



# The SeaRAS AquaSense™ System: Real-Time Monitoring of H<sub>2</sub>S at Sub µg/L Levels in Recirculating Aquaculture Systems (RAS)

Eldar Lien\*, Geir Valsvik, Jan Vidar Nordstrand, Vincent Martinez, Vegard Rogne, Ola Hafsås, Sara Queralt, Behnood Sjøstad Fathi and Morten Aga

Research and Development (R&D) Department, SeaRAS AS, Bergen, Norway

## OPEN ACCESS

### Edited by:

Olav Vadstein,  
Norwegian University of Science and  
Technology, Norway

### Reviewed by:

Maria Elena Giordano,  
University of Salento, Italy  
Carlo C. Lazado,  
Fisheries and Aquaculture Research  
(Nofima), Norway

### \*Correspondence:

Eldar Lien  
eldar.lien@searas.no

### Specialty section:

This article was submitted to  
Marine Fisheries, Aquaculture and  
Living Resources,  
a section of the journal  
Frontiers in Marine Science

Received: 11 March 2022

Accepted: 08 April 2022

Published: 06 May 2022

### Citation:

Lien E, Valsvik G, Nordstrand JV,  
Martinez V, Rogne V, Hafsås O,  
Queralt S, Fathi BS and Aga M (2022)  
The SeaRAS AquaSense™ System:  
Real-Time Monitoring of H<sub>2</sub>S at Sub  
µg/L Levels in Recirculating  
Aquaculture Systems (RAS).  
Front. Mar. Sci. 9:894414.  
doi: 10.3389/fmars.2022.894414

Hydrogen sulfide (H<sub>2</sub>S) is toxic to all species used in aquaculture. The extensive fish mortalities inflicted by H<sub>2</sub>S-poisoning can cause significant direct economic losses. Called the *silent killer* among scholars and fish farmers, H<sub>2</sub>S is highly soluble in aquatic environments and has significant damage potential; once detected, the time for mitigation actions is limited as fish stocks are rapidly impacted by the gas. The detection of H<sub>2</sub>S at low concentrations is challenging with the state-of-the-art sensors used today. Despite its significant damage potential, the industry has not been able to provide a reliable yet user-friendly system to address this. The aim of this paper is to provide a step forward in this direction by introducing a system for real-time monitoring of H<sub>2</sub>S at sub µg/L levels in RAS. The SeaRAS AquaSense System (AQS) utilizes real-time monitoring of H<sub>2</sub>S in parallel with other water quality parameters, such as CO<sub>2</sub>, O<sub>2</sub>, pH, and temperature, through a set of autonomous wireless-based sensor units installed in multiple locations in RAS. The system is calibrated and verified further by an autonomous calibration system where substrates of known values are brought to the sensor at a defined interval, offering the user data confidence that is exceptional and of high value. The H<sub>2</sub>S levels are measured in real-time at high-temporal resolution (down to 0.05 µg/L). By having units located in distinct locations in the water treatment loop, a true real-time monitoring of water treatment processes in RAS is given. Preliminary results provide new insights to the mechanisms behind H<sub>2</sub>S formation in operational RAS, and, more important, provide a basis for the development of risk-reducing actions and means for risk mitigation through degassing processes. By measuring real time simultaneously in multiple locations and in the exhaust of the degasser, a true mass balance of the system can be formulated by the given amount of feed, measured parameters by sensors and the known flow rate. By using the SeaRAS AquaSense System it is also possible to track the amount of H<sub>2</sub>S that leaks to the recirculating water per m<sup>2</sup> of biofilm in RAS. This is a critical parameter and of high priority to be considered in the context of coping with the H<sub>2</sub>S-induced risks in RAS. The finding of this work highlights that the formation of toxic H<sub>2</sub>S is inevitable in RAS-process, ergo, the focus of the industry should instead go toward how to cope with it. In this regard, the SeaRAS AquaSense System provides a step

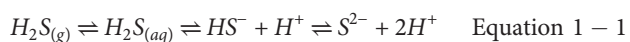
forward towards a practical solution for managing H<sub>2</sub>S-poisoning risk in aquaculture. This work suggests a need for future research on determining acceptable limits for H<sub>2</sub>S in water quality context in RAS and investigating a new insight on interaction between H<sub>2</sub>S and other water quality parameters such as CO<sub>2</sub>.

**Keywords:** RAS, hydrogen sulfide (H<sub>2</sub>S), fish mortality, real-time measuring, sustainability, aquaculture, water quality

## 1 INTRODUCTION

The formation of hydrogen sulfide, H<sub>2</sub>S (also known as dihydrogen sulfide, sulfane, hydrosulfuric acid, dihydrogen monosulfide, sulfurated hydrogen, sewer gas and sulfur hydride) is of major concern in aquatic environment nowadays; especially, within the recent growing trend in marine land-based recirculating aquaculture systems (RAS) (Rojas-Tirado et al., 2021). In recent years, H<sub>2</sub>S-poisoning in RAS has caused severe fish mortality in Atlantic salmon (*Salmo salar*) in Norway (Hjeltnes et al., 2019; Sommerset et al., 2020). These extensive fish mortalities impose direct significant economic losses and high damage potential within the market (Åtland and Stenberg, 2019). The increasing use of saline water in post-smolt RAS may explain the increasing trend in H<sub>2</sub>S-induced fish mortalities. The sulfate concentration in seawater is about 1000 times higher than that found in freshwater (Letelier-Gordo et al., 2020); this elevated sulfate level in RAS, in turn, may lead to more H<sub>2</sub>S formation. This makes post-smolt RAS and grow-out fish size productions vulnerable to H<sub>2</sub>S outbreaks, since seawater is used to prevent desmoltification and to prepare the post-smolt for a transfer to the sea (Rojas-Tirado et al., 2021).

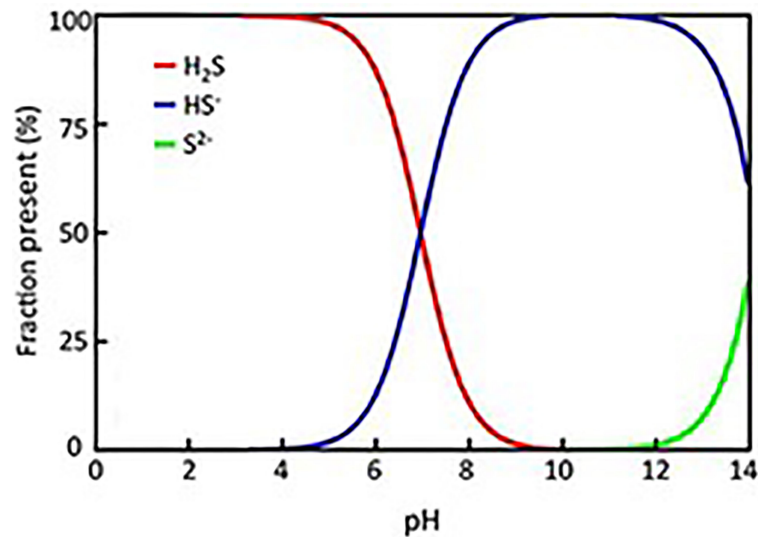
The rotten-egg odorous H<sub>2</sub>S known as *silent killer* among scholars and fish farmers is highly soluble in aquatic environment [4000 mg/L (at 20°C)] (Duan et al., 2007; Barton et al., 2014); once appears, the time for mitigation actions is limited as fish stocks are rapidly impacted by the toxic gas. The gas is flammable, colorless, and slightly heavier than air (specific gravity 1.19 at 15°C) with molar mass of 34.08 g/mol (Rubright et al., 2017). In aqueous solution, similar to that of water, it always equilibrates with its anions: bisulfide (HS<sup>-</sup>) and sulfide (S<sup>2-</sup>) – see equation 1-1 (Li and Lancaster, 2013). Unlike H<sub>2</sub>S and S<sup>2-</sup> that are substantially toxic, the anion HS<sup>-</sup> is not toxic by nature (Tanudjaja, 2021). Aqueous H<sub>2</sub>S is volatile, in other words, it inevitably equilibrates between the aquatic and gas phases (Li and Lancaster, 2013) – first left equilibrium in Equation 1-1. The dissociation process of H<sub>2</sub>S<sub>(aq)</sub> [with pK<sub>a1</sub> equals to 7.04 at 25°C and pK<sub>a2</sub> from 12 to 15 – see Li and Lancaster (2013) for a review] highly depends on the pH of that solution (the bond dissociation energy is 90 kcal/mol). In other words, pH determines the concentration of H<sub>2</sub>S<sub>(aq)</sub>. The RAS, in particular smolt and post-smolt production, is typically run under pH ranges between 6 and 8, hence, in theory, containing H<sub>2</sub>S fraction in the form of both H<sub>2</sub>S<sub>(aq)</sub> and HS<sup>-</sup> - **Figure 1**.



The H<sub>2</sub>S toxicity threshold is approximated to be as low as 0.002 mg/L for fish; although, there is no exact threshold defined

for Atlantic salmon, nor for RAS as of today (Hjeltnes et al., 2019; Sommerset et al., 2020). The H<sub>2</sub>S 96-hour LC<sub>50</sub> (lethal concentration 50) values for freshwater species found to be in range 20-50 µg/L, while, for marine species this is 50-500 µg/L (Boyd, 2014) – this is a measure that reflects the concentration of the toxic gas in water that killed 50% of test animals in 96 hours of testing time. Although there is no certain regulation set by the Norwegian Food Safety Authority (Mattilsynet) for H<sub>2</sub>S toxicity in aquaculture (Rosten et al., 2004), it is recommended that freshwater and marine species are exposed to no more than 2 µg/L and 5 µg/L of H<sub>2</sub>S gas respectively (Boyd, 2014). Tanudjaja (2021) suggested that this may be explained by the lack of enough measured H<sub>2</sub>S data during RAS process, while Rojas-Tirado et al. (2021) pointed the lack of understanding H<sub>2</sub>S formation mechanism, in particular, the importance of biofilter as potential hotspots to produce the gas. Long term exposure to H<sub>2</sub>S can lead to growth reduction, fecundity, swimming performance and a great stress on fish as it disrupts the respiration process that lead to cellular anoxia (Kierner et al., 1995). To fully investigate reasoning behind an H<sub>2</sub>S-poisoning incident, collecting data before the incident from different potential hotspots in RAS is essential in analyzing the involving factors. In addition, a system that collects data throughout RAS-process can be an important tool for further setting proper early-warning alarms in occurrence of H<sub>2</sub>S-spikes. Real-time monitoring of H<sub>2</sub>S at sub µg/L levels, parallel to other water quality parameters has not been available and therefore not yet practiced for commercial purposes. This left researchers and experts to not being able to find out the formation mechanism of sudden H<sub>2</sub>S-induced fish mortalities with the track of possible involving factors (Dalsgaard, 2019), leading to ultimately more unreliable outcomes to be further analyzed (Cämmerer et al., 2020). Many fish farms, as of today, measure several water quality parameters at fixed times and in selected tanks, leading often to mislead the results captured from peaks, and the rhythmic patterns. More frequent measuring points with higher sampling rate together with a systematic data analysis are thus needed to obtain specifically the knowledge of H<sub>2</sub>S-formation. This would also enable the industry to implement warning's alarms for such incidents that start escalating. A real-time monitoring system that can measure H<sub>2</sub>S in sub µg/L in important potential hotspots in RAS should thus be of priority to be practiced in industry.

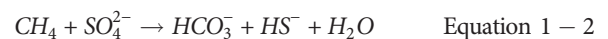
Possible mechanisms to explain H<sub>2</sub>S formation are linked to several factors: i) the natural sulfate (SO<sub>4</sub><sup>2-</sup>) content in both freshwater (~5–50 mg/L) and higher in seawater (~2700 mg/L) – therefore higher chance of the incident in seawater (Letelier-Gordo et al., 2020), ii) the existence of carbon-based compound like methane in water, iii) biodegradation processes governed by reduction-oxidation (redox) chemical reactions mainly under



**FIGURE 1** | The equilibrium speciation of aqueous hydrogen sulfide at different pH [taken from (Holmer and Hasler-Sheetal, 2014)].

anoxic (absence of oxygen) conditions in presence of accumulated organic matters and bacteria e.g., sulfate-reducing bacteria (SRB), and methane-reducing bacteria (MRB) (Li and Lancaster Jr., 2013), iv) environmental chemical conditions of the aquatic environment (water) including mobility of major anions and cations, pH, temperature, alkalinity and redox potential (Tanudjaja, 2021), v) physical properties of the aquatic environment in favor of H<sub>2</sub>S accumulation such as high turbidity, low water flow, and dirt sedimentation inside of the pipelines (Yu and Bishop, 2001; Prema, 2020) and lastly vi) technical problems that may trigger water backlash around the sediments, ergo, release all formed accumulated H<sub>2</sub>S at once (Tanudjaja, 2021). The presence of carbon-based (C-based) compounds like methane or organic matters as result of both feeding fish and waste solids produced by fish may play a key role in understanding the formation mechanism of toxic H<sub>2</sub>S. The C-based compounds reacts with sulfate (SO<sub>4</sub><sup>2-</sup>) content in water and form HS<sup>-</sup> which reacts later with existing dissociated hydrogen ion (H<sup>+</sup>) in water to form the toxic H<sub>2</sub>S. This reaction can be either directed naturally *via* chemical thermodynamics (Equation 1-2, in presence of methane) or *via* bacteria under an anoxic condition (**Table 1**). The source of methane in water may have resulted from either deep-lying and

oxygen-free, yet carbon-rich sediment stores at bottom of fjords or river-streams that interacted with geologic carbon-rich formations. One may argue that, in the case of land-based post-smolt RAS in Norway, since the intake waters are often taken from the deep fjord due to its optimal stable water quality, it may contain more methane and therefore be more prone to form H<sub>2</sub>S – Equation 1-2.



The presence of organic matter in process of RAS may also contribute to the formation of toxic H<sub>2</sub>S through biodegradation process. This chemical breakdown process of organic matters is governed by redox reactions where electrons from organic carbon (electron donor) to other compounds (electron acceptor) are transferred. Redox reactions occur in order of highest-to-lowest chemical energy thermodynamics obtained by bacteria to degrade organic matters as well as the availability of these electron acceptors in sequence: O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, Mn (IV), Fe (III), SO<sub>4</sub><sup>2-</sup>, and CO<sub>2</sub> (Mougin et al., 2007; Weiner, 2008). The greatest amount of energy obtained by bacteria is from O<sub>2</sub>, hence, the aerobic (oxic) respiration takes always place prior to anaerobic (anoxic) one and it continues until all

**TABLE 1** | Possible redox reactions as biodegradation process that together may describe the formation of the toxic H<sub>2</sub>S.

Occurring order	Process	Electron acceptors	Associated chemical reactions
1	Aerobic respiration	O <sub>2</sub>	{CH <sub>2</sub> O} + O <sub>2</sub> → CO <sub>2</sub> + H <sub>2</sub> O
2	Denitrification	NO <sub>3</sub> <sup>-</sup>	5{CH <sub>2</sub> O} + 4NO <sub>3</sub> <sup>-</sup> + 4H <sup>+</sup> → 5CO <sub>2</sub> + 2N <sub>2</sub> + 7H <sub>2</sub> O
3	Manganese reduction	Mn (IV)	{CH <sub>2</sub> O} + 2MnO <sub>2(s)</sub> + 4H <sup>+</sup> → CO <sub>2</sub> + 2Mn <sup>2+</sup> + 3H <sub>2</sub> O
4	Iron reduction	Fe (III)	{CH <sub>2</sub> O} + 4Fe(OH) <sub>3(s)</sub> + 8H <sup>+</sup> → CO <sub>2</sub> + 4Fe <sup>2+</sup> + 11H <sub>2</sub> O
5	Sulphate reduction	SO <sub>4</sub> <sup>2-</sup>	2{CH <sub>2</sub> O} + SO <sub>4</sub> <sup>2-</sup> + H <sup>+</sup> → 2CO <sub>2</sub> + HS <sup>-</sup> + 2H <sub>2</sub> O
6	Methanogenesis	CO <sub>2</sub>	2{CH <sub>2</sub> O} → CH <sub>4</sub> + CO <sub>2</sub>

{CH<sub>2</sub>O} indicates the degradation of organic matters.

Proposed reactions take place in sequence of happening from 1 as the first one to 6 as the last one.

dissolved  $O_2$  is consumed. Once  $O_2$  consumed, the bacteria start consuming  $NO_3^-$ , Mn (IV), Fe (III),  $SO_4^{2-}$ ,  $CO_2$  in sequence – **Table 1**. This basically means the formation of  $H_2S$  in biodegradation process *via* sulphate-reducing bacteria (SRB) may be favored by the presence of organic matter and the absence of oxygen,  $NO_3^-$ , Mn (IV), and Fe (III). Given this order sequence in the complex RAS setup with the dynamic fluctuation of the involving elements, it is important to monitor mass-balance changes throughout the system for further analysis. This may not only help us understand the change dynamics, but also may give us a chance to prevent such incidents *via* mitigation actions.

Overall, there can be two main contributions to form  $H_2S$  in RAS:

1. The constant formation of the toxic  $H_2S$  in the anaerobic part of the biofilm where bio-elements provide large amount of surface area (Rojas-Tirado et al., 2021). This explains the root cause for the steady-state  $H_2S$ -level measured in RAS as a result of the constant diffusion-out rate through biofilm.
2. Encapsulation of sedimented or dead organic material/loads (Rosten et al., 2004). Mechanical damage of this causes the often-deadly  $H_2S$  spikes in RAS.

Furthermore, one liter of water at the standard temperature and pressure (STP) typically contains approximately 4.5 g  $H_2S$  gas at 100% saturation. One may argue that by releasing one liter water at 100%  $H_2S$ -saturation into 1000  $m^3$  of water, it gives the concentration of 4.5  $\mu\text{g/L}$  in theory, which is at the upper limit of what today is reckoned to be acceptable.

Although the monitoring of mass balances of key factors involved is important for taking mitigation actions, it may not be enough to control and prevent  $H_2S$  poisoning incidents, as  $H_2S$  spike occurs fast, and more important, impacts the fish quickly. Therefore, there is a need for a feasible and viable solution that can predict such potential deadly incidents that can be formed in matter of seconds anywhere in RAS. In this study, the aim was to develop a real-time monitoring of  $H_2S$  that can measure the gas down to sub levels  $\mu\text{g/L}$  in different potential hotspots in RAS together with the track of some other important water quality parameters and offer this as a tool to the industry for further gaining knowledge around formation of  $H_2S$ . The proposed system in this study can let industry predict the future deadly spikes of  $H_2S$  and, hence, allow for mitigation measures in advance.

## 2 MATERIALS AND METHODS

RAS comprises a series of treatment processes to maintain water quality in intensive fish farming. These treatments include in general: i) mechanical filter such as drum filter used to remove particulate organic matter larger than 20  $\mu\text{m}$ , ii) biofilter to convert toxic ammonia ( $NH_4^+$  and  $NH_3$ ), excreted by the fish into nitrate ( $NO_3^-$ ), iii) trickling filter to degas  $CO_2$  and  $N_2$  followed by usually a protein skimmer to remove less than 20  $\mu\text{m}$  of particulate organic matter from the water through foam coagulation process and iv) oxygen enrichment system to

increase dissolved oxygen content before water gets back into the tank – **Figure 2A**.

### 2.1 AquaSense System (AQS)

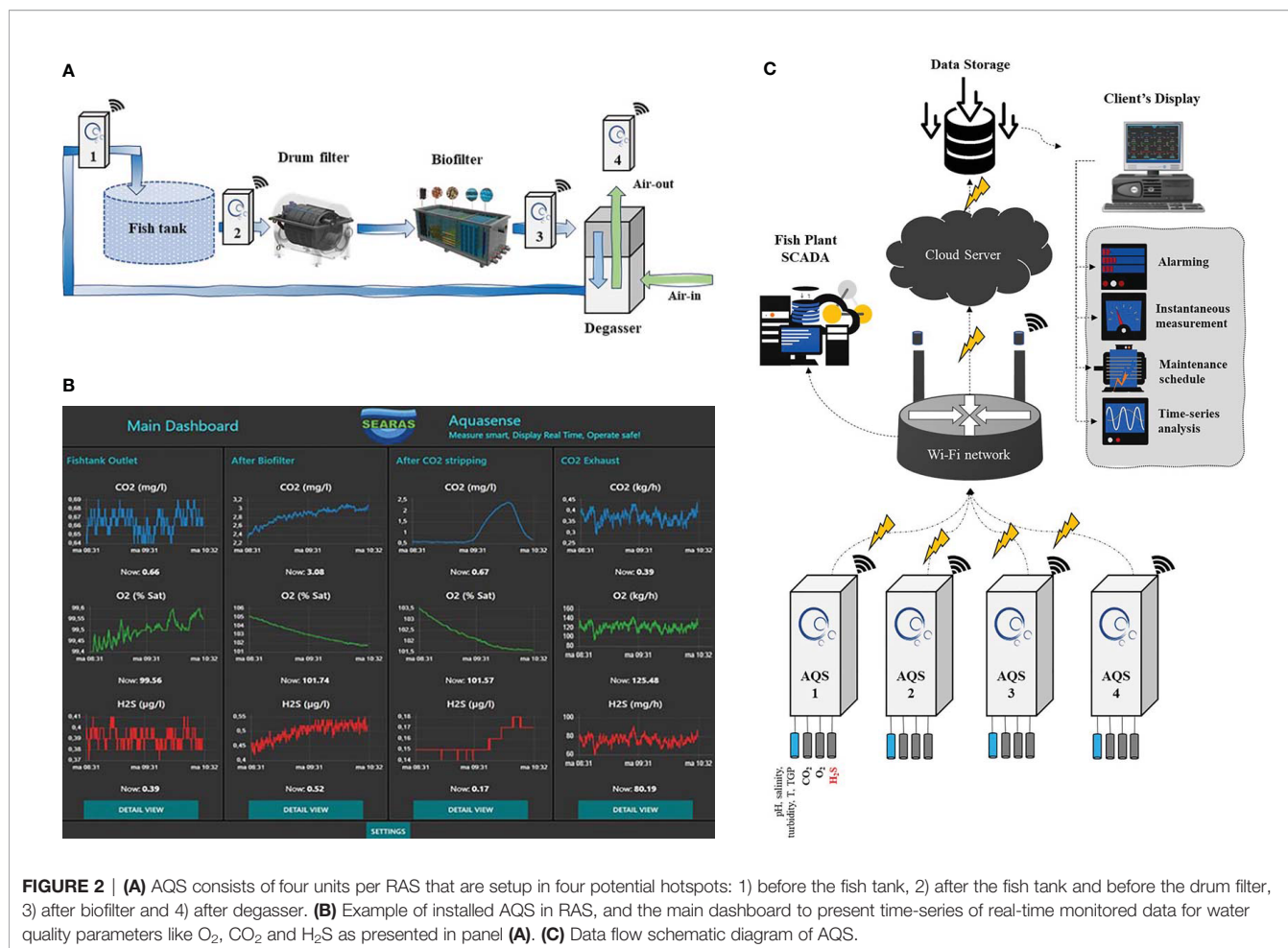
AquaSense system (AQS) is a mobile, wireless-based, and real-time monitoring system for measuring water quality parameters such as  $CO_2$  (mg/L),  $O_2$  (mg/L),  $H_2S$  ( $\mu\text{g/L}$ ), pH, salinity (S) (ppt or g/kg or ‰), temperature (T) ( $^{\circ}\text{C}$ ), total gas pressure (TGP) (%), and turbidity (NTU). It was explicitly designed to measure  $H_2S$  at high-temporal resolution (down to 0.05  $\mu\text{g/L}$ ) with a sampling rate of 1 Hz (one cycle per second) and is attributed to different locations in the closed fish tank. The sensors are not directly exposed to water, ensuring low maintenance as well as high accuracy. A system for automatic verification of calibration is implemented by bringing a calibrated gas to the sensor at regular time intervals. The typical setup for AQS would be four units per RAS to be installed in four different important hotspots – they are hereby referred as AQS1, AQS2, AQS3 and AQS4 in the text. The first unit locates before the fish tank and after degassing. The second one is setup after the fish tank before the drum filter. The third unit is installed after the biofilter. The fourth unit is installed after degasser and measures the air out of it – **Figure 2A**. Smart autonomous wireless-based sensors communicate together through an open standard network and collect data to be later stored in a shared cloud or locally. This lets the system exchange data with other system in an easy manner. **Figure 2B** shows an example of the installed AQS with the customized dashboard for the client. Data are represented in a web-based interface with options to transmit data or alarms to external systems like supervisory control and data acquisition (SCADA) – **Figure 2C**. Data is sent through Wi-Fi network to a cloud server or a fish plant SCADA. Stored data can be linked to the client's display for further analyzing, offering options to display time series analysis of measured water quality parameters or the instantaneous measurements with defined alarms – **Figure 2C**. Note that the dashboard can be setup based on customer's needs, providing more options to analyze upon request. Sensor calibration is verified at regular intervals by comparing to a reference calibrated source. This offers the user the data confidence that is exceptional and of high value.

AQS covers the ranges 0–80  $\mu\text{g/L}$  and 2–430  $\mu\text{g/L}$  of  $H_2S$  to be measured – these ranges are valid under following condition: T = 15 $^{\circ}\text{C}$ , S = 20 ‰, pH = 7, pressure = 1 atm. AQS should not be performed close to downstream of ozone ( $O_3$ ).

### 2.2 Mass Balance in AQS

By measuring water quality parameters in different locations in water flow in RAS (see **Figure 2A**), mass-balance change of these parameters can be simply computed. The biomass, amount of feeding,  $O_2$ ,  $CO_2$  and  $H_2S$  are measured and are thus known values, hence, can be used to formulate the mass-balance changes over the system. In particular, production or consumption in fish tank, in biofilter and in degasser are all calculated based on registered data in the different AQS's as well as the known flow rate. An example of mass-balance calculations can be seen in **Figure 3B**.

Individual alarm levels can be set on each of the sensors (low and high). Color codes can be defined in the display with green, yellow, and red to help decision makers (e.g., technicians) for more efficient



response taking. Trends can be displayed to present increasing or decreasing tendency of the measured parameters. Together all of these options are designed to facilitate controlling dynamic factors in the complex environment of RAS. The AQS units let industry learn from its failures, study critical factors in more efficient running of RAS and more important gain knowledge about each RAS. Ultimately, this may help industry gain more accurate insight of incidents so that early warnings can be triggered to predict possible H<sub>2</sub>S-spike releases in the system.

## 3 RESULTS

### 3.1 Real-Time Monitoring of Water Quality Parameters in RAS

The AquaSense system (AQS) consists of four units installed in four locations in the RAS: 1) AQS1 (inlet before water gets to the fish tank), 2) AQS2 (outlet water after the fish tank and before the drum filter), 3) AQS3 (after moving bed biofilter reactor), and 4) AQS4 (air out of degasser). Sensors in AQSs measure H<sub>2</sub>S (μg/L), O<sub>2</sub> (% saturation), and CO<sub>2</sub> (mg/L) over time with continuous track of pH, salinity (‰), alkalinity (mg/L), turbidity (NTU) and temperature (T) (°C). The

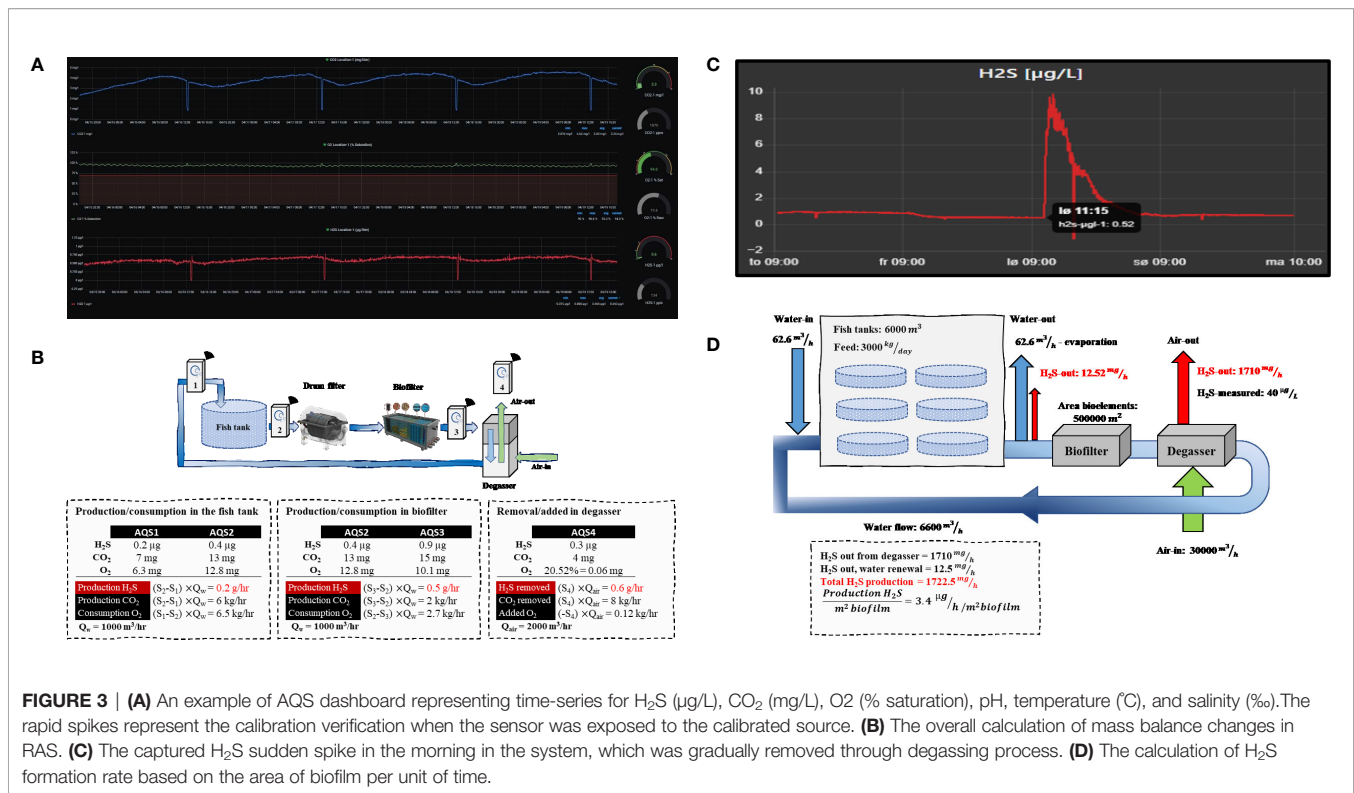
result shows a continuous dynamic change in water quality parameters (H<sub>2</sub>S, O<sub>2</sub> and CO<sub>2</sub> concentrations) throughout RAS-process.

#### 3.1.1 Measuring and Monitoring of H<sub>2</sub>S at Sub μg/L Levels in RAS

Through the setup of AQSs in RAS we were able to measure H<sub>2</sub>S at sub μg/L levels. **Figure 3A** shows the dashboard for measured and monitored data (H<sub>2</sub>S, CO<sub>2</sub>, and O<sub>2</sub>) in the RAS where we were able to successfully measure low concentration of formed H<sub>2</sub>S in the system. Time series of CO<sub>2</sub>, O<sub>2</sub> and H<sub>2</sub>S are shown for 4 days. As can be seen, H<sub>2</sub>S is continuously formed in low concentration, and the gas was successfully measured at the level of 0.5 μg/L. There is a diurnal pattern for CO<sub>2</sub>. Similar cyclic variation can also be seen for H<sub>2</sub>S.

#### 3.2 Mass Balance in AQSs

By installing the AQSs in potential critical hotspots in the water treatment loop in RAS, we were able to obtain mass balance of key parameters of the system. **Figure 3B** shows an example of mass balance calculation in RAS. Mass balance were calculated to demonstrate three hotspots in RAS: i) production or consumption in the fish tank, ii) production and consumption in



biofilter, and iii) removal or addition in degasser. More details of the calculations can be found in **Figure 3B**. The values represent typical values found in post-smolt RAS with salinity around 13 ppt (‰).

As can be seen in **Figure 3B**, the degassing capacity is of utmost importance to control the sudden increasing H<sub>2</sub>S levels in RAS. In a steady-state situation, the production of H<sub>2</sub>S equalizes the removal one.

As discussed earlier in introduction, the main production of constant H<sub>2</sub>S forms in the anaerobic part of the biofilm due to the large amount of surface area provided by the bio-elements. This diffusion rate of H<sub>2</sub>S through the biofilm is the root cause of the steady-state H<sub>2</sub>S concentration level in the RAS and it is mainly controlled by the degassing capacity of the system. The exchange of water provides only a minor contribution – this will be discussed in the next section.

### 3.3 The Impact of Degassing in Controlling H<sub>2</sub>S Spike

H<sub>2</sub>S can be released in a matter of seconds and causes a spike in the system – it is known as bomb effect. Such incidents are associated with high risks in fish farming industry today. **Figure 3C** shows such H<sub>2</sub>S incident, where a stable H<sub>2</sub>S concentration level under 1 µg/L suddenly jumped to 10 µg/L, and then gradually was aerated out of the system after 15 hours, and eventually the water was back to normal steady-state condition. This incident was captured by the AQS and highlights the responsiveness of the system in occurrence of H<sub>2</sub>S spikes. As can be seen in **Figure 3C**, the spike was controlled by smooth removal of the gas through the degassing process. It can be seen from the registered data that there is continuous H<sub>2</sub>S

formation throughout RAS-processes, ergo, the only way to cope with it, it is either through degassing or at minor extent by the water exchange.

This sudden release of H<sub>2</sub>S-spike may have been caused by mechanical breakage of the relatively small volume (some liters) of the anaerobic dead materials saturated at 100% H<sub>2</sub>S. Although conclusive remarks regarding the formation mechanism of H<sub>2</sub>S cannot be made, immediate actions can be performed in time once warning are triggered in occurrence of such spikes. This can help the industry to control and then reduce the impact of H<sub>2</sub>S-spike by means of degassing. Therefore, It is critical to have emergency procedures in action as every minute matters. AQS would then be a useful tool to let industry gain enough knowledge on H<sub>2</sub>S-spikes so that such incidents can be predicted, and hence immediate mitigation measures can be taken in advance.

### 3.4 H<sub>2</sub>S Formation Rate

It has been shown that there is a constant production rate of H<sub>2</sub>S in RAS. This may vary depending on many factors that the industry today may have not full overview of. **Figure 3D** highlights the calculation of H<sub>2</sub>S formation rate based on given amount of formed H<sub>2</sub>S per area of biofilm per unit of time. This ratio (criteria) is a specific parameter for each RAS and it is important to be followed in each RAS over time. It can also be an important criteria to consider in process of designing a RAS. As can be seen in **Figure 3D**, the main contribution to control H<sub>2</sub>S in RAS is through degassing and just to a minor extent through water exchange. This means reducing degassing capacity when CO<sub>2</sub> levels are low may not be a valuable idea as poses a risk of

H<sub>2</sub>S-accumulation in the system unless there is full control on the H<sub>2</sub>S levels to avoid such incidents.

## 4 DISCUSSION AND CONCLUSION

The formation of toxic H<sub>2</sub>S known as the silent killer, is of major concern in aquatic environments, especially within RAS, as it appears fast, leaves its impact fast and is the cause for the growing trend of reported H<sub>2</sub>S-induced fish mortalities. Despite its high toxicity, authorities have not yet set a regulation to address the toxicity level of the gas in aquaculture – freshwater and marine species are recommended to not being exposed to more than 2 µg/L and 5 µg/L respectively in literature. Although H<sub>2</sub>S-induced fish mortalities is a well-known issue, there is still scarce information regarding its formation mechanism, the potential forming spots, its toxicity threshold, and the relationship to other water quality parameters with regards to the kinetic of its formation mechanism. To answer all of these, collecting data from potential hotspots through a real-time monitoring system, studying those data in occurrence of H<sub>2</sub>S spikes, and ultimately analysis the accidents and the variation in production rates over time, should be regarded in the context of bridging this industry gap. To this end, we developed a sensor that can detect the gas in sub (µg/L) with high precision given the fact that the gas toxicity threshold proposed to be as low as 2 µg/L. This helps the researchers, experts, and fish farmers to have more concrete understanding of the gas formation mechanism to consider possible mitigation strategies for further preventing such incidents. Furthermore, since AQS can measure very low concentration of the formed H<sub>2</sub>S in the system, it can be a useful tool to let authorities find out and later set toxicity threshold for both freshwater and marine species in RAS with regards to H<sub>2</sub>S. Typical SeaRAS AquaSense system (AQS) consists of four units that were installed, in this work, in four potential hotspots in RAS: before and after fish tank, after biofilter, and in the exhaust of the degasser. Each unit measured H<sub>2</sub>S (µg/L), O<sub>2</sub> (% saturation), and CO<sub>2</sub> (mg/L) in real-time with continuous track of pH, salinity (‰), alkalinity (mg/L), turbidity (NTU) and temperature (T) (°C). The autonomous wireless-based sensors used in AQS units collect and stores data in time-resolution of one second in the water treatment loop (covering range for measuring H<sub>2</sub>S concentration in AQS are 0–80 µg/L and 2–430 µg/L – these are valid under following condition: T = 15°C, S = 20 ‰, pH = 7, pressure = 1 atm). AQS should not be performed near to downstream of ozone (O<sub>3</sub>). AQS provides valuable information, on how H<sub>2</sub>S is produced and controlled and how such spikes disperse in the loop. This real-time measuring and monitoring

of water quality parameters let us calculate the mass-balance change throughout RAS, providing a new insight on production and removal ratio of different parameters in the process including H<sub>2</sub>S. The first finding of this work highlights that the formation of H<sub>2</sub>S in RAS is inevitable as there is continuous production of the gas in the anaerobic part of the biofilm where a large surface is provided by bio-elements. H<sub>2</sub>S is something that exist in all systems, and hence the industry must learn instead how to cope with it. One may argue that the formed H<sub>2</sub>S, although low, may be still enough to lead to H<sub>2</sub>S-poisoning once accumulated in the system. The calculation of mass balance changes based on measured data in sensors in AQS units showed a significant removal of H<sub>2</sub>S gas through degassing. Degassing was demonstrated in this work as a powerful tool to control the continuous H<sub>2</sub>S formation in RAS process. The results also showed the importance of the ratio of the formed H<sub>2</sub>S per area of biofilm in the course of time. Although conclusive remarks regarding the variation of H<sub>2</sub>S per area of biofilm cannot be made, this work suggests that a thorough investigation may yields answers in future research. More research is also needed to investigate acceptable limits to control H<sub>2</sub>S-poisoning in the context of water quality in RAS and to provide insight on the interaction between H<sub>2</sub>S and other water quality parameters such as CO<sub>2</sub>.

## DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the authors do not have permission to share data. Requests to access the datasets should be directed to [www.searas.no](http://www.searas.no).

## AUTHOR CONTRIBUTIONS

This research is the result of the work of all authors. The main credit goes to SeaRAS AS as the main founder and funder of the research.

## FUNDING

This research was funded by SeaRAS AS.

## ACKNOWLEDGMENTS

Special thanks to all SeaRAS team for all the efforts they have put to develop such system.

## REFERENCES

- Åtland, Å., and Stenberg, S. K. (2019). "Water Quality," in *The Health Situation in Norwegian Aquaculture 2018*. Eds. B. Hjeltnes, B. Bang-Jensen, G. Bornø, A. Haukaas and C. S. Walde (Norway: Norwegian Veterinary Institute 2019), 96–99.
- Barton, L. L., Fardeau, M. L., and Fauque, G. D. (2014). Hydrogen Sulfide: A Toxic Gas Produced by Dissimilatory Sulfate and Sulfur Reduction and Consumed by Microbial Oxidation. *Mets. lon. Life Sci.* 14, 237–277. doi: 10.1007/978-94-017-9269-1\_10
- Boyd, C. E. (2014). *Hydrogen Sulfide, Toxic But Manageable* (Auburn, Alabama USA: Global Aquaculture Advocate), 34–36.
- Cämmerer, M., Mayer, T., Penzel, S., Rudolph, M., and Borsdorf, H. (2020). Application of Low-Cost Electrochemical Sensors to Aqueous Systems to Allow Automated Determination of NH<sub>3</sub> and H<sub>2</sub>S in Water. *Sensors* 20 (10), 2814. doi: 10.3390/s20102814
- J. Dalsgaard (Ed.) (2019). "5th NordicRAS Workshop on Recirculating Aquaculture Systems," in *Book of Abstracts* (Berlin, Germany: DTU Aqua, Danmarks Tekniske Universitet).
- Duan, Z., Sun, R., Liu, R., and Zhu, C. (2007). Accurate Thermodynamic Model for the Calculation of H<sub>2</sub>S Solubility in Pure Water and Brines. *Energy Fuel.* 21 (4), 2056–2065. doi: 10.1021/ef070040p
- Hjeltnes, B., Bang-Jensen, B., Bornø, G., Haukaas, A., and Walde, C. (2019). *The Health Situation in Norwegian Aquaculture 2018* (Ås, Norway: Norwegian Veterinary Institute), 132. (ISSN 1893-1480).

- Holmer, M., and Hasler-Sheetal, H. (2014). Sulfide Intrusion in Seagrasses Assessed by Stable Sulfur Isotopes—A Synthesis of Current Results. *Front. Mar. Sci.* 1, 64. doi: 10.3389/fmars.2014.00064
- Kierner, M. C., Black, K. D., Lussot, D., Bullock, A. M., and Ezzi, I. (1995). The Effects of Chronic and Acute Exposure to Hydrogen Sulphide on Atlantic Salmon (*Salmo Salar* L.). *Aquaculture* 135 (4), 311–327. doi: 10.1016/0044-8486(95)01025-4
- Letelier-Gordo, C. O., Aalto, S. L., Suurnäkki, S., and Pedersen, P. B. (2020). Increased Sulfate Availability in Saline Water Promotes Hydrogen Sulfide Production in Fish Organic Waste. *Aquacul. Eng.* 89, 102062. doi: 10.1016/J.AQUAENG.2020.102062
- Li, Q., and Lancaster, J. R. Jr. (2013). Chemical Foundations of Hydrogen Sulfide Biology. *Nitric. Oxide* 35, 21–34. doi: 10.1016/J.NIOX.2013.07.001
- Mougin, P., Lamoureux-Var, V., Bariteau, A., and Huc, A. Y. (2007). Thermodynamic of Thermochemical Sulphate Reduction. *J. Petrol. Sci. Eng.* 58 (3–4), 413–427. doi: 10.1016/j.petrol.2007.01.005
- Prema, D. (2020). “Water Quality Management in Aquaculture,” in *The Blue Bonanza: A Manual for on the Job Training Programme for VHSE Students on Advances in Fisheries & Aquaculture Techniques* (Kochi: ICAR-Central Marine Fisheries Research Institute), pp 4–pp78. Available at: <http://eprints.cmfri.org.in/14147/>.
- Rojas-Tirado, P., Aalto, S. L., Åtland, Å., and Letelier-Gordo, C. (2021). Biofilters are Potential Hotspots for H<sub>2</sub>S Production in Brackish and Marine Water RAS. *Aquaculture* 536, 736490. doi: 10.1016/J.AQUACULTURE.2021.736490
- Rosten, T., Åtland, Å., Kristensen, T., Braaten, B., Rosseland, B. O., and Winther, U. (2004). *Vannkvalitet Og Dyrevelferd I Oppdrett. Utredning for Mattilsynet* (Trondheim, Norway: KPMG Senter for havbruk og fiskeri).
- Rubright, S. L. M., Pearce, L. L., and Peterson, J. (2017). Environmental Toxicology of Hydrogen Sulfide. *Nitric. Oxide.: Biol. Chem.* 71, 1. doi: 10.1016/J.NIOX.2017.09.011
- Sommerset, I., Bang Jensen, B., Bornø, G., Haukaas, A., and Brun, E. (2020). *The Health Situation in Norwegian Aquaculture 2020* (Ås, Norway: Norwegian Veterinary Institute).
- Tanudjaja, Y. (2021). *Early Warning Signs and Dynamics of H<sub>2</sub>S Production In Recirculating Aquaculture System* (Trondheim: Norwegian University of Science and Technology (NTNU)).
- Weiner, E. R. (2008). *Applications of Environmental Aquatic Chemistry: A Practical Guide*. 2nd ed (Boca Raton: CRC Press).
- Yu, T., and Bishop, P. L. (2001). Stratification and Oxidation–Reduction Potential Change in an Aerobic and Sulfate-Reducing Biofilm Studied Using Microelectrodes. *Water Environ. Res.* 73 (3), 368–373. doi: 10.2175/106143001X139399

**Conflict of Interest:** Authors in this article were employed by SeaRAS. The funder had the following involvement with the study: design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Lien, Valsvik, Nordstrand, Martinez, Rogne, Hafssås, Queralt, Fathi and Aga. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.