

Oxygen mass balance in a recirculation aquaculture system for raising European Wels (*Silurus glanis* L.)

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The growth of the European Wels or sheat-fish (*Silurus glanis* L.) was evaluated in a recirculation aquaculture system situated in a greenhouse. Recirculation aquaculture system components were evaluated in terms of oxygen use and generation. Oxygen gradients revealed the main fault of the originally constructed system elements – mechanical and biological filters. The influence of organic matter accumulation and biofilm formation in the mechanical filter was found to have a strong influence on oxygen regime. An airlift fed biological filter successfully restored oxygen depleted by the infrequent cleaning of mechanical filter media and did not need backwashing during the experiment mainly due to effective mechanical cleaning, hydraulic loading decline and subsequent heavy airlift aeration.

Raceway type fish growing tanks appeared to be somewhat unpractical for use in the recirculation system due to a lack of self-cleaning effect and waste accumulation.

The constants for oxygen mass balance equations taken from literature appeared to be in good correlation with the results of the experiment.

Key words: recirculation aquaculture system, aeration, biofilter, oxygen mass balance

INTRODUCTION

Recirculation aquaculture systems (RAS) are a promising technology in the intensification of fish production. Water organisms are grown under fully controlled conditions, i.e. physical and chemical water characteristics are maintained adequate to the needs of the growing species with the help of wastewater treatment technologies allowing water reuse.

Oxygen concentration in water is the first limiting factor in such systems. Oxygen poorly dissolves in water. The saturation values 10.08 mg/l at 15 °C for growing coldwater species (salmon, trout) and 8.26 mg/l at 25 °C for warm water farming (carp, catfish) force to replenish constantly oxygen content in water to ensure an efficient fish growth. The oxygen concentration threshold for warm water species is 50% (absolute value 4.13 mg/l at 25 °C) (Ulikowski, 2004) and 60% (6.48 mg/l at 15 °C) for salmonids at a fish growing tank effluent (Goryczko, 1999).

If there is a lack of oxygen in a water system, it has to be aerated or oxygenated in order to supply oxygen not only for growing organisms but also to meet the demand of biochemical reactions in the system components.

The objectives of this work were to investigate the possibility of growing the European Wels in a simple

RAS and to determine the operation of the system components in terms of oxygen demand and balance. The system components' analysis in terms of oxygen generation and consumption was expected to reveal their operation and management peculiarities. The intention was to minimize the influence of biological water purification components on water quality in the system by exchanging a sufficient portion of water. The influence of water exchange rate on oxygen dynamics in the system was considered as well.

The European Wels was chosen as a commercially valuable species and as an attractive game fish. It is defined as occasional (usually not found) in Lithuania. Its population is to be increased here because of its low resilience (population doubling time 4.5–14 years). It is reported also as a protected fauna species in Annex III of the Bern Convention.

The optimal temperatures for growing the European Wels were found to be 20–24 °C (Pruszynski, Pistelok, 1999) which are not common for Lithuanian weather conditions. A RAS situated in a greenhouse enables to prolong the rearing period significantly due to the elevated temperature of water circulating inside the greenhouse.

As a warm water species, the European Wels is more tolerant to a poor water quality and lower dissolved oxygen concentrations reported to be as low as 0.78 mg/l in

an experimental RAS (Pruszyński, Pistelok, 1999). For efficient non-stressed fish growing, the recommended oxygen saturation should be 50% and more (Ulikowski, 2004). This stands for a lower risk of fish growing in a simple RAS, where no complex and expensive technologies are used.

MATERIALS AND METHODS

A simple RAS (Fig. 1) was constructed in the greenhouse of JSC "Kietaviškių gausa". The RAS consisted of standard components (Timmons and Losordo, 1994) employed for water treatment in order to meet the requirements for recirculated water quality parameters for rearing fish. A greenhouse enclosed system ensures the following operation advantages:

1. A longer rearing period when using an elevated temperature in the greenhouse.
2. The usage of the existing water supply system.
3. The possibility of the further use of nutrient-rich RAS water for plants hydroponically grown in the greenhouse (Adler 1998).

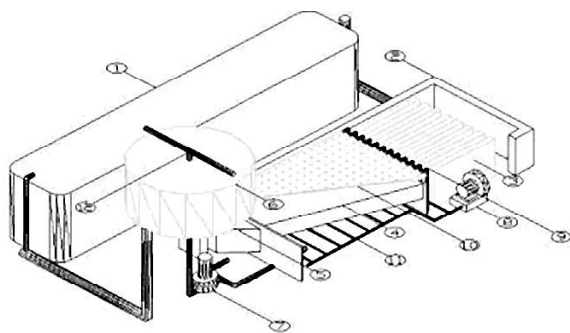


Fig. 1. Scheme of recirculating aquaculture system. 1 – raceway type fish rearing unit, 2 – concrete channel, 3 – plate mechanical filter, 4 – fixed bed biological filter, 5 – secondary filtering bed for biofilter backwash, 6 – aeration tower with packing, 7 – pump, 8 – air blower, 9 – airlifts, 10 – distribution plate, 11 – air supply manifold, 12 – water distribution rotating arm

Fish were raised in four raceway type plastic basins (only one is shown in Fig. 1) of a total volume 5.2 m³. The mechanical filtering of water in the RAS was performed by a plate filter consisting of 8 polyurethane foam plates (0.86m × 0.55m × 0.05m) with the total filtering area 1.89 m². The fixed bed biological filter was fed with filtered and aerated water by means of airlifts over a distribution plate. 50% of biofilter volume (0.84 m³) was filled with polyurethane foam pieces (specific surface area 1413 m²/m³) to provide surface for the growth of water purifying organisms. The biofilter bed was intended to be pneumatically washed by the air supplied through the manifold. The aeration tower (height 0.9 m, diameter 1.2 m, volume 1.3 m³) was filled with plastic tubes to improve oxygen mass transfer from air to water as well as to be used as a se-

condary water-purifying trickling filter. Water was distributed over the tower by a rotating arm. Water circulated by the pump at a flow rate 10 m³/h was changed every 30 min in the fish-holding tanks.

The experiment lasted 140 days (from June 25 until November 11, 2004). On June 25, the system was stocked with 3500 European Wels fry (mean weight $m = 7$ g), the total of 24.5 kg, brought from the Polish Institute for Inland Fisheries Department in Dgal. The yield of fish at the end of the growing period was 163.4 kg, with the mean weight 53 g, survival rate 88.1% and stocking density 31.42 kg/m³.

Fish were fed with commercial Aller Aqua (Denmark) trout fodder of the following composition: proteins 45%, fat 15%, carbohydrates 21%, energetic value 4924 kcal. 150 kg of fodder was used over the growing period, yielding the food conversion rate 1.08.

The daily water exchange varied greatly. The unlimited additional water supply for the system excluded the concern about operation efficiency of the RAS water purification section. Thus wastes generated in the system (TAN (total ammonia nitrogen) and organic wastes) were not monitored directly but controlled through water exchange by adding fresh water to the system.

Oxygen concentration was measured (OxyGuard MkII, OxyGuard International AS, Denmark) in the inlets and outlets of individual system components during the experiment.

A modern RAS design generally uses mass balance computations that identify and quantify the inputs, outputs and internal changes (conversions and consumptions) of the system. For design purposes, the maximum loading (stocking rate, feed rate, etc) should be used and the system is expected to operate in a "steady state" condition.

The layout of the RAS under investigation in terms of oxygen consumption and generation in different components is shown in Fig. 2. The water flow rates (m³/h), used for oxygen mass balance calculation in the components are also indicated.

Q – circulation flow rate in the system;

Q_p – make-up flow rate;

$Q_n = Q_p$ – discharge flow rate;

Q_a – emergency flow rate for aeration improvement.

Oxygen concentration measurement points:

C_{ZT} – fish rearing unit – input;

C_{MF} – mechanical filter – input;

C_{ER} – airlifts – input;

C_{BF} – biological filter – input;

C_{AB} – aeration tower – input.

Oxygen concentration differentials (g/m³), positive or negative depending on whether oxygen was generated (aeration) or used (fish and biochemical reaction in the system components):

$\Delta C_{ZT} = C_{MF} - C_{ZT}$ – fish rearing unit;

$\Delta C_{MF} = C_{ER} - C_{MF}$ – mechanical filter;

$\Delta C_{BF} = C_{AB} - C_{BF}$ – biological filter;

$\Delta C_{AB} = C_{ZT} - C_{AB}$ – aeration tower.

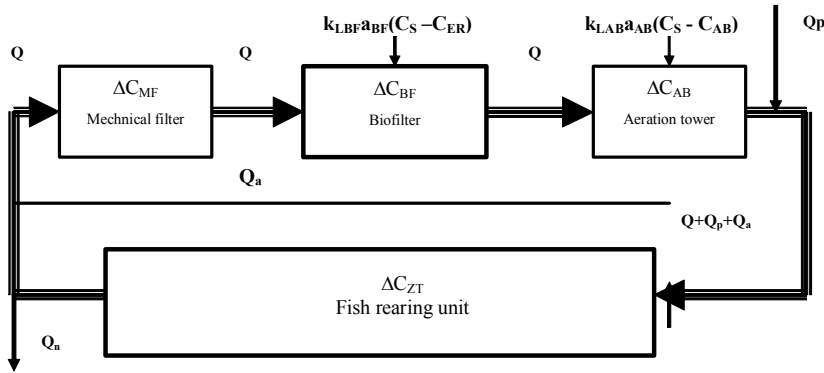


Fig. 2. Oxygen concentration indexes in the components of the system under investigation

Oxygen generated in the system, $\text{g/m}^3/\text{day}$:
in biological filter supplied with water by airlifts:

$$G_{BF} = k_{LBF} a_{BF} V_{BF} (C_s - C_{ER}), \quad (1)$$

where $k_{LBF} a_{BF}$ is the oxygen mass transfer coefficient in the airlift aeration system, day^{-1} ;

C_s is the oxygen saturation value at given temperature and pressure g/m^3 ;

C_{BF} is the oxygen concentration in water entering the biofilter, g/m^3 ;

V_{BF} is the biofilter volume, m^3 .

Similarly, oxygen in the aeration tower can be expressed:

$$G_{AB} = k_{LAB} a_{AB} V_{AB} (C_s - C_{AB}), \quad (2)$$

where $k_{LAB} a_{AB}$ is the oxygen mass transfer coefficient in the aeration tower, day^{-1}

C_{AB} is the oxygen concentration in water entering the aeration tower, g/m^3 .

V_{AB} is the volume of the aeration tower, m^3 .

Oxygen is used for fish growth (respiration), nitrification of toxic ammonia and degradation of fish waste and uneaten feed in the system and its depletion all over the system components can be expressed as follows:

$$24\Delta C = (R_{BODT} + R_{BODN} + R_{NT} + R_{NA} + R_{FISH})(1-WE), \quad (3)$$

where ΔC is the oxygen depletion value across the component (difference between the inflow and outflow values), g/m^3 ;

R_{BODT} is oxygen demand by heterotrophic organisms, dealing with dissolved organic waste elimination from the water, $\text{g/m}^3/\text{day}$;

R_{BODN} is oxygen demand by heterotrophic organisms dealing with elimination of organic waste accumulated in system components from water by hydrolysis, $\text{g/m}^3/\text{day}$;

R_{NT} is the oxygen demand of the autotrophic (nitrifying) microorganisms for ammonia oxidizing, $\text{g/m}^3/\text{day}$;

R_{NA} is the oxygen demand of autotrophic (nitrifying) microorganisms for buffering the ammonification process, i.e. for oxidizing ammonia involved in the process of organic waste ammonification, $\text{g/m}^3/\text{day}$;

R_{FISH} is oxygen used by fish stock, $\text{g/m}^3/\text{day}$;

WE is water exchange in the system determining waste elimination, decimal;

24 is the dimension uniformity constant, day/h .

Oxygen differential measurements in every RAS component were performed and equations (1)–(3) were used to evaluate their operation.

RESULTS AND DISCUSSION

Oxygen concentration dynamics in RAS components

Figure 3 shows oxygen concentration dynamics in separate RAS components and in the recirculation flow rate in the system in the course of the experiment. At the beginning of the system operation, when biofilter acclimation was performed by exchanging the system water volume up to 50% and because of the low system loading (low fish biomass and therefore low feed loading), the oxygen difference across the system components was insignificant. The system flow rate Q decreased as low as to $6 \text{ m}^3/\text{h}$ on day 85 due to the perforated water gathering manifold fouled with a biofilm. Oxygen concentration then reached a critically low value in the outflow of the rearing units ($C_{MF} = 2.8 \text{ mg/l}$) and the emergency pump was switched on with a flow rate $Q_a = 20 \text{ m}^3/\text{h}$. The total flow rate is denoted as $Q_B = Q + Q_a$ in Fig. 3.

Oxygen mass balance in separate system elements

Wastes generated in RAS originate solely from fish feed input and thus are directly proportional to it.

If the feed composition is known (proteins, fat and carbohydrates and thus its energetic value), the BOD and NOD (nitrogenous oxygen demand) values in the

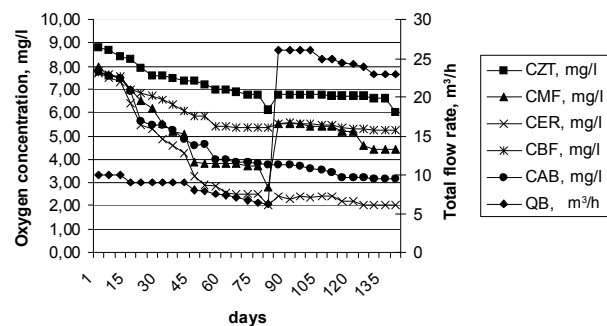


Fig. 3. Dynamics of oxygen concentration in the system components

system can be readily calculated (Bovendeur, 1989; Eels..., 1991).

Organic matter consumes oxygen as carbon is broken down (carbonaceous oxygen demand, COD) through biological processes (heterotrophic organisms' activity). The COD / dry feed matter ratio was found to be 1.4 and the biodegradability of COD originated from feed was 80% (Bovendeur, 1989). Thus, the balance on mass basis for organics is $O_2 : (R_{BODT} + R_{BODN}) = 1.1$.

The rate of BOD to feed input is expressed in relation to organic waste and is reported in the literature as 430–488 g/kg fodder (Karpinski 1995, Aquacultural... 2001). The BOD ratio in this study is taken as $488 \times 1.1 = 536$ g/kg fodder.

Oxygen used by autotrophs for nitrification – commonly defined as NOD – can be readily calculated from stoichiometric nitrogen oxidation reactions' sequence $NH_4^+ \rightarrow NO_2 \rightarrow NO_3$ where the coefficient on mass rate appears to be $O_2 : N = 4.57$. Some authors (Golz 1999) suggest to use the ratio 4.18 in biochemical reactions where pure chemical transformations instead of cell growth are to be taken into account, i.e. the balance on mass basis is $O_2 : (R_{NT} + R_{NA}) = 4.18$.

It is known that protein contains 16% of nitrogen and the fish body flesh protein content is 16% and all non-assimilated nitrogen becomes ammonia nitrogen, thus total ammonia nitrogen (TAN) excretion in the system when the food conversion rate is equal to 1.08 can be calculated as follows: 1 kg of given fodder exerts $0.45 \times 0.16 \times 1.08 - 0.16 \times 0.16 = 0.052$ kg TAN and needs 0.169 kg oxygen to oxidize it.

Accumulation of organic matter in the system negatively affects the oxygen balance in the system and highly increases its demand – the values of the components R_{BODN} and R_{NA} (according to expression (3)) are increasing. The process negatively affects the nitrification process as well while encouraging heterotroph proliferation and the overcompetition of nitrifying organisms in the biofilm (Malone et al., 1998).

1. Fish rearing unit. Reared fish are the main oxygen users here. Oxygen demand depends on metabolic rate so the oxygen usage is expressed in terms of feeding rate, i.e. the fodder used. Literature data (Bovendeur, 1989; Parker, 2000; Timmons and Losordo, 1994) report broad limits ranging from 200 g/kg to 610 g/kg of fodder used. Henceforth the value $k_{O/P} = 368$ g O_2 /kg fodder/day (Bovendeur 1989) applied for African catfish rearing in RAS. Some authors (Malone et al., 1998) report that about 30% of nitrification is performed here by the organisms suspended in water and immobilized on tank and pipe walls because of excellent mass transfer conditions due to a high oxygen concentration, good mixing and the constantly renewing biofilm. One more portion of oxygen is required to oxydize fodder remains and fish excretions because raceway type rear-ing tanks are not designed for self-cleaning. So, the overall oxygen mass balance can be expressed as follows:

$$24(Q + Q_p + Q_a)\Delta C_{ZT} = (k_{O/P}Wm + R_{BODTZT} + R_{BODNZT} + R_{NTZT} + R_{NAZT}) (1-WE), \quad (4)$$

here and henceforth subscripted indexes stand for corresponding RAS components according to Fig. 2. Here we have ZT for a fish rearing unit:

m – mass of reared fish, kg;

W – daily feeding rate, % from m .

Oxygen usage dynamics is shown in Fig. 4. The measured mean daily usage of oxygen in the units is expressed as $M_{O2ZT} = 24Q_B(C_{ZT} - C_{MP})$, kg/day; M_{O2ZTSK} is the calculated oxygen demand in the rearing units according to expression (4) and neglecting biochemical oxygen demand components. The fish growing curve FW is shown here as it directly indicates the amount of the fodder fed and, therefore, the oxygen used. Oxygen consumption exceeds the amount needed for fish biomass. This can be explained by an additional oxygen demand by feed and fish excretion remnants accumulated in tanks which were not self-cleaning. Then the components R_{BODN} and R_{NA} increase. When the difference from the calculated value diminished (days 80 to 85), signs of oxygen shortage in the fish tanks appeared and the emergency pump was launched. It recirculated the flow directly from the fish tank effluent (flow Q_a , Fig. 2), i.e. the main portion of untreated water was recirculated and the difference between M_{O2ZT} or M_{O2ZTSK} grew up due to an increase of biochemical oxygen demand components (Fig. 4).

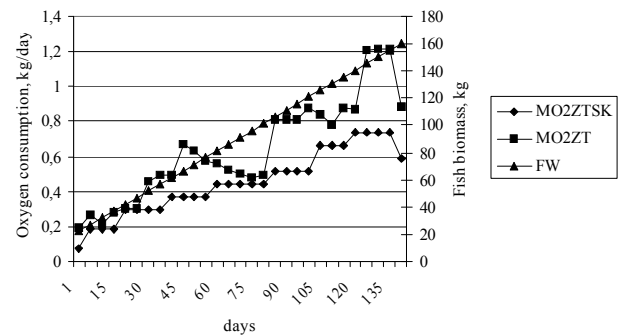


Fig 4. Calculated and measured oxygen consumption in fish rearing tanks

2. Mechanical filter. Multiple filtering polyurethane plates were used for water filtering. The construction of the filter allows an intensive development of the biofilm. The high organic load suggested the first stage biological filtration mode with a heterotrophic biofilm with a minor role of nitrifying organisms and their components in oxygen demand balance R_{NTMF} or R_{NAMF} according to expression (3):

$$24Q\Delta C_{MF} = (R_{BDSTMF} + R_{BDSNMF} + R_{NTMF} + R_{NAMF}) (1-WE), \quad (5)$$

The oxygen consumption dynamics is shown in Fig. 5. M_{O2MF} and $M_{O2MFval}$ denote oxygen consumption on non-cleaned and cleaned filter plates respectively. The

construction of the filter favoured waste accumulation and promoted biofilm development. The filter did not cause hydraulic loss and therefore the plates did not require cleaning up to the 50th day of the experiment because accumulation of nutrients was low in the system. Oxygen difference during this period was insignificant – $\Delta C_{MF} = 0.4 \text{ mg/l}$, and later on, when the biofilm began to grow rapidly, oxygen consumption on the plates risen up to $0.22 \text{ kg O}_2/\text{day}$ while the flow in filter Q declined constantly from $6 \text{ m}^3/\text{h}$ to $3 \text{ m}^3/\text{h}$ at the end of the experiment (Fig. 5).

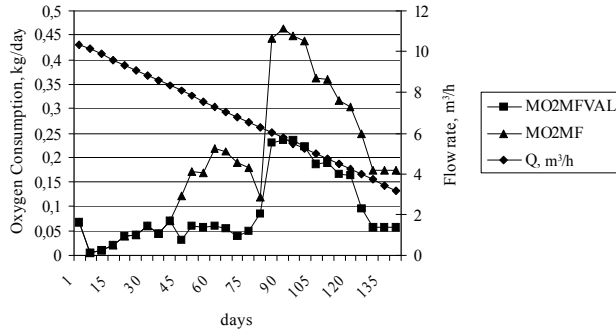


Fig 5. Oxygen consumption in mechanical filter

3. Nitrifying biofilter. Normally it is considered as a second stage biological filter with an emphasis on nitrification and therefore exerting mainly NOD due to a lower organic loading, i.e. components standing for BOD (R_{BODTBF} and R_{BODNBF}) are smaller here. Water was supplied here by airlifts which aerated it. So, a combination of expressions (1) as oxygen generation and (3) as oxygen consumption gives the following mass balance for oxygen:

$$\begin{aligned} 24Q\Delta C_{MF} + kLa_{BF}V_{BF}(C_s - C_{ER}) = \\ = (R_{BDSTBF} + R_{BDSNBF} + R_{NTBF} + R_{NABF}) \\ (1-WE), \end{aligned} \quad (6)$$

The oxygen consumption and generation dynamics is shown in Fig. 6. The drawback of the biofilter construction prevented an effective backwash of the filter bed. The airlift-supplied aerated water flow damped successfully oxygen depletion in the biofilter bed due to waste accumulation and biofilm growth (components R_{BODN} and R_{NA}), and examination of the filter bed did not show anaerobic zones in it at the end of the experiment. The flow to the filter Q declined continuously but biofilter bed hydraulic loading remained constant because the airlift ensured a constant flow $Q_{ER} = 10 \text{ m}^3/\text{h}$. The airlifts aeration efficiency was very low up to the 20th day when biofilter acclimation was in progress and there was no significant oxygen demand in the system. Later on, the demand for oxygen grew up, but the flow declined, so the airlift aerating capabilities markedly exceeded the oxygen demand.

4. Aeration tower. No significant biofilm developed here throughout the experiment, thus oxygen consumption can be neglected here. According to (2), only oxy-

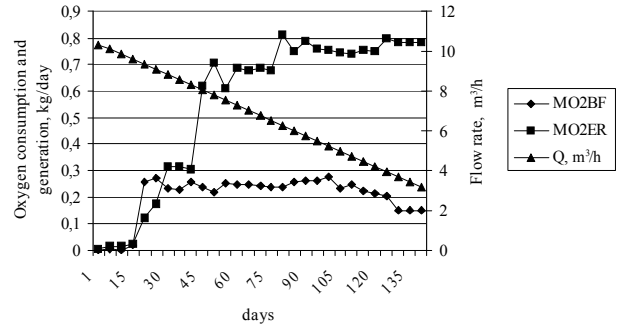


Fig 6. Oxygen consumption and generation in airlift-fed bio-filter

gen generation occurs here. The mass balance for the aeration tower is as follows:

$$24Q\Delta C_{AB} + kLa_{AB}V_{AB}(C_s - C_{AB}) = 0. \quad (7)$$

The dynamics of oxygen generation and flow rate Q_B are shown in Fig. 7. Oxygen addition took place mainly here (up to $M_{O2AB} = 2.032 \text{ kg O}_2/\text{day}$).

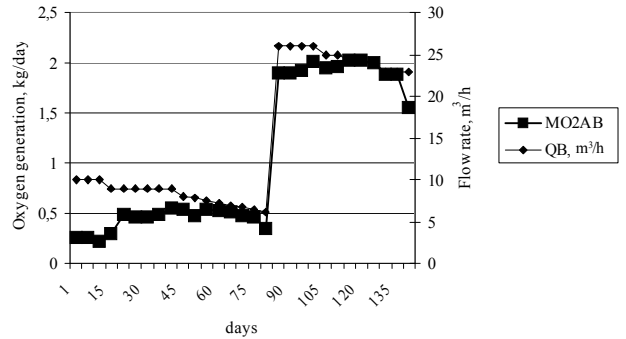


Fig 7. Oxygen generation in aeration tower

Figure 8 shows a comparison of oxygen generation by the aeration tower and airlifts ($G_{O_2} = M_{O2ER} + M_{O2ZAB}$), oxygen usage in the system components ($P_{O_2} = M_{O2ZT} + M_{O2MF} + M_{O2BF}$) and the theoretically calculated oxygen demand (PT_{O_2}). The total calculated oxygen demand $PT_{O_2} = P_Z + P_{BOD} + P_{NOD}$. It is the sum of demands for fish P_Z for organic waste P_{BOD} and for nitrification P_{NOD} . The above described ratio constants $\text{kg O}_2 / \text{kg feed}$ were used for calculation. The water exchange rate in the system A_{H_2O} was taken into ac-

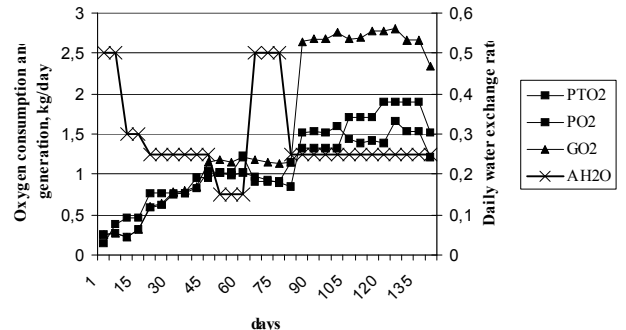


Fig 8. Oxygen generation and consumption in the system

count, and the wastes were considered to be removed from the system at a rate equal to water exchange rate.

CONCLUSIONS

Effective organic waste (feed remains and fish excretions) elimination is critical for oxygen mass balance in recirculation aquaculture systems. Aeration capabilities are limited in an intensive growing system due to a low concentration of oxygen in the air (21% on volumetric basis) and because not eliminated organics excretes significant amounts of BOD in the system. Attention should be paid to the following peculiarities of the RAS components:

1. Accumulation of organic matter and biofilm growth on the plates of the mechanical filter consumed large amounts of oxygen. Water leaving the mechanical filter prior to filter plate cleaning had a 20% oxygen saturation vs. 40% after cleaning.

The construction of the involved plate mechanical filter ensures good operation results – filtration and, possibly, partial nitrification, but its cleaning is labor-intensive, so it is hardly suitable for larger commercial units of RAS without additional investigation and construction refinement.

2. Airlift water supply to the biological filter effectively rebuilt oxygen concentration in the water leaving the mechanical first-stage filtration. Oxygen concentration rose from 20% up to 45% saturation in most critical moments when the peak oxygen depletion in the mechanical filter occurred.

3. The water recirculation rate must be at least 4 to 5 turnovers per hour in order to maintain an adequate oxygen concentration level in the water at fish growing densities of 30 kg/m³. The aeration tower generates the main oxygen mass in the system. The higher water turnover rate improved oxygen generation here.

4. Sufficient make-up water supply (25–50% of system volume daily) eliminated an adequate amount of soluble BOD and suspended solids from the system thus greatly improving oxygen regime in the components of the system. Fish rearing units must be self-cleaning ones not to allow the accumulation of organic waste and thus minimize the water exchange rate in the system.

The simple RAS under investigation does not require high capital cost investment and after eliminating the drawbacks has a good potential for avoidance of seasonal factor in aquaculture, fry rearing in no season and thus shortening the production cycle, or rearing fish juveniles up to more viable stages.

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DEGUONIES BALANSAS UŽDAROS APYTAKINĖS AKVAKULTŪROS SISTEMOS KOMPONENTUOSE AUGINANT ŠAMUS (*SILURUS GLANIS* L.)

Santrauka

Uždaros apytakinės akvakultūros sistemos įgyja vis didesnę populiarumą akvakultūros sektoriuje. Jos turi daug privalumų, todėl intensyviai tiriamos, siekiant sumažinti energetinius poreikius gaminamai produkcijai. Pirminis ribojantis veiksnys – tai ištirpusio vandenyje deguonies kiekis, nulemiantis ne tik auginamų organizmų kiekį, bet ir biocheminius virsmus uždaroje apytakinėje sistemoje.

Sukonstruota ir preliminariai įvertinta jos komponentų deguonies naudojimo požūriū nesudėtinga recirkuliacinė akvakultūros sistema. Eksperimento, trukusio 140 dienų, metu įvertintas europinio šamo (*Silurus glanis* L.) augimas apytakinėje akvakultūros sistemoje. Apytakinėje sistemoje, kurios tūris 5,2 m³, išauginta 3100 šamų šiųmetukų, iš viso 163,4 kg, vidutinis svoris – 53 g, tankis – 31,42 kg/m³, išėiga – 88,1%. Visą

eksperimento laiką buvo stebimas deguonies balansas sistemos sudėtinėse dalyse – auginimo talpose, mechaniniame ir biologiniame filtruose, aeravimo elementuose – aeravimo bokšte ir panardinto filtro aeravimo sistemoje.

Deguonies masės balansas sistemos komponentuose leidžia preliminariai įvertinti jos funkcionavimą ir, kaip parodė tyrimas, esant galimybei padidinti vandens atsinaujinimą sistemoje iki 50%, tampa vieninteliu ribojančiu veiksniu gamybos procese.

Tyrimo metu nustatyta, kad: 1) efektyvus organinių liekanų šalinimas iš sistemos yra kritinis rodiklis, užtikrinantis sėkmingą sistemos funkcionavimą, todėl būtina efektyvi auginimo

talpų savivala, efektyvi mechaninė filtracija, neakumuliojanti nuosėdų; 2) aeracija efektyviausiai vykdoma aeravimo bokšte su įkrova, ir jos efektyvumui padidinti būtinas recirkuliacijos debitas, lygus 4–5 sistemos tūriams per valandą; 3) esant pakankamam papildomo vandens tiekimui (25–50% sistemos vandens tūrio per dieną) iš sistemos pašalinama daug tirpaus BDS ir suspenduotų dalelių, o tai ženkliai pagerina deguonies režimą sistemos komponentuose.

Raktažodžiai: akvakultūra, aeracija, biofiltras, deguonies balansas