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Novel Biological Recycling Water Purification System for Use in Fish Toxicology Studies

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Contamination of commercial catfish producing ponds by pesticides may have substantial impact on production and consumer health. The contamination may be due to the past use of the land, to water run-off of adjacent farmland, or from accidental direct contamination of these ponds by aerial application. Data shows that pesticides affect growth rates, feeding, reproduction, and hatching activities of fish (Murty 1943a). Toxicological testing of agrichemicals could prove beneficial to both the catfish farmer and the consumer. A major problem with fish toxicity testing has been maintaining an adequate supply of healthy, acclimated fish in the laboratory setting from which test populations can be obtained. The build-up of metabolic waste (ammonia) in holding tank environments leads to a stressful situation for the fish, resulting in mortality or erroneous toxicity data (Murty 1943b; Tucker 1985).

Ammonia is the principal nitrogenous waste product of catfish and is excreted primarily as the toxic unionized ammonia from the gills (Plumb 1979). Unionized ammonia may be lost to the environment by volatilization, or ammonia-nitrogen may be nitrified to non-toxic nitrate by aerobic bacteria with the toxic intermediate in the reaction being nitrite. The purpose of this study was to develop a novel biological filter system which facilitates the nitrification process, thereby removing or controlling the build-up of toxic metabolic waste products in holding tanks in the laboratory. This system would provide a healthy, non-stressful environment for blue channel catfish (*Ictalurus Punctatus*) fingerlings prior to their use in LC50 determinations of various insecticides, herbicides, and fungicides currently employed in the vicinity of catfish ponds or farms.

MATERIALS AND METHODS

The aquaculture system (see Figure 1) was composed of one 500-L glass holding tank (35.6x40.6x61 cm) and one 114-L plastic filter tank (71.1x50.8x35.6 cm). Dechlorinated water, obtained by

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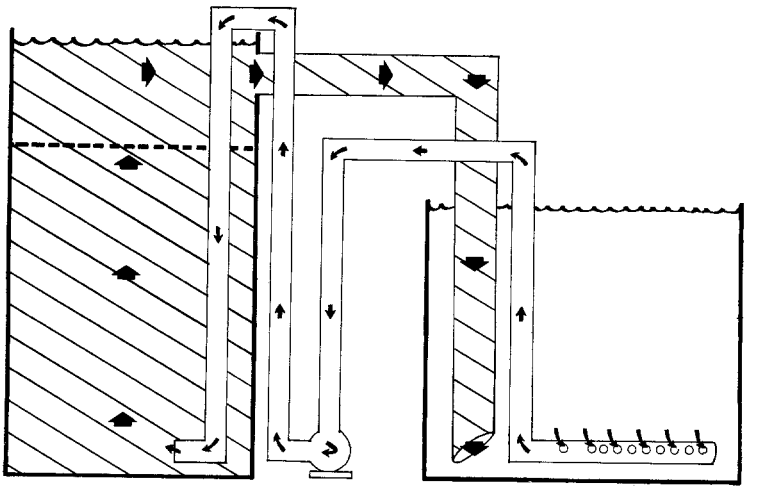

Figure 1 Schematic of Recycling Purification System

filtering tapwater through a bacteriostatic water treatment unit (National Safety Associates Inc., Memphis, Tennessee) was used at all times. The ph was 7.5 and hardness 85.5-mg/$CaCO_3$.

The water was pumped from the bottom of the holding tank by a 1/2 hp pump (Little Giant, TE-3-MD-HC) at a rate of 18.9 liters per minute to the bottom of the filter tank through 1.3-cm PVC piping. The water then rose through 0.08-m^3 of plastic matrix (Biodek®, Munters Corp., Fort Myers, Florida) contained in the plastic filter tank. This matrix provided the desired surface area for the growth of a number of bacterial species utilized to convert the ammonia and nitrite produced in the holding tank to the non-toxic nitrate. After the water had risen through the filter matrix, it then passed through a 5.1-cm PVC pipe down into the holding tank.

A commercial nitrification bacterial suspension (Aqua-Bacta-Aid®, Water Quality Science Inc., Bolivar, Missouri) was placed in the system on the inflow side of the filter at the concentrations of 10-mg/L initially and 3-mg/L on a daily basis thereafter. The suspension consisted of two major groups of bacteria: obligate aerobes and facultative aerobes. The obligate aerobes were composed of the following bacterial species: *Bacillus subtilis, Nitrosomonas sp., Nitrobacter winogradsky, Pseudomonas denitrificans, Pseudoomonas stutzeri,* and *Rhodopseudomonas palustris.* Within this group, the *Nitrosomonas sp.* were responsible for the oxidation-nitrogen to nitrate-nitrogen, and the *Pseudomonas sp.* in combintion with the *Bacillus subtilis* degraded solid organic waste. The remaining species of this group produced nutrients which were utilized by the other bacteria above.

Ammonia and nitrite concentrations as well as pH were measured

daily using Hack Chemical (Loveland, Colorado) colorimetric methods. These tests utilized the drop-count method of titration with a reported accuracy of ± 5%. Initial water hardness was also measured at 85.5mg/L CaCO3. Water loss due to evaporation was replaced on a weekly basis and no chemical buffer solutions were used.

Blue channel catfish (*Ictalurus punctatus*) fingerlings (2-7cm in length and supplied by the U.S. Wildlife and Fish Hatchery, Tupelo, Mississippi) were put into the aquaculture system at a loading of 6gm/L or a total of approximately 360 catfish fingerlings. The fingerlings were fed trout feed every 3 days but were fasted 24-h prior to toxicity testing. The room was on a 16:8-h light:dark illumination cycle and the temperature was maintained at approximately 21 C°.

Preliminary studies were performed utilizing DDT and alachlor (Lasso®, Monsanto Co., St. Louis, Missouri). Although these tests were preliminary in nature, it was thought that the data obtained would provide a measure for evaluating the health of the holding tank population. Two consecutive 96-h DDT LC50 determinations were conducted with five concentrations of DDT: 0.01, 0.02, 0.04, 0.08, and 0.16-mg/L. A vehicle control, reagent grade acetone (0.016-ml/L), and a water control were also included. Ten fish were used per concentration with five fish placed in each test tank (34.1-L). A total of 14 test tanks were used. Preparation of test tanks followed the Environmental Protection Agency static aquatic testing protocol (Environmental Protection Agency 1975). Alachlor testing was conducted in the same manner as DDT, but due to a limited test population, only one 96-h LC50 was performed for alachlor.

RESULTS AND DISCUSSION

Daily pH, nitrite and ammonia concentrations were monitored over a 25-day period (See Figure 2). Nitrite concentration fell from an initial 1.65-mg/L to 0.2-mg/L or less over the 25-day period. Ammonia levels never exceeded 0.65-mg/L with a mean concentration of 0.32-mg/L for the 25-day period. Downward shifts in pH were preceded by rising ammonia levels. It should be noted that fluctuations in pH, ammonia, and nitrite levels were preceded by a reduction in the holding tank population on days fish were removed for toxicity testing. Holding tank mortality for the 25 day period was 0%.

The system provided a stable, non-stressful environment to maintain fish prior to lethality testing. Evidence of this was reflected by 0% mortality, stable low ammonia and nitrite levels, and reproducible DDT 96-h LC50's. There was no evidence of nitrite, induced methemoglobin caused by high levels of nitrite which gives a characteristic brown color to the fish (Huey 1980) and no bacterial or fungal infections, often present in stressed populations (Plumb 1979).

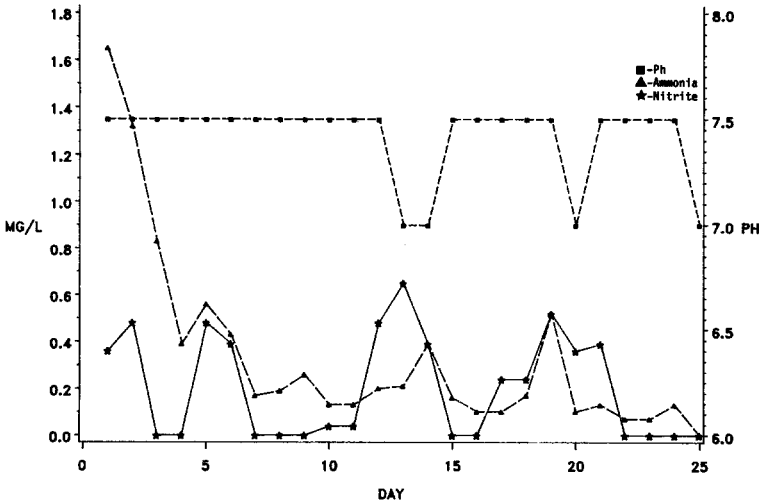

Figure 2. Tank nitrite and ammonia concentration and ph vs time.

The 96-h LC50 with the 95% confidence limits for the two DDT and one alachlor tests are seen in table 1 below.

Table 1. Fingerling catfish 96-h LC_{50} of DDT[1] and alachlor[2]

Test	LC_{50} mg/L[3]	95% Confidence Interval (mg/L)
DDT		
1	0.09	0.06 to 0.16
2	0.08	0.04 to 0.15
ALACHLOR		
1	1.76	1.63 to 1.92

[1]DDT concentration range (5 levels, 0.01 to 0.16-mg/L)

[2]Alachlor (Lasso®, pre-emergent, herbicide, Monsanto, Co., St. Louis, Missouri) concentration range (5 levels, 1.25 to 2.05-mg/L)

[3]LC_{50} Analysis (Finney 1971)

In addition to providing a stable environment, the recirculating system used required minimal man-hours to maintain and was relatively inexpensive to construct. In comparison with the flow-through systems which require an uninterrupted supply of dechlorinated water, or daily removal and replacement of the water, this system is much less labor intensive. Flow-through systems often require settings that are physically and/or econmically prohibitive. The system described is compatible with most laboratory settings.

In summary, the aquaculture system described here provided an economical method for maintaining an aquatic test population in a stable, non-stressed environment. A healthy, acclimated test population is vital for reproducible and accurate results in aquatic toxicity testing (Murty 1943a). Since sublethal levels of ammonia are known to affect the growth rates of catfish (Murty 1943b), further modifications of the biological filter system may suggest its use on a larger scale, such as in the commercial production of catfish. This system may produce increased yields and decreased mortality of catfish raised for commercial purposes. Finally, the aquaculture system detailed here may be used for the maintainence of other aquatic species and should prove to be an asset in the growing field of aquatic toxicity research.

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