Efficacy of a pilot-scale wastewater treatment plant upon a commercial aquaculture effluent
I. Solids and carbonaceous compounds

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ABSTRACT

A pilot-scale wastewater treatment station was built and operated at a commercial recirculating aquaculture facility in order to initiate, characterize and optimize the operation of a treatment strategy for effluent recovery and reuse. The treatment train consisted of sedimentation, denitrification, ozonation, trickling filter treatment, and chemical flocculation. The study consisted of four different sets of treatment conditions, differentiated by alternative use of 6 or 4 lpm flow and recycling rates, ozone doses between 36.6 and 82.5 mg O$_3$/l water, and 6- or 9-min ozonation time. The effects of treatment on solids and dissolved organic compounds are reported here. Over 70% of solids were removed by sedimentation under all experimental conditions. At the end of treatment, up to 99% of TSS was removed due to the combined action of ozonation and chemical flocculation. COD removal was not significantly different among experimental conditions by sedimentation (59.2–62.7%, $p > 0.05$), but was positively correlated with ozone dose (slope = 0.452, r^2 = 0.99), yielding total COD removal η(CODt) of 19.8–40.7%. Of these amounts, 60.4–66.5% of COD was removed with foam, while the balance was mineralized. The ozone reactivity was 83.7% at a dose of 82.5 mg O$_3$/l water. The ozone consumption coefficient Y(O$_3$/CODox) for COD oxidized was 1.92–2.23 g/g O$_3$ COD and 0.70–0.78 g O$_3$/g COD when total COD removed was considered. Overall, 87.9–92.4% of COD was removed by the treatment train, to an average of 44 mg/l at the highest ozone dose, a value 3.3–3.9 times less than in fish tanks. Under the same conditions, cBOD$_5$ was reduced by 88%, 3.8–4.1 times less than in fish tanks. The water's biodegradability was increased by over 20%. DOC did not change significantly through the treatment train, and fluctuated through the system due to methanol addition to support denitrification. Work with the pilot station showed that the treatment strategy employed could support effective recovery and recycling of aquaculture effluent, although salts and refractory organics may accumulate in the system.

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1. Introduction

A major advance from extensive pond-based aquaculture toward intensive production was achieved by development of recirculating aquaculture systems (RAS), allowing increased rearing densities, reduced land usage and water consumption, and greater control over water quality (Ackefors, 1999; Waller, 2001). Water quality is maintained through water treatment and water exchange, the rate of exchange needed depending on the efficiency of water treatment (Waller, 2001). Most commercial RAS contain a primary loop of recirculation, but lack a secondary treatment loop needed to allow complete reuse of water (e.g., ammonia removal, oxygenation/carbon dioxide stripping, and dissolved and suspended solids removal devices). After secondary treatment, water may be returned to the primary recirculation system or discharged, decreasing water demand and minimizing impact to the receiving environment. Two major types of secondary treatment may be applied for water recovery. The first category includes systems such as settling ponds, lagoons, wetlands or aquaponics to remove organics by encouraging aquatic macrophyte or phytoplankton development (Naegel, 1977; Rakocy et al., 1993). A second category of methods uses techniques analogous to secondary or tertiary treatment of domestic and industrial wastewater discharges; several authors have reported successes from laboratory and pilot-scale closed RAS (Rosenthal and

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Nomenclature

A^*	specific ozone absorption
BRA	Blue Ridge Aquaculture
$cBOD_5$	carbonaceous biochemical oxygen demand (mg/l)
$c(M)_0$	influent pollutant concentration (mg/l)
$c(M)_e$	effluent pollutant concentration (mg/l)
C_{G0}	influent gas concentration (mg/l)
C_{Ge}	effluent gas concentration (mg/l)
C_{Le}	effluent liquid concentration (mg/l)
CF	chemical flocculation
COD	chemical oxygen demand (mg/l)
COD_{ox}	chemical oxygen demand removed by oxidation (mg/l)
COD_t	total chemical oxygen demand (i.e., removed by oxidation and foam) (mg/l)
$COD/cBOD_5$	water biodegradability index (mg/l)
DO	dissolved oxygen (mg/l)
DO_3	dissolved ozone (mg/l)
DOC	dissolved organic carbon (mg/l)
DR	denitrification reactor
DRi	denitrification reactor influent
F	foam
$F(O_3)$	ozone dose or feed rate (mg/l s)
FBBR	fluidized bed biological reactor
FE	final effluent (after chemical flocculation)
FSS	fixed suspended solids (mg/l)
FTS	fixed total solids (mg/l)
I^*	specific ozone dose or input (g O_3/g COD)
MB	mixing basin
MSe	microscreen filter effluent
MSF	microscreen filter
NO_2^--N	nitrite-nitrogen (mg/l)
NO_3^--N	nitrate-nitrogen (mg/l)
NTU	nephelometric turbidity units
Ore	ozone reactor effluent
OFR	overflow rate
OR	ozone reactor
ORi	ozone reactor influent
Q_G	gas flow rate (l/s)
Q_L	liquid flow rate (l/s)
$r(COD)$	COD removal rate (mg/l s)
$r(COD_{ox})$	rate of COD removed by oxidation
$r(COD_t)$	total COD removal rate
$r(O_3)$	ozone consumption rate (mg/l s)
$r_A(O_3)$	ozone absorption rate (mg/l s)
rpm	rotations per minute
RAS	recirculating aquaculture system
RBC	rotating biological contactor
RI	raw (untreated) influent
SB	sedimentation basin
SBe	sedimentation basin effluent
SBi	sedimentation basin influent
SC	stripping chamber
SSB	sludge from sedimentation basin
tH	hydraulic retention time (min)
tO_3	residual total percent of ozone dose measured as residual (water and gas)
TAN	total ammonia nitrogen (mg/l)
TF	trickling filter
TFe	trickling filter effluent
TFi	trickling filter influent
TS	total solids (mg/l)
TSS	total suspended solids (mg/l)
V_L	liquid volume (m^3)
VSS	volatile suspended solids (mg/l)
VTS	volatile total solids (mg/l)
Y	ozone consumption coefficient (g O_3/g COD)

Greek symbols

$\eta(COD_{ox})$	degree of oxidized COD removal (%)
$\eta(COD_t)$	degree of total COD removal (%)
$\eta(O_3)$	ozone transfer efficiency (%)

Black, 1993; Thoman et al., 2001; Gelfand et al., 2002). Hybrids of the two technologies have been employed to treat the effluent from commercial closed RAS facilities (van Gorder, 2002). The most appropriate treatment train depends on wastewater characteristics, economics, and the ultimate destination of the effluents.

1.1. Problem statement and design concept

Increased tilapia (hybrid *Oreochromis* sp.) production at Blue Ridge Aquaculture (BRA, Martinsville, VA, USA) is constrained by the availability of high quality influent water. Meanwhile, BRA discharges an estimated 2290 m^3 of wastewater per day to the municipal sewer system, equivalent to an average of 3.00 kg feed/m^3 discharge cumulative food burden. This effluent also loses heat energy, as water temperature is maintained at about 28–30 °C to optimize tilapia growth. Concerned about the reliability and costs of their wells and the city water system, BRA seeks an alternative solution. Developing a wastewater treatment system that recovers and reuses the water presently discharged could minimize these problems.

Previous pilot-scale research at BRA (B. Brazil, unpublished data) employing foam fractionation, mechanical filtration, and biological treatment for nitrate removal demonstrated improvement in quality of recovered water, although not all targeted water quality improvements were achieved. Hence, a new, more comprehensive design was developed. Following an ozone treatability study (Sandu, 2004), a pilot-scale wastewater treatment station was built in order to initiate, characterize and optimize the operation of this more complex treatment strategy. The need to eliminate settable solids, colloids, dissolved organic substances and nitrogenous compounds led to selection of a sequential treatment process employing physical, biological, chemical, and again, biological steps. The treatment train (Fig. 1) included: a primary sedimentation basin, mechanical filtration using a microscreen drum filter, denitrification using a fluidized bed biological reactor with methanol added to provide carbon and energy for cellular growth, ozonation and foam fractionation in a bubble-contact ozone reactor, dissolved ozone quenching in an air-bubble stripping chamber, aerobic biological treatment using a trickling filter, and jar test-scale chemical flocculation, followed by sand filtration.

1.2. Objective

The objective of this study was to determine the effectiveness of the wastewater treatment train with regard to treatment of solids, turbidity, COD, $cBOD_5$, and DOC.
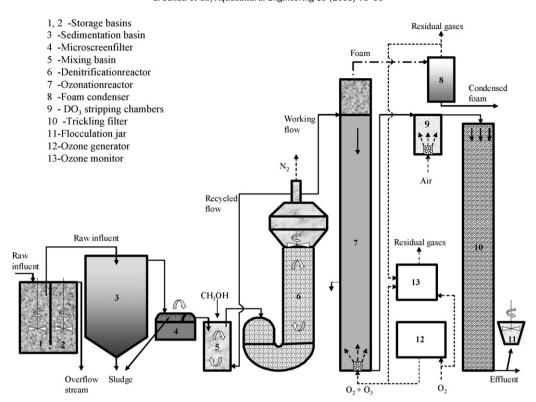
Fig. 1. Schematic diagram of pilot-scale wastewater treatment train at Blue Ridge Aquaculture. The details are not drawn to scale.

2. Methods

2.1. Treatment train design

At BRA, tilapia are produced in RAS that include fish tanks, sedimentation (multi-tube clarifier) basins, nitrification biofilters (rotating biological contactors), and U-tubes for oxygenation. There is a hatchery and an assemblage of three greenhouses for broodstock holding and juvenile production. A volume of 2290 m^3/day is flushed at BRA during a 24-h period. Water exchange is executed discontinuously among the various production units, influenced by the availability of replacement water, labor, and need for water exchange. Wastewater flowing through the treatment train was estimated as 18.4% from the greenhouses and 81.6% from grow-out systems. A capacity to store wastewater was needed in order to provide a continuous wastewater feed to the pilot station. Hence, two tanks were installed with a total capacity of 2.7 m^3 (1.0 and 1.7 m^3, respectively), providing a reserve for up to 4 h of operation of the pilot station. The tanks were connected at the bottom to promote influent homogeneity. Each tank was equipped with a low speed propeller (90 rpm) to prevent sedimentation. A flow switch-controlled submersible pump located in the main effluent discharge pipe for the BRA facility fed the first storage tank. When the storage tanks were full and the pumped stream exceeded the pilot station needs, excess wastewater was released from the second tank through an overflow stand-pipe.

A submersible pump located in the first storage tank fed the wastewater to the sedimentation basin (Fig. 1). To prevent clogging, the feed was discontinuous, with the pump operating two of every 5 min. The sedimentation basin was cylindrically shaped, 76-cm diameter, 115-cm depth, with a conical bottom (45° side angle), and a volume of 400 l. These characteristics provided a 55–75 min residence time for an average overflow

rate of 1.15 and 1.75 cm/min, respectively, assuring removal of 85–88% of suspended solids (Wong and Piedrahita, 2000). A submerged pump linked to a timer (operating 15 s during every 5 min) periodically flushed a volume of 4.5 l of sludge accumulated at the bottom, which corresponded to a continuous flow rate of 0.9 lpm. Because of flow variations caused by intermittent feed and by sludge removal, the overflow rate varied around the design values in the sedimentation basin. The treatment stream (i.e., the working stream) exited the top of the basin by gravity, through a connection located across from the influent point, entering the microscreen filter for additional solids removal.

The microscreen filter had 120 μm mesh. Its vessel had a volume of 160 l. It was equipped with a vacuum system that removed captured solids from the screen surface, and disposed of them.

From the microscreen filter, the working stream entered a mixing basin at the top. The mixing basin was a truncated conical PVC vessel, 65 cm tall, 34 cm average diameter, and holding a volume of 55 l of water. At the bottom, the mixing basin also received the recycled stream from the denitrification reactor. From the mixing basin, a submersible pump passed water onward to the denitrification reactor. Methanol was introduced continuously into the mixing basin by a peristaltic pump. The flow rates varied between 81 and 54 ml/h at wastewater working streams rates of 6 and 4 lpm, respectively.

The fluidized bed biological denitrification reactor had a J-shaped cylindrical PVC vessel, with an enlarged fiberglass capsule attached to the top. The PVC cylinder was 360 cm long (300 cm on the long arm, 30 cm along the curvature and 30 cm on the short arm), 15 cm diameter, and 64 l volume. It held an initial volume of 15 l of 0.7 mm diameter silica sand. Along the length of the reactor were four transparent PVC windows for inspection. Water from the mixing basin was pumped to the short arm of the reactor, exerting

backpressure on the sand bed, which expanded and fluidized. A capsule at the top of the denitrification reactor had a truncated conical shape at the lower part, with the base connected to the PVC body. It continued upward with a cylindrical section of 34 cm diameter, and a cone-shaped lid at the top. The capsule had 60 cm total height, and a volume of 30 l. The large diameter of the capsule reduced water velocity, preventing washout of sand. Inside the capsule was a flat-bladed propeller, driven by a submersed, electrical motor, which sheared excess biofilm from sand particles reaching its level, further preventing loss of sand at the top of the reactor. A flow-splitting device at the top of the reactor's capsule (i.e., a 4 in. cylinder with connections and valves) allowed controlled, partial return of the flow to the mixing basin, while the rest of the flow (the working stream) went onward to the ozone reactor. An opening at the top allowed release of biologically produced nitrogen to the atmosphere. Regardless of the recycled and working streams, the flow injected to the denitrification reactor was always 10 lpm (0.91 cm/s). The need for maintaining a minimum flow rate to sustain sand fluidization and the need for sufficient residence time for ozonation in the next process unit provided the rationale for using recirculation, rather than concerns about completion of denitrification in a single pass.

The ozone reactor was operated in a counter-current configuration. The reactor was a fine-bubble diffuser column 510 cm long and 10 cm diameter. The height of the water in the column before gas injection was 450 cm, corresponding to a 35-l volume. The gas flow rate was constant, expanding the water height to 485 cm. The remaining space (25 cm) accommodated foam formation. The reactor had a connector at the top for evacuation of foam and gases, one for receiving the influent stream from the denitrification reactor, and a valve at the bottom for exiting water. A collection port for sampling was inserted into the reactor wall 168 cm from the bottom. Ozone was produced from pure oxygen using one or two corona discharge ozone generators (Pacific Ozone Technology, Brentwood, California, Model G21, and Model CD1500P, Clear-Water Tech, San Luis Obispo, CA, USA), as necessary. The gas was injected at the bottom of the reactor using two 20 cm × 4 cm × 4 cm silica stone diffusers (Aquatic Ecosystems, Inc., Apopka, FL, USA). The ozone concentration in the gas phase was measured with an ozone monitor (PCI Ozone & Control Systems, Inc., Caldwell, NJ, USA, Model HC-400). Valves were used to direct the gas stream from the diffusers to the ozone monitor for inflow ozone concentration measurements. By reversing the valves' positions, the gas stream went to the diffusers and was dispersed as bubbles into the reactor. At the top of the reactor, the residual gases were collected along with foam and directed to a foam collector for separation. The foam collector was a cone-shaped condensing tank, with a volume of 50 l. The foam/gas mixture was introduced at the top, as it exited from the ozonation reactor. A port at the top allowed the separated gases to be evacuated, while the condensed foam remained and was drained at the bottom by a U-shaped pipe, which prevented escape of gases. Residual ozone in this gas stream was measured with the ozone monitor, using 1 lpm

for sample measurement. All parts that came into contact with ozone were made from ozone-resistant materials.

The working stream flowed gravitationally to the stripping chamber, placed on a high platform such that the top had the same water level as the ozone reactor. The chamber was a 55-l PVC truncated conical basin (65 cm deep and 34 cm average diameter), with inflow at the bottom and outflow at the top. An air pump injected approximately 10 lpm through a submersed, 4 cm × 4 cm × 20 cm silica stone diffuser, removing residual dissolved ozone and other dissolved gases from the stream.

The stream from the stripping chamber flowed gravitationally to the trickling filter, a rectangular reactor (425 cm tall × 45 cm × 45 cm) made from PVC. The trickling filter was filled with Biodeck® 60° cross-flow plastic bundles. A wall vertically split the interior of the trickling filter into two equal chambers, allowing the use of one or both as necessary. The water was distributed at the top by five 2.54-cm diameter pipes (perforated laterally and end-plugged), and further distributed by a perforated PVC plate placed beneath them. A plastic tray located at the bottom of the trickling filter collected the exiting stream, allowing sampling and flow rate monitoring. The effluent stream then was released.

2.2. Operation and analytical techniques

This study was designed to test four different combinations of working (treatment) conditions, under two wastewater stream flow rates. Two ozone doses were tested for each flow rate (Table 1). After construction of the pilot station, operations began with solids removal in the sedimentation basin and the micro-screen filter and acclimation of the denitrification reactor. The working stream flow rate used for acclimation was 6 lpm, with a recycled flow rate of 4 lpm. Methanol was added to the mixing basin from the beginning, at a stoichiometric ratio of 3.6 mg methanol/mg nitrate-nitrogen (NO_3^--N), based on the average influent NO_3^--N concentration of 50 mg/l in BRA effluent. In the first month, effluent from the denitrification reactor was released from the system. Samples were collected twice per week at 4:00 p.m. from the sedimentation basin effluent, mixing basin, and denitrification reactor effluent, beginning with the second week of operation.

Beginning with the 30th day of operation, the ozone reactor was connected to the system in flow-through mode of operation. Subsequently, the effluent stream from the denitrification reactor was treated with ozone (6-min hydraulic residence time), passed through the stripping chamber and then through the trickling filter, completing the treatment train. This moment marked the start of trickling filter acclimation. At the maximum working flow of 6 lpm, the entire trickling filter was exposed. The trickling filter was designed to remove 40 mg/l $cBOD_5$ and 6 mg/l TAN when fully acclimated. Tests assessing acclimation were applied to the trickling filter influent and effluent. COD was measured to assess organics removal. Acclimation was considered complete around

Table 1
Conditions applied to the pilot station for four experimental treatments

Treatment	Water flow (lpm)	Gas flow[a] (lpm)	O_3 concentration (mg O_3/l gas)	O_3 dose (mg O_3/l water)	Oz. time (min)	Recircul. DR[b] (lpm (%))	TF used[c] (%)
1	6	10	22	36.6	6	4 (40)	100
2	6	10	33	55.0	6	4 (40)	100
3	4	10	33	82.5	9	6 (60)	50
4	4	10	22	55.0	9	6 (60)	50

[a] Flow of the O_2/O_3 mixture.
[b] Recirculation rate in the denitrifcation reactor.
[c] Proportion of cross-section of trickling filter used.

day 81 of trickling filter operation when it treated about 50% of influent TAN and 30–35% of COD, while the denitrification reactor had been found to remove about 95% of the NO_3^--N two weeks earlier. These findings indicated that both bioreactors had reached a steady-state mode of operation. Additionally, a consistent brown biofilm was observed to coat the entire sandbed in the denitrification reactor, and bed expansion had increased significantly.

Maintenance operations proved necessary to keep the pilot station working properly. Every 48 h, the sedimentation basin was skimmed manually to remove floating solids. Solids deposition on connecting hoses and tank walls throughout the treatment train required weekly cleaning.

2.2.1. Treatment 1

This experimental treatment was conducted under the same conditions as the acclimation: 6 lpm working flow, 4 lpm recycling in the denitrification reactor, and 22 mg ozone/l gas injected into the ozonation reactor (Table 1). The equivalent dose was 36.6 mg O_3/l water. This represented the highest working flow rate, and the lowest dose and time of exposure to ozone tested. The system was operated under these conditions for four weeks, during which three sets of tests were conducted at 10-day intervals.

Each set of tests included three consecutive runs over a 24-h period. Samplings began at 2:00 p.m., 10:00 p.m. and 6:00 a.m. At the beginning of each sampling, solids settleability tests were performed on the raw influent. The collection of sample aliquots was timed to match the residence time of water in each treatment unit, allowing sampling of the same parcel of the stream as it passed through particular collection points. Because the total volume of the treatment train was about 800 l and the working flow rate in this stage was 6 lpm, the sampling stretched over a two hours and 15 min period. Another two tests were conducted on days 10 and 20 at 8 p.m., when $cBOD_5$, COD, and DOC were measured; sample locations were those for $cBOD_5$.

A spectrophotometer was used for determining turbidity and DO_3. Raw influent settleability tests were determined following the Inhoff Cone Standard Method 2540F (APHA et al., 1998). These analyses were performed on site. In addition, a set of sample aliquots were passed through 1.5 μm filters for total suspended solids (TSS) determination. These filters, along with a set of refrigerated aliquots and another set acidified below pH 2 with H_2SO_4 were transported to the Civil and Environmental Engineering Laboratories at Virginia Polytechnic Institute and State University for further processing. TSS determinations were completed there, along with VSS and FSS, using Standard Methods 2540D and 2540 E, respectively. Fresh samples were analyzed for TS, VTS, and FTS following Standard Methods 2540 B and 2540E, respectively. For $cBOD_5$ determinations, fresh aliquots were collected, refrigerated on ice, and processed according to Standard Method 5210B. COD was determined by using the closed reflux procedure (Standard Method 5220C), and DOC by using a total carbon analyzer (Dorhmann model DC 80, Santa Clara, CA, USA, Standard Method 5310A) on samples passed through 0.45 μm filters. A jar-test chemical flocculation treatment was applied to the trickling filter effluent. $FeCl_3$ was used as the flocculating agent, because it operates over a wide pH window and because the trickling filter effluent often had pH higher than 8. Preliminary tests involving doses of 33, 50 and 75 mg/l $FeCl_3$ showed that a dose of 50 mg $FeCl_3$/l effluent was appropriate. Foam samples were assayed for COD, $cBOD_5$, TS, VSS, FTS, TSS, VTS, FSS, and turbidity. Volumes of sludge from the sedimentation basin and foam streams were measured.

2.2.2. Treatment 2

At the beginning of this treatment, a second ozone generator was connected in parallel with the existing one. The oxygen flow (10 lpm) then was split equally between the two generators. By receiving a lower oxygen flow rate, each generator increased its ozone production efficiency, achieving a total concentration of 33 mg ozone/l of gas stream (a dose of 55 mg O_3/l water). This treatment lasted for 18 days, with 24-h sampling runs occurring at 6-day intervals. The tests that included $cBOD_5$ were performed on days 4 and 16.

2.2.3. Treatment 3

The conditions for this treatment were set by reducing the working flow to 4 lpm and increasing the recycle flow rate in the denitrification reactor to 6 lpm. This modification increased the residence time of water in each component of the treatment train, extending the sampling time to 3 h and 20 min. Water residence time in the ozone reactor increased from 6 to 9 min. The ozone concentration was kept at 33 mg/l gas, but extension of residence time increased the dose to 82.5 mg O_3/l water. The smaller working flow rate led to closing half of the cross-section of the tricking filter in order to keep the hydraulic loading rate above the 1.8 m/h necessary for this type of filter (Grady et al., 1999). Treatment 3 conditions provided the highest ozone dose to interact with the treatment stream (Table 2). The treatment lasted for 21 days, following the same sampling protocol as in Treatment 2, except for timing of sample collection as a parcel of water passed through the system. The first three days of the stage were allowed for the system to adjust to the new conditions.

2.2.4. Treatment 4

The conditions for this treatment were set by returning to 22 mg/l ozone gas, keeping the flow rate at 4 lpm. This treatment was designed to assess application of a dose of 55 mg O_3/l water (e.g., similar to Treatment 2), but during a 9-min exposure period. The treatment lasted four weeks, with sampling days as in Treatment 1. Due to the different working flow, the sampling events through treatment train were timed as in Treatment 3.

2.2.5. Data analysis

Parameters regarding ozone performance were calculated using steady-state equations presented by Gottschalk et al. (2000), and included: ozone dose or feed rate, $F(O_3)$ (in mg/l s); ozone consumption rate, $r(O_3) = r_L$ (mg/l s); ozone absorption rate, $r_A(O_3)$ (mg/l s); pollutant removal rate, $r(M)$ (mg/l s); specific ozone dose or input, I^* (g O_3/g M); specific ozone adsorption, A^* (g O_3/g M); ozone transfer efficiency, $\eta(O_3)$ (%); degree of pollutant removal, $\eta(M)$ (%); and ozone reaction coefficient, $Y(O_3/M)$ (g O_3/g ΔM); where all parameters are as defined in Nomenclature.

Table 2

Waste stream characteristics for BRA effluent, collected on different days at 6:00 a.m., 2:00 p.m., and 10:00 p.m. each day

Parameter	Average	Minimum	Maximum
COD (mg/l)	573	88	1102
TS (mg/l)	1812	108	2426
VTS (mg/l)	731	118	1363
FTS (mg/l)	1081	844	1299
TSS (mg/l)	558	53	993
VSS (mg/l)	461	44	824
FSS (mg/l)	97	8	169
DO (mg/l)	0.45	0.06	3.2
Set.S.[a] (ml/l)	24.0	4.0	30.0

Average values represent non-flow-weighted averages (12% for samples from 2:00 p.m. and 44% each for the other two).

[a] Settleable solids.

2.3. Statistical analyses

To assess system performance for removing pollutants and improving water quality, we compared parameter values before and after each water treatment unit process, as well as after the entire water treatment train. The null hypothesis was that there was no difference in concentrations across units or between different sets of operating conditions (i.e., experimental treatments). We computed 95% confidence intervals about the mean percent removal for different parameters after each of the five main units in the treatment train.

To more accurately estimate the daily removal for a certain parameter, we used a weighted average of the three measurements to obtain the daily mean. Weights were based on amounts of water that entered the system, and were 18.4% for the 2:00 p.m. measurements, and 40.8% for each of the other two measurements.

ANOVA tests for differences of means compared parameter values among the four treatment conditions. Values of parameters in the influent stream were used as covariates in the ANOVA model. Because inclusion of covariates did not improve the model, they are not further considered here. To observe the relationship between ozone dose and several water quality parameters, linear regressions were performed. All statistical analyses were conducted using SAS, Version 8.2 (SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1. BRA waste stream characterization

The BRA waste stream was characterized over 8-h sampling periods on 12 different days. The results indicated that solids, COD, and nitrate were the most significant waste components in terms of mass discharge. Depending which fish culture systems were being flushed or cleaned, waste stream characteristics varied (Table 2). Using Metcalf and Eddy's (1991) criteria for classification of untreated domestic wastewaters, BRA effluent had weak strength in the morning and medium strength in the afternoon and overnight. Evaluations indicated that 12% of the waste stream came from auxiliary systems such as fingerling and broodstock production units (i.e., morning waters), and 88% from grow-out system water exchanges. BRA effluent showed a higher fixed solids fraction (60%) than is typical for a domestic wastewater. The high fixed suspended solids (FSS) was probably due to the accumulation of minerals in RAS waters, which enter the systems with feed (and then the water as metabolic byproducts), and from various chemicals added to the water to manage water quality (e.g., $NaHCO_3$ and $NaCl$). Tests conducted on settled samples indicated a low biodegradability index ($cBOD_5/COD$ ratio of 0.25–0.30). This could be explained by the presence of uneaten, fine-particle feed components, and by poor digestibility of polysaccharides in feed that subsequently entered the systems with feces. Both of these forms are generally unsuitable for bacterial consumption. Additionally, solids that settled in the sumps could support a rich and diverse bacterial community that utilizes readily metabolized materials, accounted for as $cBOD_5$. BRA released daily 4150 kg of TS (1674 kg as volatile), 1278 kg TSS (1057 kg as volatile), and 1312 kg COD.

3.2. Solids removal

Settleable solids tests indicated that BRA effluent settles well, resulting in a separation of 4–30 ml settled solids/l water. A brownish color persisted after sedimentation, indicating a large number of colloidal particles and dissolved macromolecules in the water column.
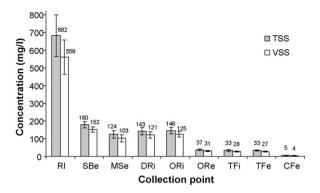

Fig. 2. Treatment train profile for total suspended solids (TSS) and volatile suspended solids (VSS) variation in Treatment 3. Treatment conditions: working stream flow rate = 4 lpm, ozone reactor residence time = 9 min, ozone dose = 82.5 mg O_3/l water. Mean values ± standard deviations shown ($n = 6$).

Raw influent non-flow-weighted averages were 1812 mg/l for TS, 731 for VTS, and 1081 for FTS (Table 2). Sedimentation removed 21.7–27.7% of TS, 44.6–52.0% of VTS, and 8.7–10.8% of FTS. High VTS removal could be explained by a large portion of this fraction being trapped in solids that settled, while most of the fixed fraction was dissolved. A large fraction (60%) of FTS associated with poor FTS settleability suggested that the stream was rich in dissolved minerals.

Complete treatment removed 98.5–99.1% of the TSS. Highest removal was observed at the largest ozone dose (Stage 3), yielding an average of less than 5 mg/l TSS in the final effluent. Fig. 2 presents a treatment train profile of TSS and VSS dynamics in Treatment 3, with values shown representing means for tests conducted at 10:00 p.m. and at 6:00 a.m.; samples from 2:00 p.m. generally yielded much lower values. TSS removal in the main treatment units are shown in Table 3. In the raw influent, 81.7–83.7% of TSS was volatile, the remainder representing the fixed fraction, values typical of those for aquaculture sludge solids (Chen et al., 1996; Summerfelt et al., 1999).

In the sedimentation basin, removal of TSS was 70.9–72.9% and VSS between 68.8 and 72.0%. Across Treatments 3 and 4, there was no statistically significant improvement in TSS removal across the sedimentation basin as retention time increased from 55 to 75 min. This unexpected finding could be due to discontinuous feeding of the sedimentation basin, to solids floatation, and to a large percentage of fine, unsettleable solids in wastewater. Alternatively, solids may have needed less than 55 min to settle. Persistent brownish color and high turbidity of water in the sedimentation basin indicated that stable fine particles could impair sedimentation. The problem of unsettleable colloids is typical of aquaculture wastewaters (Chen et al., 1993; Tetzlaff,

Table 3
Mean TSS removal performance for unit process designed to remove solids

Treatment	TSS (mg/l)		TSS removal per treatment unit (%)			Overall removal (%)
	RI	FE	SB	OR	CF[a]	
1	552.8	8.3	70.9[a]	75.6[a]	78.9[a]	98.5[a]
2		7.3		76.6[ab]	80.7[ab]	98.7[ab]
3	563.3	4.7	72.9[a]	77.7[b]	86.0[b]	99.1[b]
4		6.0		76.9[ab]	82.9[ab]	98.9[ab]

The values represent non-flow-weighted averages for an entire experimental treatment. Means with the same superscript letters (a and b) are not significantly different ($p > 0.05$).

[a] Chemical flocculation using 50 mg/l $FeCl_3$.

2001). Pumping of effluent twice before reaching the sedimentation basin could have increased the number of fine, stable, hard-to-remove particles.

In the microscreen filter, TSS removal ranged from 28.8 to 38.9%. Accumulation of solids was observed inside the microscreen basin, despite vacuuming of solids from the rotating screen. The accumulation could be due to a high density of particles that should normally settle in the sedimentation basin that were held in the water column by gas bubbles and passed onward to the microscreen filter.

TSS increased 18.2–29.4% in the denitrification reactor, with largest increases in the first two experimental treatments, presumably to bacterial growth. Across the ozone reactor, 129–157 mg/l TSS in the influent was reduced to 31–35 mg/l in the effluent (Table 3). The difference was statistically significant only between Treatments 1 and 3. Comparable efficacy across treatments could be due to rapid separation of solids at the top of the ozone reactor, with but a small amount removed in the lower part of the ozone reactor via mineralization. With this explanation, different residence times exerted little influence on the amount of solids separated; neither did ozone doses, which probably were sufficient to destructively solubilize suspended solids. The volume of condensed foam depended on the ozone dose and residence time, representing an equivalent of 3.6 and 6.9% of the working stream in Treatments 1 and 3, respectively (Table 3). A mass balance of the foam showed that 58.3–66.3% of TS, 71.6–77.9% of VTS, and 80.7–86.4% of TSS were removed, although 81.2–86.6% of VSS was not recovered in foam. The significant reduction of volatile solids could be due to partial mineralization of organic matter. The disappearance of the suspended fraction was probably due to disintegration of cells and other solids. Destruction of bioflocs by ozonolysis also was reported by Kamiya and Hirotsuji (1998) and Wang and Pai (2001) for wastewaters. Rueter and Johnson (1995) and Krumins et al. (2001) found that light ozonation had a flocculent effect, increasing TSS concentration that could be separated afterward. These results collectively suggest that there is a critical ozone dose determining how solids in wastewater are affected. Below the critical dose, ozone acts as a flocculent; above it, solids are solubilized (Grasso and Weber, 1988). Despite massive solids destruction, the foam in this study still had a non-flow-weighted average concentration of 935 ± 137 mg/l VTS in all experimental stages, indicating solubilization and oxidation of the organic fraction of the solids.

Following ozonation, the trickling filter added 7.7–12.5% TSS to the stream due to cell growth. TSS values decreased with the organic loading of the stream (i.e., from Stages 1 and 2 to Treatments 3 and 4).

Chemical flocculation treatment removed 78.9–86.0% of TSS (Table 3). A linear, positive correlation was observed between TSS removal during chemical flocculation and the ozone dose applied (slope = 0.155; r^2 = 0.91), despite lack of correlation between these two parameters in the ozone reactor. Grasso and Weber (1988) suggested that improved TSS removal occurs because of the flocculent-enhancing effect of ozone upon colloidal and particulate matter in wastewater.

3.3. Turbidity

A dark brownish color in the raw effluent was due to suspended solids, colloids, and dissolved humic matter, most of which disappeared after combined physical and chemical treatment. The raw effluent had many large, rapidly settling particles, complicating a precise determination of turbidity. Repeated measurements showed average treatment values ranging from 131.9 to 139.5 NTU (Table 4). After sedimentation, turbidities in the two working

Table 4
Turbidity variation in different treatment units and percent removal over the entire treatment train

Treatment	Turbidity (NTU)					Overall removal (%)
	RI	SB	DR	OR	CF	
1	131.9	69.8[a]	59.1[a]	23.9[b]	5.7[a]	95.7[a]
2				21.0[ab]	4.5[ab]	96.6[ab]
3	135.5	67.7[a]	52.1[a]	18.3[a]	4.2[b]	97.0[b]
4				20.8[a]	4.3[ab]	96.8[ab]

The mean values represent non-flow-weighted averages for an entire experimental treatment. Means with the same superscript letters (a and b) are not significantly different ($p > 0.05$).

streams differed significant ($p = 0.046$), indicating that longer sedimentation had a beneficial effect on turbidity. Mean total turbidity reduction ranged between 5.7 NTU in Treatment 1 and 4.2 NTU in Treatment 3 after chemical flocculation.

An example of turbidity dynamics over the treatment train for Stage 3 is presented in Fig. 3. Initial turbidity of the greenhouse effluent was less than one-third that of the growout facility, and the two-fold difference persisted through the treatment train. For both waste streams, sedimentation, ozonation and chemical flocculation had greatest impact on turbidity reduction. Initial turbidity in the growout facility stream was reduced sequentially through the sedimentation basin, ozone reactor, and chemical flocculation by an average of 46.7, 73.0, and 75.0%, respectively. The turbidity of greenhouse stream was reduced by an average of 32.6, 65.7, and 81.8%, respectively.

The impact of ozonation on particle stability is most commonly quantified as change in turbidity (Grasso and Weber, 1988). Ozone reacted with the mix of pollutants in BRA effluent, resulting in mineralization or destabilization of most organic matter, which then became prone to fractionation. Foam resulting from ozonation of the greenhouse stream had higher turbidity than foam from ozonation of the growout system effluent, probably due to higher concentrations of solid and dissolved organics in the greenhouse stream.

Efficient reduction of turbidity during chemical flocculation likely resulted from ozone interacting with pollutants in the previous step, as suggested by the correlation with TSS. Improved flocculation after ozonation is explained by polymerization of meta-stable organics and subsequent adsorption and inter-particle bridging or charge neutralization (Grasso and Weber, 1988). This results in particle stabilization, with newly formed polymers having polyvalent ions from the flocculation agent at their core. With 95.7–97.0% average removal in our study, turbidity was one of the most dramatically improved parameters.
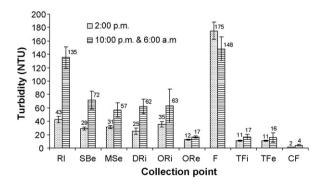

Fig. 3. Treatment train profile for turbidity variation in Treatment 3. Treatment conditions: working stream flow rate = 4 lpm, ozone reactor residence time = 9 min, ozone dose = 82.5 mg O_3/l water. Mean values $\pm$ standard deviations shown (n = 3 for 2:00 p.m. and n = 6 for 10:00 p.m. and 6:00 a.m.).

Table 5
Raw influent, sedimentation basin, and chemical flocculation COD mass non-flow-weighted average concentration, percent COD removal in different units, and overall percent COD removal across the treatment train for each treatment

Treatment	COD (mg/l)			%COD removal/unit				Overall removal (%)
	RI	SB	CF	SB	OR	TF	CF	
1	562.2	229.1	68.2	59.2	19.8[b]	28.9	44.9	87.9
2			54.6		27.8[ab]	31.1[a]	47.8[a]	90.3[a]
3	584.6	218.0	44.4	62.7	40.7[a]	34.4[b]	50.1	92.4
4			54.9		30.0[a]	32.5[ab]	44.8[a]	90.6[a]

Means with the same superscript letters (a and b) are not significantly different ($p > 0.05$).
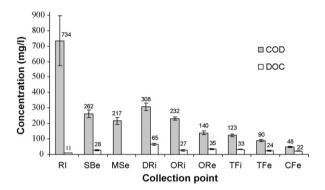

Fig. 4. Treatment train profile for COD and DOC variation in Treatment 3. Mean values ± standard deviations shown ($n = 6$).

3.4. COD

COD in the raw influent was between 562.2 and 584.6 mg/l, decreasing to 44.4–68.2 mg/l after chemical flocculation (Table 5). The sedimentation basin removed an average 59.2 and 62.7% of COD for the two experimental flows, a statistically significant difference ($p = 0.02$). These removal percentages were lower than expected for the overflow rate used in this study, indicating that a large proportion of organics could have been dissolved or trapped in the unsettled colloids. However, subsequent treatment steps – such as ozonation, two forms of biological oxidation, and chemical flocculation – removed most of the remaining COD. The highest average percent COD removal was 92.4% in Stage 3. A profile of COD dynamics through the train in Treatment 3 is presented in Fig. 4; the values represent averages only for samples collected at 10:00 p.m. and 6:00 a.m.

Percent COD removal in the ozone reactor showed a positive, linear relationship with ozone dose applied (slope = 0.452; $r^2 = 0.99$). A smaller correlation was observed between percent COD removal in chemical flocculation and ozone dose (slope = 0.116; $r^2 = 0.74$). Table 6 shows that total COD removal, $\eta(COD_t)$, in Treatment 3 at an ozone dose of 82.5 mg/l (40.7%) was more than double that in Treatment 1 at an ozone dose of 36.6 mg/l (19.8%). Similar COD removals in Treatments 2 and 4 (27.8 and 30.0%, respectively) indicate that high ozonation achieves the same COD removal in less time than low ozonation. Paraskeva et al. (1998) also found that ozone dose was the main factor determining COD removal, although foam removal was not addressed in their study.

Table 6
Parameters characterizing ozonation tests and COD variation during the four treatments with respect to system definition, experimental procedure, and results assessment

Parameter	Units	Experimental condition			
		1	2	3	4
t_H	min	6	6	9	9
V_L	l	36	36	36	36
Q_L	l/min	6	6	4	4
Q foam	% of Q water	3.6	4.0	6.9	5.4
COD in	mg/l	230	221	224	221
COD out	mg/l	185	160	133	155
COD_t rmvd.	mg/l	45	61	91	66
Foam COD	mg/l	756	928	899	792
COD rmvd. as foam	mg/l (%)	27.2 (60.4)	37.0 (60.6)	60.5 (66.5)	42.5 (64.4)
COD ox.	mg/l (%)	17.8 (39.6)	24.5 (39.4)	30.7 (33.5)	23.8 (35.6)
O_3 dose in water	mg O_3/l	36.6	55.0	82.5	55.0
C_{G0}	mg/l	22	33	33	22
C_{Ge}	mg/l (%)	0.62 (2.8)	3.73 (11.3)	4.22 (12.8)	2.32 (10.5)
C_{Le}	mg/l (%)	0.07 (0.32)	0.55 (1.66)	1.17 (3.54)	0.64 (2.91)
tO_3 residual	%	3.14	12.96	16.32	13.41
O_3 reacted	%	96.86	87.04	83.68	86.59
$F(O_3)$	mg/l s	0.102	0.153	0.153	0.102
$r(O_3)$	mg/l s	0.098	0.131	0.127	0.086
$r_A(O_3)$	mg/l s	0.099	0.136	0.133	0.091
$r(COD_t)$	mg/l s	0.125	0.169	0.169	0.122
$r(COD_{ox})$	mg/l s	0.049	0.068	0.057	0.044
I^*	g O_3/g COD	0.159	0.249	0.368	0.249
A^*	g O_3/g COD	0.155	0.221	0.321	0.223
$\eta(O_3)$	%	97.18	88.70	87.21	89.45
$\eta(COD_t)$	%	19.8	27.8	40.7	30.0
$\eta(COD_{ox})$	%	7.74	11.09	13.71	10.77
$Y(O_3/COD_t)$	g O_3/g COD	0.78	0.77	0.75	0.70
$Y(O_3/COD_{ox})$	g O_3/g COD	1.98	1.92	2.23	1.94

The tricking filter performed well, removing an average of 47.7 mg/l in Treatment 1, 40.4 mg/l in Treatment 2, 35.3 mg/l in Treatment 3, and 41.3 mg/l in Treatment 4. Comparing mass to percent COD removals in the tricking filter (Table 5), Treatment 3 showed lowest amount of COD removed, and the highest percent removal. A statistically significant difference was observed between Treatments 1 and 3, presumably due to more efficient total COD removal during ozonation in Treatment 3 (Table 6), leaving less COD to the tricking filter.

Chemical flocculation removed a maximum of 50.1% of COD on average in Stage 3, while 44.8–47.8% were removed in the other three stages (Table 5). There was a statistically significant difference between Treatments 1 and 3, but not between Treatments 2 and 4. Although differences were not large, results suggested that waste-waters treated with more ozone responded better to chemical flocculation, as reported by Grasso and Weber (1988). Alternatively, better flocculation in Treatment 3 could result from lower COD content of the stream exiting the trickling filter, allowing more flocculating agent to be effectively available for reaction. This explanation is supported by the larger percent TSS removal obtained by chemical flocculation for Treatment 3 (Table 3).

3.5. Reaction of ozone with COD

As noted above, ozone was suspected responsible for strong foam formation by BRA wastewater. This linkage was supported by the positive correlation (slope = 0.081; $r^2 = 0.96$) between the ozone dose and the volume of foam removed, expressed as a percent of working stream flow (Table 6). Table 6 also shows other parameters characterizing ozonation and COD response. The COD concentration in foam did not appear to be dependent on the concentration of COD in the ozone reactor influent. This was probably due to the different degree of foam dilution and COD mineralization, $\eta(COD)$, at various ozone doses.

Two ozone dose rates, $F(O_3)$, characterized this study at steady state (0.102 mg/l s in Treatments 1 and 4, and 0.153 mg/l s in Treatments 2 and 3). The best ozone absorption [$r_A(O_3)$, mg/l s] and consumption [$r(O_3)$] rates were observed in Treatment 1 [$r_A(O_3) = 97.0\%$ and $r(O_3) = 96.0\%$ from $F(O_3)$] under conditions of lowest ozone dose and longest hydraulic residence time, t_H. At the other extreme set of conditions (Treatment 3), $r_A(O_3)$ was 86.9%, and $r(O_3)$ was 83.0% of $F(O_3)$. Treatments 2 and 4, in which the same dose of ozone was applied at different hydraulic loading rates, had similar values for these two parameters. These findings indicate that t_H and ozone concentration could be equally important for the reaction under the conditions we tested. However, comparison of results from Stages 1 and 3 suggests that less ozone in gas and longer t_H could result in better ozone absorption and consumption. This interpretation is supported by the values of ozone transfer efficiency [$\eta(O_3)$, %], which represents a ratio between the specific ozone absorption (A^*, g O_3/g COD) and the specific ozone dose (I^*, g O_3/g COD). $\eta(O_3)$ was 97.2% in Stage 1 and 87.2% in Stage 3, indicating closer values of A^* and I^* in Stage I. Findings of better transfer at lower ozone concentration agree with results of the treatability study (Sandu, 2004). Gottschalk et al. (2000) attributed lower $\eta(O_3)$ at high ozone concentration to faster depletion of COD, reducing the chance of ozone reacting (i.e., A^* becomes smaller relative to I^*). Our results indicated that the near-zero dissolved ozone concentration in Treatment 1 could be due to rapid ozone reaction with pollutants, such that the transfer rate limited the reaction rate. In contrast, in Treatment 3 (and to a smaller extent, in Treatments 2 and 4), residual dissolved ozone was observed, indicating that the reaction regime was kinetically controlled. Besides faster COD reduction under conditions of intense ozonation at a certain t_H, Beltran et al. (2001b) suggested

that formation of compounds more refractory toward ozone also could decrease $\eta(O_3)$. Despite lower $\eta(O_3)$, Treatment 3 showed the largest quantity of ozone transferred, performance attributed to Henry's law (Masschelein, 1985; Mazzey et al., 1995).

We used COD to assess reactions of organic pollutants with ozone. Parameters estimated were rate of COD removal [$r(COD)$, mg/l s], degree of COD removal $\eta(COD)$, and ozone consumption coefficient [$Y(O_3/COD)$]. Because of partial removal of COD in foam, these parameters were determined separately for total COD removed (CODt) and for COD oxidized (CODox). This double determination allowed quantification of the advantages of foam removal for the ozonation process (Table 6). For the extreme conditions tested, total removal rates $r(CODt)$ were 0.125 and 0.169 mg/l s in Treatments 1 and 3, corresponding to degrees of removal [$\eta(CODt)$] of 19.8 and 40.7%, respectively. In terms of COD oxidized, $\eta(CODox)$ was 7.7% in Treatment 1 and 13.7% in Treatment 3. Expressed as percentages of total COD removed, amounts oxidized were 39.6% in Treatment 1 and 33.5% in Treatment 3, with intermediate values for the other two stages. The smaller percent COD oxidized in Treatment 3 could be explained by $\eta(O_3)$ being 10% lower than in Treatment 1, and consequently, a smaller portion of the ozone dose reacted. Additionally, $Y(O_3/CODox)$ was larger in Treatment 3 (2.23 g O_3/ g COD) than in other stages (1.92–1.98 g O_3/g COD), indicating decreased ozone efficiency with increased dose. An explanation for this phenomenon was provided by Beltran et al. (1999a), as referred to above. When total COD removed was considered in determining Y, values between 0.70 and 0.77 g O_3/g CODt were obtained. Values obtained for Y as a function of COD oxidized are typical of those from wastewater experiments. Our results suggest that by generating foam, the efficacy of ozonation could be enhanced up to three-fold with regard to COD removal. Maximum COD removal, 40.7% under conditions of 87.2% ozone transfer efficiency, suggests that an ozone dose above that of Treatment 3 could be used to reach the maximum limit of feasibility for ozonation of wastewater, suggested by Kirk et al. (1975) to be 50–70% COD removal. However, a feasibility limit should be determined in a case-by-case basis and must take into account additional factors such as initial COD loading, final effluent quality desired, pollutant composition and resistance to ozone attack, techniques for separating foam, and costs associated with low ozone transfer efficiency.

3.6. cBOD5

A treatment train profile of cBOD5 dynamics in Treatment 3 is presented in Fig. 5. Starting from 77.3 to 74.5 mg/l in settled
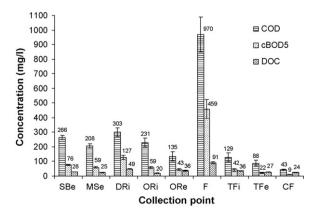

Fig. 5. Treatment train profile for COD, cBOD5 and DOC variation in Treatment 3. Mean values ± standard deviations shown ($n = 2$).

Table 7
$cBOD_5$ dynamics through the pilot plant: initial and the final $cBOD_5$ mass non-flow-weighted average concentrations, percent $cBOD_5$ removal in different treatment units, total removal for the treatment train, and values and biodegradability assessment for the OR

Treatment	$cBOD_5$ (mg/l)		$cBOD_5$ removal/unit (%)				$cBOD_5$/COD		
	SB	CF	OR	TF	CF	Total	OR_i	OR_e	Increase (%)
1	77.3	15.0	19.0	45.7	42.6	79.9	0.315	0.330	4.5[b]
2		15.5	24.8	41.1	41.4	80.6	0.270	0.285	5.2[ab]
3	74.5	9.0	27.6	48.6	58.1	88.1	0.250	0.315	20.6[a]
4		12.5	20.4	38.0	47.2	82.9	0.250	0.285	12.2[ab]

Means with the same superscript letters (a and b) are not significantly different ($p > 0.05$).

influent, $cBOD_5$ was reduced by a maximum of 88.1% in Treatment 3, to 9 mg/l average concentration after chemical flocculation (Table 7). $cBOD_5$ increased because of methanol addition before denitrification, but was reduced to approximately its initial level in the denitrification reactor effluent. The observation that $cBOD_5$ was not reduced to lower levels in that effluent indicates that sufficient (or even excess) methanol was added to fully support denitrification, despite fluctuations of NO_3^--N concentration in the influent. Results showed that the biodegradability index (i.e., the $cBOD_5$:COD ratio) was between 0.24 and 0.29 in the sedimentation basin effluent, much lower than that of a typical domestic wastewater of 0.5–0.6. Low biodegradability appears to be common in aquaculture effluents (Herbst, 1994; Easter et al., 1996), probably due to the presence of particulate and dissolved organic materials derived from uneaten feed, fish feces and mucus. Mainly proteins, lipids and polysaccharides, these high molecular weight compounds are not appropriate substrates for bacteria. To render them suitable for bacterial consumption, these substances should be hydrolyzed, commonly by fermentation. However, the effluent from the aquaculture systems had no time to ferment because it entered the treatment train shortly after its release. Under these circumstances, use of an external carbon source to support denitrifiers became necessary. In contrast, in sewage, most of the soluble organic matter is in the form of acetate and other short-chain fatty acids, because there is more time for fermentation as the wastewater flows to the treatment plant (Grady et al., 1999).

During ozonation, 19.0–27.6% of the $cBOD_5$ was removed (in Treatments 1 and 3, respectively), but the biodegradability index increased by 4.5 and 20.6%, respectively (Table 7). The difference in results between Treatments 1 and 2 was statistically significant. The increase of biodegradability was technically due to a greater decrease of COD than for $cBOD_5$ under certain conditions of reaction. For example, the difference can be observed in Fig. 5 by comparing the values of these parameters at collection points before and after the ozone reactor. Improvement of wastewater biodegradability by ozonation has been reported by Medley and Stover (1983), Rivas et al. (2000), Beltran et al. (2001a), and Tosik and Wiktorowski (2001), although Rosenthal and Kruner (1985) observed that at high ozonation levels, $cBOD_5$ removal from aquaculture wastewater ceased. In a similar situation, Beltran et al.

(1999b) suggested that improved biodegradability becomes limited at excessive ozone doses because chemical oxidation produces highly oxidized products with little metabolic value for microorganisms. Hence, large amounts of ozone could be wasted upon easily biodegradable reaction intermediates, and system efficiency could be decreased. In our study, increase of ozone dose and biodegradability improvement proved beneficial for removing $cBOD_5$ in the trickling filter and even in chemical flocculation. Treatment 3, in which the highest ozone dose was applied, showed the best results in this respect, with 48.6% $cBOD_5$ removal by trickling filter and 58.1% $cBOD_5$ removal by chemical flocculation. This suggests that none of the ozone doses tested were above the level at which biodegradability was impaired.

In foam, the biodegradability index ranged between 0.46 and 0.58 (Fig. 5). This high degradability of organics was probably due to efficient contact and reaction of ozone with organics in the thin walls of bubbles even after they rose above the water surface. Intense reaction in foam also could help explain the destruction of TSS and COD, which were not in significant amounts in condensed foam. Regardless of the reason, the foam may prove useful as a substrate for controlled fermentation, generating volatile fatty acids that could replace methanol in the denitrification reactor. This approach would require additional installations (i.e., fermentation reactors), but would reduce the costs of operation as savings for methanol and condensed foam removal.

3.7. DOC

Tests for DOC showed no significant difference between influent and effluent concentrations; all values were between 20.8 and 24.5 mg/l. In sedimentation basin effluent, DOC represented 11.3% of COD concentration in Treatment 1 and 10.7% in Treatment 3, but after chemical flocculation, the ratios increased to 32.9 and 49.6%, respectively (Fig. 5). The change of ratio was due to significant decrease of COD, with little overall variation of DOC. This suggests that an increase in the oxidation state of organics occurred as the stream passed through the treatment units, notably during ozonation (Yu and Yu, 2000). Large fluctuations of DOC concentrations were observed through the treatment train (Table 8). Average DOC concentrations in the mixing basin are presented in order to assess the consumption of

Table 8
Dissolved organic carbon dynamics through the pilot plant: initial DOC mass non-flow-weighted average concentrations, average DOC generation (+) or loss (−) and percent DOC variation in different treatment units

Treatment	SB	MB		DR		OR		TF		CF	
	(mg/l)	mg/l	(%)	mg/l	(%)	mg/l	(%)	mg/l	(%)	mg/l	(%)
1	23.4	69.9	(+198.7)	28.5	(−59.2)[a]	33.3	(+14.4)[a]	23.3	(−26.8)[a]	21.7	(−6.9)[a]
2		38.4	(+25.8)[b]	24.8	(−29.1)[a]	23.1	(−7.3)[a]				
3	23.0	59.7	(+159.6)	27.2	(−54.4)[a]	35.6	(+23.6)[ab]	23.1	(−30.6)[a]	21.3	(−7.9)[a]
4		37.6	(+27.7)[b]	22.1	(−33.9)[a]	20.6	(−7.2)[a]				

Means with the same superscript letters (a and b) are not significantly different ($p > 0.05$).

added methanol in denitrification reactor. Methanol addition increased the organic substrate accounted for as DOC by an average of 198.7 and 159.6% for each of the two working flows. The addition of methanol at a constant flow rate was probably in excess when the NO_3^--N was at a low concentration in the influent stream. Nevertheless, there were cases (i.e., at high NO_3^--N concentration in the influent) in which the denitrification process used some of the effluent DOC in addition to the added methanol, resulting in lower DOC in the denitrification reactor effluent. These findings contradict those of Balderston and Sieburth (1976) and Arbiv and van Rijn (1995), who suggested that organics from aquaculture wastewaters include mostly long-chain carbon molecules unsuitable for sustaining denitrification. Overall, on a non-flow-weighted average basis, 59.2–54.4% of the denitrification reactor influent DOC (native plus methanol) was removed by cell growth during denitrification from each working stream. There was no statistically significant difference between the two NO_3^--N concentrations or percent removals, suggesting that the stoichiometric ratio of 3.6 mg CH_3OH/mg NO_3^--N was sufficient. Also, in situations when the stream NO_3^--N was around 50 mg/l, the concentrations of COD before entering the mixing basin and after denitrification were approximately the same. The ratio used in this study was consistent with those of Jeris and Owens (1975), Jeris et al. (1977) and Semon et al. (1997), ranging between 2.9 and 4.2 mg CH_3OH/mg NO_3^--N. Authors that recommended a ratio of 3:1 conducted their studies on wastewaters with much higher DOC, but did not specify the role of this form of DOC in denitrification. In this study, then, natural DOC was suitable for bacterial growth, which could explain the near-complete denitrification achieved when NO_3^--N concentrations above 50 mg/l were present in the influent. Alternatively, some of the NO_3^--N could have been converted to ammonia through assimilative reduction for use in cell synthesis, thereby increasing the ratio by making less NO_3^--N available for denitrification.

Following ozonation, stream DOC increased by 14.4–27.7% (i.e., from ozonation average influent of 27.2–28.5 to 33.3–37.6 mg/l). DOC appears to be a byproduct of the ozonation process (Summerfelt et al., 1997; Kamiya and Hirotsuji, 1998). Wang and Pai (2001) attributed increase of DOC by up to 120% to decomposition of microorganisms and other unsettled solids; by filtering the same wastewater before ozonation, they obtained a 80% reduction of DOC after 2 h of ozonation. Because in our study results were obtained from a similar wastewater rich in microorganisms and solid biofloc, we infer that effluent DOC increased through a similar mechanism.

The degree of DOC generation in the ozone reactor was not linear with increase in ozone dose. The largest average percent increases were found in Treatments 2 and 4, the lowest increase in Treatment 1, and an intermediate increase in Treatment 4 (Table 8). Percent increases in Treatments 1 and 3 were statistically different, but those in Treatments 2, 3 and 4 were not. More DOC was generated than destroyed at lower ozone doses. As ozone dose increased, DOC reached a maximum as the two processes' rates equalized at approximately the ozone dose used in Stages 2 and 4, 55 mg O_3/l. Beyond this point, DOC destruction became predominant, resulting in a decline of DOC accumulation. Wang and Pai (2001) reported similar results, suggesting that the peak of DOC generation during ozonation coincided with the moment when all microorganisms were decomposed. Following that point, organic compounds accounted for as DOC were removed more rapidly by mineralization.

DOC removal in the trickling filter ranged between 20.6 and 23.1% and did not appear to depend on ozone dose. This finding indicated that at this point, a major part of DOC was not amenable to bacterial consumption. Studying post-ozonation DOC characteristics, Carlson and Amy (1997) showed that only a part of DOC is readily biodegradable (called DOC_{rapid}), while the remainder biodegrades more slowly (called DOC_{slow}); they reported that formation of DOC_{slow} was not sensitive to ozone dose, consistent with results from our study.

DOC was reduced by 6.9–7.9% during chemical flocculation, but the final effluent had an overall DOC approximately equal to that of the stream that entered the treatment train. However, there was probably a compositional difference, in that the final DOC was less biodegradable. Establishing the impact of this final DOC on fish if the stream is reused remains a subject for further investigation.

4. Engineering considerations

4.1. Summary of findings and recommendations

Work with the pilot station showed that the treatment strategy employed could support recovery and recycling of BRA effluent. The findings will be useful for design of a scaled-up system. Although settable solids, colloids, dissolved organic substances and nitrogenous compounds could be eliminated to an acceptable degree, refinements of the systems and operations are still needed.

Downstream treatment units would benefit from better solids separation at the beginning of the treatment train, because it will be harder and more expensive to remove solids from subsequent units. Despite an overall 99% removal of solids at the system level, sedimentation basin performance of around 70% removal was not considered satisfactory. TSS removal of 85% should be a minimum target for sedimentation, considering current technology used in primary wastewater treatment. Such performance could eliminate the need for microscreen filtration. Otherwise, for the case of BRA, higher solids concentration will interfere with operation of subsequent units. For example, high solids concentration would divert ozonation from removing dissolved and colloidal organics, and would affect operation of the denitrification reactor. Improvement of sedimentation could be achieved by better effluent release management (i.e., more uniform volumetric discharge), but also by more efficient sludge removal, preventing solids floatation. Design of the sedimentation process could be determined by the need for storing excess effluent; because a sufficient and continuous source of effluent is useful for effective operation of the treatment station, the need for storage may determine the size of the sedimentation basin. The discharged effluent should be passed as few times as possible, preferably none, through high-speed pumps before reaching the sedimentation basin, in order to prevent break-up of solids into colloidal particles.

COD was removed throughout the treatment system by sedimentation, ozonation, biological oxidation, and chemical flocculation, from an initial concentration of 562.2–584.6 mg/l in the raw influent, to 44.4–68.2 mg/l after chemical flocculation. These values were much lower than 145–173 mg/l COD found in the fish tanks. During ozonation, removal of COD was correlated with ozone applied within the range of 36.6–82.5 mg O_3/l water. Consequently, the performance of the ozonation unit was significantly different between the treatment stages. BRA effluent is rich in organics that react easily with ozone and form abundant foam, confirming findings from the treatability study (Sandu, 2004). From a maximum COD removal of 40.7% at 82.5 mg O_3/l water during a 9-min ozonation period, 64.4% of COD was removed by foam and the balance was mineralized, 83.7% of the injected ozone reacted, TSS was reduced by 77.7%, turbidity by 65.0%, and the water biodegradability index increased by 20.6%. Larger doses of ozone showed beneficial effects on performance of the trickling filter and chemical flocculation. The only potential problem appeared to be a slight accumulation of DOC during ozonation,

also observed during the treatability study. Ozone utilization decreased with ozone dose, but better overall COD removal was achieved at higher doses due to stronger foam formation. At 82.5 mg O_3/l water, ozone reactivity was 83.7%. The ozone reaction coefficient for COD oxidized [$Y(O_3/COD_{ox})$] was between 1.92 and 2.23 g O_3/g COD, and for total COD removed [$Y(O_3/COD_t)$], it was between 0.70 and 0.78. The difference indicates that due to foam elimination, the efficiency of the ozonation process could be enhanced by up to three-fold. The results suggested that high ozone doses are necessary in order to achieve a lower final COD concentration and better organic biodegradability. Study of the ozonation process suggested that doses as high as 100 mg O_3/l wastewater might be employed in a scaled-up system. To overcome the problem of wasting ozone, improvements could be achieved by diffusing smaller bubbles to improve ozone transfer and reaction efficiency. Improved removal of COD as foam also could be expected with this modification.

The biodegradability of BRA effluent is less than one-half that of domestic wastewater. A maximum of 88.1% $cBOD_5$ removal was obtained during this study, corresponding to a concentration of 9 mg BOD_5/l. This is around one-fourth that of concentrations in the fish tanks. Increasing ozone dose could reduce this $cBOD_5$ concentration further. A greater ozone dose also could reduce loading of the trickling filter and favor nitrification, with beneficial effects on final effluent.

DOC treatment management probably could be enhanced with automated synchronization between nitrate concentration and the dosage of methanol provided to the system (i.e., 3.6 mg CH_3OH:1 mg NO_3^--N).

This study indicated that the units and management practices evaluated would work successfully on a scaled-up version of the treatment station after refinement of management procedures. However, organics refractory to ozone treatment, heavy metals, halogens, and other substances could accumulate in the system. There is little information suggesting maximum concentrations tolerated by tilapia, especially for combinations of stressors. Our recommendation would be to control the levels of these constituents by periodically exchanging water to dilute concentrations experienced by fish. The volumes required and the frequency of exchanges will be determined by adaptive management once the system becomes operational. A scaled-up system will produce concentrated effluents such as sludge from sedimentation, foam from ozonation and chemically bonded sludge from flocculation. The fate and possible utilization of these residuals should be considered in the design of a full-scale operating system.

Acknowledgements

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