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Degradation of Furazolidone in Fresh- and Seawater

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Abstract

The degradation of furazolidone (FZ) in fresh- and seawater in plastic tanks placed in a hatchery was studied. Reverse-phase high-performance liquid chromatography with ultra-violet spectrophotometric detection was used for the quantification of FZ residues. The half-life calculated from the intersection of the plot of ln (FZ concentration) vs. time (over 18 days) with the plot of ln (50% initial FZ concentration) was 170 h for freshwater (average pH 7.4, average temperature 26.7 °C) and 135 h for seawater (average pH 8.2, average temperature 26.7°C).

Introduction

Furazolidone (FZ), 3-(5-nitrofurfurylideneamino)-2-oxo-oxazolidine, is a nitrofuran compound (Fig. 1) which possesses a broad antimicrobial spectrum (Lambert and O'Grady 1992). It is a bright deep yellow crystalline powder which is light sensitive (Sugden *et al.* 1983; Samuelsen 1990) and not very soluble in water. Its solubility at pH 6 is approximately 40 mg $\cdot$ l^{-1}, and decreases with increase in alkalinity (Anonymous 1983). FZ is used for the treatment of diseases in poultry, cattle and swine (Ernst and Van Der Kaaden 1980; Sugden *et al.* 1983; Perkins *et al.* 1991) as well as in fish and shrimp (Heaton and Post 1968; Samuelsen *et al.* 1991; Anderson 1992; Baticados and Paclibare 1992; Tonguthai and Chanratchakool 1992).

In recent years, the use of nitrofurans has been banned in some countries because many of them are either mutagenic or carcinogenic (Samuelsen 1990

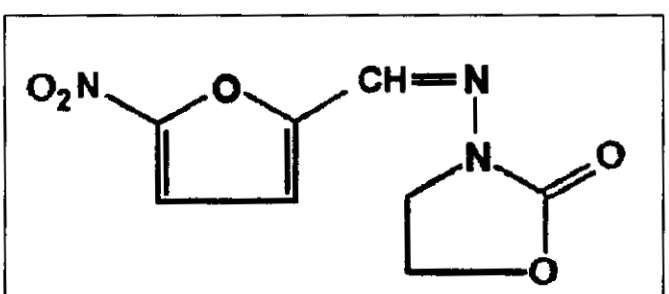

Fig. 1. Furazolidone

and Oka *et al.* 1995). The United States Food and Drug Administration, which has one of the most stringent regulations on the approval of drugs for public use, does not allow the registration of any nitrofuran for aquaculture and has been attempting to ban all uses of nitrofuran since 1976 (Schnick 1991). However, the use of nitrofurans is still allowed in some European countries, for example in Norway, where FZ is used as a therapeutic antibacterial agent (Samuelsen 1990), and in Japan, where it is used in fish breeding to stimulate growth and to prevent infection (Samuelsen 1990). Nitrofurans, in particular FZ, have been widely used in Malaysia for animal husbandry and aquaculture. However, since 1996, a government ban has been imposed on their usage. Despite these restrictions, nitrofurans are still widely used in many countries within the Association of Southeast Asian Nations.

FZ is highly degradable when present in sediments (Jacobsen and Berglind 1988; Samuelsen 1989; Bjorklund *et al.* 1990, 1991; Hektoen and Berge 1990; Lunestad 1991 and Samuelsen *et al.* 1991). Lunestad (1991) reported that FZ was readily degraded in natural seawater when illuminated (half-life of 10 days) but when kept in the dark was only slightly reduced. Still, information on FZ residues in the aquatic environment under tropical conditions is scarce. This paper describes the degradation of FZ in fresh- and seawater under tropical conditions, and may help provide information on its persistence and impact on the environment.

Materials and Methods

FZ was analyzed using a reverse-phase high-performance liquid chromatography technique. The mobile phase composition and the operating conditions used were similar to that described by Choo (1997).

Chemicals and reagents

Methanol and acetonitrile (HPLC grade; Lab-scan Analytical Sciences, Co. Dublin, Ireland). Oxalic acid dihydrate and sodium hydrochloride (Analytical grade; Merck, Darmstadt, Germany). The water used for the preparation of reagents was of ultrapure grade obtained from the Alpha-Q Water System (Millipore Corporation, Milford, U. S. A.) with resistivity of 18.2 megaohm-cm. Pure FZ was obtained from Sigma Chemical Co. (St. Louis, U. S. A.).

Apparatus

The HPLC system consisted of a Waters 600E pump (Millipore Corporation, U. S. A.) connected to a Waters 715 Ultra WISPS autosampler and injector, a Waters 486 UV detector and a Waters Maxima 825 chromatography software and workstation. The column used was a Novapak end-capped C_{18} steel column (3.9 x 150 mm with spherical packing material of 4 μm size) which was connected to a Guard-Pak™ μ Bondapak™ C_{18} HPLC pre-column insert (both items from Millipore Corporation, Waters).

A Corning pH meter 220, an Atago refractometer and a LCD display Ama-digit ad 16th thermometer were used to measure the pH, salinity and temperature of the water, respectively, while an exposure meter used for photography and set at ASA 100 was used to estimate light intensity in the hatchery.

Chromatography

The mobile phase was prepared from methanol, acetonitrile and 0.01M aqueous oxalic acid (1:1.5:7.5 respectively). The pH of the mobile phase was adjusted to 4.60 with 6N NaOH, and filtered through an FH 0.5 µm Millipore filter and degassed with a stream of helium (He) gas at 100 ml· min^{-1} for 15 minutes. A small stream of He (20 ml· min^{-1}) was passed through the mobile phase throughout the analyses. The detection of FZ was made at a wavelength of 360 nm, a temperature of 30^0C, a flow rate of 1.0 ml· min^{-1} and a detector sensitivity of 0.01 a.u.f.

Experiment

The experiment was carried out in 20L plastic tanks of 37.5 x 21.0 x 27.0 cm. The tanks were filled with 15L of fresh- or seawater and placed indoors in a hatchery at the Fisheries Research Institute, Penang, where natural lighting (approximately 12h light and 12h darkness) was maintained. The freshwater , sourced from the chlorinated municipal tap water supply, was pumped into a holding reservoir where it remained for 3-4 days to allow chlorine to dissipate, after which it was supplied for use in the hatchery through pipes. The seawater was sourced from the coastal waters off Glugor, where the Fisheries Research Institute was formerly located. It was pumped into a sedimentation tank where particulate matter was allowed to settle for a week or so before it was filtered through a high- pressure sand filter and piped to the hatchery. Ten tanks were used in the experiment altogether. One tank was used as the freshwater control and another as the seawater control. No FZ was added to the controls. Four tanks were filled with freshwater and another four with seawater. FZ dissolved in 1L of ultrapure water was poured into the experimental tanks so that the concentration in the tanks measured about 1mg·l^{-1}. At this concentration, FZ could easily dissolve in the fresh- and seawater. The tanks were topped up with fresh- or seawater to compensate for evaporation whenever the volume fell below the 15L mark. All the tanks were provided with gentle aeration. About 1 ml of water was taken from each tank daily for FZ analysis. The samples were filtered through a Millex-HV_{13} Millipore filter and 50 µl injected into the column.

The standard curve was prepared with Alpha-Q ultrapure water. The stock solution (40 mg· l^{-1}) was prepared by dissolving the FZ in methanol, and the working standards by diluting the stock solution with the mobile phase. The working standards were filtered through a Millex- HV_{13} Millipore filter. Calibration curves were prepared from standard solutions ranging from 0.02, 0.05, 0.1, 0.3, 0.5 and 1.0 mg· l^{-1}.

Method detection limit (MDL) and recovery of FZ were determined using four replicates of 0.02 mg l^{-1} of FZ solution. MDLs were calculated using the formula described by Hollis (1994).

$MDL = s^{*}t_{(n-1,\ 1\text{-alpha}=99)}$

where (n-1, 1-alpha) = student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of trials

s = standard deviation of trials

Results and Discussion

The average lighting condition in the hatchery, the average pH, temperature and salinity measurements of the fresh- and seawater in the experimental tanks are shown in Table 1. Measurements were taken at 0900h daily for 18 days.

The FZ standard curve has a high degree of linearity (average r=0.99971 ñ 0.00009). The average recovery of FZ was 98.2±3.6%, and the MDL was 0.008 mg l^{-1}. In both the fresh- and seawater controls, no peaks were recorded from the HPLC runs in the region where the FZ peak was detected. The retention time of FZ from the standard solutions was 3.38±0.02 minutes, and from both fresh- and seawater 3.38±0.01 minutes. The concentration of FZ in the tanks from the beginning to the end of the experiment is shown in Table 2. The first half-life of FZ in freshwater (intersection of the horizontal line drawn through 50% FZ concentration with the FZ degradation freshwater curve) was 166 h and, in seawater, 131 h (Fig. 2). The results of the first half-lifes were

Table 1. Lighting conditions in the hatchery, and average measurements of pH, temperature and salinity (with their standard deviations) of fresh- and seawater in the experimental tanks.

	Fresh Water	Sea Water
Lighting	2 - 2.8	2 - 2.8
pH	7.37±0.05	8.20±0.02
Water temperature (°C)	26.7±1.0	26.7±0.8
Salinity		28

Table 2. Average concentration (from four replicates) and the standard deviation of FZ. Ten tanks were used altogether: five freshwater and five seawater - one freshwater and one seawater tank used as negative control and the remaining tanks treated with 1 mg·l^{-1} FZ.

Days	Average FZ Concentration (mg·l^{-1} ± S.D)	Average FZ Concentration (mg·l^{-1} ± S.D)
	Freshwater	Seawater
0	1.32 ± 0.24	0.99 ± 0.09
3	0.91 ± 0.05	0.70 ± 0.12
6	0.68 ± 0.03	0.47 ± 0.10
9	0.60 ± 0.03	0.33 ± 0.06
12	0.48 ± 0.05	0.24 ± 0.05
15	0.40 ± 0.09	0.17 ± 0.03
18	0.32 ± 0.08	0.10 ± 0.01

very close to the half-lifes calculated from the intersection of the plot of ln (FZ concentration) vs. time (over 18 days) with the plot of ln (50% initial FZ concentration). For the study in freshwater, a linear plot ($y=-0.0757x + 0.1668$; $R^2=0.9838$) was obtained, giving a half-life of 170 h. In seawater, the equation of the linear plot was $y=-0.1245x - 0.0025$ ($R^2=0.9972$), which gave a half-life of 135 h.

Student's T-tests (Steel and Torrie 1980) were carried out to determine whether the % FZ degradation in fresh- and seawater at different time intervals were significantly different. The results indicated that the degradation rate of FZ in fresh- and seawater was not significantly different from day 0-12, but on the 15th and 18^{h} day, degradation was significantly ($P<0.05$) faster in seawater (Fig. 2). However, this study does not show whether the degradation

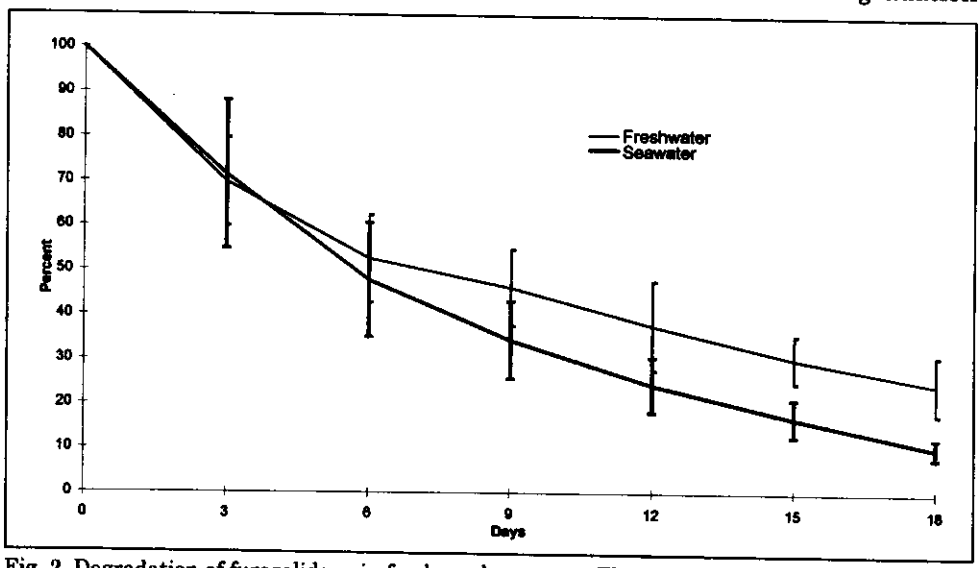

Fig. 2. Degradation of furazolidone in fresh- and seawater. The graphs show the mean FZ values from four samples, and the vertical lines indicate their standard deviations

observed is purely chemical or biological, or whether it includes clearance from other mechanisms like volatilization and adsorption.

Lunestad (1991) reported a half-life of 10 days for FZ in natural seawater (15°C) when illuminated. The difference in water temperature (26.7°C) could have accounted for the different half-life of FZ in seawater in this study.

Due to its short half-life in water as well as in sediments, FZ does not persist in the aquatic environment. However, nitrofurans, as a group of antibiotics, are generally considered unsafe for humans because of their carcinogenic capability (Plumb 1992). On this ground, the ban on its use in Malaysia is perhaps justified since there may be a likelihood for this drug to be misused, as guidelines/regulations for its application and the requirement of a withdrawal time do not yet exist in Malaysia.

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