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APPLICATION OF NATURAL ZEOLITES AND MACROPHYTES FOR WATER TREATMENTIN RECIRCULATION AQUACULTURE SYSTEMS

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Abstract

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The macrophytes enrich the water with oxygen and help to remove nutrients. The zeolite is also a natural way to combat pollution. The aim of this study was to investigate the effectiveness in waste water treatment process in RAS, applying filter with natural zeolites and macrophytic plants from genus *Lemna* and *Elodea*. The temperature and pH were measured daily with a portable combined meter in the newly constructed control and experimental RAS. Ammonium, nitrite, nitrate, total nitrogen and phosphor us were measured spectrophotometrically. At the end of the trial the fish were weighed and specific growth rate and FCR (feed conversion ratio) were calculated. The addition of zeolite and macrophytic plants as a part of filtration system of experimental RAS, decreased significantly the quantity of nitrogen and phosphorus compounds, compared to their amount in conventional one. The better water quality in experimental system, due to the presence in the filter of zeolites and macrophytic plants, influenced positively the growth of rainbow trout and feed utilization.

Key words: RAS, zeolite, *Elodea*, *Lemna*, water treatment

Introduction

Nitrogenous compounds (ammonia, nitrite, and nitrate) are considered as major contaminants in aquaculture wastewater. Toxic effects from their presence in the water include impairment of physiological parameters factors, such as growth rate, oxygen consumption and disease resistance in fish species (Chan, 2003; Aiyuk et al., 2004). Zeolites are able to remove ammonium and ammonium nitrogen in water (Marking and Bills, 1982). It has a large pore space and has many applications, such as gas absorption, odor control and water filtration for municipal and residential drinking water and aquariums (Pak et al., 2002). Thus make them attractive for wastewater treatment in aquaculture and they were object of different research in the past for removal of dangerous metabolites from living environment – in fish hauling and holding tanks (Bower and Turner, 1982), in recirculation systems and in shrimp pond (Silapajarn et al., 2006).

Macrophytes in aquaculture are used as biofilter, fish feeding, non-pollutant agent, turbidity reducing agent. Aquatic plants contribute to nutrient transformation by a setting of physical, chemical and microbial processes, besides removing nutrients for their own growth.

Different researches retraced possibility aquatic plant from genera *Lemna* (Wang et al., 2002; Mkandawire et al., 2004; Goulet et al., 2005; Stout and Nusslein, 2005) and *Elodea*(Bishop and Eighmy, 1989; Mkandawire and Dudel, 2007) to be used for phytoremediation process.

Recirculation aquaculture systems (RAS) represent a new and unique way to farm fish. They possess variety of advantages, compared with other technological options in aquaculture. The most important from them includes maximization of the production, combined with the limited need of water and land and almost full control of environmental parameters during the cultivation, etc.

High costs, associated with waste treatment of RAS have triggered the growth of efforts from aquaculture scientists to find the cheaper and economically feasible approach for filtration of wastewater in these systems. There are different studies, which explore the possibility toxic waste compounds to be removed from the water of RAS by zeolites or assimilated by macrophytes. There is a gap in knowledge in this field, which dealing with wastewater treatment in RAS, when both – zeolites and plants are used.

The aim of this study was to investigate the effectiveness in waste water treatment process in RAS, applying filter with natural zeolites and macrophytic plants from genus *Lemna* and *Elodea*.

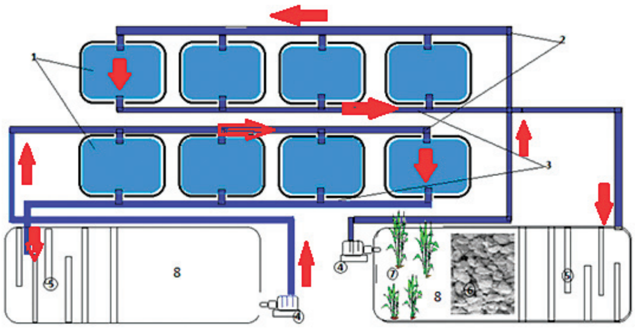

Fig. 1. Control and experimental systems, used during the trial

Materials and Methods

The trial was conducted at Experimental base of aquaculture in Agricultural Faculty of Trakia University, Stara Zagora.

Fish

Healthy, without visible injuries rainbow trout were chosen and transported from Fish farm "Bukovetz", Tvardica. The period of acclimatization lasted one week. The average initial live weight of rainbow trout from both experimental variants was as follows:

- Conventional RAS (cont. RAS) – 31.19 ± 0.81 g ;
- Experimental RAS (exp. RAS) –30.56 ± 0.69 g.

The stocking density, used during the trial was 200 pcs.m^{-3}. The fish were fed with extruded trout feed, with content of 40% crude protein. The daily ratio was determinate on the base of water temperature and live weight of cultivated fish. The fish were fed manually, three times per day. The trial lasted 40 days.

RAS

The experiment was conducted in two models of recirculation systems. Each of them consisted of 4 tanks, with volume of 0.06 m^3 (Figure 1). The first recirculation system (experimental) waste water was treated by a mechanical filter (sedimentation tank), biofilm bioreactor, where natural zeolites and macrophytic plants from genus *Lemna* and *Elodea* were situated. Zeolites were delivered from deposit „Beliplast", Kyrdjali district from the company „Bentonit" AD. The zeolite was recharged with a solution of NaCl (10%) every 5 days and replaced fully in the middle of the experiment. Macrophytes were harvested and transported from Lake Zagorka in Stara Zagora. The macrophytes were determined by Flora Reipublicae Popularis Bulgaricae, vol. II (Jordanov et al., 1963). The conventional RAS (control) consisted from mechanical filter (sedimentation tank) and biological filter (Figure 1). Continuous aeration in both biological filters was assured during the trial. The turn over in systems were maintained at 8 l.min^{-1}, which assured completely three times per hour hanging of water in every tank, from each system.

Daily fresh water in volume of 20 l was added to every system for compensation of water loses from evaporation and cleaning process.

Hydrochemical parameters

Water pH, dissolved oxygen (ppm) and temperature (°C) were measured daily. The measurement of this parameters was made by portable pH meter (Model HQ30D) and for ammonium, nitrites (mg.l^{-1}), nitrates (mg.l^{-1}), total nitrogen (mg.l^{-1}) and phosphate (mg.l^{-1}) was used spectrophotometer (DR 2800 – Hach Lange®), with appropriate cuvette tests.

Table 1

Methods and range of tests, used for monitoring the water quality parameters during the experiment

Quality parameters	Determination method	Measuring range
Ammonia	Indophenol blue	0.015–2 mg.l^{-1}
Nitrite – nitrogen	Diazotization	0.015–0.6 mg.l^{-1}
Nitrate – nitrogen	2.6 dimethylphenol	5–35 mg.l^{-1}
Total nitrogen	Koroleffdigestion$^+$ 2.6 dimethylphenol	5–40 mg.l^{-1}
Phosporus (ortho + total)	Phosphormolybdenumblue	0.05–1.5 mg.l^{-1}PO_4–P 0.15–4.5 mg.l^{-1}PO_4

The samples for their determination were taken once every five days. The measurement ranges and methods of used tests during the trial are shown in Table 1.

Measurement of experimental fish

The live weight (g) and standard length (SL, cm) of cultivated rainbow trout were measured in the start and in the end of trial.

Specific growth rate was calculated (SGR) as [(lnWf.–lnWi)/T]*100, where Wf – final weight, Wi – initial weight, T-time interval (number of days).

Food conversion ratio FCR was calculated as follows F/(Wf–Wi), where F – the amount of feed administrated, (Wf–Wi) – growthgain;

Survival rate (%) and consumable weight (g) were also calculated in the end of experiments by the following formulas:

$$\text{Survival rate (\%)} = \frac{\text{Number of surviving fish}}{\text{Number of initial fish}} \times 100.$$

Weight of eviscerated fish(%) = [weight of fish – (weight of gills + internal organs)]

Data analysis

The received from the trial data were statistically analyzed by T-test (Microsoft office, 2010).

Results and Discussion

Hydrochemical parameters

Temperature of the water

During the experimental period the values of water temperature were similar in two recirculating systems (Figure 2)

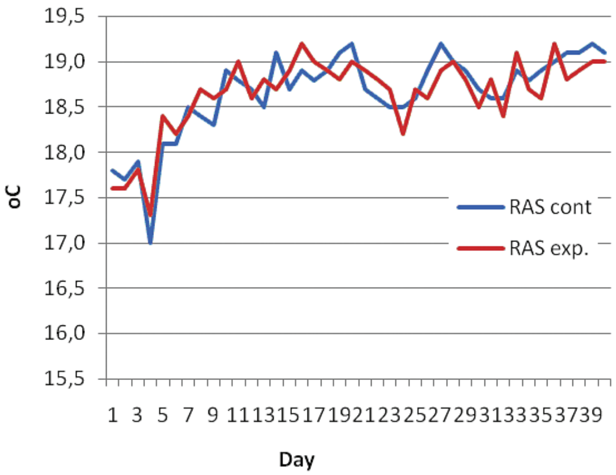

Fig. 2. The temperature of water in experimental and control RAS

and ranged between 17°C and 19.2°C. However, the temperature during the experiment was near to favorable temperature range for raised in the systems fish, because its value for rainbow trout is assumed to be 12 to 18°C (Raleigh et al., 1984). Continuously oxygenation of water in cultivation tanks avoided the possible negative effect on trout of slightly higher temperature in tanks in the end of the experiment (Staykov, 2001). The macrophytes from experimental recirculation system showed good development and photosynthesis at measured temperatures (Hodgson, 1970).

Dissolved Oxygen

The oxygen content (Figure 3) in both systems was around permissible value of this parameter for the water used in trout production – 8 mg.l^{-1} (Zaikov, 2006). The content of measured gas in water of the experimental RAS was higher, compared with the quantity, determinate for the control system. The received data are in confirmation with these ones, obtained from different researchers, where macrophytic plants were used like a biofilter in aquaculture and significantly increase the quantity of oxygen in cultivation units (Ferdoushi et al., 2008; Velichkova and Sirakov, 2013).

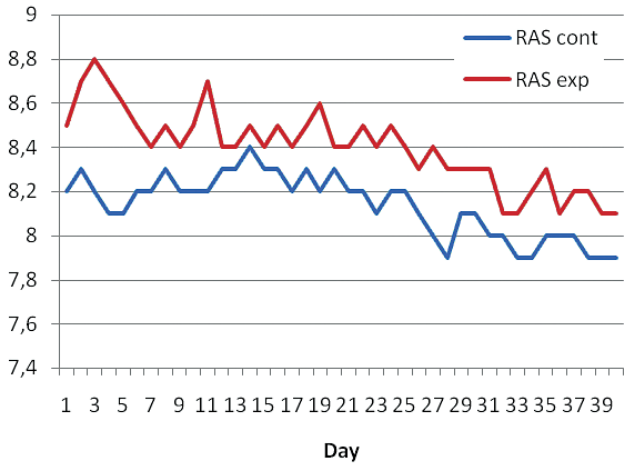

Fig. 3. Dissolved oxygen in control and experimental RAS (mg.l^{-1})

pH of the water

During the experiment, the measured pH was slightly alkaline in both tested systems (Figure 4). The minimal value of pH for the experimental recirculation system was 7.7 and for the control RAS was 8.1. The maximal values were respectively 8.4 in the experimental system and 8.5 in the control one. Extremely low or high values of pH cause damage to the tissues of the fish, especially bleeding may occur in the gills and in the lower part of their body (Wagner et al., 1997). The pH of water used in aquaculture can affect

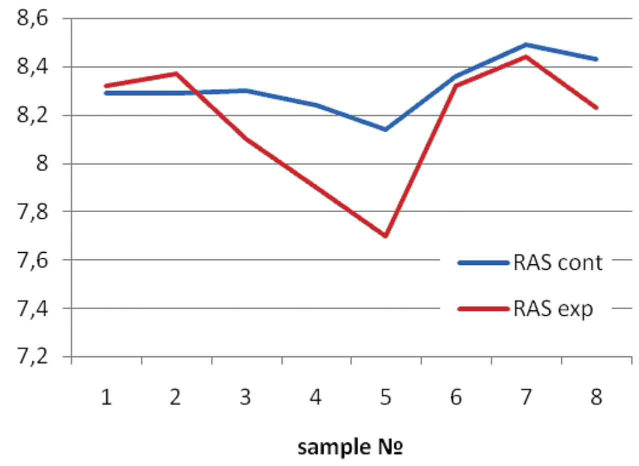

Fig. 4. Measured pH in experimental and control RAS

fish health directly. For most species pH between 6 and 9 is good (Tucker, 1993). Below pH 6.5 fish species slow growth is observed (Lloyd and Jordan, 1964; Lloyd, 1992). The higher pH (9.0 or greater) leads to a stress. The combination of higher temperatures (19.9–22.8°C) and pH level greater than 9.4 resulted in mortality of rainbow trout (Wagner et al., 1997). The water in the experimental RAS showed better pH profiles during the trial, compared with those, determinate in the conventional system.

Nitrogen compound and Phosphates

Ammonium ion

The maximum concentration of ammonium ions in the water of the control recirculation system was 0.1 mg.l^{-1}, while in the experimental RAS – 0.064 mg.l^{-1}. Minimal values of this parameter were respectively 0.045 mg.l^{-1} and 0.015 mg.l^{-1}. The average value of ammonium ions in the tanks of the experimental recirculation system was lower with 79.5%, compared to their concentration in the control RAS. The difference was statistically significant (P≤0.05) (Table 2).

Table 2

Hydrochemical parameters in experimental and control RAS during the trialperiod (mg.l^{-1})

Hydrochemical parameters	RAS cont.	RAS exp.	p
	x ± Sx	x ± Sx	
Ammonium ion (NH_4^+)	**0.083 ± 0.05**	**0.017 ± 0.015**	**
Nitrite (NO_2^-)	**0.061 ± 0.31**	**0.041 ± 0.30**	*
Nitrate (NO_3^-)	**6.31 ± 0.34**	**5.69 ± 0.21**	*
Total Nitrogen (TN)	**15.91 ± 1.90**	**10.5 ± 1.98**	***
Phosphates (PO_4^-)	**0.31 ± 0.0197**	**0.21 ± 0.0154**	*

P ≤ 0.05*, P ≤ 0.01**, P ≤ 0.001***, P ≥ 0.05 – ns

The major factor, controlling the toxicity of ammonia in the water is pH, which together with temperature regulate the proportion of un-ionized ammonia. This ion is the primary degradation product of nitrogen metabolism in fish and is produced by metabolic activity of the nerve and muscle tissue, deamination of amino acids by the liver and by enzyme activity of the micro flora of the gut. Ninety percent of ammonia is excreted at the gills in freshwater fish. Un-ionized ammonia is toxic and readily diffuses across the gill membrane into the circulatory system, whereas ionized ammonia is a larger, hydrated and charged molecule and can only cross the membrane by active transport through charge-lined micro pores of hydrophobic membrane components.

The lower concentration of ammonium ion in experimental RAS due to potential of zeolite for removing ammonia from water, especially at salinities lower than 10 ppt, but the main obstacle in using zeolite is its uselessness after some hours, because of the loss of ion exchanging capacity (Emadi et al., 2001). This obstacle easy surmounted regenerating the zeolite. NH_4^+ plays an important role as a source of nitrogen for macrophytic plants and could be removed from them (Kronzucker et al., 1997; Cao et al., 2004; Nimptsch and Pflugmacher, 2007; Wang et al., 2008).

Nitrites

During the trial the minimal value of nitrites in the water of both tested system was 0.01 mg.l^{-1}. The maximum value of nitrites – 0.07 mg.l^{-1}, was measured in the control recirculation system and in the experimental, containing the zeolites and macrophytes like filter elements it was 0.05 mg.l^{-1}. When the nitrite content in the water is greater, than 0.5 mg.l^{-1} in Atlantic salmon and channel catfish could be observed a disease, called methemoglobinemia, when hemoglobin is oxidized by nitrite to form methemoglobin, affecting the respiratory process (Helfrich and Libey, 1990). Kruopova et al. (2008) showed the effect of sub chronic nitrite exposure at concentration 0.01 mg.1^{-1} on rainbow trout was manifested with changes in segmental hyperplasia of the respiratory epithelium of secondary lamellae and elevated glucose and decreased potassium. The cases of mortality in rainbow trout during the recent trial could be resulted from nitrite exposure of fish at concentration higher, than 0.01 mg.l^{-1}, which lead the continuous stress in raised fish The availability of nitrite in both tested system by our opinion could be the result from inefficient nitrification process from *Nitrobacter* bacteria, which are convert the nitrite to nitrate. Nevertheless the average value of the nitrite in the water of the experimental recirculation system was approximately with 32.8% lower, compared to the content of this parameter in the control RAS and the difference was statistically proven (P ≤ 0.05). These lower nitrite values in the water of experimental RAS could be result of lower ammonia level in the experimental RAS, which were converted to nitrite. Two main reasons for lower value of nitrites in exp. RAS could be distinguished – indirect (removal of ammonia from zeolites and macrophytes) and direct (nitrite assimilation from water plant).The last one is confirmed by Boyd and Queiroz (1997), who found, that aquatic plants in biofilter systems were capable of removing 94% of nitrite.

Nitrate

The maximum value of nitrates 11 mg. l^{-1} in the water of the control system and 8.6 mg.l^{-1} in the experiment. The average concentration of nitrates in recirculation system, using zeolites and macrophytes like a biofilter was lower with around 10%, compared with the concentration, measured in the control RAS and difference was statistically proven ($P \leq 0.05$) (Table 2). The obtained result from the research of Mažeikiene et al. (2008), exploring absorption potential of ammonium ion and nitrate from zeolite, showed, that it can be very useful sorbent for NH_4^+, but it is not suitable for nitrate absorption from water. The nitrate removing in experimental RAS was made mostly by macrophytes. The made conclusion in the recent study is in confirmation of data, received from Alabaster and Lloyd (1980), which showed, that aquatic macrophytes could be highly effective in nitrate removal from water, containing high concentrations of it, like fish-farms, agricultural and industrial effluents.

Total nitrogen

During the experiment, the measured concentrations of the total nitrogen in the water were higher in the control recirculation system, as compared to those ones in the experimental system (Table 2). The average content of total nitrogen in the experimental variant was with 34% lower, compared to its content in the control recirculation system (Table 2) ($P \leq 0.001$).The data, obtained from the present study are in confirmation of the data, received from Schulz et al. (2003), which observed, that a plant community in wetland, where water from rainbow trout farm are passed for treatment, decreased the total nitrogen with 30%.

Phosphates

The content of phosphates in the water of the experimental variant was lower, compared to those, and received for the control RAS (Table 2). The maximum value of phosphates in the control and experimental recirculation system was respectively 0.6 mg.l^{-1} and 0.5 mg.l^{-1}. The average value of phosphates was with 32.2% lower in experimental RAS, as compared its concentration in the control system (Table 2). The decreased quantity of phosphates in the water of the experimental RAS could be result of their removal from zeolite, as well as presenting in the system macrophytes. The clinoptilolite material (zeolite) was found to have a phosphate adsorption capacity of 2.15 g.kg^{-1} (Sakedevan and Bavor, 1998). Ferdoushi et al. (2008) conducted an experiment with macrophytes as biofilter in fish pond, found that phosphates are higher in variants without macrophytes (3.5 mg.l^{-1}). Our results are in contrast to those, obtained by Boyd and Queiroz (1997), who stated, that the aquatic plants in the trickling filter systems are able to remove 97% of the phosphorous compounds in the water.

Removal rates were usually significantly correlated to plant biomass and productivity. Mkandawire and Dudel

Table 3

Technological parameters in fish, cultivated in control and experimental RAS

Technological parameters	RAS cont.	RAS exp.	p
	x ± Sx	x ± Sx	ns/*
	Start of trial		
Initial fish weight (g)	31.19 ± 2.5	30.56 ± 2.2	ns
Standard length (cm)	13.5 ± 0.4	13.2 ± 0.69	ns
Weight of eviscerated fish (%)	85.1 ± 7.6	80.6 ± 14.04	ns
	End of trial		
Survival rate (%)	75	85	*
Final weight (g)	42.4 ± 4.1	51.37 ± 2.5	*
Standard length (cm)	15.4 ± 0.27	16.5 ± 0.22	*
Weight of eviscerated fish (%)	88.8 ± 0.6	90.2 ± 1.1	ns
SGR (%/day)_	0.74 ± 0.16	1.27 ± 0.18	***
FCR	2.5	1.5	*

$P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***, $P \geq 0.05$ – ns

(2007) found that *Lemna* species have many unique properties, suitable for phytoremediation: they have fast growth and primary production, high bioaccumulation capacity, ability to transform or degrade contaminants, resistant to extreme contaminant concentration, and can be applied on multiple pollutants simultaneously.

Technological parameters

The live weight, standard length (SL) and weight of eviscerated fish from both experimental variant did not shown statistically proven differences in the start of trial ($P \geq 0.05$) (Table 3). The survival of rainbow trout, cultivated in recirculation system, containing zeolite and macrophytes like filter element, showed with 11.7% higher survival rate, compared with this found out for the fish in control RAS. The final weight, standard length, weight of eviscerated fish and specific growth rate in fish from experimental RAS showed respectively with 18%, 6.6%, 1.6% and 41.7% higher value, compared with these, estimated for trout from the control variant ($P \leq 0.05$) ($P \leq 0.001$) (Table 3). The better growth in fish from the experimental system, reflected also in calculated FCR, which showed with 40% lower value, compared with this one, calculated for the trout from the control variant ($P \leq 0.05$). The technological parameters in rainbow trout cultivated in exp. RAS were better compared with these found out for fish in control RAS due to the good water quality. The zeolite and macrophyte decreased the concentrations of harmful metabolites, which have a negative effect on fish growth and feed utilization This results are in confirmation of data received from Ariyaratne (2010), Rakocy and Allison (1984), who found out, that the water quality is much better in the presence of macrophytes in the reservoir and the fish has better growth, compared to this without aquatic plants.

Conclusion

The addition of zeolite and macrophytic plants *Lemna minor* and *Elodea canadensis* as a part of the filter for water treatment in the experimental recirculation system for growing of rainbow trout, decreased significantly the quantity of nitrogen and phosphorus compounds, compared to their quantity in conventional RAS. The better water quality in experimental variant due to the presence of zeolite and macrophytes, influenced positively the growth of fish and feed utilization. The final weight, standard length, weight of eviscerated trout and specific growth rate in fish from experimental RAS showed, respectively with 18%, 6.6%, 1.6% and 41.7% higher value, compared with these, estimated for fish from control variant.

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