatment process. Distillery spent wash disturbed the respiratory metabolism resulting in decreased oxygen consumption in common carp (Ramakritinan *et al*., 2005). Alterations of biochemical parameters in blood (e.g. glucose, lactic acid and glycogen), liver and muscle of common carp related to treatment with distillery spent wash were reported by Saroja *et al*. (2013). Similarly, the finding of Kumar and Gopal (2001) indicated that levels of lactic acid, protein, glycogen, glucose in tissues (e.g. liver, brain, kidney and muscle) and blood of spotted snakehead fish (20 g/fish) were significantly affected by distillery spent wash. Kumar and Gopal (2001) also reported that there were significant differences in hematological parameters, such as red blood cells, white blood cells, hematocrit, and hemoglobin. Additionally, histological lesions of common carp exposed to distillery spent wash exhibited in gills, intestines and kidneys of common carp and guppy (Kumar *et al*., 1995; Kumar and Gopal, 2001).

Nam Phong River, which is in the northeast of Thailand, provides an important water resource for irrigation, electricity

generation, agriculture, aquaculture and many industries including papers, sugar, cassava and liquor. The pollution crisis caused by overload of untreated and improperly treated wastewater and untreated residue solids in Nam Phong River was documented in 1997. Over 500,000 fish and a huge number of shellfish and other aquatic organisms were killed by water pollution resulting from wastewater and by products spilled from these industries and run-offs from agricultural areas into the river (Faculty of Science, 1987, 1991; Inmuong, 1998). Nowadays, Nile tilapia (*Oreochromis niloticus*) cage farming is widespread in Nam Phong River; unfortunately, the levels of distillery spent wash contaminating the water for fish have not been documented. Hence, it is necessary to investigate the acute toxicity of distillery spent wash on the mortality of Nile tilapia fingerlings under laboratory conditions in order to determine the toxic effect of distillery spent wash on tilapia. The findings can be used as preliminary data for strategies in water management or utilization for aquaculture in the future.

MATERIALS AND METHODS

Experimental animals

Nile tilapia (*Oreochromis niloticus*) fingerlings were obtained from a hatchery located in Khon Kaen Province, Thailand, and transported to the laboratory of the Department of Fisheries, Faculty of Agriculture, Khon Kaen University. Fish were acclimatized in a 500-L fiberglass tank with aeration for 4 days. Totally 410 fish were randomly distributed into the experimental system (32-L aquarium equipped with aeration) for 14 days before the beginning of the preliminary toxicity test and the definitive toxicity test (10 fish/aquarium). During acclimatization, fish were fed with commercial feed (32% crude protein) twice daily at 10% body weight/day. Water temperature, pH, salinity, electrical conductivity (EC) and dissolved oxygen (DO) were maintained at 25.1±0.2ºC, 7.1±0.1, 0.14±0.04 parts per thousand (ppt), 0.42±0.06 milliSiemens/centimeter (mS/cm) and 5.0±0.3 mg/L, respectively. Daily water exchange was at 10%, using dechlorinated tap water. Feeding was stopped 24 hours prior to starting the preliminary and definitive toxicity tests. No feeding and water exchange occurred during the experiments.

Distillery spent wash

Distillery spent wash which has been treated by anaerobic digestion process and collected from a liquor factory in Khon Kaen Province, was analyzed to determine its physico-chemical characteristics. It was used to prepare the stock solution (1,000 ml/L). The experiment was divided into two steps, including the preliminary toxicity test and the definitive toxicity test.

The preliminary toxicity test

The preliminary toxicity test of distillery spent wash was aimed to determine the lowest concentration which would kill all the tilapia (LC_{100}), and the highest concentration which would kill none of the tilapia (LC_0), to establish a narrower concentration range in the definitive toxicity test. Static bioassay was conducted at 1, 3,

6, 12, 24, 48, 72 and 96 h with a 32-L test solution. The test solutions for each treatment were prepared by diluting the stock solution (distillery spent wash) with dechlorinated tap water as a diluent, with two replications. The treatments were: 0 (control: dechlorinated tap water), 1, 2, 5, 10, 12, 15, 20, 22 and 25 ml/L. Ten tilapia (body weight = 5.2±0.14 g/fish) were randomly distributed into each 32-L aquarium. Tilapia mortality rate (%) was recorded at each interval. A fish was considered dead when no opercular movement was observed, according to Noga (2010).

The definitive toxicity test (LC_{50} determination)

Based on the results of the preliminary toxicity test, the definitive toxicity test consisted of seven treatment concentrations as 0 (control: dechlorinated tap water), 12, 14, 16, 18, 20 and 22 ml/L, with 3 replicates per treatment. The procedure to prepare test solution for each treatment was similar to that in the preliminary toxicity test. Ten tilapia (body weight = 5.6±0.45 g/fish) were randomly distributed into each 32-L aquarium. Static bioassay (Reish and Oshida, 1987) was conducted at 24, 48, 72 and 96 h with a 32-L test solution. Behavioral changes, including general behavior, abnormal coloration and respiratory distress, and mortality of fish were observed and recorded during the experiment (Noga, 2010). Tilapia mortality rate (%) was recorded at each interval.

Water quality analysis

Water quality of distillery spent wash (stock solution) was determined through analysis of pH, temperature, salinity, electrical conductivity (EC), total alkalinity, total hardness, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS), total dissolved solids (TDS), suspended solids (SS), total nitrogen, total phosphate and total potash according to APHA *et al.* (2012). For the preliminary and the definitive toxicity tests, water quality including water temperature, dissolved oxygen (DO), pH, salinity and EC were measured every 24 h until the end of the experiments.

Statistical analysis

Median lethal concentration values (LC_{50}) were determined from mortality percentage of fingerlings and concentrations of the distillery spent wash through Finney's Probit Analysis LC_{50} determination method (Finney, 1971). Confidential limits (Upper and Lower) were calculated, and SPSS 16 was also used to determine LC_{50} value of distillery spent wash with the help of Probit analysis. Water quality data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Significant differences were determined at $P < 0.05$.

RESULTS

The physico-chemical characteristics of dechlorinated tap water and distillery spent wash used for stock solution (1,000 ml/L) are presented in Table 1.

In the preliminary toxicity tests, mortality rate of tilapia with exposure for 96 h increased with increasing concentrations of

Table 1. Physico-chemical characteristics of dechlorinated tap water and distillery spent wash (stock solution; 1,000 ml/L) treated by anaerobic digestion process.

Parameter	Unit	Dechlorinated tap water	Distillery spent wash
Color	-	Colorless	Dark brown
Dissolved oxygen	mg/L	6.21	0.33
pH	-	7.68	7.83
Temperature	^{o}C	25.7	28.9
Electrical conductivity (EC)	mS/cm	0.24	6.89
Total alkalinity	mg/L as $CaCO_3$	78	2,647
Total hardness	mg/L as $CaCO_3$	81	3,538
Chemical oxygen demand (COD)	mg/L	ND	45
Biochemical oxygen demand (BOD)	mg/L	ND	<5
Total solids (TS)	mg/L	ND	12,900
Total dissolved solids (TDS)	mg/L	ND	10,503
Suspended solids (SS)	mg/L	ND	373
Total nitrogen (as N)	%	ND	0.11
Total phosphate (as P_2O_5)	%	ND	0.01
Total potash (as K_2O)	%	ND	0.36
Salinity	ppt	0.11	3.44

ND= Not determined

distillery spent wash, which ranged from 0 to 25 ml/L (Table 2). The highest concentration of distillery spent wash which did not kill any tilapia (96 h LC_0) was 12 ml/L. On the other hand, the lowest concentration of distillery spent wash which killed all tilapia (96 h LC_{100}) was 25 ml/L. Furthermore, mortality of fish exposed to15 ml/L occurred initially at 48 h interval (10%) and 100% mortality of fish exposed to 22 ml/L appeared after 24 h exposure. As a result of the preliminary toxicity tests, the concentrations of distillery spent wash ranging from 0 to 22 ml/L were selected as test concentrations for the definitive toxicity test (96 h LC_{50}).

In the definitive toxicity test (LC_{50} determination), mortality of tilapia increased with increasing concentrations of distillery spent wash (Table 3). No mortality of tilapia was observed during the 96 h exposure of fish in the control treatment (0 ml/L) and in 12 ml/L treatment. Total mortality occurred only in fish exposed to 22 ml/L of distillery spent wash for 96 h. The results based on Finney's Probit Analysis and SPSS 16 analysis showed that the median lethal concentrations (LC_{50}) of distillery spent wash to tilapia at 24, 48, 72 and 96 h of exposure were 18.547, 18.024, 17.965 and 17.965 ml/L, respectively (Table 4).

The behavioral and swimming patterns of tilapia in the control group and group exposed to 12 ml/L of distillery spent wash were normal and no mortality occurred during the experimental period. During the initial stages of exposure, tilapia in 14, 16, 18, 20 and

Table 2. Mortality rate (%) of Nile tilapia (5.2±0.14 g/fish; N = 20) exposed to distillery spent wash (ml/L of distillery spent wash in test solution) from 1 to 96 hours during the preliminary toxicity test.

Concentration (ml/L)	Mortality rate (%)								
	N	1h	3h	6h	12h	24h	48h	72h	96h
0 (control)	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	20	0.0	0.0	0.0	0.0	0.0	10.0	10.0	10.0
20	20	0.0	0.0	0.0	30.0	55.0	70.0	70.0	70.0
22	20	0.0	0.0	30.0	65.0	100.0	100.0	100.0	100.0
25	20	0.0	0.0	40.0	90.0	100.0	100.0	100.0	100.0

Table 3. Mortality rate (%) of Nile tilapia (5.6±0.45 g/fish; N = 30) exposed to distillery spent wash (ml/L of distillery spent wash in test solution) in the definitive toxicity test (96 h LC_{50}).

Concentration (ml/L)	Mortality rate (%)				
	N	24h	48h	72h	96h
0 (control)	30	0.0	0.0	0.0	0.0
12	30	0.0	0.0	0.0	0.0
14	30	0.0	3.3	3.3	3.3
16	30	13.3	13.3	13.3	13.3
18	30	26.7	43.3	46.7	46.7
20	30	80.0	86.7	86.7	86.7
22	30	100.0	100.0	100.0	100.0

Table 4. Correlation between distillery spent wash and mortality rate (%) of Nile tilapia (5.6±0.45 g/fish) on time (24-96 h) in the definitive toxicity test (96 h LC_{50}).

Point	Concentration (ml/L), (95% confidence interval end point)			
	24h	48h	72h	96h
LC_1	14.540 (13.007–15.474)	13.757 (12.202–14.725)	13.704 (12.147–14.673)	13.704 (12.147–14.673)
LC_5	15.714 (14.568–16.441)	15.007 (13.840–15.762)	14.952 (13.783–15.708)	14.952 (13.783–15.708)
LC_{10}	16.340 (15.387–16.970)	15.674 (14.701–16.329)	15.617 (14.641–16.274)	15.617 (14.641–16.274)
LC_{15}	16.762 (15.930–17.337)	16.123 (15.271–16.721)	16.066 (15.212–16.665)	16.066 (15.212–16.665)
LC_{50}	18.547 (18.041–19.071)	18.024 (17.501–18.560)	17.965 (17.442–18.499)	17.965 (17.442–18.499)
LC_{85}	20.332 (19.727–21.232)	19.925 (19.304–20.827)	19.863 (19.245–20.760)	19.863 (19.245–20.760)
LC_{90}	20.754 (20.089–21.780)	20.375 (19.692–21.401)	20.312 (19.633–21.333)	20.312 (19.633–21.333)
LC_{95}	21.380 (20.614–22.603)	21.041 (20.256–22.264)	20.978 (20.196–22.195)	20.978 (20.196–22.195)
LC_{99}	22.554 (21.575–24.169)	22.291 (21.290–23.907)	22.226 (21.229–23.834)	22.226 (21.229–23.834)

22 ml/L of distillery spent wash displayed rapid opercular movement and frequent gulping of air at the surface of the water. These abnormal activities of fish increased after 24 h exposure.

Water quality conditions of the test solution per treatment during the definitive toxicity are presented in Table 5. There were significant differences in pH, salinity and EC among treatments ($P<0.05$). The increasing concentrations of salinity and EC varied with increasing dilution of distillery spent wash. The temperature and DO were not significantly different. Notably, DO slightly tended to decrease with increasing concentrations of distillery spent wash due to oxygen consumption of tilapia and microorganisms in the sediment for aerobic degradation throughout the experiment relating to low pH in the test solutions.

Table 5. Mean values of water quality parameters after 24 h of experiment until the end of experiment in the definitive toxicity test (mean±standard deviation).

Concentration (ml/L)	Temperature (°C)	DO(mg/L)	pH	Salinity (ppt)	EC (mS/cm)
0 (control)	24.9±0.3^{a}	3.38±0.23^{a}	7.0±0.1^{a}	0.16±0.03^{f}	0.39±0.12^{e}
12	24.9±0.3^{a}	3.33±0.23^{a}	6.8±0.1^{b}	0.29±0.06^{e}	0.86±0.19^{d}
14	25.1±0.5^{a}	3.29±0.21^{a}	6.7±0.2^{bc}	0.37±0.04^{d}	0.94±0.15^{cd}
16	24.9±0.3^{a}	3.27±0.20^{a}	6.7±0.2^{cd}	0.45±0.07^{c}	0.98±0.16^{bcd}
18	24.9±0.3^{a}	3.25±0.20^{a}	6.6±0.1^{de}	0.50±0.09^{bc}	1.04±0.16^{abc}
20	24.9±0.3^{a}	3.23±0.19^{a}	6.5±0.1^{ef}	0.55±0.10^{b}	1.11±0.19^{ab}
22	24.9±0.4^{a}	3.21±0.18^{a}	6.5±0.1^{f}	0.62±0.11^{a}	1.18±0.21^{a}
P-value	0.541	0.475	<0.001	<0.001	<0.001

Means in the same column having different superscripts are significantly different ($P < 0.05$).

DISCUSSION

The median lethal concentrations at 96 h (LC_{50}) of untreated distillery spent wash in guppy (*Lebistes reticulatus*) and common carp (*Cyprinus carpio*) were reported as 53.2 and 27.3 ml/L (Kumar *et al*., 1995; Patil and Ghole, 2010), respectively, which were higher than that obtained in the present study. Ramakrishnan (1991) reported that with common carp, *C. carpio*, 96 h LC_{50} of distillery (sugar-mill) spent wash was 8.0 ml/L, which are considerably lower than the present study. This result indicated that differences in observed concentrations of distillery spent wash in previous studies compared to the present study were due to the differences in raw materials used for alcohol production, fermentation process, alcohol distillation, treatment process and species of experimental animal (Khangarot *et al*., 1985; Mohana *et al*., 2003; Pant and Adholeya, 2007; Patil and Ghole, 2010; Shukla and Shukla, 2012a,b). For the behavioral response of the fish exposed to distillery spent wash, fast, erratic and jerky movements were observed in the studies of Kumar *et al*. (1995) and Patil and Ghole (2010) but were not observed in the present study. Fish displayed less movement with

increase in exposure duration, and showed increased weakness, remaining motionless most of the time. Prior to death, fish swimming decreased and later fish settled to the bottom of the aquaria, with their opercula moving slower until they finally stopped. The gill coloration of fish became pale and the gills were clogged with sediment of distillery spent wash. This observation was similar to the finding of Ramakritinan *et al*. (2005) suggesting that the high concentration of suspended solids present in distillery effluent clogged up the gills and induced the excretion of mucus covering the gills and bodies leading to the inhibition of the oxygen transfer. The mortality occurring in the present study was obviously associated with interfering with respiratory system of tilapia. Moreover, Patil and Ghole (2010) reported that gills of fish were directly damaged by exposing to distillery effluent for 96 h, which increased with increasing the strength of the distillery effluent and indicated that damaged gills of fish exposed distillery effluent treated by anaerobic and oxidized process were significantly lesser as compared to fish exposed untreated distillery effluent. For water quality, the toxicity of distillery spent wash was synergistic to water quality as an environmental condition. Kumar and Gopal (2001) and Saroja *et al*. (2013) reported that fish (spotted snakehead fish and common carp) exposed to distillery spent wash for 96 h had high level of blood glucose and accumulation of lactic acid to deal with stressful situation relating to consequence of exposure to unfavorable condition (El-Sayed, 2006). Thus, distillery spent wash should be suitably treated or diluted before disposal to the ecosystem or utilization in aquaculture.

CONCLUSION

The results showed that LC_{50} of distillery spent wash to tilapia for 96 h of exposure was 17.965 ml/L. Besides, exposure to over 12 ml/L of distillery spent wash could have adverse impacts on fish health. This finding suggests distillery spent wash adversely affected Nile tilapia. Therefore, distillery spent wash should be properly treated before spilling into the environment. In case of aquaculture, the toxicity of distillery spent wash to fish should be taken into consideration prior to using it as source nutrients for phytoplankton production in earthen ponds. Level of toxicity of distillery spent wash could vary depending on the raw materials used and ethanol production and treatment processes.

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