Project acronym:	FutureEUAqua
Project title:	Future growth in sustainable, resilient and climate friendly organic and conventional European aquaculture
Grant number:	H2020-BG-2018-1: Project no. 817737
Coordinator:	NOFIMA, Norway
Website:	www.futureeuaqua.eu

Deliverable D4.5:

Water quality descriptors

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Dissemination level:	CO
Deliverable type:	R - Report
Approval Task/WP:	03.06.2022
Approval Project Management Board:	14.06.2022
Submission date:	14.06.2022

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Societal challenge, Blue Growth, Grant Agreement No 817737.

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Executive summary

Different descriptors of water quality exists and are already in use at commercial recirculating aquaculture systems. Generally, these measurements address physical-chemical water quality parameters for which simple and reliable test kits etc. are easily accessible and readily applied at the farms.

Further development in farming technology and the need for improved control for sustaining stability in water quality and thereby stable, continuous fish production have recently highlighted the need for methods and means for monitoring of additional water quality parameters, collectively referred to as “microbial water quality”.

In this report, new and innovative water quality descriptors including methods and approaches to “microbial water quality” are described including a suggestion for an industrial application.

Introduction

Land-based recirculating aquaculture systems (RAS) are intensive fish production systems, which in addition to reduced environmental impact and nutrient discharge from fish farming (d'Orbcastel et al., 2009; Martins et al., 2010) offers possibilities of controlling many parameters important for the production and well-being of the fish. For these reasons, RAS is becoming increasingly popular for commercial farming of many species (Dalsgaard *et al*. 2013) including also Atlantic salmon smolt and grow-out (Dalsgaard *et al*. 2017).

Being able to avoid or eradicate parasites, to control production parameters like temperature, salinity and oxygen, and also chemical water parameters like pH, total ammonia nitrogen (TAN) and nitrite are all production related benefits serving as levers for more commercial RAS. With the technology becoming mature, these issues are largely solved, and the progressive optimization of RAS operations can now focus on parameters related to what can overall been termed microbial water quality (MWQ; Rojas-Tirado, 2018).

Micro-particles (<35 um) are an important characteristic of the water quality in aquaculture systems (Brinker & Rösch, 2005). Being smaller than most mechanical removal devices such as drum filters can remove, they easily accumulate to high levels in RAS. Previous studies have indicated that micro-particles can have direct negative effects on RAS performance (Michaud et al., 2006) and fish health (Bullock et al., 1997; Clark E. R., 1985) although Becke et al. (2018) found no major effects on rainbow trout (*Oncorhynchus mykiss*) gills exposed to high suspended solids loads throughout a grow-out period. Accumulation of micro-particles results in an increase in particulate surface area as smaller particles have larger surface to volume ratio than larger particles (Fernandes et al., 2017; McMillan et al., 2003). Micro particles can provide surface area for bacteria to colonize (Attramadal et al., 2012; Bullock et al., 1997) and bacteria-inhabited particles could potentially be used by specific or opportunistic pathogenic bacteria (Attramadal et al., 2012; Blancheton et al., 2013) which may cause serious problems for the fish. Positive correlation between particle surface area and bacterial activity measured by Bactiquant® in RAS has previously been demonstrated (Pedersen et al., 2017). Gregersen et al., 2019 recently also reported positive correlation between micro particle levels and bacterial activity using hydrogen peroxide degradation rate (Pedersen et al., 2019), another new measure for microbial activity.

In a mature and stable RAS running at constant load, delicate balances between system organic and particle load, systems operation, and bacterial activity and probably composition are supposedly maintained and MWQ parameters in consequence at fairly constant levels.

Whether such constant MWQ levels exist in commercial RAS, which components and operations that influence them, as well as the establishment of a baseline for normal operation ranges are most important issues for the further development and operation of commercial RAS, fresh as well as saline systems.

Commercial RAS are currently lacking validated tools to measure microbial water quality, including the abundance and activity of microbes in the system. These tools are needed to identify unstable conditions, and subsequently perform control measures to maintain a good microbial water quality for stable fish production. Knowledge on ranges and limits for microbial water quality variables are needed and validated, recently developed methods for quantification needs to be implemented in the industry.

In this report, new and innovative water quality methods, descriptors, and approaches, in particular relating to microbial water quality, will be presented and discussed and the applicability in the industry are addressed.

Traditional physical-chemical water quality parameters

In most RAS a series of physical-chemical water quality parameters are traditionally measured continuously, daily, or weekly, the regularity depending on the variation expected and the immediate importance of the parameter in question.

Among these are continuously measured parameters like temperature, oxygen content, pH, and perhaps CO_2 content. Generally, these measurements are connected to a monitoring and alarm system that permanently tells the operator about existing levels and activates an alarm (and a response) if beyond given, set limits.

Nitrogenous substances

Nitrogenous substances (total ammonia nitrogen (TAN), nitrite (NO_2-N) and nitrate (NO_3-N)) indicating biofilter performance and the turnover of toxic ammonia is generally measured manually on a sample collected at relevant sites in the system at daily or two-day intervals. Comparing the actual and the previously recorded values tells the farmer about current biofilter performance and whether potentially harmful levels are arising. In case this happens, relatively few operational handles are immediately available to the farmer, that has to rely on feeding reductions and increased water exchange. Despite the well documented importance of biofilter oxygen content (Suhr & Pedersen, 2010) as well as alkalinity (Rusten *et al*., 2006; Pedersen *et al*., 2011) on TAN turnover many RAS do not perform these measurements continuously/regularly although it might provide valuable information of importance for biofilter performance and TAN conversion.

Organic matter

The organic matter content (particulate and dissolved) of the water in a RAS is quite important and a relevant parameter since the amount of organic matter available is giving information about the amount of substrate available for microbial growth. In addition, the actual level can be compared to the one generally observed in a system and by that indicate whether the system is getting out of balance and there could be need for corrective measures. Despite this, organic matter is seldomly measured or monitored at RAS farms, probably because measuring it is manual and laborious.

Chemical oxygen demand (COD) is a relatively fast measurement that can be performed using test-kits that tells you the total amount of organic matter in the water. By measuring both a raw (i.e., unfiltered) and a filtered sample you can calculate the particulate as well as the dissolved COD. By that, the farmer can tell whether it is the particulate or the dissolved fraction that, in case, are increasing and make it easier to decide on which specific corrective measures to apply.

Biological oxygen demand (BOD) is a somewhat laborious measurement and since the oxygen consumption in the sample is normally measured over 5 days (BOD_5) the information is already somewhat outdated when you receive it. Nevertheless, since it tells you the amount of readily degradable organic matter it provides very important information about the level of substrate available for immediate microbial growth within the RAS. As for COD, performing analysis on a raw as well as a filtered sample provides information on the particulate as well as the dissolved fraction, making it easier to decide on which specific corrective measures to apply.

The BOD to COD ratio (BOD_5/COD) is an indicator of the proportion of readily degradable organic matter to total organic matter. In e.g., wastewater this ratio is often quite high (>0.5) indicating very biologically reactive water, that will serve as immediate substrate for massive microbial growth.

In a well-operated, balanced RAS the ratio in the water is generally much lower and values around 0.1-0.2 should be expected. In case it is higher, there is a latent risk of uncontrolled microbial growth that might well hamper fish growth and well-being.

UV transmittance

The relative transmittance of UV light through a water sample; UV Transmittance or short: UVt, is a measurement of the relative amount of ultraviolet light (of wavelength 254 nm) that is able to pass through 10 mm of water. The relative amount (% UVt) that passes is an indicator of transmittance and thus water quality. In addition to particles several compounds in the water (e.g., humic acids, colloids, and metals) can absorb the UV light and thus reduce UVt. In some systems UVt relates more or less directly to the amount of particles (Figure 1) and thus turbidity (see below), whereas in others not so obvious since the colouration from e.g., humic acids influences the UVt but not the turbidity.

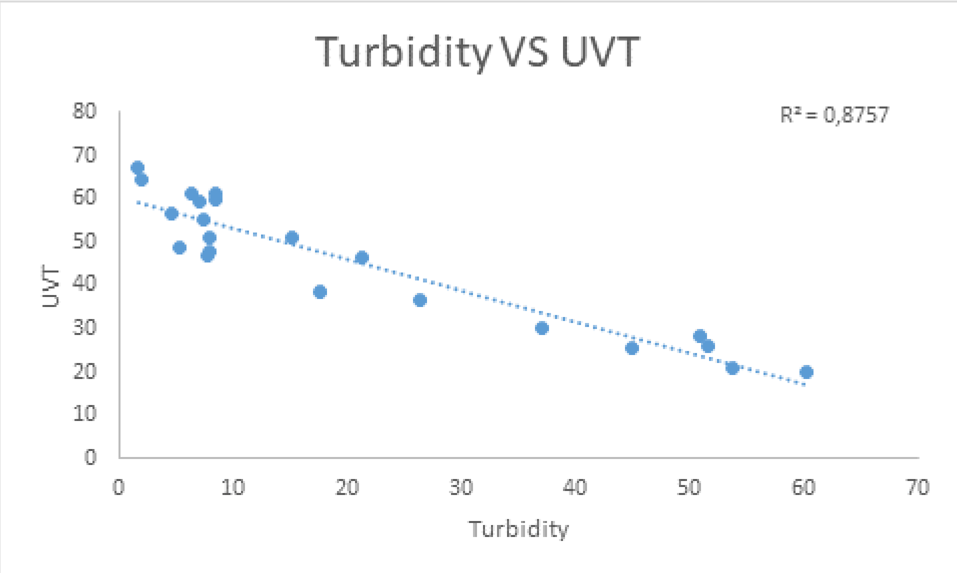


Figure 1. Correlation between turbidity and UVt in a set-up of 12 independent RAS.

Turbidity

Turbidity is a measure of the cloudiness of a fluid, generally caused by micro- and colloidal particles. It can thus be used to determine the concentration of suspended particles in a water sample. Basically, the method measures the incident light scattered at right angles from the sample when a light is shined through the water sample. The scattered light is captured by a photodiode, producing an electronic signal that is converted to a turbidity. The higher the intensity of scattered light, the higher the turbidity. Turbidity is often expressed as FNU (Formazin Nephelometric Units; ISO).

The measurement is done by a relatively cheap and easy-to-use meter, a turbidity meter provided with a turbidity sensor. This set-up can be either hand-held or in a fixed arrangement and by coupling it to a turbidity analyser continuous in-line turbidity measurements can be performed for particle density monitoring over time. A relatively simple set-up that we in this project have documented actually provides the farmer with a lot of relevant and important information on the (microbial) water quality in the RAS. Please see the later chapter Industrial application for details.

We have demonstrated, that although turbidity *per se* is not necessarily the important measure, it correlates quite well with the parameters that is important/decisive but that are much most costly and difficult to measure on a regular basis. Many different fabrics are available on the market, and Fig. 2 is just a random example of such equipment.

From: www.wtw.com

Figure 2. A sensors and analyser for turbidity measurement in water

New and innovative water quality parameters

Microparticles

Suspended particles have adverse effects in RAS. They increase turbidity and reduce overall water quality, directly or indirectly affecting the fish and their appetite/growth. In addition, they act as substrate for fast growing, opportunistic bacteria and microorganisms that may become harmful especially if the fish are stressed (Attramadal et al. 2014).

Suspended particles in intensive recirculating aquaculture systems (RAS) consist largely of organic matter basically originating from the feed and deriving from faeces (Dalsgaard & Pedersen, 2011) and uneaten feed as well as from biofilter flocks and slough off. Bacteria attach to particle surfaces (reviewed by Davey & O'Toole, 2000), and the heterotrophic bacterial carrying capacity in RAS is generally considered to be high (Attramadal et al. 2012, 2014), increasing with increased loading of suspended organic particles.

Since most/all mechanical filters and filtration will not remove particles below 30-50 µm, these fine particles will accumulate in RAS (Fernandez et al., 2015; Davidson et al., 2011; Fig. 3) and generally only be balanced by the water renewal (make-up water). Since fine particles represent a large surface area to volume ratio (A/V ratio; Brinker & Rösch, 2005; Fig. 4), accumulation of these in the system will in total provide a huge surface area for bacteria to colonize.

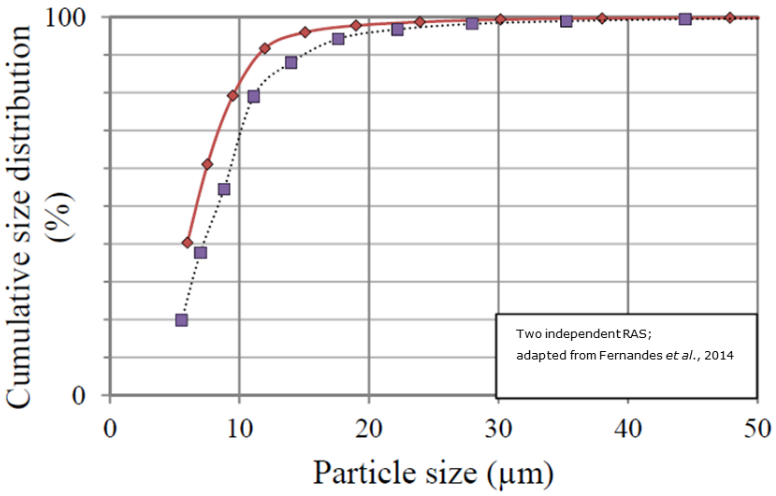


Figure 3. The cumulative particulate area in relation to particle size in a RAS.

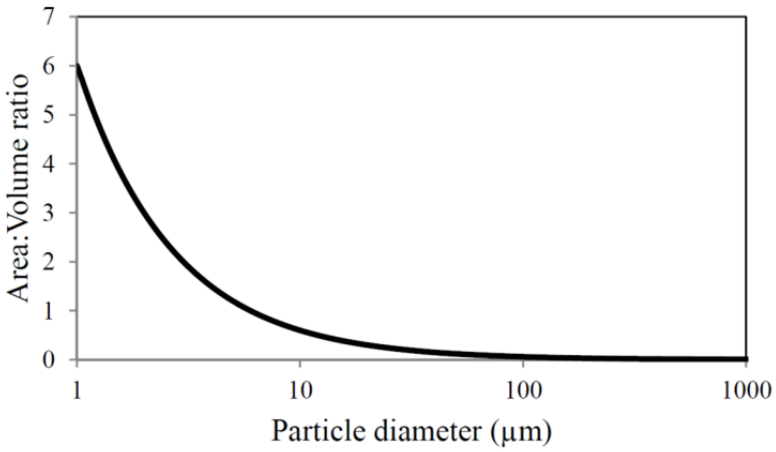


Figure 4. The Area/Volume ratio of particles of a given diameter

The number and the size distribution of particles are thus important measures for assessing microbial water quality in RAS, in addition to specific particle information also indicating the surface area available for microbial growth.

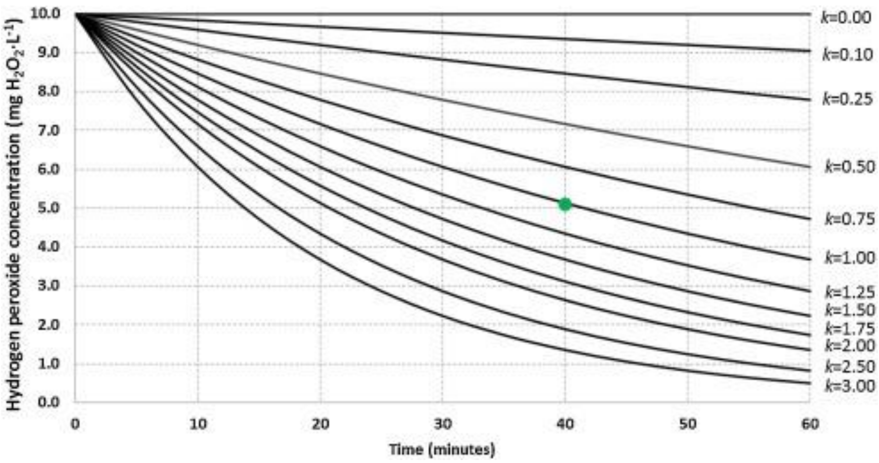
Unfortunately, access to scientific equipment able to measure and count fine particles (e.g., a Coulter Counter™ or Accu-Sizer™) is a prerequisite for assessment. Due to the cost and the need for caretaking etc. it is probably not equipment that will be implemented on most commercial farms. Rather, they might have to rely on sporadic assistance from e.g., Universities to get such specific information on the fine particles in their system(s).

Microbial activity

Traditional methods, such as plate counting to obtain colonies forming units (CFU) has been used to predict changes in relative numbers of bacteria in aquaculture water (Leonard et al., 2000; Brambilla et al., 2008). Plate counting includes various agars and incubation regimens, and thus results will be culture dependent and depend on whether or to what extend the specific bacteria can grow on the specific media used. Beside this bias also the laborious process and the long response time makes this approach not suitable/applicable for RAS, and faster and culture-independent methods are needed.

H_2O_2 degradation rate

DTU Aqua has developed and through this project refined a new, simple, rapid and cultivation-free method for determining microbial activity in RAS water. The basic principle, described in Pedersen et al., 2019, is based on measuring the degradation rate of H_2O_2 under standardized conditions. At the analytic conditions applied, degradation of H_2O_2 is governed by and directly related to microbial enzymatic activity (mainly catalases and peroxidases) and H_2O_2 decomposition follows an exponential first order decay (Richard et al., 2007). Thus, adding a small amount of H_2O_2 (400 µl 1000 ppm) to 40 ml RAS water at 22°C and mixing and measuring the H_2O_2-concentration over time (e.g., 1 hour) provides one with the data to calculate the decomposition rate; k. The higher the enzyme activity (i.e., the microbial activity) the faster the decomposition i.e., the higher the value of k. This is schematically illustrated in Figure 5.



From Pedersen et al. 2019

Figure 5. Theoretical/modelled decomposition scenarios of H_2O_2 calculated based on an initial nominal H_2O_2 concentration of 10 mg/L and various decomposition rates (k; in h^{-1}).

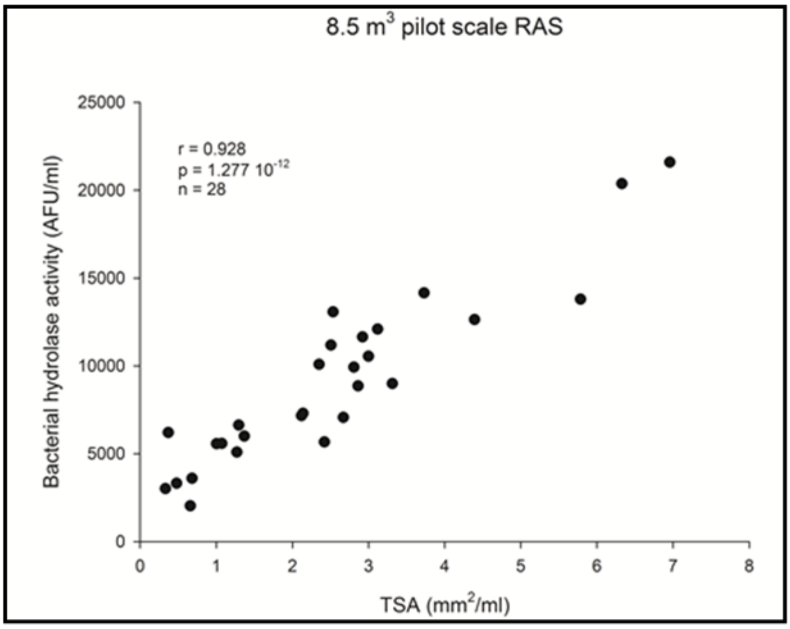
We have demonstrated that the rate of H_2O_2 decomposition is positively correlated to the composition and quantity of microorganisms and the total enzymatic activity of the microbiota. We have now applied the method to numerous water samples from different RAS and find it very useful and applicable to farms that have access to a small lab bench and some simple equipment. Anyway, to make the method widely used in commercial settings some simplification or provision of standardized, pre-prepared materials / set-ups would be beneficial.

Bactiquant®

Bactiquant® (Mycometer A/S, Denmark) is a standardized way of measuring microbial activity. The principle is based on enzymatic degradation of a fluorogenic enzyme substrate that releases fluorophores when hydrolysed. The amount of fluorophores are subsequently measured on a fluorometer (Bactiquant®), and the results are expressed in standardized Bactiquant® values (BQV/ml) that directly relates to bacterial activity at given conditions. The more fluorescense the more bacteria are present in the sample.

Once bought, the equipment and method is reliable and user-friendly in application. A small laboratory bench and some simple equipment and skills are needed, and then the method is quite easily used at farm conditions. There is limited time consumption, results are timely and culture independent.

In semi-intensive RAS, a linear relationship between the total surface area of particles (TSA) and the bacterial activity measured using Bactiquant® have been previously demonstrated as appears from Figure 6.



From: Pedersen et al., 2017

Figure 6. Relationship between particulate surface area (TSA) and the bacterial activity measured using Bactiquant®

Flow-cytometry

Flow cytometry is a quite advanced instrument for sophisticated measurements of cells and e.g., bacteria. The operation principle (Fig. 7) is based on measurement of the light scattering features of the sample, that can be caused by addition of dyes or specific antibodies added to investigate the specific purpose. It is an advanced tool for scientific purposes but can for instance in short time tell the number of bacteria in a sample (by staining with SYBR® green) as well as the number of live and dead cells (double staining with SYBR® green and propium iodide). It is a very interesting and powerful method for detailed studies on e.g., microbial abundance and effects of specific components of a RAS, but probably too advanced for practical application at commercial farm settings. Still is has been extremely useful for this work on developing new methods and approaches to monitor, control, and understand components of microbial water quality in RAS.

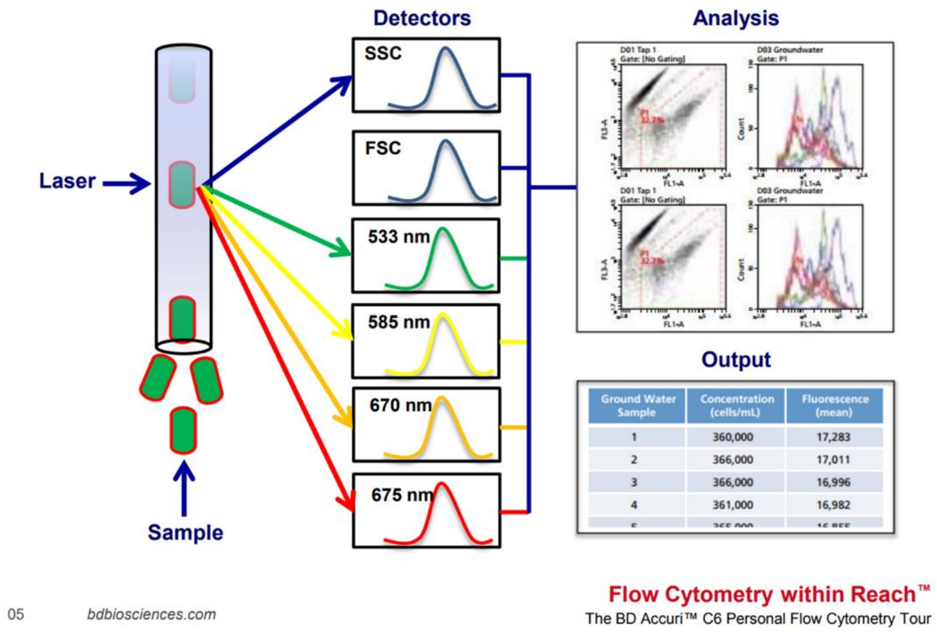


Figure 7. The basic principle of flow cytometry. From: bdbiosciences.com

Industrial application and take-home suggestion

Many measurements have been done also on commercial farms in order to develop new, innovative ways and methods to describe water quality parameters in addition to the physical-chemical ones traditionally monitored in RAS. In particular, micro particles and the concept of microbial water quality have been pursued since this is the next step and information needed to further develop system operation and provide means for a stable production in intensive RAS (de Jesus Gregersen *et al*., 2019; de Jesus Gregersen *et al*., 2020; Aalto *et al*., 2022)

Recirculating aquaculture systems rely on efficient water treatment, and changes in the operational parameters can affect water quality, and consequently, fish growth and welfare and thereby the economy of the farm. Many fish species, and in particular salmon, are very sensitive to changes in water quality. Biofilters have a central role in the RAS water treatment, hosting microbes that respond and balance chemical water quality. The microbiology of RAS is however, not limited to the microbial biofilms on biofilter carrier elements but includes free-living and particle-attached microbes in the water phase, and biofilms growing on surfaces throughout the system. The abundance and activity of these groups are part of the collective concept “microbial water quality”, which has a direct impact on fish performance and overall system water quality (Rojas-Tirado et al., 2018). Understanding the processes, dynamics, and interrelationships between different indicators and associated measurements, will enable us to define the ranges and limits of a stable RAS. This in turn provides means of improved system control through adaptive operation and measures targeting microbial and chemical water quality, sustaining stable conditions for healthy fish production.

One key challenge has been the identification of new “warning signals” that informs the farmer whether critical water quality parameters are potentially sliding out of acceptable range. In this report different water quality descriptors and methods have been described but are still simple and practical, real-life operational measurements could reasonably be asked for by the farming industry.

In this concluding section, an affordable and applicable suggestion are made:

As figure 6 shows, there is a linear relationship between particulate surface area (TSA) and bacterial activity (Bactiquant®) in RAS water. As described in the chapters above, none of these are easily measured nor collected over time at a commercial farm. However, in FutureEUAqua we have demonstrated that a nice linearity actually also exists between turbidity and microbial activity (H_2O_2 degradation rate (Fig. 8) in RAS. This indicates that a continuous or at least regular measurement of turbidity is actually a very useful approximation of microbial activity in the water.

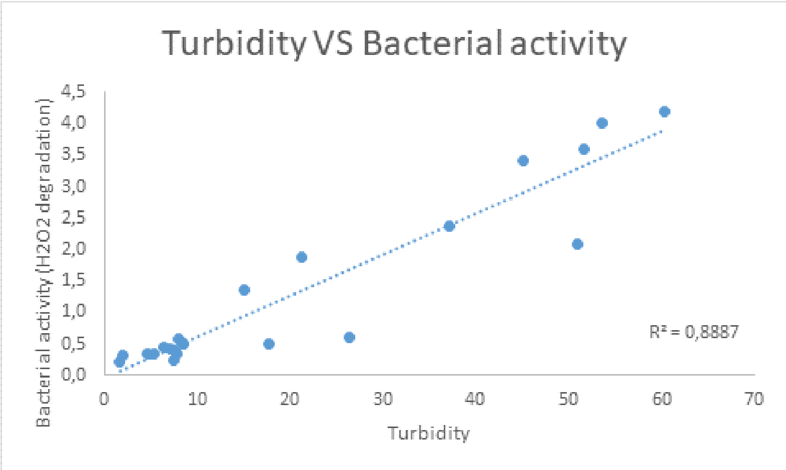
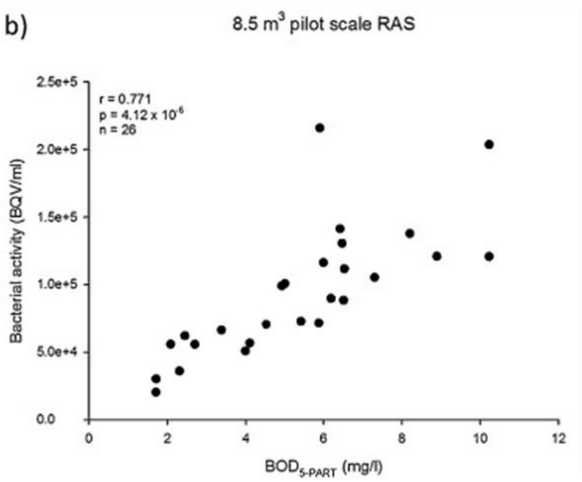


Figure 8. Correlation between turbidity and bacterial activity (H_2O_2 degradation rate)

Since turbidity is directly related to (micro) particles and by that to microbial activity (as shown above) and thus probably also to (readily available) particulate organic matter (i.e., $BOD_{5\ PART}$) as shown in Fig. 9, simply measuring turbidity continuous or regularly may provide a kind of "summary" or proxy for many of the more complicated/challenging and laborious measurements.



From: Pedersen et al., 2017

Figure 9. Relationship between BOD_5 (particulate) and microbial activity (BQV)

By applying such measurement, it will be possible to get coherent data-sets from individual farms and by that describe "normal" ranges, stable levels, and safe margins for operation. Over time, it will then also be possible to define "not normal" levels indicating instability and/or instability which might be on its way or about to come.

It will be most interesting to test this suggestion of using turbidity as an operational proxy for other important water quality parameters experimentally at full-scale in the industry.

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Research output: Contribution to journal › Journal article › Research › peer-review

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