Ecological Engineering 37 (2011) 873–882

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

The efficiency of a closed-loop chemical-free water treatment system for cyprinid fish farms

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ARTICLE INFO

Article history:
Received 4 March 2010
Received in revised form 12 January 2011
Accepted 23 January 2011
Available online 10 March 2011

Keywords:
Glass fibre filters
Fish farming
Recirculation
Ultrasound
Ultraviolet lamps
Water treatment

ABSTRACT

The study presented has been carried out to evaluate the treatment performance, fish production and water consumption of a closed-loop chemical-free water treatment system for small-scale cyprinid fish farms. The closed-loop system consisted of a 36 m^3 experimental pond (Pond A) with initial carp load of 1 kg/m^3 (34 *Cyprinus c. carpio*); of a treatment train (TT) with a roughing filter (RF), glass fibre filters (GFF), and UV-C units (UV); and of an ultrasound unit (US) installed in the corner of the pond. The average circulation of the water in the closed-loop system was 2.3 times per day. Pond A was compared with a control pond (Pond B) of the same dimensions and fish load but with no TT or US. The TT was efficient in the removal of total suspended solids, biochemical oxygen demand, chemical oxygen demand, total coliforms (TC), and faecal coliforms (FC), reaching 35%, 42%, 33%, 37%, 91% and 91% removal, respectively. The majority of pollutant removal took place in the RF, while the GFF contributed mostly to the removal of TC and FC. UV did not contribute to the removal of bacteria, mostly due to low TC and FC inputs. The removal of nutrients in the TT (ammonia, nitrites, nitrates, total phosphorous and orto-phosphate) was not efficient. Despite this, Pond A had markedly lower nutrient concentrations compared to Pond B, and all the mean values of the measured parameters except nitrites and total phosphorous in Pond A were below legislation limit. Specific growth rate and fish body weight increase in Pond A were higher than in Pond B (0.3%/day, 0.2%/day, 152% and 115%, respectively) indicating better rearing conditions in Pond A. However, fish showed with 2.8 in Pond A and 3.3 in Pond B poor feed conversion rate over warm months. Higher water consumption in Pond A was due to various interventions during the pilot operation that can be reduced in normal operation. The results showed that the closed-loop system presented could be useful for semi-natural fish farming of 1–2 kg $fish/m^3$. However, the system should be improved with regular sedimented algae removal to avoid nutrient accumulation.

1. Introduction

Aquaculture has been increasing worldwide in production at a rate of between 8% and 10% per annum for the previous decade. Although the European sector has not matched these high levels, it still has seen significant increases (Dallimore, 2008). The profile of aquaculture in EU has also changed as a result of extension to eastern Europe. In western Europe, aquaculture is represented by intensive and semi-intensive systems with mostly marine fish species, while in the eastern countries traditional aquaculture is extensive, in ponds, with primarily freshwater fish species (Popa, 2008). Extensive aquaculture utilizes large amounts of freshwater from rivers, groundwater or lakes. The environmental risks of fish farming are well recognized. When decomposing, the not-ingested and surplus feed and fish excreta can reduce the oxygen content and increase nutrient concentration in the receiving water, thus leading to eutrophication (Gál et al., 2003). In recent years, scientists and river managers have also recognized the negative impacts of altered flow regimes on river ecosystems, due to fish farms. In order to alleviate or minimize these impacts, a number of different methods and approaches have been developed to define a regulated flow regime that will maintain healthy ecosystems and their benefits, while enabling concurrent human use of the water (Smolar-Žvanut et al., 2008).

One of the most important limiting factors in the global expansion of the aquaculture industry is the outbreak of infectious fish diseases. Coliforms and other bacteria present in water may penetrate all fish organs, even the muscle, reaching concentrations exceeding those observed in the surrounding water (WHO, 1981). Once a disease takes hold, options are limited to

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doi:10.1016/j.ecoleng.2011.01.004

chemical treatment, drug therapy, or the culling of infected populations. The drugs are effective but can lead to an increase in chemically resistant pathogens in ponds, requiring repeated applications that can be labour-intensive and increase production costs (Zimba and Grimm, 2008). In the past, most treatments in aquaculture used chlorine to oxidize the organic matter and to inactivate microbes. Chemicals used in a system may have undesirable effects on the water, the biological filter, the fish or employees (Yanong, 2003). Existing water disinfection processes, such as chlorination, have frequently failed to comply with the Safe Drinking Water Standards (e.g., Drinking Water Directive, Council Directive 98/83/EC) because of the formation of disinfectant by-products (Modak, 2008; Zimba and Grimm, 2008). The problem of disinfectant by-products underscores the need to develop a safe and reliable disinfection process to overcome the drawbacks inherent in the use of chemical disinfectants.

It is obvious that the global fish and aquaculture industry has come under increasing pressure to optimize production efficiency while reducing environmental load (Sardar et al., 2007). Sustainable water treatment technologies are therefore needed to reduce nutrient and chemical discharge into receiving waters in order to minimize both environmental impact and water abstraction to preserve the survival and reproduction of aquatic organisms in different hydraulic habitats and to enable "good ecological and chemical status" by 2015 covering all surface and ground waters (Popa, 2008; Smolar-Žvanut et al., 2008). In order to improve rational water use, which is especially important in areas with limited water supply, and to lower the environmental impact, intensive aquaculture systems with recirculation are being developed (Graber and Junge, 2009) with efficient control of water supply and quality, together with new methods of fish feeding (vegetarian feeds), which could offer better rearing conditions, and thus contribute to an increase in fish production. The growing concerns and regulations associated with a clean environment encourage the aquaculture industry to adapt recirculation systems to reduce and treat the wastewater discharge (Kim et al., 2000) and thus improve the ecological quality of surface waters according to European water policy.

This paper describes a monitored pilot operation under field conditions, i.e. a small-scale land-based cyprinid fish farm with a diversion of recirculating water into a closed-loop system consisting of three water treatment devices: glass fibre filters (GFF), ultrasound (US) and UV-C (UV) with a roughing filter (RF) as a pretreatment stage. GFF are used as a filter material for the filtration of different types of less-loaded waters (drinking waters, oligotrophic waters, etc.) (Salonen, 1979; Varjus et al., 2004). The application of GFF in aquaculture systems has not yet been documented, to our knowledge. US and UV are widely used for algae control (Ahn et al., 2003; Alam et al., 2001; Bosma et al., 2003; Oyib, 2009; Van Hannen and Gons, 1997; Zhang et al., 2006) and disinfection of water in different systems (Demirci and Krishnamurthy, 2007; Guo et al., 2009; Joyce et al., 2003; Stamper et al., 2008). In aquaculture, UV disinfection is commonly used (Yanong, 2003), while the application of US is still in research phases (Zimba and Grimm, 2008). The UV lamps emit ultraviolet light (a wavelength of approximately 254 nm is considered optimal) that penetrates cells and damages genetic material and proteins (Modak, 2008; Yanong, 2003). The major disadvantages of UV disinfection are: (1) UV intensity decreases sharply with its passage in water and its decrease is even more significant with high water turbidity; (2) microorganisms attached (hidden) to the suspended particles may escape UV irradiation, reducing the UV treatment efficiency; and (3) microbial DNA, once damaged by UV, can be repaired via enzyme repair systems (e.g., photolyase and excision repair), resulting in the survival of the microorganisms (Modak, 2008). In many cases, a water disinfection process using UV in conjunction with US is applied (Joyce et al., 2006; Modak, 2008; Yong et al., 2008). Sonication improves the UV disinfection kinetics of wastewater by breaking up large suspended particles (Yong et al., 2008), resulting in increased efficiency of combined UV–US treatment (Joyce et al., 2006). Due to water recirculation, the closed-loop system presented was also expected to enable water savings in accordance with European water policy. In contrast to the investigated closed-loop system, in recirculating aquaculture systems biological wastewater treatment is commonly in use, mainly as biofilm reactors, biofilters, moving beds and an activated sludge process. However, due to the relatively high expense of those technologies (especially for small fish farms) and unstable operation, recirculation aquaculture systems are not widespread (Wik et al., 2009). Besides the investigated closed-loop system, constructed wetlands (CW) could be sustainable cost effective alternatives (Sindilariu et al., 2009) with the usage of various sand filters (Brovelli et al., 2009) or free floating aquaponic systems (Graber and Junge, 2009). However, filters made from glass fibres can be considered an upgraded version of CW using quartz sand filters, but with the added advantage of a highly increased inner surface area. Compared with sand filters, fibres could also have major advantages due to lower volumes, easier handling and maintenance, and reduced construction costs. Furthermore, the presented multi-functional integrated technology has all the benefits of physical water treatment without any use of chemicals, since UV and US act as disinfectant and algae inhibitors, while water circulation can contribute to water savings.

The objective of this study was to evaluate the treatment performance, fish production and water consumption of a pilot closed-loop system for small-scale land-based cyprinid fish farms consisting of GFF, US and UV with a RF as a pre-treatment stage. Our hypothesis was that GFF, US and UV devices can restrain suspended solids as well as dissolved nutrients, counteract algae growth and act as a disinfectant.

2. Materials and methods

2.1. Description of a pilot system

The research was carried out at the experimental fish farm located in Ajdovščina in Slovenia. The experiment was performed in two fish ponds (length 9 m, width 5 m, depth 0.8 m, volume 36 m^3) of which one served as an experimental (Pond A), and one as a control pond (Pond B) (Fig. 1). A commercially available US transducer (LG Sonic® Tank, range 50 m, power 12 W, with dual core multi frequency technology, 20–200 kHz) was installed, floating in the corner of Pond A. From Pond A the water was pumped by a bypass in a treatment train (TT) consisting of the RF as a pre-treatment step, the GFF, and the UV. The TT with Pond A form a closed-loop system, in which the water was treated first by the RF with the dimensions 2.25 m^2 (1.5 × 1.5 m) and height 1.1 m. The RF was filled from the bottom up with gravel with a grain size of 4/8 mm to a height of 0.5 m, 8/16 mm sand to a height of 0.3 m, and with sand gravel with a grain size of 6/22 mm in ratio of 1:1 up to a height of 1.1 m. The RF was followed by eight units of submersed GFF with length 175 cm, diameter 30 cm, weight 5 kg, covering 13.2 m^2, a filtration capacity of particles <0.1 mm and with a flow rate capacity of 0.5 m^3/h per unit, and two UV units running in parallel with a power of 40 Watt, wavelength 210–400 nm and with a flow rate capacity of 4 m^3/h per UV. In UV sterilization, water passed over the UV-emitting lamps encased in a quartz sleeve and flowed back to Pond A. Both ponds had constant aeration (disk diffuser). Beside aeration, Pond B did not receive any treatment. At the start of the experiment, both ponds were filled with the groundwater from the

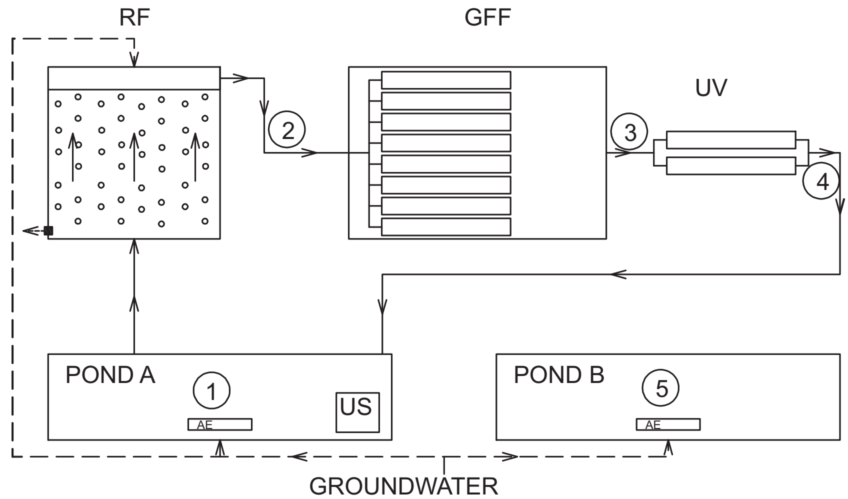

Fig. 1. Experimental set up of the pilot system consisting of an experimental pond (Pond A), a control pond (Pond B), an aeration unit (AE), a roughing filter (RF), glass fibre filters (GFF), UV-C units (UV) and an ultrasound transducer (US). Circled numbers mark sampling points—1: in Pond A, 2: after the RF, 3: after the GFF, 4: after the UV which coincides with the effluent from the treatment train, and 5: in Pond B.

nearby source. Groundwater was also added in the ponds during the experiment when water conditions threatened fish population regarding threshold values (pH > 10, NO_2-N > 0.6 mg/L, dissolved oxygen (DO) < 3 mg/L) and to compensate for evaporation losses. The groundwater was also consumed for maintenance of the TT and to clean the ponds during the time of fish measurements. The RF was cleaned weekly with flush-back into the sewage. UV devices were cleaned and calibrated when the transmission rate dropped below 30%. The water flow rate through the TT was approximately 4 m^3/h over the whole testing period.

2.2. Monitoring of the pilot system

The pilot system was monitored from May 2007 to June 2008. Sampling points are marked in Fig. 1. DO, pH, electric conductivity (EC), and temperature were measured twice a day (8 am and 8 pm) in both ponds and once a day (8 am) after the UV, using WTW Multiline/F portable meters, throughout the monitoring period. Water from both ponds, and effluents from the RF, the GFF and the UV were sampled two to three times per month from May 2007 to November 2007 and from February 2008 to May 2008 for total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), orto-phosphate (PO_4-P) and total phosphorous (TP) according to Standard Methods (APHA et al., 2005). Microbiological parameters of total coliforms (TC) and faecal coliforms (FC) were analyzed once per month in both ponds and after the RF, the GFF and the UV by the Most Probable Number (MPN) method (APHA et al., 2005) from June 2007 to September 2007 and from February 2008 to March 2008. The samples for chlorophyll *a* analyses were taken in both ponds once a month from May 2007 to May 2008. Due to the fact that planktonic algae live primarily near the surface of stagnant water bodies (Wetzel, 2001), chlorophyll *a* samples were collected just below the surface. Samples were collected at one station near the middle of the pond, which was assumed to be adequate for a simple characterization of a possible trend in chlorophyll *a*. Extraction of the chlorophyll *a* was performed as follows: a 10 mL suspension was filtered using a Whatmann glass fibre filter (GF/F), diameter 47 mm, cat. no. 1825-047. The filtration was made using an apparatus made of a filter holder, a Buchner flask, and a vacuum pump. Five replicates were performed for each sample. For the determination of chlorophyll *a*, the GF/F were ground up in 10 ml of a 90% acetone solution and incubated for 24 h, at 6 °C in darkness to prevent the pigment denaturing. After incubation, the solution was placed in a 3.5 mL glass cuvette and the optical density (OD) of the supernatant was measured at three wavelengths: 663, 645, and 630 nm. A solution of acetone at 90% was used as a blank. The chlorophyll *a* concentrations were calculated according to the equation of Scor/Unesco (1969) chlorophyll *a* concentration (mg m^{-3}) = (11.64 OD_{663} − 2.16 OD_{645} − 0.1 OD_{630}) * (volume of acetone (mL)/volume of sample (L)).

The starting fish load in both ponds was 1 kg/m^3 or 34 carp (*Cyprinus c. carpio* Linnaeus 1758) in each pond. The fish body weight (BW) increase was measured at the beginning of the pilot operation in May 2007, in September 2007 and at the end of the experiment in June 2008. The fish were hand fed with DAN-EX KARPFEN 32/2 fish food on average 0.5 kg once per day in spring, summer and autumn and on average 0.09 kg once per day in winter, based on fish needs. The fish were fed less in the winter due to reduced fish metabolism at low temperatures. The amount of fish food was the same for both ponds. In the case of fish mortality, the dead fish were registered, veterinary inspected by the Fisheries Research Institute of Slovenia, weighed and removed from the pilot system. The specific growth rate (SGR) according to Chao et al. (2005) and food conversion rate (FCR) were calculated. In the calculations the mortality was adequately considered.

3. Results

3.1. Water quality of the pilot system

Table 1 summarizes physical, chemical and microbiological parameters of the water in both ponds, and of the effluent from the TT. There was no marked difference in the mean values of DO, pH and EC between Pond A and Pond B. There was also no obvious difference in mean morning and evening DO, pH and EC values between the ponds. However, in the summer, morning DO values were higher in Pond A compared to Pond B (Fig. 2), while pH values show the opposite pattern. In Pond B, there was a wide range in pH values (6.0–11.5); higher pH values were measured especially in warmer periods of the year. Mean EC was higher in Pond

Table 1
Mean (±1 standard deviation) and range for measured parameters in the experimental pond (Pond A), in the control pond (Pond B) and after the treatment train (TT) of Pond A in comparison with Slovene, Italian and Austrian legal requirements. Exceeded legislation values are marked in bold.

	Unit	*n*	Pond A		Pond B		TT		Limit value according to legislation		
			Mean ± stand. dev	Range	Mean ± stand. dev.	Range	Mean ± stand. dev	Range	Slovene (1)	Italian (2)	Austrian (3)
Dissolved oxygen	mg/L										
8 am		195	9.8 ± 2.4	1.0–20.8	9.2 ± 3.1	2.1–19.5	7.3 ± 2.5	3.4–12.3	≥ 5	≥ 5	5–9
8 pm		195	10.1 ± 2.6	1.6–15.3	10.0 ± 3.1	2.3–19.2	.	.	≥ 5	≥ 5	5–9
pH											
8 am		195	8.0 ± 0.5	6.8–9.6	8.1 ± 0.8	6.0–11.5	8.0 ± 0.4	7.4–8.7	6–9	6–9	6.5–8.5
8 pm		195	8.1 ± 0.5	6.8–9.4	8.3 ± 0.8	6.3–10.4	.	.	6–9	6–9	6.5–8.5
Electric conductivity	μS/cm										
8 am		195	415 ± 80	216–620	340 ± 102	141–610	431 ± 84	319–590	–	–	–
8 pm		195	420 ± 81	220–580	346 ± 107	160–834	.	.	–	–	–
Temperature	°C										
8 am		195	14.9 ± 6.5	1.3–29.7	13.8 ± 7.2	0.3–26.7	17.2 ± 6.7	4.8–25.3	–	max 28	16–26
8 pm		195	16.5 ± 6.8	1.8–29.5	15.9 ± 8.0	1.2–31	–	–	–	max 28	16–26
TSS	mg/L	25	14.3 ± 11.3	0.5–35.0	**178.0** ± 115.3	14.5–372.0	3.5 ± 4.9	0.2–20.5	≤ 25	25	–
BOD_5	mg/L	25	2.9 ± 1.9	0.9–6.5	**53.8** ± 28.1	14.3–87.2	1.4 ± 0.9	0.4–3.0	≤ 6	6	–
COD	mg/L	25	21.2 ± 9.1	9.3–37.7	230.8 ± 131.1	31.8–407.0	13.8 ± 7.4	6.8–28.8	–	–	–
NH_4-N	mg/L	25	0.07 ± 0.02	0.04–0.14	**0.39** ± 0.72	0.06–3.09	0.07 ± 0.05	0.04–0.26	≤ 0.16	0.16	–
NO_3-N	mg/L	25	0.79 ± 1.01	0.01–3.37	0.43 ± 0.78	0–2.57	0.95 ± 1.12	0.06–3.81	–	–	–
NO_2-N	mg/L	25	**0.04** ± 0.06	0–0.23	**0.28** ± 0.57	0–2.06	**0.03** ± 0.07	0–0.27	≤ 0.01	0.01	0.02–0.03
PO_4-P	mg/L	25	0.35 ± 0.42	0.03–1.69	0.72 ± 0.89	0.03–2.93	0.40 ± 0.45	0.03–1.60	–	–	–
TP	mg/L	25	**0.31** ± 0.27	0.08–0.69	**2.22** ± 0.62	1.52–2.71	0.30 ± 0.22	0.19–0.68	≤ 0.4	0.14	–
Chlorophyll *a*	mg/m^3	13	60 ± 82.3	6.7–304.4	909 ± 1321	78.1–4880.5	–	–	–	–	–
TC	log MPN/100 mL	10	2.1 ± 1.1	0.6–3.4	2.0 ± 1.1	0.3–3.4	0.6 ± 0.9	0.3–2.7	–	–	–
FC	log MPN/100 mL	10	2.1 ± 1.1	0.3–3.4	1.6 ± 1.3	0.3–3.2	0.4 ± 0.2	0.3–0.8	–	–	–

(1) According to Slovenian standards for cyprinid surface waters concentrations presented in Decree on the quality required of surface waters supporting fresh-water fish life (Official Gazette of Slovenia, No 46/2002).
(2) Italian legislation denotes water quality required supporting fresh-water fish life (Legislative Decree no. 152 of 3 April 2006, Official journal no. 88 of 14 April 2006).
(3) Austrian legislation denotes water quality requirements for carps (Bohl, 1982).
– no data.

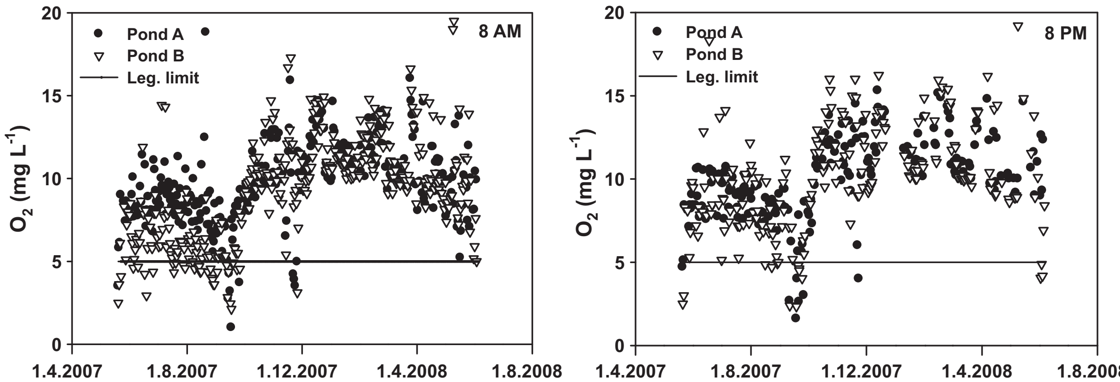

Fig. 2. Dissolved oxygen concentrations measured daily at 8 am and 8 pm in the experimental pond (Pond A) and in the control pond (Pond B) during the experimental period with legislation limit stated in Table 1.

A compared to Pond B; however, in Pond B it had wider range and standard deviation. In Pond A and in the effluent from the TT, mean EC and its range were similar. Temperatures in Pond A and in Pond B were the highest during summer with maximum of 29.7 and 31 °C, respectively, and the lowest during winter with minimum of 1.3 and 0.3 °C, respectively (Table 1). In January 2008, both ponds were briefly frozen.

Mean values of TSS, BOD_5, COD, NH_4-N, NO_2-N, PO_4-P and TP were lower in Pond A compared to Pond B; however, mean values of NO_3-N were lower in Pond B (Table 1). Mean values of TSS, BOD_5, and COD and were lower in the effluent from the TT compared to the Pond A, while the concentration of NO_3-N was slightly lower in Pond A, indicating an accumulation of nitrates in the TT. In Pond A and in the effluent from the TT, mean values of NO_2-N, PO_4-P and TP were in the same range. Standard deviations for all parameters were in the same range as the mean values, showing high fluctuation over time (Table 1). Chlorophyll *a* values were markedly lower in Pond A, compared to Pond B, due to the US in Pond A (Table 1). Mean log values of TC were in the same range in both ponds, while an unexpected higher FC contamination in Pond A was noticed. The effluent from the TT had a markedly lower bacterial content compared to both ponds (Table 1). The bacterial contamination in both ponds was much lower during winter/spring than during summer (Fig. 3).

The mass inputs and outputs of pollutants from Pond A per m^2 are compared in Table 2. It is clearly seen that in the closed-loop system, Pond A is a source of pollution, namely the loads of TSS, BOD, and COD, and bacteria are markedly higher at the output from Pond A compared to the input. NO_2-N and TP loads are slightly higher at the output from Pond A, while the inputs and outputs for NH_4-N, NO_3-N and PO_4-P were similar.

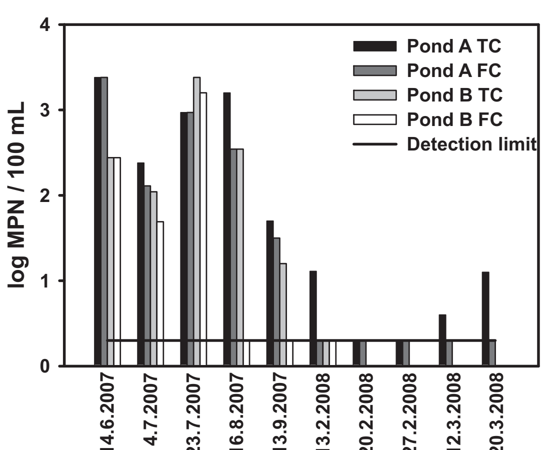

Fig. 3. Seasonality of bacterial contamination in the experimental pond (Pond A) and in the control pond (Pond B). TC: total coliforms, and FC: feacal colifirms.

3.2. *Removal efficiency of the treatment train*

The TT showed elimination of TSS, BOD_5, and COD (Table 3). The mean mass removal percentages for listed parameters were rather low (from 33% for COD to 42% for BOD_5), which can be explained by fairly low mass inputs into the TT, which have to be reduced to even lower levels, and high variability in mass loads. The mass loads of NH_4-N, NO_2-N, TP and PO_4-P were consistent through the TT and there was an increase in NO_3-N load resulting in negative mass removal. The majority of pollutant removal took place in the RF, especially for TSS, BOD and bacteria. The TT was highly efficient in the elimination of TC and FC and reached 91% removal. GFF contributed mainly to the removal of bacteria. The removal was high both for TC and FC; however, the deviations in bacteria numbers at the outflow from the TT were more consistent for FC compared to TC (Table 3).

3.3. *Fish monitoring*

Table 4 summarizes results of fish monitoring during the experimental period. The BW increased from May to September by 152% (25.8 kg) in Pond A and by 115% (21.9 kg) in Pond B at 72 kg of total feed load per pond. From October 2007 to June 2008, BW increased by 122% (8.8 kg) in Pond A and by 127% (10.2 kg) in Pond B at 43 kg of total feed load per pond. The total feed load was for each pond 115 kg over the 389 days of the experiment. SGI was in warmer months in Pond A 0.67%/day and in Pond B 0.55%/day, while it was

Table 2
Mass input into the experimental pond (Pond A) from the treatment train and mass output from Pond A into the treatment train.

	Unit	*n*	Mass input into pond A	Mass output from pond A
TSS	$g/m^2/day$	25	4.7	24
BOD_5	$g/m^2/day$	25	2.2	4.9
COD	$g/m^2/day$	25	22	36
NH_4-N	$g/m^2/day$	25	0.12	0.13
NO_3-N	$g/m^2/day$	25	1.9	1.6
NO_2-N	$g/m^2/day$	25	0.056	0.065
PO_4-P	$g/m^2/day$	25	0.68	0.62
TP	$g/m^2/day$	25	0.45	0.51
TC	MPN $10^6/m^2/day$	10	0.85	10
FC	MPN $10^6/m^2/day$	10	0.032	7.6

Table 3
Mean (±1 standard deviation) mass input (IN) to the treatment train (TT) of the roughing filter (RF), glass fibre filters (GFF) and the UV-C lamp (UV); mass outputs (OUT) from individual units and mass removal of TT (given in weight or MPN units and percentage).

	Unit	*n*	IN	OUT RF	OUT GFF	OUT UV	Mass removal of TT	Mass removal of TT (%)
TSS	kg/day	25	1.06 ± 0.87	0.30 ± 0.47	0.24 ± 0.34	0.21 ± 0.20	0.81 ± 0.86	35%
BOD_5	kg/day	25	0.22 ± 0.16	0.12 ± 0.09	0.11 ± 0.08	0.10 ± 0.06	0.10 ± 0.13	42%
COD	kg/day	25	1.63 ± 0.75	1.01 ± 0.52	0.97 ± 0.59	0.98 ± 0.46	0.51 ± 0.30	33%
NH_4-N	g/day	25	6.0 ± 3.0	6.3 ± 3.5	5.9 ± 3.5	5.4 ± 3.2	0.2 ± 3.5	-6%
NO_3-N	g/day	25	73 ± 96	85 ± 95	84 ± 98	86 ± 102	−10 ± 14	−81%
NO_2-N	g/day	25	2.9 ± 4.6	2.2 ± 5.5	2.3 ± 5.2	2.5 ± 5.5	0.7 ± 2.0	7%
PO_4-P	g/day	25	28 ± 31	31 ± 37	31 ± 35	30 ± 33	0.4 ± 6	−12%
TP	g/day	25	23 ± 19	22 ± 23	22 ± 18	20 ± 13	−1.6 ± 5	−24%
TC	MPN 10^6/day	10	480 ± 760	130 ± 280	10 ± 22	40 ± 130	430 ± 670	91%
FC	MPN 10^6/day	10	340 ± 660	40 ± 82	1.6 ± 1.4	1.4 ± 1.5	430 ± 720	91%

Table 4
Results of fish monitoring from May 2007 till June 2008 in the experimental pond (Pond A) and in the control pond (Pond B).

	Unit	May 07/September 07 (spring/summer)		October 07/June 08 (winter/spring)	
		Pond A	Pond B	Pond A	Pond B
Fish load	kg/m^3	0.68–1.7	0.76–1.64	1.7–1.92	1.64–1.94
Fish biomass[a]	kg	17–42.8	19–40.9	39.1–47.9	38.2–48.4
Fish biomass growth	kg	25.8	21.9	8.8	10.2
Average fish weight	kg	0.74–1.86	0.64–1.36	1.86–2.28	1.36–1.72
Body weight increase	%	152	115	122	127
Specific growth rate	%/day	0.67	0.55	0.08	0.09
Food conversion rate		2.8	3.3	4.9	4.2
Feed load total	kg	72	72	43	43
Mortality[b]	%	32.3–8.7	11.8–6.7	0	0

[a] The fish biomass was measured in May 2007, September 2007 and June 2008.
[b] Fish mortality was detected in May 2007 and in September 2007; the dead fish were removed from the pilot system.

similar in colder months (0.08%/day in Pond A, 0.09%/day in Pond B). In warmer months FCR was in Pond A 2.3 and in Pond B 3.3, while in colder months FCR was 4.9 and 4.2, respectively. Within the first 10 days of the experiment, 32.3% of the fish died in Pond A and 11.8% of the fish died in Pond B. In September 2007, 8.7% fish in Pond A and 6.7% fish in Pond B died.

3.4. Daily water circulation and water consumption

The circulation of the water in the closed-loop system fluctuated during the experimental period from 0 to 4.6 cycles per day, with an average of 2.3 times per day (Fig. 4). There was no recycling of the water when the closed-loop system was switched off due to maintenance work (cleaning of the filters, the UV and the ponds). Recirculation rates varied due to variable hydraulic conductivity, especially before and after the RF cleaning procedure. The total amount of consumed groundwater for Pond A and for the maintenance of the TT was 518 m^3, of which 290 m^3 was consumed for the maintenance of the TT (mostly cleaning the RF) and 80 m^3 for compensation of evaporation losses and water addition due to exceeded threshold values, while the total amount of consumed groundwater for Pond B was 292 m^3, of which 118 m^3 was consumed for compensation of evaporation losses and water addition due to exceeded threshold values. High amounts of groundwater were consumed for the cleaning of the ponds in September 2007 (98 m^3 in Pond A and 129 m^3 in Pond B) and in June 2008 (50 m^3 in Pond A and 45 m^3 in Pond B) (Fig. 5).

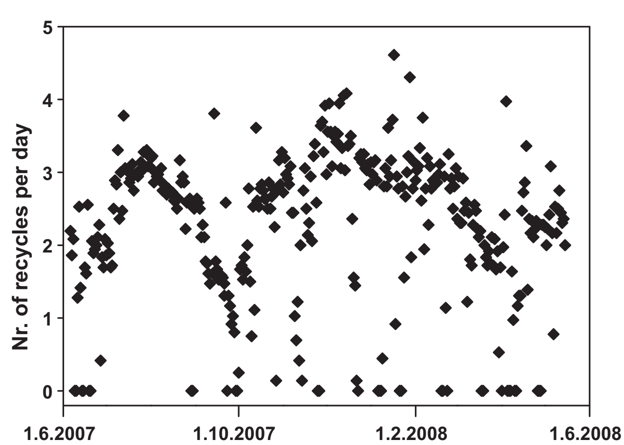

Fig. 4. Daily water recirculation in the closed-loop system during the experimental period.

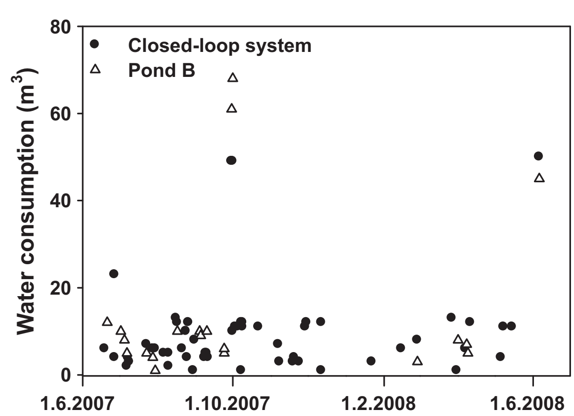

Fig. 5. Water consumption for the closed-loop system and for Pond B.

4. Discussion

4.1. Performance efficiency of the pilot system

DO values below the threshold of 3 mg/L were present in both ponds in less than 1.5% of measured values, mostly due to the constant aeration that was necessary to preserve the living conditions for fish. However, low DO values were detected more often in Pond B in the warmer period of the year. In general, oxygen and pH conditions were better in Pond A compared with Pond B, probably due to the efficient control of algae by US. The efficiency of US was also manifested as a clear visual effect on the water transparency and was demonstrated with markedly lower chlorophyll *a* values in Pond A in comparison to Pond B, which have already been reported on algal study in the pilot system presented (Krivograd Klemenčič and Griessler Bulc, 2010). The ratio between PO_4-P and TP was almost 1 in Pond A, indicating that no P in particulate form was present, whereas in Pond B this ratio was 0.3 showing the prevalence of particulate form, i.e. phytoplankton. Low morning DO values indicate a lack of oxygen at nights, most probably because of the respiration of algal flora in the early and middle summer and its decomposition in the late summer, while low evening DO values indicate intensive decomposition in late summer (Fig. 2). According to Wetzel (2001), decomposition of algae in late summer could reduce the oxygen content of the entire water body to near anoxia. Daily pH values varied in both ponds. The threshold value of pH 10 was exceeded only in Pond B in summer, mainly in the evenings. In May 2008, pH in Pond B reached 11.5, which can be explained by the extraction of CO_2 from the water through assimilation into algal biomass at a rate faster than can be replaced through atmospheric CO_2 diffusion, respiration and fermentation processes; readjustment of carbonate equilibrium leads to an increase in pH level (Wetzel, 2001). When DO and pH values in the ponds exceeded threshold values, groundwater was added in order to prevent fish mortality. The measured EC values in both ponds were in the typical range for natural water bodies in Slovenia (Krivograd Klemenčič and Toman, 2010); the difference in EC between the ponds was negligible. Temperatures in both ponds show high seasonal dynamics, with freezing temperatures during winter and heating of the ponds during summer. Higher evening temperatures compared to the morning temperatures in both ponds were due to direct exposure of the ponds to solar radiation.

The TT was significantly efficient in the removal of TSS, BOD_5, COD and bacteria, but the removal of nutrients was low or negative. Besides the efficient elimination of TSS, BOD_5, and COD through the TT, it seems that US could partly contribute to the lower concentrations of listed parameters in the closed-loop system, namely by efficient algae control. Mean NH_4-N levels were markedly lower in Pond A, compared to Pond B. A high NH_4-N value of 3.09 mg/L in Pond B was measured only once (26.9.2007) and contributed to a higher mean value and standard deviation. However, when excluding extremely high NH_4-N concentration, the mean NH_4-N concentration in Pond B also remains above legislation limit of 0.16 mg/L. Total ammonia nitrogen (TAN) consists of un-ionized ammonia (NH_3) and ionized ammonia (NH_4^+); the former of which is highly toxic to fish. The proportion of TAN in the un-ionized form is dependent upon the pH and temperature of the water. At higher pH and water temperatures, the percentage of toxic unionized ammonia could be high (Alam and Al-Hafedh, 2006); however, in September 2007 the measured pH value in Pond B was near neutral and the water temperature was below 20 °C. For this reason, we assume that NH_3 could not threaten fish health. At that time, 8.7% (Pond A) and 6.7% (Pond B) of the fish died. The mortality for Pond B was lower than for Pond A; therefore, a connection with a toxic impact of NH_3 and NH_4-N values cannot be proved. Lower NH_4-N concentrations and higher NO_3-N concentrations in Pond A compared to Pond B, and higher concentrations of NO_3-N in the effluent from the TT compared to Pond A are most probably the consequence of nitrification occurring in the TT, while denitrification was negligible due to aerobic operation. This was also confirmed by negative mass removal for NO_3-N through the TT. However, NH_4-N concentrations entering the TT (i.e. from 0.04 to 0.14 mg NH_4-N/L) might be too low to support a significant growth of nitrifying bacteria in the RF and the GFF, as stated by Yang et al. (2001) that values below 0.5 mg NH_4-N/L are limiting for abundant bacterial growth. Despite this, our results indicate that some nitrification was still carried out in the TT. Aquatic species can tolerate high concentrations of NO_3-N (>100 mg/L), while NO_2-N can be harmful to them. However, the concentrations of NO_2-N and NO_3-N were lower in both ponds than observed by Alam and Al-Hafedh (2006) in green water fish tanks, which were in the range of 0.63–0.87 mg NO_2-N/L and 31.51–61.04 mg NO_3-N/L. In this study, nitrate levels were also below the values reported to affect the early-life stages of fish (Kincheloe et al., 1979). The groundwater used to replenish the ponds was polluted by agriculture and presented an additional source of NO_3-N for both ponds. The mean values of NO_3-N in groundwater (1.78 ± 0.70 mg NO_3-N/L) were higher than the mean values of NO_3-N in both ponds, revealing NO_3-N uptake by algae in the ponds. However, due to depletion of algae by US the uptake of NO_3-N was in Pond A noticeable lower and concentrations of NO_3-N noticeable higher. Lower values of NO_3-N, and NO_2-N in both ponds in winter could be attributed to the lack of fertilization of agricultural areas in winter season. NO_2-N concentrations in Pond A were below the threshold value of 0.6 mg/L, but they often exceeded legislation limit in warm months. Mean NO_2-N concentrations were much lower in Pond A compared to Pond B where threshold values were occasionally exceeded. Exceeded limit NO_2-N values in Pond A can be explained with the reduction of the nitrification processes in the summer season due to the lack of oxygen at nights, higher feeding load, high temperature and agricultural pollution. Vymazal (2001) reported that nitrification processes will continue until concentrations of DO decline under 2 mg O_2/L. Below this concentration, diffusion rates of oxygen to the bacteria becomes critical. During the day, DO levels in both ponds probably increased, but water samples for chemical analyses were sampled at 8 am when DO levels were still low (Fig. 2). At the time the threshold value in the Pond B was exceeded, groundwater was added to the pond to prevent fish mortality. Phosphate levels also differed markedly between the ponds. Mean TP in Pond A exceeded only the Italian legislation limit, while in the Pond B mean TP exceeded all cited legislation limits. In both ponds, the legislation limits were exceeded mostly in warm months. TP was lower in Pond A compared to Pond B probably due to sedimentation of planktonic algae by US and weekly RF flush-back. A slight increase in mass output from Pond A indicating a release of particulate phosphorous from the pond, while PO_4-P decreased in Pond A due to algae uptake. Inputs and outputs of TP were consistent through the TT indicating no removal efficiency mostly due to limited amount of sorption sites (Arias and Brix, 2005).

TC and FC values were higher in both ponds in warm months showing seasonal dynamic of bacterial contamination reported also by De Donno et al., 2002. Higher bacteria values at that time could be attributed to higher temperatures, higher feeding activity of the fish and higher amount of added polluted groundwater in that time of the year. TC and FC mass removal efficiencies of the TT were high (91% for both TC and FC). UV was expected to contribute to the removal of bacteria; however, this was not the evident case. Low removal of bacteria in the UV was due to already low bacterial input into the UV, which was due to the efficient elimination of bacteria in the RF and the GFF. The bacterial contamination levels observed in Pond A were unexpectedly high despite efficient disinfection by the TT, even higher than in Pond B. The reason could be that bacteria which passed through the TT were efficiently reproducing due to low generic times (Métris et al., 2005) and favourable retention time in Pond A (on average 9 h). According to Modak (2008) microbial DNA can be repaired via repair systems after passing the UV, resulting in survival of bacteria. Higher bacterial contamination levels in Pond A compared to Pond B were probably due to successful removal of bacteria consuming algae by US. According to the list of algae (Krivograd Klemenčič and Griessler Bulc, 2010) several algal taxa present in Pond A belonged to mixotrophic organisms (Graham and Wilcox, 1999), which are significant consumers of bacteria (Zubkov and Tarran, 2008). Despite the differences in bacterial contamination between the ponds, the observed levels of TC and FC in both ponds were much below the results normally noted in fish ponds (Davis and Goulder, 1993; Harnisz and Tucholski, 2010) and in winter were mostly below detection limits (Fig. 3). The reason for the low bacterial contamination on general could be in low fish stocking in the presented experiment. Nevertheless, the composition and abundance of bacterial communities in fish ponds are very important, since the qualitative and quantitative content of microorganisms in the digestive tract of fish depends on the level of hygiene and microbiological quality of the environment (Buras et al., 1987; Harnisz and Tucholski, 2010).

In general, mass inputs into Pond A from the TT for NH_4-N, NO_3-N, TP, and PO_4-P were similar to the outputs from the pond, although Pond A was a source of those nutrients accumulated mostly in sedimented algae. The results showed that the TT was noticeable efficient in the mass removal of TSS, BOD_5, COD and bacteria, but the removal of nutrients was low or negative. Despite this, Pond A had markedly lower nutrient concentrations compared to Pond B, as a result of sludge removal from the closed-loop system by flush-back of the RF and algae sedimentation by US. The results showed that the RF was found to be an important treatment unit to prevent clogging and enable the operation of the GFF. Similarly to this, Vollertsen et al. (2009) reported that sand filters were used to prevent clogging of sorption filters that followed after the sand filters in a stormwater treatment pond. Efficient removal of suspended matter but no removal of dissolved NH_4-N, NO_3-N, NO_2-N and PO_4-P in the RF indicates that the main treatment process is filtration because no evident adsorption processes and no microbial denitrification to remove NO_3-N, NO_2-N were present. However, a microbial activity was evident with an increase in nitrates in the RF, where the majority of microbial activity was carried out in the top layer of the filter (Aslan and Cakici, 2007), resulting in a sludge layer on the top, which was removed during flush-back of the filter. UV did not contribute to the removal of pollutants, except FC. Fluctuations in the mass loads of the measured parameters during the whole experimental period were high, indicating unstable operation of the closed-loop system due to the dynamic properties of the system. Since the required feed and the waste produced by the fish depends on fish type, age and size, the resulting characteristic time of the recirculation system stabilization may range up to several months (Wik et al., 2009). Water quality fluctuations, such as temporary increases in ammonia or nitrite, can, by themselves, result in disease or significant losses. These environmental fluctuations often lead to suppressed immune systems and greater susceptibility to pathogens (i.e. disease-causing organisms, such as bacteria, parasites, fungi, and viruses) and disease outbreaks (Yanong, 2003), which could be the reason for the fish mortality in the experiment presented.

4.2. The comparison of measured parameters with legal requirements

Slovenian and Italian legislation only refer to fresh water quality for cyprinid species, whereas Austrian legislation defines water quality requirements for the cultivation of carp. The limit values for fish cultivation are usually higher than the requirements for fresh water. According to the legislations presented in Table 1, the mean values of DO, pH, EC and temperature in both ponds met the standards; however, in single measurements DO and pH in both ponds exceeded the legislation limit of pH 5 mg/L, and 8.4 (9) especially in warmer months and mostly in Pond B. pH values that exceeded legislation limits were higher in Pond B compared to the exceeded values in Pond A, indicating that the TT contributed to the mitigation of pH values due to its buffering capacity. According to Slovenian standards for cyprinid surface waters, the concentration limits for nitrites were exceeded in Pond A, while the mean values of TSS, BOD_5, TP and ammonium were below the limits. In contrast, in Pond B the limits according to Slovene legislation for nitrites, TSS, BOD_5, ammonium, and TP were exceeded. The same is also true for the comparison of measured parameters with the Italian legislation, with the exception of TP. The latter has a noticeably lower limit in Italian legislation and was therefore also exceeded in Pond A. Among TSS, BOD_5, NH_4-N, NO_2-N and TP, Austrian legislation on carp cultivation (Bohl, 1982) limits only NO_2-N. Although the Austrian limit concentration for nitrites is higher compared to Slovenian and Italian legislation, the mean values of NO_2-N in the closed-loop system and in Pond B did not meet the given limits. None of the discussed legislation limits the amount of microorganisms in cyprinid surface waters or in waters for fish cultivation; however, it is very important to monitor their hygienic and sanitary quality in order to avoid risk to public health. Fish from faecal polluted waters can be contaminated by enteric human pathogens and may pose a potential risk to public health (De Donno et al., 2002). According to the legislation limits, the closed-loop system met the legislation requirements more often and consistently compared to Pond B.

4.3. Fish biomass

During the summer, the most relevant period for fish farming, the BW increase was higher in Pond A than in Pond B. SGR was for Pond A on average higher (0.3%/day) than in Pond B (0.2%/day) indicating better rearing conditions. However, fish showed poor food conversion efficiency in both ponds. Based on the Slovene data from seminatural fish farms with 1.5 food conversion rate on average (Kalin, 1984; Bravničar, 2003), it can be concluded that in the experiment presented fish were overfed mostly due to quick hand feeding. In Pond A fish production was 1.04 kg/m^3/year, which was 19 times higher than in the Slovene carp farms with 0.055 kg/m^3/year on average, according to Bravničar (2003). In Pond B fish production was 0.88 kg/m^3/year, which was 16 times higher than in the same Slovene carp farms (Bravničar, 2003). The results showed that Pond B more frequently (8 times) exceeded the threshold values regarding DO values in the main production period (summer 2007) than Pond A (4 times). Pond A was efficient at a stocking density of 1–2 kg fish/m^3, although a higher fish load could lead to conditions that threaten fish population regarding Jana (1998) who found that carp are more sensitive to low levels of DO and high levels of ammonia than some other fish species (tilapia or catfishes) and need a larger water area for growth. Within the first days of the experiment fish died in both ponds. Based on veterinary inspection, the reason was fish parasites that occurred after transportation to the experimental side. The mortality in

September 2007 possible occurred due to the fluctuations of measured parameters.

4.4. Daily water circulation and water consumption

Water recirculation through the closed-loop system was variable due to the changing hydraulic conductivity of the filters and unstable operation of the pumps. Therefore, in further studies, the recycling should be optimized to reach the best removal rates and constant water flow through the closed-loop system. Constant supervision of flow meters by the maintenance stuff is especially important in the case of full scale implementation of such system. Additionally, the re-designing of RF should be considered in order to decrease the clogging and thus cleaning periods and water usage. In Pond A, groundwater consumption during the experimental period was higher in total due to the maintenance of the TT (mostly cleaning the RF) than in Pond B, where water consumption was based mostly on fish demands regarding pH, NO_2-N and DO and on compensation of evaporation losses. Less groundwater was consumed for both ponds in winter due to low fish metabolism, low amount of added fish food, and the absence of algae bloom. From November 2007 to March 2008, 87 m^3 was consumed for Pond A and cleaning of the RF, while only 3 m^3 of groundwater was added to Pond B (Fig. 5). However, an automatic control system could probably lower the consumption rate for the RF cleaning. The amount of groundwater consumption for the cleaning of the ponds in September 2007 could be lower with more efficient cleaning equipment with no difference between the ponds. Water use efficiency in Pond A (water consumption of 518 m^3 (0.05 L/m^2/day)) was 0.045 kg/m^3 inflow water, while water use efficiency in Pond B (water consumption of 292 m^3 (0.02 L/m^2/day)) was 0.076 kg/m^3 inflow water. The water consumption for Pond A was four times less and for Pond B 6.7 times less in comparison with fish farms in Slovenia (Bravničar, 2003).

5. Conclusions

Chemical-free treatment of aquaculture water for recirculation purposes is a promising tool to support the further development of sustainable aquaculture industry without excessive water demands and chemical use. The study presented evaluated the performance efficiency, fish production and water consumption of the closed-loop chemical-free water treatment system for small-scale cyprinid fish farms. Our hypothesis that combination of glass fibre filters (GFF), ultrasound (US) and UV-C (UV) devices can restrain suspended solids as well as dissolved nutrients, counteract algae growth and act as a disinfectant was partially confirmed. The results showed that the closed-loop system was efficient in removal of total suspended solids, biochemical oxygen demand, chemical oxygen demand and bacteria but the removal of nutrients was not considerably efficient. The US successfully inhibited algae growth while the GFF and the UV did not show treatment performance as expected. The majority of pollutant removal took place in the pretreatment unit of roughing filter. GFF contributed to the removal of faecal coliforms and total coliforms, while UV did not contribute to further water disinfection. Legislation limits in the closed-loop system were met for all the measured parameters except nitrites and total phosphorous. In the experimental pond, higher fish production was achieved compared to the control pond due to better rearing conditions. The closed-loop system presented could be useful for semi-natural fish farming with fish load of 1–2 kg/m^3. Water consumption due to recirculation was for the experimental pond 4 times less and for the control pond 6.7 times less in comparison with extensive cyprinid fish farms. Higher water consumption in the experimental pond was because of various interventions during the pilot operation that can be reduced in normal operation. The system presented can be an efficient alternative chemical-free solution for the removal and inactivation of microbes and algal cells and the linked harmful potential in fish farms. However, the system could be improved with sedimented algae removal. The further research will focus on the same closed-loop system where a vertical constructed wetland will replace the fibre filters to improve nutrient reduction.

Acknowledgements

This work was carried out within the project CHEM-FREE (Development of a chemical-free water treatment system through integrating glass fibre filters, ultrasound and UV-C; Contract No. COOP-CT-2006-032719; duration: 1.7.2006–30.6.2008), a Cooperative Research Project (CRAFT) funded within the Horizontal Research Activities involving SMEs of the EU 6th Framework Programme. The authors are also grateful for the support of the Fisheries Research Institute of Slovenia.

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