Application of a fluidized bed reactor charged with aragonite for control of alkalinity, pH and carbon dioxide in marine recirculating aquaculture systems

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Abstract

Control of alkalinity, dissolved carbon dioxide (dCO₂), and pH are critical in marine recirculating aquaculture systems (RAS) in order to maintain health and maximize growth. A small-scale prototype aragonite sand filled fluidized bed reactor was tested under varying conditions of alkalinity and dCO₂ to develop and model the response of dCO₂ across the reactor. A large-scale reactor was then incorporated into an operating

marine recirculating aquaculture system to observe the reactor as the system moved toward equilibrium. The relationship between alkalinity dCO_2 , and pH across the reactor are described by multiple regression equations. The change in dCO_2 across the small-scale reactor indicated a strong likelihood that an equilibrium alkalinity would be maintained by using a fluidized bed aragonite reactor. The large-scale reactor verified this observation and established equilibrium at an alkalinity of approximately 135 mg/L as $CaCO_3$, dCO_2 of 9 mg/L, and a pH of 7.0 within 4 days that was stable during a 14 day test period. The fluidized bed aragonite reactor has the potential to simplify alkalinity and pH control, and aid in dCO_2 control in RAS design and operation. Aragonite sand, purchased in bulk, is less expensive than sodium bicarbonate and could reduce overall operating production costs.

Key Words: pH; Carbon Dioxide; Control; Aragonite; Alkalinity.

1. Introduction

Control of alkalinity, pH, and carbon dioxide in marine recirculating aquaculture systems is fundamental to maintaining efficiency of nitrification in the biofilter and to maintaining the health of culture animals (Timmons and Ebeling 2007). Alkalinity in the form of bicarbonate ions (HCO_3^-) is required for proper biofilter operations since it is consumed by nitrifying bacteria during the conversion of ammonia to nitrate (Timmons and Ebeling 2007). Hardness and alkalinity are often directly associated in aquaculture since both can be provided by calcium carbonate dissociation. Calcium can be associated with deleterious effects such as nephrocalcinosis (Chen et al. 2001) but is also a requirement

for specific life stages (e.g., larvae) of some fish, or for proper growth of crustaceans for instance (Boyd 1990).

Alkalinity can be adjusted in recirculating aquaculture systems (RASs) by the addition of a buffering compound such as sodium bicarbonate (Loyless and Malone 1997; Timmons and Ebeling 2007) or by system volume replacement (Seginer et al. 2008). Dissolved carbon dioxide ($d\text{CO}_2$) is typically removed by using degassing towers or aeration (Moran 2010; Summerfelt et al. 2000; 2003; Timmons and Ebeling 2007). Carbon dioxide and pH control are directly linked due to shifts in the carbonate buffering system (Boyd 1990). The relationship between carbon dioxide, carbonic acid (H_2CO_3), bicarbonate ions (HCO_3^-), and carbonate ion (CO_3^{2-}) concentrations relative to pH in aquaculture systems is thoroughly reviewed by Boyd (1990) for ponds and Timmons and Ebeling (2007) for RASs.

Previously, a system was developed and applied to pretreatment of highly acidic waters (e.g., coal mine drainage, or acidified lake water) prior to use in fish culture systems that consists of an upflow fluidized bed of limestone sand (calcite, CaCO_3) (Watten et al. 2005; Sibrell et al. 2006). The build-up of $d\text{CO}_2$ in high density RAS operations due to respiration has the potential to acidify water within the system. The build-up is of particular concern when pure oxygen is being used since there is little stripping of $d\text{CO}_2$ in oxygenation systems compared to aeration systems (Summerfelt et al. 2000; Timmons and Ebeling 2007). In addition, the biofilter nitrification process is alkalinity consuming

and adds hydrogen ions resulting in acidification of the culture water while removing its buffering capacity (Timmons and Ebling 2007).

This study was conducted to determine whether a fluidized bed reactor similar to that applied by Watten et al. (2005) and Sibrell et al. (2006) in freshwater applications could be incorporated into a marine recirculating aquaculture system to aid in the control of pH, dCO_2 , and alkalinity. Sand composed of the aragonite form of $CaCO_2$ was selected for the reactor media rather than calcite sand due to its relatively higher dissolution rate (Chen et al. 2001). The overall performance of two pilot reactors (small-scale and large-scale) on two RASs stocked with fish was reported based on changes in water chemistry.

2. Materials and Methods

2.1. Small-Scale Pilot Reactor

A small-scale fluidized-bed pilot reactor was constructed from a 1.5 m length of clear 10.2 cm diameter PVC pipe (Schedule 40) that was fitted with a 2.5 cm diameter elbow centered in the bottom and attached using a Uniseal[®] bulkhead fitting. Water was pumped through the reactor with a 1/3 hp submersible pump fitted with a bypass valve to allow water flow to the reactor to be adjusted to 6.25 ± 0.03 L/min (mean $\pm$ SE; n=24) to produce a hydraulic retention time (HRT) within the fluidized aragonite bed of 1.41 ± 0.01 minutes (mean $\pm$ SE; n=24). The reactor was initially filled with water and aragonite sand (Aragamax[™] sugar sized sand, CaribSea, Inc., Fort Pierce, Florida) added to a height of 0.4 m (0.32 m^3). The aragonite sand used had a mean particulate diameter by

weight of 211 μm determined by particle sieve analysis (Figure 1). Upon initiation of flow the aragonite bed was expanded to $1.1 \text{ m} \pm 0.01 \text{ m}$ (mean $\pm$ SE; $n=24$). The small-scale pilot reactor was operated on a 43 m^3 marine recirculating aquaculture system (Weirich et al. 2009) with four 7.8 m^3 tanks two of which were stocked with a total of approximately 890 Florida Pompano *Trachinotus carolinus* averaging 578 g giving an overall system biomass load of 11.7 kg/m^3 . The fish were fed a ration of 2% body weight per day of a 45% protein 16% lipid commercial floating salmonid diet (Nelson and Sons, Inc., Silver Cup, Steelhead 3.5 mm) based on total biomass in the system at the initiation of tests.

The recirculating system consisted of two propeller washed bead filters (Aquaculture Systems Technology, LLC, New Orleans, LA.) filled with 0.7 m^3 of floating plastic bead media. The submersible pump supplying the reactor was alternately installed in the side box of one of two 7.8 m^3 culture test tanks each fitted with the Cornell style dual drain system. One of the two tests tanks, Tank 1, was stocked with 41 kg/m^3 of pompano ($n=590$; average weight 540 g), the other, Tank 2, was not stocked with fish allowing for comparison of two different influent dCO_2 concentrations during the trials.

Reactor performance was determined in each of the two tanks empirically by manipulating total alkalinity of the entire system by controlling additions of sodium bicarbonate. Four individual trials were conducted over the course of several weeks during which the total alkalinity of the system was targeted at 70, 100, 130, and 200 mg/L as CaCO_3 (Table 1). At each alkalinity level the influent and effluent water from

the reactor was sampled three times from each tank to determine alkalinity, pH, and dCO_2 . Alkalinity was measured by titration using the bromocresol green-methyl red indicator under the assumption that the primary contributor to alkalinity under the conditions of the tests was bicarbonate (HACH[®] method 10244, Hach Company, Loveland, CO). A handheld pH meter that was calibrated against 7.00 and 10.01 pH buffers each day prior to use was employed for pH measurement (YSI EcoSense pH100, Yellow Springs Instruments, Inc., Yellow Springs, OH). Water flow rate out of the reactor was measured and adjusted prior to each trial and after each sample was collected using a timer and calibrated 20 L bucket. Dissolved carbon dioxide was measured by the method of Moran et al. (2010) that was verified periodically using a head space unit and a CO_2 infra-red gas analyzer (GD-444, CEA Instruments, Inc., Westwood, NJ) (Pfeiffer et al., 2011). Salinity was measured by conductivity (YSI Model 30 SCT meter, Yellow Springs Instruments, Inc.). The system was operated at a salinity of 8.3 ± 0.04 g/L (mean $\pm$ SE; n=24) and temperature of 28.3 ± 0.02 °C (mean $\pm$ SE; n=24) during all four trials.

During a separate test run changes in Ca^{++} , K^+ , Mg^{++} , Na^+ , P^+ , Mn^{++} , Zn^{++} cations as the water passed through the aragonite reactor were measured by inductively coupled plasma (ICP) spectrometry using an Optima 2100-DV ICP-optical emission spectrometry spectrometer (PerkinElmer, Shelton, Connecticut). The reactor was run for several minutes influent water was collected and after an appropriate amount of time passed for the associated water to have passed completely through the reactor a sample of effluent water was collected. The test conditions of the small-scale aragonite reactor during

sample collection for this ion analysis were: salinity = 8.2 g/L, alkalinity = 126 mg/L, influent pH = 6.5, influent dCO₂ = 34 mg/L, and HRT = 1.25 minutes.

Multiple regression analysis was used to establish the relative change in hydrogen ion concentration (% Δ [H⁺]), and in alkalinity in mg/L as CaCO₃ (% Δ [ALK]), or the percent change in dCO₂ across the reactor (SAS 9.2, SAS Institute Cary, N.C.).

Graphical representations of the predictive models were developed using GraphPad Prism 4.03 for Windows (GraphPad Software, Inc., La Jolla, Ca.). The relative change in H⁺ was calculated by assuming that hydrogen ion activity was equivalent to H⁺ concentration and therefore, % Δ [H⁺] = ((10(-pHe)-10(-pHi))/10(-pHi)) $\times$ 100%, where; pHi = influent pH, and pHe = effluent pH.

2.2. Large-Scale Pilot Reactor

A 0.6 m diameter CycloBio[®] (Pentair Aquatic Ecosystems, Apopka, FL) fluidized sand bed biofilter operated with a water level of 2.3 m was used as the vessel for the large-scale pilot reactor (reactor vessel volume = 0.67 m³) (Summerfelt 2006). The reactor vessel was filled with water, and then aragonite sand (0.12 m³) was added to the filter to a compacted bed depth of 0.41 m. Water was pumped into the reactor with a 0.5 hp submersible pump fitted with a bypass valve to allow water flow to the reactor to be adjusted to 132 Lpm. This produced an expanded bed depth of approximately 1.0 m. Water flow rate and the quantity of aragonite used in the reactor were directly scaled up from the small-scale pilot reactor based on a target HRT (expanded bed) of 1.2 minutes. Measurement of the expanded bed height was difficult in the opaque reactor vessel and

was done with a submersible camera only during the initial set up of the vessel so as not to disrupt reactor operation during trials.

The large-scale pilot reactor was installed on a 20 m³ marine recirculating aquaculture system that included nine 1.3 m³ tanks serviced a moving bed bioreactor and a static bed filter each of which was filled with 0.7 m³ of MB³[™] biomedica (W-M-T Inc, Baton Rouge, La.). Six of the system's tanks were stocked with Florida Pompano at a mean rate of 56.6 kg/m³ (SD=3.9) representing a mean number of 128 fish per tank (SD=5) with a mean weight of 444 g per fish (SD=40) the remaining three tanks were not stocked with fish. The fish were fed a ration of 500 grams of a 45% protein 16% lipid commercial floating salmonid diet (Nelson and Sons, Inc., Silver Cup, Steelhead 3.5 mm) per tank per day for a total of 3 kg per day for the entire system. The system was operated at a higher salinity of 26.0 ± 0.3 g/L (mean $\pm$ SE; n=35) and temperature of 28.3 ± 0.1 °C (mean $\pm$ SE; n=35) during the three trial runs.

Three trials were run to assess the reactors ability to come to equilibrium on the operating marine recirculating system relative to pH, dCO₂, and alkalinity. Trial 1 began with the system at an initial alkalinity >200 mg/L as CaCO₃ when the reactor was turned on. Trial 2 began with the system at an initial alkalinity of >200 mg/L as CaCO₃ (adjusted by the addition of sodium bicarbonate) without turning the reactor on. Trial 3 began with the system at an initial alkalinity of <70 mg/L as CaCO₃ when the reactor was turned on. Trial 1 was run for 16 days to ensure that a stable equilibrium was established. Trial 2 was suspended when the reduction in system alkalinity approached 50 mg/L as CaCO₃ so

that biofilter performance of the system would not be compromised. Trail 3 was run for six days. No sodium bicarbonate for alkalinity adjustment was added to the system during the three trials.

Best fit models were calculated for alkalinity, pH, and dCO₂ as the independent variables and run day as the dependent variable using stepwise regression with MaxR improvement as the selection method (SAS 9.2, SAS Institute Inc., Cary, NC)

3. Results

3.1. Small-Scale Pilot Reactor

The modeled percentage change in [ALK] as a function of influent pH and [dCO₂] produced the relationship (Figure 2):

$$\% \Delta [\text{ALK}] = 4.955 - 0.672 (\text{influent pH}) + 0.008 (\text{influent } [\text{dCO}_2] \text{ mg/L})$$

$$(r^2 = 0.8635; p < 0.0001)$$

The modeled percentage change in [H⁺] as a function of influent [ALK] and influent [dCO₂] produced the relationship (Figure 3):

$$\% \Delta [\text{H}^+] = - 0.618 + 0.003 (\text{influent } [\text{ALK}] \text{ mg/L as CaCO}_3) - 0.019 (\text{influent } [\text{dCO}_2] \text{ mg/L})$$

$$(r^2 = 0.9190; p < 0.0001)$$

The modeled percentage change in the $[dCO_2]$ as a function of influent $[dCO_2]$ and $[ALK]$ produced the relationship (Figure 4):

$$\% \Delta [dCO_2] = 0.532 - 0.003 (\text{influent } [ALK] \text{ mg/L as CaCO}_3) + 0.016 (\text{influent } [dCO_2] \text{ mg/L})$$

$$(r^2 = 0.9144; p < 0.0001)$$

Changes in concentration of three monovalent and divalent cations were measured as water passed through the small-scale aragonite reactor, Ca^{++} , P^+ , and Mn^{++} increased 15.8%, 17.0% and 200% respectively (Table 2). Only the Zn^+ ions remained unchanged and did not show an increase of some magnitude. The remaining three cations, K^+ , Mg^{++} , and Na^+ , changed slightly (<2%) on a percentage basis.

3.2. Large-Scale Pilot Reactor

Operating the large-scale reactor on the system with a starting alkalinity at either 200 or 70 mg/L as $CaCO_3$ showed a general trend for the system to reach an equilibrium alkalinity, pH, and dCO_2 within a week to 10 days of operation (Figure 5). The equilibrium alkalinity was in the vicinity of 130 mg/L as $CaCO_3$ for Trial 1 and Trial 3. In contrast, if no means of controlling alkalinity was used (e.g., no addition of sodium

bicarbonate) and the reactor was not operated the system alkalinity and pH dropped, while dCO_2 increased (Figure 5).

4. Discussion

Empirical trials of the small-scale pilot reactor indicated that an increase in alkalinity and pH with a concomitant reduction in $[dCO_2]$ was possible in a marine recirculating aquaculture system using a fluidized bed of aragonite sand. The degree of change that could be expected is dependent upon the presence of dCO_2 and a low pH. However, the modeled data indicated that at a pH as high as 7.0 and zero measurable influent dCO_2 , an increase in alkalinity of up to 25% was possible within the small-scale pilot reactor.

Scaling up the process using a CycloBio™ fluidized bed filter as the reactor proved to be possible and produced results consistent with those seen in the small-scale prototype.

This was true even though the large-scale reactor was operated in a system with 26 g/L salinity instead of 8.2 g/L used for the small-scale reactor tests.

Our test system design is similar to that of Sibrell et al. (2006) with the exceptions that:

1) our tests were conducted in recirculating aquaculture system rather than as a pre-treatment system; 2) aragonite was used rather than calcite due to the higher dissolution rate, 3) CO_2 was not injected to accelerate dissolution since our inflow water to the reactors carried CO_2 from respiration of fish in the systems; and 4) our systems were saltwater rather than freshwater. Their study indicated that increasing settled bed depth increased the limestone dissolution rate due to an increased contact time, with a bed depth of 0.41 m resulting in an alkalinity increase of 200 mg/L. In our reactors, the

fluidized bed depths were maintained at or over 1.0 m but our contact time (HRT) in the bed was measured in seconds whereas in Sibrell's system HRT was measured in minutes. Also, their inflow alkalinity was less than 10 mg/L and in this study the inflow alkalinity was between about 70 and 200 mg/L. Thus, it is promising that the substantial percent changes in alkalinity, dCO_2 and pH through our reactors was observed since the reaction parameters were close to the equilibrium values. Optimization of a reactor based on this design concept could provide substantial savings in water treatment by reducing or eliminating the addition of more costly reagents such as sodium bicarbonate or sodium hydroxide necessary for optimal biofilters nitrification.

One caveat to application of aragonite or any other calcium carbonate to a recirculating system is the possibility of increased dissolved calcium providing an environment that enhances the possibility of nephrocalcinosis, particularly when high dCO_2 concentrations exist in the culture tanks. Chen et al. (2001) reported in a case study that calcifications found in the kidneys of Nile Tilapia *Oreochromis niloticus* were potentially due to the farms change of practice from adding sodium bicarbonate to calcium carbonate (agricultural-grade lime stone) in their recirculating system. The concentration of dCO_2 in the system was very high and ranged between from 34 to 51 mg/L. This linkage was anecdotal and was not verified as a causal effect through experimental analysis. A causal relationship has also been shown for nephrocalcinosis related to elevated dCO_2 in Atlantic Salmon *Salmo salar* smolts exposed to varying levels of dCO_2 (Fivelstad et al 2003; Hosfeld et al. 2008). However, it is not well understood how calcium impacts nephrocalcinosis relative to hypercapnia in marine systems where calcium levels can be

substantially higher than in the freshwater systems. European flounder, *Platichthys flesus*, however does appear to regulate divalent ion homeostasis by regulating bicarbonate ions through intestinal secretion and concomitant hydrogen ion secretion at the gills (Wilson and Grosell 2003).

The relationship between calcium, carbon dioxide and pH in freshwater and marine systems is a subject that should be investigated in more detail to instruct the proper application of control systems such as this aragonite reactor. This study concentrated effects relative to alkalinity provided through the carbonate buffer system (i.e., bicarbonate). However, other substances contribute to alkalinity in aquaculture that may or may not have been accounted for, or measured, by the methods used.

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Table 1. Mean ($\pm$ SE) reactor conditions during small-scale aragonite reactor test trials (n=3 for each tank in each trial). Tank 1 was the high dissolved carbon dioxide [dCO₂] test condition and Tank 2 was the low [dCO₂] test condition for each trial. The system salinity during the trials was 8.3 ± 0.04 g/L (mean $\pm$ SE; n=24) and temperature was 28.3 ± 0.02 °C (mean $\pm$ SE; n=24).

Trial	Tank	N	Alkalinity (mg/L as CaCO ₃)	pH	Influent dCO ₂ (mg/L)	Flow (L/min)	Bed Height (cm)	HRT (sec)
1	1	3	195 $\pm$ 0.3	6.92 $\pm$ 0.009	19.3 $\pm$ 0.40	6.38 $\pm$ 0.04	117 $\pm$ 0.0	1.50 $\pm$ 0.010
	2	3	190 $\pm$ 2.3	7.29 $\pm$ 0.006	8.0 $\pm$ 0.01	6.25 $\pm$ 0.02	105 $\pm$ 0.0	1.37 $\pm$ 0.003
2	1	3	135 $\pm$ 0.6	6.76 $\pm$ 0.006	19.9 $\pm$ 0.24	6.13 $\pm$ 0.00	105 $\pm$ 0.0	1.40 $\pm$ 0.000
	2	3	134 $\pm$ 1.7	7.15 $\pm$ 0.003	7.8 $\pm$ 0.09	6.03 $\pm$ 0.00	100 $\pm$ 0.0	1.35 $\pm$ 0.000
3	1	3	100 $\pm$ 2.2	6.60 $\pm$ 0.003	21.3 $\pm$ 0.38	6.47 $\pm$ 0.01	115 $\pm$ 0.3	1.45 $\pm$ 0.003
	2	3	94 $\pm$ 1.3	6.98 $\pm$ 0.003	8.3 $\pm$ 0.10	6.22 $\pm$ 0.00	105 $\pm$ 0.3	1.38 $\pm$ 0.003
4	1	3	72 $\pm$ 2.3	6.48 $\pm$ 0.000	20.1 $\pm$ 0.64	6.24 $\pm$ 0.02	110 $\pm$ 0.0	1.44 $\pm$ 0.004
	2	3	69 $\pm$ 1.8	6.84 $\pm$ 0.006	8.3 $\pm$ 0.21	6.29 $\pm$ 0.05	110 $\pm$ 0.9	1.42 $\pm$ 0.023

Table 2. Influent and effluent concentrations (mg/L) of ions potentially affected by the operation of the small-scale aragonite reactor (salinity=8.2 g/L, alkalinity = 126 mg/L, influent pH = 6.5, and influent dCO₂ = 34 mg/L, HRT =1.25 min).

Sample Location	Ion Concentration (mg/L)						
	Ca ⁺⁺	K ⁺	Mg ⁺⁺	Na ⁺	P ⁺	Mn ⁺⁺	Zn ⁺⁺
Influent	117.4	104.2	305.2	2316	5.9	0.001	0.03
Effluent	136.0	104.8	308.2	2358	4.9	0.003	0.03

Figure 1. Particle size distribution of aragonite sand (Aragamax™ sugar sized sand, CaribSea ,Inc., Fort Pierce, Florida) used in both the small-scale and large-scale aragonite reactors.

Figure 2. The modeled percent change in alkalinity [ALK] in mg/L as CaCO_3 as a function of influent pH and dissolved carbon dioxide [dCO_2] in mg/L ($r^2 = 0.8635$; $p < 0.0001$). The system salinity during the trials was 8.3 ± 0.04 g/L (mean $\pm$ SE; $n=24$) and temperature was 28.3 ± 0.02 °C (mean $\pm$ SE; $n=24$).

Figure 3. The modeled percentage change in [H^+] as a function of influent alkalinity [ALK] and influent dissolved carbon dioxide [dCO_2] ($r^2 = 0.9190$; $p < 0.0001$). The system salinity during the trials was 8.3 ± 0.04 g/L (mean $\pm$ SE; $n=24$) and temperature was 28.3 ± 0.02 °C (mean $\pm$ SE; $n=24$).

Figure 4. The modeled percentage change in the dissolved carbon dioxide [dCO_2] in mg/L as a function of influent [dCO_2] and alkalinity [ALK] in mg/L as CaCO_3 ($r^2 = 0.9144$; $p < 0.0001$). The system salinity during the trials was 8.3 ± 0.04 g/L (mean $\pm$ SE; $n=24$) and temperature was 28.3 ± 0.02 °C (mean $\pm$ SE; $n=24$).

Figure 5. Response of alkalinity [ALK], dissolved carbon dioxide [dCO_2], and pH in a marine recirculating aquaculture system stocked with Florida Pompano fitted with a large-scale aragonite reactor. Two trial runs of the reactor with high and low starting

[ALK] (mg/L as CaCO_3), and a trial run in the same system without the large-scale aragonite reactor connected and no attempt to control [ALK] are presented.

Fig 1.

Fig 2.

Fig 3.

Fig 4.

Fig 5.