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Farmed tilapia *Oreochromis mossambicus* involved in transport and biouptake of arsenic in aquacultural ecosystems

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Abstract

The present study couples the Michaelis–Menten (M–M) type flux and the Fick's type of dynamic mass transfer flux to arrive at the Best equation to quantitatively model the transport and biouptake mechanism of the gills of freshwater tilapia (*Oreochromis mossambicus*) exposed to waterborne arsenic (As). We conducted a 15-day uptake/depuration bioassay to examine the accumulation kinetics of As in tilapia gills by incorporating a bioconcentration model to obtain the steady-state and dynamic bioconcentration factors. A diffusion-based permeability can be calculated using the physiological and allometric-related parameters. The bioaffinity parameter and the limiting uptake flux in M–M equation are acquired by fitting the experimental values from published literature. The biouptake rate incorporating with bioavailability number is examined to better understand the effects of variabilities of field circumstances on biouptake flux. A linear relationship between As biouptake rate and As concentration in ambient water is obtained. The fitted bioaffinity parameter and limiting uptake flux were 3.07 mg l^{-1} and 2.17 mg l^{-1} day^{-1}, respectively, suggesting a low As binding affinity of tilapia gills yet a relative high binding capacity was obtained. The As permeability through tilapia gills membrane decreased from 1.42 μm day^{-1} to a steady-state value of 0.82 μm day^{-1} after 2 months, indicating the nonequilibrium aspects of biouptake processes involved.

Keywords: Tilapia; Arsenic; Biouptake; Bioavailability

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1. Introduction

Arsenic (As) is widespread in the environment as a consequence of both anthropogenic and natural processes (Liao et al., 2003). It is ubiquitous but potentially a toxic trace element. Inorganic, as well as organic forms of As, are present in the environment, and the former seems to be more toxic and slightly more accumulated in some freshwater aquatic species than the latter (Oremland and Stolz, 2003). Trivalent As may show an adverse effect on aquatic biota and is considered more toxic than the inorganic pentavalent form (Hall and Burton, 1982).

Chen et al. (2001) indicated that long-term exposure to ingested inorganic As in artesian well water has been found to induce blackfoot disease (BFD), a unique peripheral vascular disease. Recently, a number of studies on acquired and genetic susceptibility to As have been carried out in the BFD-endemic areas of southwestern Taiwan to find out the cause of BFD (Chen et al., 2001). Nowadays, most of the people living in these areas do not drink groundwater because tap water has been made available; however, groundwater is still used for aquaculture.

Farming tilapia (*Oreochromis mossambicus*) is a promising practice in the BFD area because of its high market value. These fish are maintained in ponds for at least 6 months (from April to October) before harvest. At present, however, data on the biouptake mechanisms of As in tilapia are limited. The accumulation of metals in aquatic organisms has been linked to decreased survival and reduced reproductive ability (Pelgrom et al., 1995; Wong and Wong, 2000; Liao et al., 2003). If As levels in pond water are high, it may have severe health effects on the cultured fish, reducing their market prices and leading to closure of fish farms.

The bioconcentration factor (BCF), which relates the concentration of metals in water to their concentration in an aquatic animal at equilibrium, is generally used to estimate the propensity to accumulate metals in the organism (Hemond and Fechner-Levy, 2000). Fish are targets for BCF assessments because of their importance as a human food source and the availability of standardized testing protocols. In our previous studies, the 24- to 96-h LC_{50} of As to tilapia ranged from 69.06 to 28.68 mg l^{-1}, indicating a low risk of toxicity to tilapia in the aquacultural ecosystem (Liao et al., 2003; Liao and Ling, 2003). The As concentrations in various tissues of tilapia in BFD area, however, are relatively higher than the background levels. A probabilistic risk assessment further indicated that the consumption of cultured tilapia from the BFD area possibly poses a potential risk to human health (Liao and Ling, 2003). Thus, the ability to predict and quantify the uptake process, as well as elimination process, is important for improving the assessment of risk to fish and humans caused by As in aquatic ecosystems.

The term biouptake describes the processes by which the substance is actually taken into the body of the fish. The amount that is actually accumulated will depend on the balance between uptake rate, metabolism of the chemical and excretion rate (Heath, 1995). Sijm and van der Linde (1995) further indicated that biouptake kinetics gives information on how fast chemicals are taken up. It is generally recognized that there are three possible routes for a substance to enter a fish: gills, food and skin. Owing to direct contact with ambient water, gills are proposed to be the first and most important targets of waterborne metals (Playle, 1998; Wong and Wong, 2000; Tao et al., 2000). Several studies also

claimed that the major route of biouptake for metals that concentrate in fish is across the gill epithelium (Pelgrom et al., 1997; Bury et al., 1999). Nichols et al. (1996) simulated the biouptake flux of three organic chemicals by rainbow trout and channel catfish and proposed that the uptake flux across fish skin contributes from 1.7% to 8.3% of total flux. They concluded that gill uptake is expected to dominate total biouptake from water. Szebedinszky et al. (2001) demonstrated that initial uptake of cadmium from water into the gills is followed by subsequent transfer to the blood for distribution throughout the body. Hence, to clarify the movement of a metal into an organism, the mechanism of metal uptake through the gills is fundamental in aquatic toxicology.

Generally, when referring to the mechanism of metal uptake, two common approaches are used, i.e., free ion activity model (FIAM) and biotic ligand model (BLM). Jansen et al. (2002), however, indicated that these two models completely neglect the nonequilibrium aspects of the processes involved. They further revealed that equilibrium distributions are not generally achieved and more common is some steady state in which fluxes are constant but not zero. A more physically based model should be adopted to investigate the biouptake mechanisms. Bryan (1979) firstly assumed the metal uptake by fish gills to be a simple diffusion. Various authors have suggested thereafter that the waterborne metals are normally taken up via the fish gills by passive diffusion and can be described by Fick's first law (McKim et al., 1985; Barber et al., 1988; Erickson and McKim, 1990; Randall et al., 1991; Sijm et al., 1993; Del Vento and Dachs, 2002). Recently, many research efforts in biouptake flux have been exercised on a combination of both the diffusional mass transfer flux and the actual biological uptake flux (Bosma et al., 1997; Harms and Bosma, 1997; Van Leeuwen, 1999; Jansen et al., 2002). The advantage of this approach is that, once the physical–chemical and biological parameters are known, the uptake dynamics at the fish gills can be determined.

The purpose of this study is to determine the dynamic behavior of As biouptake in tilapia. An uptake/depuration bioassay has been conducted to examine the accumulation kinetics of As in tilapia. We developed the Best equation, which couples a Michaelis–Menten (M–M) type and a Fick's type of mass transfer flux of As to farmed tilapia. The major physicochemical and biological parameters controlling absorption of As from the ambient water at the fish gills have been carefully adopted from published literature. In addition, the biouptake rate in company with bioavailability number will be examined to better accommodate the effects of variabilities in field circumstances on biouptake flux. This would be helpful in generating ambient water quality criteria.

2. Materials and methods

2.1. Experimental section

The present laboratory study was designed to examine the accumulation ability of As in the gills of tilapia. The As contamination level was determined by a preliminary test exposing tilapia to different As concentrations of 0.25, 0.5, 1, 2, 4 and 6 mg l^{-1}. Six fish (mean body length=17.67±1.65 cm (mean±S.D.) and mean weight=148.72±6.5 g wet wt.) were used for each designed concentration. The median lethal tolerance (LT_{50}) of

tilapia at ≤1 mg l^{-1} As was longer than 21 days. Therefore, we conducted an uptake experiment in an As concentration of 1 mg l^{-1} for 7 days. The elimination of As from tilapia tissue was then tested over 8 days, following transfer of the fish to As-free reconstituted dilution water. The As concentrations used in this experiment were 20–50 times higher than that in the environment, which was necessary to produce high levels in the gills of tilapia.

The experiments were carried out with 60 fish (mean body length=17.9±1.54 cm (mean±S.D.) and mean weight=154.75±7.2 g wet wt.). They were supplied by Taiwan Fisheries Research Institute, Lukang, Chunghwa, and analyzed to be uncontaminated by As. The fish were visibly free of any deformities, lesions or disease. Fish were allowed to acclimate to laboratory condition for 2 weeks before exposure. During the acclimation period as well as during the experimental period, the fish were fed once a day, 7 days a week at a rate of 0.5% of fish biomass. This low level was chosen to avoid As contamination of feed remaining in the aquaria.

All experiments were carried out in 54-l indoor rectangular fiberglass aquaria full with 50 l of As concentration of 1 mg l^{-1}. The sodium arsenite ($NaAsO_2$) stock solution was prepared with deionized water. Dissolved oxygen in each tank was maintained at close to saturation by aeration (7.21±0.1 mg l^{-1}). The temperature in each aquarium was maintained at 24.7±0.24 °C, using submerged heaters. The pH values were maintained at 7.75±0.02. The photoperiod was 16-h light: 8-h dark with an intensity of 1400±100 lux. All the experiments were assigned to two replicate tanks. To maintain the ideal experimental condition, we removed the feces every 3 h and collected forage debris 1 h after feeding. The whole As solution was replaced daily in each tank to avoid the regression of ambient water quality and keep the constant As concentration level. We checked the water level in each aquarium every 6 h, if the water level dropped drastically, we supplied with distilled water to keep the constant level.

We detected As concentrations in each test media, exposure of water characteristics during the test were measured three times weekly in one selected replicated aquarium for analysis of As. The 10-ml water samples were acidified (pH<1) with 5 ml 1 N HNO_3 and then stored at −4 °C in the dark until they were analyzed. No fish died during the acclimated period. No mortality occurred during the As exposures, and no weight losses were observed. To conduct analysis of As uptake by the fish, three fish were sequentially removed from solutions after 0, 1, 2, 4 and 7 days of exposure and after 0, 5 and 8 days of depuration. Removed fish were individually wrapped in a plastic bag. Dissections were performed on a clean bench on thawed material, using a titanium knife and Teflon forceps. To obtain a homogeneous sample, the dissected gill samples were cleaned with deionized water, minced and blended, freeze-dried overnight and then ground to fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500-mg portion of the powder was digested in 10 ml concentrated HNO_3 (65% wt.) overnight at room temperature. The resulting solution was evaporated and the residue redissolved in 0.1 N HCl.

A Perkin-Elmer Model 5100PC atomic absorption spectrometer (Perkin-Elmer, Shelton, CT, USA) equipped with a HGA-300 graphite furnace atomizer was used to analyze As of fish gills. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference material (Dog fish muscle, DORM-2, NRC-CNRC, Canada). Recovery rate was 94.6±3.6%, and the

levels of detection were 0.62 μg As l^{-1} for water samples and 0.05 μg As g^{-1} for tissue samples.

2.2. Bioconcentration model

The rate of change of the As concentration in gills of tilapia is assumed to follow a first-order one-compartment model (Spacie and Hamelink, 1982)

$$\frac{dC_f(t)}{dt} = k_u C_w(t) - k_d C_f(t) \quad (1)$$

where $C_f(t)$ is the As concentration in tilapia gills (μg g^{-1}), $C_w(t)$ is the dissolved As concentration in water (μg ml^{-1}), k_u is the gill uptake constant of As in tilapia (ml g^{-1} day^{-1}), and k_d the gill depuration constant of As in tilapia (day^{-1}). Depuration constant can be determined by fitting concentration $C_f(t)$ to a first-order decay curve $\ln C_f(t)=c+k_d t$, where c is a constant. Depuration half-life ($t_{1/2}$) was calculated as $\ln 2/k_d$. Uptake constant in tilapia gills was determined by fitting concentration data to the integrated form of the kinetic equation for constant water exposure, using iterative nonlinear regression

$$C_f(t) = \frac{k_u}{k_d} C_w\left(1 - e^{-k_d t}\right) \quad (2)$$

The steady-state bioconcentration factor BCF_M can be calculated by definition as the ratio of (k_u/k_d).

2.3. Metal biouptake modeling

For the biouptake of metals from aquatic environments, both chemical and biological internalization characteristics are of importance. We consider both the diffusional mass transfer from the bulk medium toward the actual biological uptake sites and the actual biological uptake flux. Uptake, transport, accumulation, rate of metabolism, excretion and effects of metals in living organisms are involved in the relationship between metal exposure level, tissue-specific bioaccumulation, growth and mortality of organisms in aquatic ecosystems. Between metal uptake and eventual effects, there are several steps, such as the distribution over different tissues, and this requires a kinetic biocompartmental modeling approach. We consider that (i) the actual biouptake follows a Michaelis–Menten type of steady-state flux, and (ii) the supply of free metal follows a Fick's type of mass transport of free metal to organisms.

The steady-state biouptake flux couples interfacial Michaelis–Menten kinetics with a mass transfer toward the biosurface. For a bioactive metal ion M and a planar biosurface, the two steps can be represented by (i) the Michaelis–Menten equation for the uptake flux J_u (Morel and Hering, 1993),

$$J_u = J_{u,max}\left(\frac{C_w}{K_M + C_w}\right) \quad (3)$$

where $J_{u,max}$ is the limiting uptake flux (μg ml^{-1} day^{-1}), C_w is the bulk water concentration of M (μg ml^{-1}), and K_M is the bioaffinity constant of M (μg ml^{-1}); and (ii)

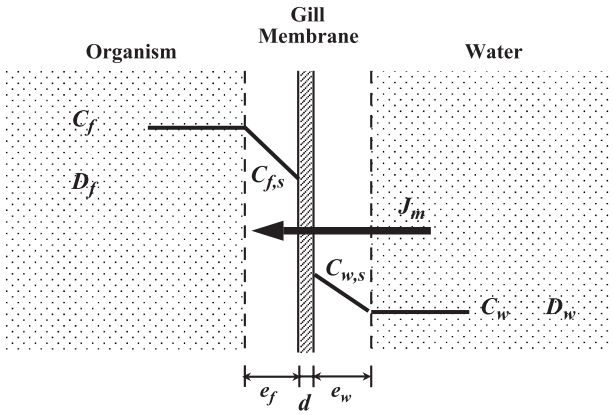

Fig. 1. Schematic representation for the transport flux of As through the gill membrane. The meanings of the symbols are given in the text.

the convective diffusion equation for the transport flux of M, J_m in the medium and in the organism matrix can be described by Fick's first law, as shown in Fig. 1

$$J_m = -\frac{D_w}{e_w}(C_w - C_{w,s}) = \frac{D_f}{e_f}\rho_f(C_f - C_{f,s}) \tag{4}$$

where D_w is the diffusion coefficient of M in water (m^2 day^{-1}), $C_{w,s}$ is the concentration of M in water-side biosurface (μg ml^{-1}), and e_w is the steady-state diffusion layer thickness in water-side (m), D_f is the diffusion coefficient of M in organism (m^2 day^{-1}), C_f and $C_{f,s}$ are the concentration of M in organism and at organism side biosurface (μg g^{-1}), e_f is the steady-state diffusion layer thickness in organism-side (m), and ρ_f is the organism biomass (g ml^{-1}). Inasmuch as the diffusion flux through the water side and organism side must be equal, from Eq. (4) we obtain

$$J_m = k\left(\frac{C_f}{BCF_M} - C_w\right) = k(C_f^* - C_w) \tag{5}$$

where k is the mass transfer rate (day^{-1}), BCF_M is the steady-state bioconcentration factor of organism (ml g^{-1})

$$BCF_M = \frac{C_{f,s}}{C_{w,s}} \tag{6}$$

and C_f^* is the normalized metal concentration in organism (μg ml^{-1}) and can be calculated as

$$C_f^* = \frac{C_f}{BCF_M} \tag{7}$$

For the steady-state condition, we develop an overall uptake flux, J, by combining the action of mass transfer and biological transport expressed in Eqs. (3) and (5) as

$$J = J_{u,max}\frac{C_f^* + K_M + J_{u,max}k^{-1}}{2J_{u,max}k^{-1}}\left\{1 - \left[1 - \frac{4C_f^* J_{u,max}k^{-1}}{\left(C_f^* + K_M + J_{u,max}k^{-1}\right)^2}\right]^{1/2}\right\} \quad (8)$$

This equation is known as the Best equation. The overall flux is finally dependent on the four physical–chemical properties, i.e., mass transfer rate (k), normalized metal concentration in organism (C_f^*), limiting uptake flux ($J_{u,max}$) and bioaffinity constant of the metal (K_M).

The Best equation can be rewritten as (Bosma et al., 1997),

$$J^* = \frac{1 + B_n^{-1}}{2(1 - C^*)}\left\{1 - \left[1 - 4C^*\frac{1 - C^*}{1 + B_n^{-1}}\right]^{1/2}\right\}, \quad (9)$$

with

$$J^* = J/J_{u,max}$$

$$C^* = C_f^*/\left(C_f^* + K_M + J_{u,max}k^{-1}\right)$$

$$Bn = k/\left(J_{u,max}K_M^{-1}\right) \quad (10)$$

where J^* denotes the biouptake rate, C^* the bioavailability, and B_n the bioavailability number.

2.4. Mass transfer rate estimation

To estimate the mass transfer rate (k), we consider the rate of metal concentration change in the bulk water medium and the organism (Fig. 1). Applying mass balance both in water side and organism side, we obtain

$$\frac{dC_f^*(t)}{dt} = -\frac{P(t)}{e_f}[C_w(t) - C_f^*(t)] \quad (11)$$

$$\frac{dC_w(t)}{dt} = \frac{P(t)}{e_w}[C_w(t) - C_f^*(t)] \quad (12)$$

with initial conditions

$$C_f^*(t = 0) = C_{f,0}^*$$

$$C_w(t = 0) = C_{w,0}$$

where $P(t)$ (m day^{-1}) is the permeability of metal M, and t is the time to permeate in day. We obtain an implicit solution of $P(t)$

$$P(t) = \frac{e_w e_f}{(e_w + e_f)t} \ln\left[\frac{(C_{w,0} - C^*_{f,0})(e_w + e_f R(t))}{(C_{w,0} e_w + C^*_{f,0} e_f)(1 - R(t))}\right] \tag{13}$$

where $R(t) \equiv C^*_f(t)/C_w(t)$ is the normalized dynamic bioconcentration factor. If the initial metal concentration in organism can be neglected (i.e., $C^*_{f,0} = 0$), Eq. (13) can be rewritten as

$$P(t) = \frac{e_w e_f}{(e_w + e_f)t} \ln\left[\frac{(e_w + e_f R(t))}{(e_w - e_w R(t))}\right] \tag{14}$$

The mass transfer rate $k(t)$ can thus be obtained from dividing $P(t)$ by the diffusion path length, $(e_w + e_f)$, as

$$k(t) = \frac{e_w e_f}{(e_w + e_f)^2 t} \ln\left[\frac{(e_w + e_f R(t))}{(e_w - e_w R(t))}\right] \tag{15}$$

The diffusion path length in water side (e_w) depends on the distance between two gills lamellae. Sijm and van der Linde (1995) proposed that the distance between gills lamellae of a fish (d, m) is given by

$$d = 20.5 \times 10^{-6} (1000W)^{0.114} \tag{16}$$

where W is fish weight (kg). Del Vento and Dachs (2002) suggested that the water side diffusion path length equals to half the distance between gills lamellae,

$$e_w = 10.25 \times 10^{-6} (1000W)^{0.114} \tag{17}$$

The diffusion path length in organism side (e_f) can then be approximately estimated from Sijm and van der Linde (1995)

$$e_f = 0.3 \times e_w \times \mathrm{BCF_M} \tag{18}$$

It can be seen obviously from the above derivation that the mass transfer rate $k(t)$ depends strongly on fish size and the accumulation ability of the fish. Hence, we use the 15-day uptake/depuration bioassay to examine both the steady-state BCF, i.e., $\mathrm{BCF_M}$ and the normalized dynamic BCF, i.e., $R(t)$ of juvenile *O. mossambica* exposed to 1 mg l^{-1} As.

2.5. Statistical analyses

We employed the nonlinear option of the Statistica® software (StatSoft, Tulsa, OK, USA) to perform all curve fittings. The Statistica® was also used to calculate the coefficient of determination (r^2) and statistical analyses (analysis of variance and student's t-test).

3. Results

3.1. Biokinetic parameters

The nonlinear regression resulting from the best fit of Eq. (2) to uptake and depuration concentration data is shown in Fig. 2. The regression equation to exposure concentration data is $C_f(t)=3.21(1\text{-}e^{-0.081t})$ ($r^2=0.96$ and 0.99 for uptake and depuration, respectively), indicating that the gill concentration profile was best fitted by the one-compartment model. The biokinetic parameters of the depuration constant and the uptake constant were estimated to be 0.08 ± 0.01 day^{-1} and 0.26 ± 0.09 ml g^{-1} day^{-1} (mean$\pm$1 S.E.), respectively, whereas BCF_M was calculated to be 3.21 ml g^{-1}, showing that gills have the potential to accumulate As when tilapia are exposed to a threshold waterborne As concentration. Depuration half-life ($t_{1/2}$) was calculated to be 8.56 day, indicating that it will take a long time to eliminate As from the gills.

3.2. Mass transfer rate

Table 1 lists the physiological and bioconcentration parameters used to estimate the mass transfer rate of the 15-day As exposure experiment for tilapia. Incorporating these

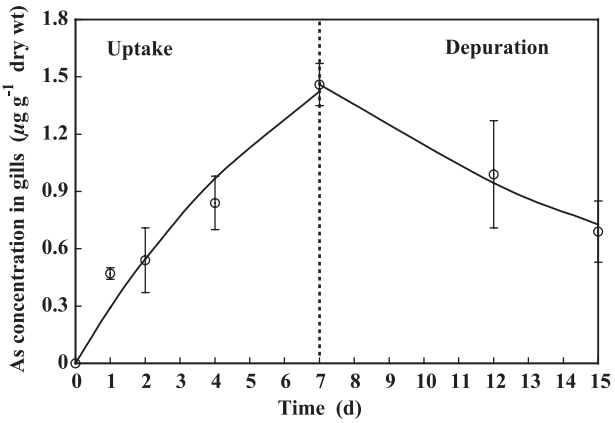

Fig. 2. Uptake and depuration of As by tilapia during a 15-day exposure experiment. Measurements are shown with open circles (○), and fitted model is shown in solid line. Error bars represent one standard deviation from the mean.

Table 1
Physiological and bioconcentration parameters for tilapia *O. mossambica* during the 15-day exposure experiment

Parameters	Value
Tilapia weight, W (kg)[a]	0.155
Gills weight, W_g (kg)[b]	0.006
Gills surface area, A (m^2)[c]	0.027
Diffusion path length in water, e_w (m)[d]	1.82×10^{-5}
Diffusion path length in gills, e_f (m)[c]	1.75×10^{-5}
Diffusion coefficient in water, D_w (m^2/s)[c]	5.04×10^{-11}
Diffusion coefficient in gills, D_f (m^2/s)[c]	1.51×10^{-11}
Gills density, ρ_f (kg/m^3)[e]	1025
Steady-state bioconcentration factor, BCF_M[a]	3.21

[a] Observation data obtained from 15-day exposure experiment.
[b] Calculated from Suhendrayatna et al. (2001b).
[c] Calculated from Sijm and van der Linde (1995).
[d] Calculated from Eq. (17).
[e] Data adopted from Del Vento and Dachs (2002).

parameters, as well as the biokinetic parameters into Eq. (15), yielded the time-varying permeability and mass transfer rate (Fig. 3). The As permeability through tilapia gill membrane decreased from 1.42 μm day^{-1} to a steady-state value of 0.82 μm day^{-1}, whereas the mass transfer rate decreased from 0.039 to 0.024 day^{-1} after 2 months, indicating the nonequilibrium aspects of the biouptake processes involved. The bioavailability numbers, as well as biouptake rates, were simulated according to Eq. (10) (Fig. 4A and B). The biouptake rates increased fast in the first 20 days, reached its maximum value of 0.010 at day 38 and slightly declined thereafter. The bioavailability

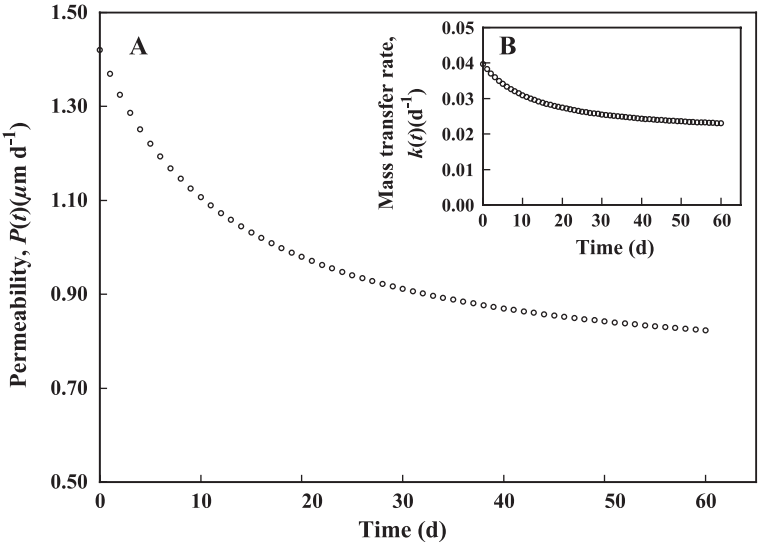

Fig. 3. A 60-day simulation carried out for (A) time-dependent permeability and (B) mass transfer rate.

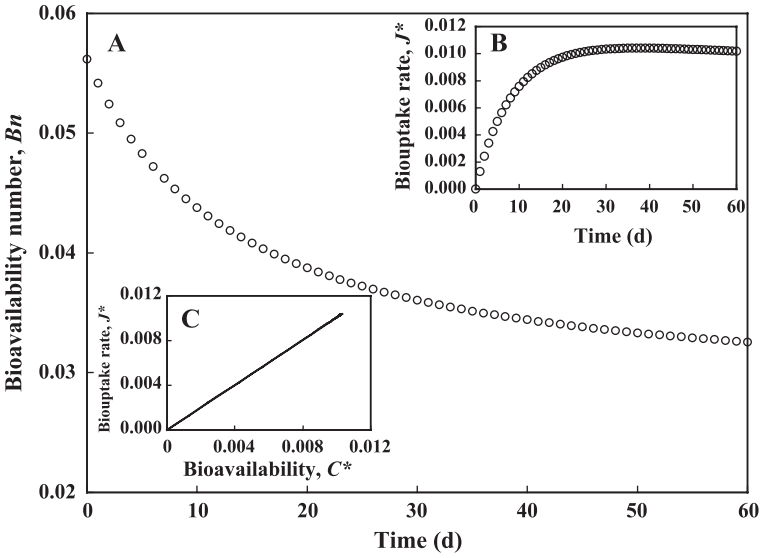

Fig. 4. A 60-day simulation carried out for (A) time-dependent bioavailability number, B_n; (B) biouptake rate, J^*; and (C) the dimensionless biouptake rate J^* versus the dimensionless bioavailability C^*.

numbers ranged from 0.057 to 0.033, suggesting a low ratio of the mass transfer rate to the bioaffinity. With the low bioavailability numbers, a linear relationship between As biouptake rate and As concentration in ambient water is obtained as $J^*=1.0095\ C^*$ ($r^2=0.99$; Fig. 4C).

4. Discussion

4.1. Bioaccumulation of waterborne As in gills of tilapia

In the present study, exposure of tilapia to 1 mg l^{-1} As for 7 days resulted in a BCF_M value of 3.21 for tilapia gills, which was in good agreement with that of other study (i.e., a BCF_M value of 3.20) under an identical experimental setup (Suhendrayatna et al., 2001a). The BCF represents the capacity of a species to accumulate a compound to a greater extent that is greater than the background level. Metal bioaccumulation in fish tissues is dependent upon exposure dose and time, as well as other biological and environmental factors. McGeer et al. (2003) summed up a body of metal bioaccumulation data for aquatic biota, indicating that an inverse relationship exists between BCF and exposure concentration. In addition, several studies demonstrated that metal acclimation mechanisms (i.e., physiological homeostasis) resulting from a long sublethal metal exposure period of fish were observed, which allow the fish to counteract the toxic effects of metal in an integrative way (Pelgrom et al., 1995, 1997; Galvez et al., 1998; Chang et al., 1998). Hence, the relatively higher value of BCF obtained from field surveys may result from long-term exposure of tilapia to a much lower background As concentration (17.8–49.0 μg

l^{-1}). On the contrary, the higher waterborne As concentration used in the exposure experiment may be toxic to tilapia, thus affecting accumulation of As by the tested fish (Suhendrayatna et al., 2002). Another possible explanation for the low bioaccumulation of As in tilapia gills under laboratory conditions may depend on the chemical composition of the water. With the pH of the bulk solution around 7.75, the pH of the fish gill microenvironment is slightly lower (Tao et al., 2000). The microlayer chemistry was, therefore, possibly favorable for metal desorption from those particles that adhere to the mucus layer of the gills (Tao et al., 2000).

Donohue and Abernathy (1999) reported that the total As in marine fish, shellfish and freshwater fish tissues ranged from 0.19–65, 0.2–125.9 and 0.007–1.46 μg g^{-1} dry wt., respectively. It is generally recognized, however, that the total body/tissue concentration of metal in aquatic biota varied little over a wide range of exposure concentrations and exposure conditions within species (McGeer et al., 2003). Chen et al. (2001) further indicated that tilapia could potentially be able to regulate the concentrations of metals in their tissues over time by combining the processes of absorption, excretion, detoxification and storage. Our results show that the total As accumulated in tilapia gills (3.21 μg g^{-1}) was close to the range of a previous field survey (Liao et al., 2003; 4.28–5.79 μg g^{-1}) and to that reported by Suhendrayatna et al. (2001b) (4.8±0.3μg g^{-1}) under different exposure concentrations and different exposure periods. This result implies that the overall capacity of tilapia gills to bind As is approximately constant; however, further studies are necessary to confirm this inference.

4.2. Biouptake parameters

Suhendrayatna et al. (2001a, 2002) conducted a series of experiments to examine organ accumulation of As by *O. mossambica* exposed to different levels of sodium arsenite for 7 days. Eq. (3) was used to fit the uptake flux of As by tilapia versus waterborne arsenite concentration (Fig. 5), resulting in the estimated limiting uptake flux $J_{u,max}$=2.17±0.38 mg l^{-1} day^{-1} and a bioaffinity constant of K_M=3.07±2.21 mg l^{-1}, with a regression coefficient (r^2) of 0.96. The high value of regression coefficient reveals that the M–M equation yields a good fit to the experimental data, indicating saturation kinetics of As uptake in tilapia gills. The limiting uptake flux and bioaffinity constant differ widely among metals and among fish species. To the best of our knowledge, however, information on biouptake parameters of As in tilapia gills is limited. We therefore compare the present results with metal biouptake data of some species of teleost fish from other studies (Table 2). The ratio of $J_{u,max}$ to K_M, the specific affinity introduced by Harms and Bosma (1997), is also given in Table 2 to depict how much the gill membrane reduces the metal concentrations at its surface.

Michaelis and Menten first used the M–M equation to describe the kinetics of enzymatic reactions in 1913 (Brown and Markich, 2000), and Morel and Hering (1993) introduced this equation to describe a two-step process of trace metal uptake by microorganisms. In the present study, we applied the M–M equation to involve the movement of As across the apical membrane into the tilapia gills and intracellular trafficking of As to the basolateral membrane. The shape of the curve obtained from M–M kinetics is hyperbolic, suggesting an initial carrier-mediated uptake process at the gill

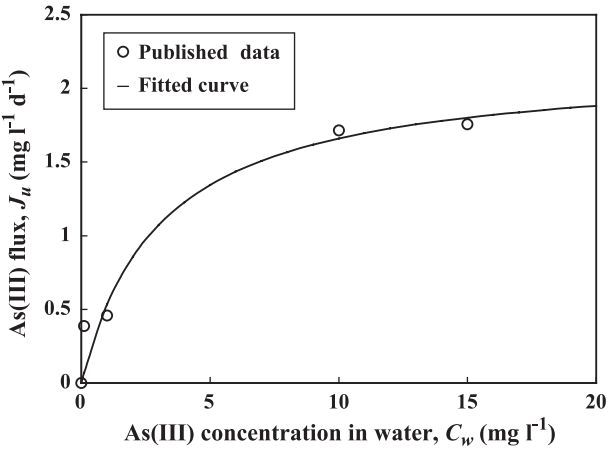

Fig. 5. Curve of published concentration-flux data (Suhendrayatna et al., 2001a, 2002) by Michaelis–Menten equation.

surface, followed by the rate-limiting extrusive step across the basolateral membrane (Bury et al., 1999).

The M–M model allows a mechanistic description of the transport process in fish gills to be characterized by two biouptake parameters, i.e., K_M and $J_{u,max}$. It can be seen in Table 2 that the gills exhibit relative differences in both the binding affinity and the binding capacity for various metals, similar to that proposed by Reid and McDonald (1991). The K_M value represents the inverse of binding affinity of the transport metal. For As binding to tilapia gills, the K_M value was the highest among various metals and different species of teleost fish, except for that of Ag binding to the gills of rainbow trout (Table 2). The binding affinity of a metal for biological ligands is a function of ligand chemistry and the type of bond formation, yet the characteristics of actual binding sites on fish gills are not entirely known (Reid and McDonald, 1991). With a low binding affinity,

Table 2
Biouptake parameters of various metals in some species of teleost fish

Species	Metal	K_M (mg l^{-1})	$J_{u,max}$ (mg l^{-1} day^{-1})	$J_{u,max}/K_M$ (day^{-1})	References
Tilapia (*O. mossambicus*)	Ca	0.001	2.11	2110	Chang et al. (1997)
		0.056	–	–	Flik et al. (1993)
	Cd	0.015	0.22	14.47	Wong and Wong (2000)
Rainbow trout (*O. mykiss*)	Zn	0.278	0.38	1.36	Hogstrand et al. (1998)
		1.17	0.52	0.44	Galvez et al. (1998)
	Ca	1.35	41.76	30.98	Hogstrand et al. (1998)
	Cu	0.76	0.08	0.11	Campbell et al. (1999)
	Ag	6.76	–	–	Bury et al. (1999)
Common carp (*C. carpio*)	Zn	0.22	3.57	16.23	Van Ginneken et al. (1999)
	Cd	0.038	1.01	26.58	Van Ginneken et al. (1999)
Tilapia (*O. mossambicus*)	As	3.07	2.17	0.71	This study

however, we could expect a less permissive As entry into the branchial membrane of tilapia.

The specific affinity (i.e., $J_{u,max}/K_M$) to some extent stands for the rate of gill–metal binding. The specific affinities in Table 2 show that the class A metals, an oxygen-seeking metal group (i.e., Ca), exhibits a strong tendency to bind to the gills of teleost, whereas the class B metals, a sulphur- or nitrogen-seeking metal group (i.e., Cd, Cu and Ag), have relative low gill–metal binding affinities. The specific affinities of borderline metals (i.e., Zn and As) are closer to that of class B metals. These results imply that the dominant metal receptors at the gill surface of teleost consist of oxygen-rich centers, and Zn and As may share similar binding sites with the class B metals.

4.3. Permeability, bioavailability and biouptake rate

Del Vento and Dachs (2002) derived a model to describe the biouptake dynamics of persistent organic pollutants (POPs) by phytoplankton based on a stagnant two-film theory. This model originated in Fick's first law, combined the bioconcentration factor in the organism matrix with the chemical flux through both water and organism sides, to obtain a predictive equation for the biouptake flux of a POP between phytoplankton and water. Inasmuch as this model is developed by physical perspective instead of any biological and/or chemical characteristics, it can equally be applied to characterize the biouptake dynamics of metal by fish. According to the mathematical expression derived by Del Vento and Dachs (2002), the permeability of As through tilapia gills membrane can be estimated to be 2.84 μm day^{-1} with the parameters given in Table 1. The permeability calculated here has the same order of magnitude with that obtained from the present study, which can in some extent validate the mathematical formulation developed by the present work.

The bioavailability number (B_n) represents a comparison between mass transfer and biouptake (Bosma et al., 1997). In the present study, the B_n were all less than unity, indicating that the uptake processes were controlled by diffusion under our experimental concentration (i.e., 1 mg As l^{-1}). Furthermore, the B_n reduced faster within the first 20 days, suggesting a high value of bioaffinity constant and a low specific affinity, which are in line with the results given in the above section. The low dimensionless concentrations (C^*) throughout the simulated period represented a low bioavailability and resulted in the low biouptake rate. A higher bulk concentration of As (i.e., greater than unity) is needed to obtain higher biouptake rate and bioavailability.

In conclusion, the obvious objective of the present study is to develop a dynamic expression of biouptake processes instead of the traditionally steady-state condition. Our results revealed that, for a less bioaccumulated and bioavailable circumstance, such as As biouptake by tilapia, the equilibrium distributions are not generally achieved and the time-varying biouptake processes involved. The proposed dynamic model successfully meets the need of clarifying the nonequilibrium aspects of biouptake mechanisms. We recommend that future research focus both on other potential sources of As bioaccumulation for tilapia (e.g., absorption of As to the body surface and uptake by the gut) and on a more thorough evaluation of the species (e.g., arsenite or arsenate) and phases (e.g., particulate or dissolved) of As in tilapia farming ponds.

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