Chapter 3
Recirculating Aquaculture Technologies

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Abstract Recirculating aquaculture technology, which includes aquaponics, has been under development for the past 40 years from a combination of technologies derived from the wastewater treatment and aquaculture sectors. Until recently, recirculating aquaculture systems (RAS) farms have been relatively small compared with other types of modern aquaculture production. The last two decades have seen a significant increase in the development of this technology, with increased market acceptance and scale. This chapter provides a brief overview of the history, water quality control processes, new developments and ongoing challenges of RAS.

Keywords Recirculating aquaculture systems (RAS) · Wastewater treatment · Biofilter · Denitrification · Membrane technology

3.1 Introduction

Recirculating aquaculture systems (RAS) describe intensive fish production systems which use a series of water treatment steps to depurate the fish-rearing water and facilitate its reuse. RAS will generally include (1) devices to remove solid particles from the water which are composed of fish faeces, uneaten feed and bacterial flocs (Chen et al. 1994; Couturier et al. 2009), (2) nitrifying biofilters to oxidize ammonia

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The original version of this chapter was revised: The chemical formula "NO2" has been corrected to "NO_2" on page 37. The correction to this chapter is available at https://doi.org/10.1007/978-3-030-15943-6_25

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excreted by fish to nitrate (Gutierrez-Wing and Malone 2006) and (3) a number of gas exchange devices to remove dissolved carbon dioxide expelled by the fish as well as/or adding oxygen required by the fish and nitrifying bacteria (Colt and Watten 1988; Moran 2010; Summerfelt 2003; Wagner et al. 1995). In addition, RAS may also use UV irradiation for water disinfection (Sharrer et al. 2005; Summerfelt et al. 2009), ozonation and protein skimming for fine solids and microbial control (Attramadal et al. 2012a; Gonçalves and Gagnon 2011; Summerfelt and Hochheimer 1997) and denitrification systems to remove nitrate (van Rijn et al. 2006).

Modern recirculating aquaculture technology has been developing for more than 40 years, but novel technologies increasingly offer ways to change the paradigms of traditional RAS including improvements on classic processes such as solids capture, biofiltration and gas exchange. RAS has also experienced important developments in terms of scale, production capacities and market acceptance, with systems becoming progressively larger and more robust.

This chapter discusses how RAS technology has developed over the past two decades from a period of technological consolidation to a new era of industrial implementation.

3.1.1   History of RAS

The earliest scientific research on RAS conducted in Japan in the 1950s focused on biofilter design for carp production driven by the need to use locally limited water resources more productively (Murray et al. 2014). In Europe and the United States, scientists similarly attempted to adapt technologies developed for domestic waste-water treatment in order to better reuse water within recirculating systems (e.g. activated sludge processes for sewage treatment, trickling, submerged and down-flow biofilters and several mechanical filtration systems). These early efforts included primarily work on marine systems for fish and crustacean production, but were soon adopted in arid regions where the agriculture sector is restricted by water supply. In aquaculture, different solutions have been designed to maximize water use including highly intensive recirculating systems that incorporate water filtration systems such as drum filters, biological filters, protein skimmers and oxygen injection systems (Hulata and Simon 2011). Despite a strong conviction by pioneers in the industry about the commercial viability of their work, most of the early studies focused exclusively on the oxidation of toxic inorganic nitrogen wastes derived from protein metabolism. The trust in technology was reinforced by the successful operation of public as well as domestic aquaria, which generally feature over-sized treatment units to ensure crystal-clear water. Additionally, extremely low stocking densities and associated feed inputs meant that such over-engineering still made a relatively small contribution to capital and operational costs of the system compared to intensive RAS. Consequently, the changes in process dynamics associated with scale-change were unaccounted for, resulting in the under-sizing of RAS treatment units in order to minimize capital costs. As a consequence, safety margins were far too narrow or non-existent (Murray et al. 2014). Because many of the pioneering

scientists had biological rather than engineering backgrounds, technical improvements were also constrained by miscommunications between scientists, designers, construction personnel and operators. The development of a standardized terminology, units of measurement and reporting formats in 1980 (EIFAC/ICES 1980), helped address the situation, though regional differences still persisted. It was not until the mid-1980s that cyclic water quality parameters became well recognized as being important in pond production, e.g. periodically measuring the concentrations of pH, oxygen, TAN (total ammonia nitrogen), NO_2 (nitrate), BOD (biochemical oxygen demand) and COD (chemical oxygen demand).

In the latter part of the last century, numerous articles were published on the early development of RAS. Rosenthal (1980) elaborated on the state of recirculation systems in Western Europe, while Bovendeur et al. (1987) developed a water recirculation system for the culture of African catfish in relation to waste production and waste removal kinetics (a design was presented for a water treatment system consisting of a primary clarifier and an aerobic fixed-film reactor that demonstrated satisfactory results for high-density culture of African catfish). This work was part of the rapid development in fish culture systems up to the mid-1990s in Northern and Western Europe (Rosenthal and Black 1993), as well as in North America (Colt 1991). New classifications, such as the classification according to how water flows through an aquaculture system, provided key insights with respect to the water quality processes that are important for fish production (Krom and van Rijn 1989). In subsequent work by van Rijn (1996), concepts were introduced focused on the biological processes underlying the treatment systems. The conclusions from this work were that incorporating methods for reducing the accumulation of sludge and nitrate resulted in more stable water quality conditions within the culture units. During this period, RAS production increased significantly in volume and species diversity (Rosenthal 1980; Verreth and Eding 1993; Martins et al. 2005). Today, more than 10 species are produced in RAS (African catfish, eel and trout as major freshwater species and turbot, seabass and sole as major marine species) (Martins et al 2010b), with RAS also becoming a crucial element in the production of larvae and juveniles of diverse species.

While maximum sustainable yields of many aquatic wild stock species have been or will soon be reached, and many species are already overfished, RAS is considered a key technology that will help the aquaculture sector meet the needs for aquatic species over the coming decades (Ebeling and Timmons 2012).

3.1.2 A Short History of Aquaponics in the Context of RAS

Aquaponics is a term that has been 'coined' in the 1970s, but in practice has ancient roots – although there are still discussions about its first occurrence. The Aztec cultivated agricultural islands known as *chinampas* (the earliest 1150–1350CE), in a system considered by some to be the first form of aquaponics for agricultural use (Fig. 3.1). In such systems, plants were raised on stable, or sometime movable and floating islands placed in lake shallows wherein nutrient rich mud could be dredged

Fig. 3.1 Chinampas (floating gardens) in Central America – artificial island construction as antecedent of aquaponic technology. (From Marzolino/Shutterstock.com)

from the chinampa canals and placed on the islands to support plant growth (Crossley 2004).

An even earlier example of aquaponics started on the other side of the world in south China and is believed to have spread within South East Asia where Chinese settlers from Yunnan settled around 5 CE. Farmers cultivated and farmed rice in paddy fields in combination with fish (FAO 2001). These polycultural farming systems existed in many Far Eastern countries to raise fish such as oriental loach (*Misgurnus anguillicaudatus*) (Tomita-Yokotani et al. 2009), swamp eel (fam. Synbranchidae), common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*) (FAO 2004). In essence, however, these were not aquaponic systems but can be best described as early examples of integrated aquaculture systems (Gomez 2011). In the twentieth century, the first attempts to create practical, efficient and integrated fish production systems alongside vegetables were made in the 1970s with the work of Lewis and Naegel (Lewis and Wehr 1976; Naegel 1977; Lewis et al. 1978). Further early systems were designed by Waten and Busch in 1984 and Rakocy in 1989 (Palm et al. 2018).

3.2   Review of Water Quality Control in RAS

RAS are complex aquatic production systems that involve a range of physical, chemical and biological interactions (Timmons and Ebeling 2010). Understanding these interactions and the relationships between the fish in the system and the equipment used is crucial to predict any changes in water quality and system performance. There are more than 40 water quality parameters than can be used to determine water quality in aquaculture (Timmons and Ebeling 2010). Of these, only a few (as described in Sects. 3.2.1, 3.2.2, 3.2.3, 3.2.4, 3.2.5, 3.2.6 and 3.2.7) are traditionally controlled in the main recirculation processes, given that these processes can rapidly affect fish survival and are prone to change with the addition of feed to the system. Many other water quality parameters are not normally monitored or controlled because (1) water quality analytics may be expensive, (2) the pollutant to be analysed can be diluted with daily water exchange, (3) potential water sources containing them are ruled out for use or (4) because their potential negative effects have not been observed in practice. Therefore, the following water quality parameters are normally monitored in RAS.

3.2.1   Dissolved Oxygen (DO)

Dissolved oxygen (DO) is generally the most important water quality parameter in intensive aquatic systems, as low DO levels may quickly result in high stress in fish, nitrifying biofilter malfunction and indeed significant fish losses. Commonly, stocking densities, feed addition, temperature and the tolerance of the fish species to hypoxia will determine the oxygen requirements of a system. As oxygen can be transferred to water in concentrations higher than its saturation concentration under atmospheric conditions (this is called supersaturation), a range of devices and designs exist to ensure that the fish are provided with sufficient oxygen.

In RAS, DO can be controlled via aeration, addition of pure oxygen, or a combination of these. Since aeration is only capable of raising the DO concentrations to the atmospheric saturation point, the technique is generally reserved for lightly loaded systems or systems with tolerant species such as tilapia or catfish. However, aerators are also an important component of commercial RAS where the use of expensive technical oxygen is reduced by aerating water with a low dissolved oxygen content back to the saturation point before supersaturating the water with technical oxygen.

There are several types of aerators and oxygenators that can be used in RAS and these fall within two broad categories: gas-to-liquid and liquid-to-gas systems (Lekang 2013). Gas-to-liquid aerators mostly comprise diffused aeration systems where gas (air or oxygen) is transferred to the water, creating bubbles which exchange gases with the liquid medium (Fig. 3.2). Other gas-to-liquid systems include passing gases through diffusers, perforated pipes or perforated plates to
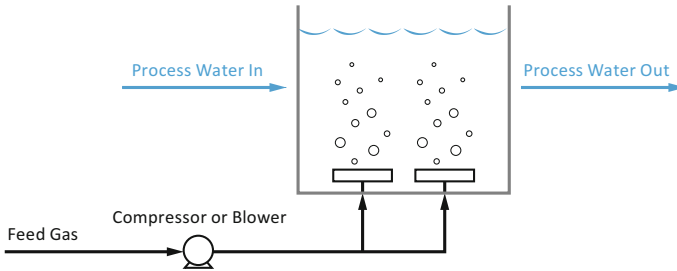
Fig. 3.2 Diagrams of two gas-to-liquid transfer examples: diffused aeration and Venturi injectors/ aspirators
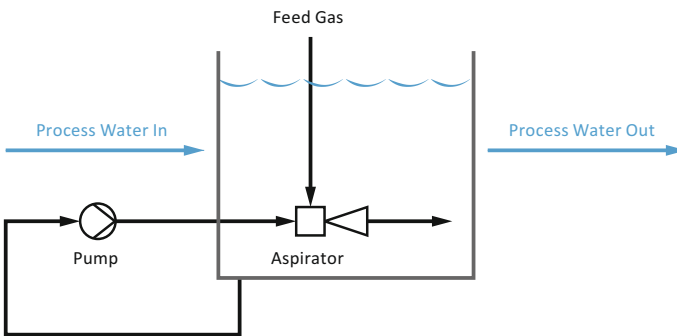

Fig. 3.3 Diagrams of two liquid-to-gas transfer examples: the packed column aerator and surface splashers in an enclosed tank. The packed column aerator allows water to trickle down an enclosed vessel, usually packed with structured media, where air is forced through using a fan or blower. Surface splashers found in pond aquaculture can also be used in enclosed atmospheres enriched with gases – normally oxygen – for gas transfer

create bubbles using Venturi injectors which create masses of small bubbles or devices which trap gas bubbles in the water stream such as the Speece Cone and the U-tube oxygenator.

Liquid-to-gas aerators are based on diffusing the water into small droplets to increase the surface area available for contact with the air, or creating an atmosphere enriched with a mixture of gases (Fig. 3.3). The packed column aerator (Colt and Bouck 1984) and the low-head oxygenators (LHOs) (Wagner et al. 1995) are examples of liquid-to-gas systems used in recirculating aquaculture. However, other liquid-to-gas systems popular in ponds and outdoor farms such as paddlewheel aerators (Fast et al. 1999) are also used in RAS.

Considerable literature is available on gas exchange theory and the fundamentals of gas transfer in water, and the reader is encouraged not only to consult aquaculture and aquaculture engineering texts, but also to refer to process engineering and wastewater treatment materials for a better understanding of these processes.

3.2.2   Ammonia

In an aqueous medium, ammonia exists in two forms: a non-ionized form (NH_3) that is toxic to fish and an ionized form (NH_4^+) that has low toxicity to fish. These two form the total ammonia nitrogen (TAN), wherein the ratio between the two forms is controlled by pH, temperature and salinity. Ammonia accumulates in the rearing water as a product of the protein metabolism of the fish (Altinok and Grizzle 2004) and can achieve toxic concentrations if left untreated. Of the 35 different types of freshwater fish that have been studied, the average acute toxicity value for ammonia is 2.79 mg NH3/l (Randall and Tsui 2002).

Ammonia has been traditionally treated in recirculation systems with nitrifying biofilters, devices that are designed to promote microbial communities that can oxidize ammonia into nitrate (NO_3). Although the use of nitrifying biofilters is not new, contemporary RAS has seen a streamlining of biofilter designs, with just a few, well-studied designs having widespread acceptance. Other highly innovative techniques to treat ammonia have been developed over the past few years, but are not widely applied commercially (examples noted below).

Ammonia is oxidized in biofilters by communities of nitrifying bacteria. Nitrifying bacteria are chemolithotrophic organisms that include species of the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrobacter* and *Nitrococcus* (Prosser 1989). These bacteria obtain their energy from the oxidation of inorganic nitrogen compounds (Mancinelli 1996) and grow slowly (replication occurs 40 times slower than for heterotrophic bacteria) so are easily outcompeted by heterotrophic bacteria if organic carbon, mostly present in biosolids suspended in the culture water, are allowed to accumulate (Grady and Lim 1980). During RAS operation, good system management greatly relies on minimizing suspended solids through adequate solids removal techniques (Fig. 3.4).

Nitrifying biofilters or biofilter reactors have been roughly classified into two main categories: suspended growth and attached growth systems (Malone and Pfeiffer 2006). In suspended growth systems, the nitrifying bacterial communities grow freely in the water, forming bacterial flocs which also harbour rich ecosystems where protozoa, ciliates, nematodes and algae are present (Manan et al. 2017). With appropriate mixing and aeration, algae, bacteria, zooplankton, feed particles and faecal matter remain suspended in the water column and naturally flocculate together, forming the particles that give biofloc culture systems their name (Browdy et al. 2012). The main disadvantage of suspended growth systems is their tendency to lose their bacterial biomass as process water flows out of the reactor, thus requiring a means to capture and return it to the system. In attached growth systems, solid forms (sand grains, stones, plastic elements) are used as substrates to retain the bacteria inside the reactor and thus, do not need a post-treatment solids capture step. Generally, attached growth systems provide more surface area for bacterial attachment than suspended growth systems, and do not produce significant solids in their outflow, which is one of the main reasons why attached growth biofilters have been so commonly used in RAS.
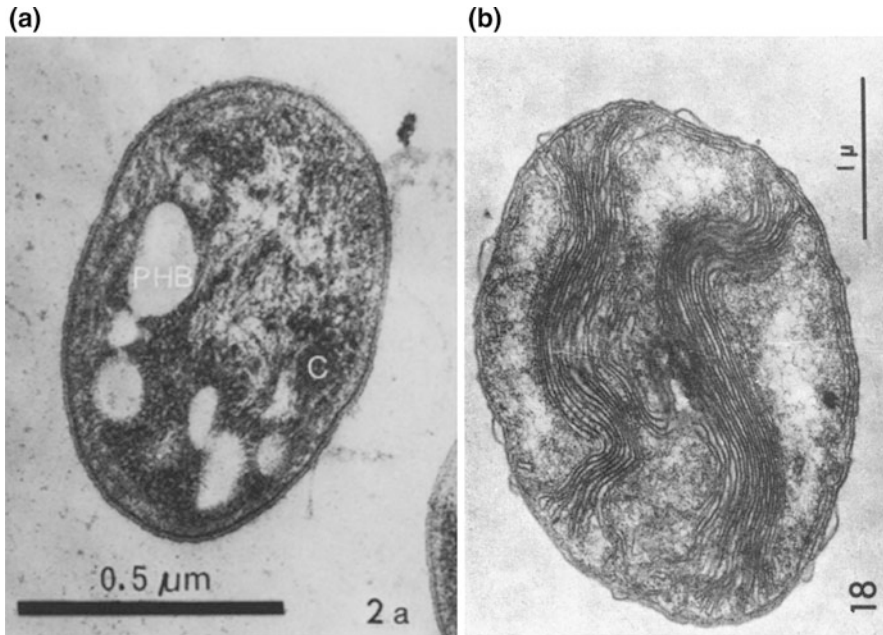
Fig. 3.4 Nitrifying bacteria *Nitrosomonas* (left), and *Nitrobacter* (right). (Left photo: Bock et al. 1983. Right photo: Murray and Watson 1965)

Efforts have been made to classify biofilters and to document their performance in order to help farmers and designers specify systems with a better degree of reliability (Drennan et al. 2006; Gutierrez-Wing and Malone 2006). In recent years, the aquaculture industry has opted for biofilter designs which have been widely studied and thus can offer predictable performance. The moving bed bioreactor (Rusten et al. 2006), the fluidized sand filter bioreactor (Summerfelt 2006) and the fixed-bed bioreactor (Emparanza 2009; Zhu and Chen 2002) are examples of attached growth biofilter designs which have become standard in modern commercial RAS. Trickling filters (Díaz et al. 2012), another popular design, have seen their popularity reduced due to their relatively high pumping requirements and relatively large sizes.

3.2.3   Biosolids

Biosolids in RAS originate from fish feed, faeces and biofilms (Timmons and Ebeling 2010) and are one of the most critical and difficult water quality parameters to control. As biosolids serve as a substrate for heterotrophic bacterial growth, an increase in their concentration may eventually result in increased oxygen consumption, poor biofilter performance (Michaud et al. 2006), increased water turbidity and

even mechanical blockage of parts of the system (Becke et al. 2016; Chen et al. 1994; Couturier et al. 2009).

In RAS, biosolids are generally classified both by their size and their removal capacity by certain techniques. Of the total fraction of solids produced in a RAS, settleable solids are those generally bigger than 100 μm and that can be removed by gravity separation. Suspended solids, with sizes ranging from 100 μm to 30 μm, are those which do not settle out of suspension, but that can be removed by mechanical (i.e. sieving) means. Fine solids, with sizes of less than 30 μm, are generally those that cannot be removed by sieving, and must be controlled by other means such as physico-chemical processes, membrane filtration processes, dilution or bioclarification (Chen et al. 1994; Lee 2014; Summerfelt and Hochheimer 1997; Timmons and Ebeling 2010; Wold et al. 2014). The techniques for controlling settleable and suspended solids are well known and developed, and an extensive literature exists on the subject. For example, the use of dual-drain tanks, swirl separators, radial flow separators and settling basins is a popular means to control settleable solids (Couturier et al. 2009; Davidson and Summerfelt 2004; De Carvalho et al. 2013; Ebeling et al. 2006; Veerapen et al. 2005). Microscreen filters are the most popular method for suspended solids control (Dolan et al. 2013; Fernandes et al. 2015) and are often used in the industry to control both settleable and suspended solids with a single technique. Other popular solids capture devices are depth filters such as the bead filters (Cripps and Bergheim 2000) and rapid sand filters, which are also popular in swimming pool applications. Moreover, design guidelines to prevent the accumulation of solids in tanks, pipework, sumps and other system components are also available in the literature (Davidson and Summerfelt 2004; Lekang 2013; Wong and Piedrahita 2000). Lastly, fine solids in RAS are commonly treated by ozonation, bioclarification, foam fractionation or a combination of these techniques. The last few years in RAS development have focused on a greater understanding of how to control the fine solids fraction and to understand its effect on fish welfare and system performance.

3.2.4 Carbon Dioxide (CO$_2$)

In RAS, the control of dissolved gases does not stop with supplying oxygen to the fish. Other gases dissolved in the rearing water may affect fish welfare if not controlled. High dissolved carbon dioxide (CO_2) concentrations in the water inhibit the diffusion of CO_2 from the blood of fish. In fish, increased CO_2 in blood reduces the blood's pH and in turn, the affinity of haemoglobin for oxygen (Noga 2010). High CO_2 concentrations have also been associated with nephrocalcinosis, systemic granulomas and chalky deposits in organs in salmonids (Noga 2010). CO_2 in RAS originates as a product of heterotrophic respiration by fish and bacteria. As a highly soluble gas, carbon dioxide does not reach atmospheric equilibrium as easily as oxygen or nitrogen and thus, it must be put in contact with high volumes of air with a low concentration of CO_2 to ensure transfer out of water (Summerfelt 2003). As a

general rule, RAS which are supplied with pure oxygen will require some form of carbon dioxide stripping, while RAS which are supplied with aeration for oxygen supplementation will not require active CO_2 stripping (Eschchar et al. 2003; Loyless and Malone 1998).

In theory, any gas transfer/aeration device open to the atmosphere will offer some form of CO_2 stripping. However, specialized carbon dioxide stripping devices require that large volumes of air are put in contact with the process water. CO_2 stripper designs have mostly focused on cascade-type devices such as cascade aerators, trickling biofilters and, more importantly, the packed column aerator (Colt and Bouck 1984; Moran 2010; Summerfelt 2003), which has become a standard piece of equipment in commercial RAS operating with pure oxygen. Although the development of packed column aeration technology has advanced over past years, most of the research done on this device has been focused on understanding its performance under different conditions (i.e. freshwater vs seawater) and design variations such as heights, packing types and ventilation rates. The effect of the hydraulic loading rate (unit flow per unit area of degasser) is known to have an effect on the efficiency of a degasser, but further research is needed to have a better understanding of this design parameter.

3.2.5 Total Gas Pressure (TGP)

Total gas pressure (TGP) is defined as the sum of the partial pressures of all the gases dissolved in an aqueous solution. The less soluble a gas is, the more 'room' it occupies in the aqueous solution and thus, the more pressure it exerts in it. Of the main atmospheric gases (nitrogen, oxygen and carbon dioxide) nitrogen is the least soluble (e.g. 2.3 times less soluble than oxygen and more than 90 times less soluble than carbon dioxide). Thus, nitrogen contributes to total gas pressure more than any other gas, but is not consumed by fish or heterotrophic bacteria, so it will accumulate in the water unless stripped. It is also important to note that oxygen will also contribute to high TGP if the gas transfer process does not allow excess gases to be displaced out of the solution. A classic example of this are ponds with photoautotrophic activity in them. Photoautotrophs (usually plant organisms that carry out photosynthesis) release oxygen into the water while a quiet water surface may not provide enough gas exchange for excess gas to escape to the atmosphere and thus, supersaturation may occur.

Fish require total gas pressures equal to atmospheric pressure. If fish breathe water with a high total gas pressure, excess gas (generally nitrogen) exits the bloodstream and forms bubbles, with often serious health effects for the fish (Noga 2010). In aquaculture this is known as gas bubble disease.

Avoiding high TGP requires careful examination of all areas in the RAS where gas transfer may occur. High-pressure oxygen injection without off-gassing (allowing excess nitrogen to be displaced out of the water) may also contribute to high TGP. In systems with fish which are very sensitive to TGP, the use of vacuum

degassers is an option (Colt and Bouck 1984). However, maintaining a RAS free from areas of uncontrolled gas pressurization, using carbon dioxide strippers (which will also strip nitrogen) and dosing technical oxygen with care, is enough to keep TGP at safe levels in commercial RAS.

3.2.6 Nitrate

Nitrate (NO_3) is the end product of nitrification and commonly the last parameter to be controlled in RAS, due to its relatively low toxicity (Davidson et al. 2014; Schroeder et al. 2011; van Rijn 2013). This is mostly attributed to its low permeability at the fish gill membrane (Camargo and Alonso 2006). The toxic action of nitrate is similar to that of nitrite, affecting the capacity of oxygen-carrying molecules. The control of nitrate concentrations in RAS has traditionally been achieved by dilution, by effectively controlling the hydraulic retention time or daily exchange rate. However, the biological control of nitrate using denitrification reactors is a growing area of research and development in RAS.

Tolerance to nitrate may vary by aquatic species and life stage, with salinity having an ameliorating effect over its toxicity. It is important for RAS operators to understand the chronic effects of nitrate exposure rather than the acute effects, as acute concentrations will probably not be reached during normal RAS operation.

3.2.7 Alkalinity

Alkalinity is, in broad terms, defined as the pH buffering capacity of water (Timmons and Ebeling 2010). Alkalinity control in RAS is important as nitrification is an acid-forming process which destroys it. In addition, nitrifying bacteria require a constant supply of alkalinity. Low alkalinity in RAS will result in pH swings and nitrifying biofilter malfunction (Summerfelt et al. 2015; Colt 2006). Alkalinity addition in RAS will be determined by nitrification activity in the systems, which is in turn related to feed addition, by the alkalinity content of the make-up (daily exchange) water and by the presence of denitrifying activity, which restores alkalinity (van Rijn et al. 2006).

3.3 Developments in RAS

The last few years have seen an increase in the number and sizes of recirculating aquaculture farms, especially in Europe. With the increase in acceptance of the technology, improvements over traditional engineering approaches, innovations and new technical challenges keep emerging. The following section describes the

key design and engineering trends and new challenges that recirculating aquaculture technology is facing.

3.3.1   Main Flow Oxygenation

The control of dissolved oxygen in modern RAS aims to increase the efficiency of oxygen transfer and decrease the energy requirements of this process. Increasing the oxygen transfer efficiency can be achieved by devising systems which retain oxygen gas in contact with water for longer, while a decrease in energy requirements may be achieved by the use of low-head oxygen transfer systems or using systems which do not use electricity at all, such as liquid oxygen systems connected to oxygen diffusers operating only by pressure. A defining factor of low-head oxygenators is the relatively low dissolved concentration that can be achieved compared to high-pressure systems. To overcome this limitation, low-head oxygenation devices are strategically placed to treat the full recirculating flow instead of using a smaller bypass of highly supersaturated water, thus ensuring sufficient mass transport of oxygen. Using oxygenation devices installed in the main recirculating flow generates savings in electricity consumption because the use of energy-intensive high-pressure systems that are necessary to achieve high DO concentrations in small flows is avoided. Low-head oxygenation systems may also reduce the amount of pumping systems needed, as high-pressure oxygenation systems are commonly placed on a bypass in the pipelines going to the fish tanks. In contrast, low-head oxygenation devices tend to be comparatively larger because of their need to handle larger flows and thus, their initial cost may be higher. Examples of devices that can treat the totality of the flow include the low-head oxygenator (LHO) (Wagner et al. 1995), operated by gravity as water is firstly pumped into a biofilter and a packed column (Summerfelt et al. 2004), low-head oxygen cones, variants of the Speece Cone (Ashley et al. 2008; Timmons and Losordo 1994) operated at low pressure, the deep shaft cones (Kruger Kaldnes, Norway), also a variant of the Speece cone designed to reach higher operating pressures by means of increased hydrostatic pressure resulting from placing the devices lower than the fish tanks and pump sumps, the U-tube oxygenator and its design variants such as the Farrell tube or the patented oxygen dissolver system (AquaMAOF, Israel) and the use of diffused oxygenation in deep fish tanks (Fig. 3.5).

3.3.2   Nitrifying Biofiltration Alternatives

Although nitrifying biofilters continue to be the main commercially accepted method of ammonia removal in commercial RAS, new nitrogen removal technologies have been developed over recent years. Some of these technologies consider alternative biological pathways to remove ammonia from the culture water, while others aim to
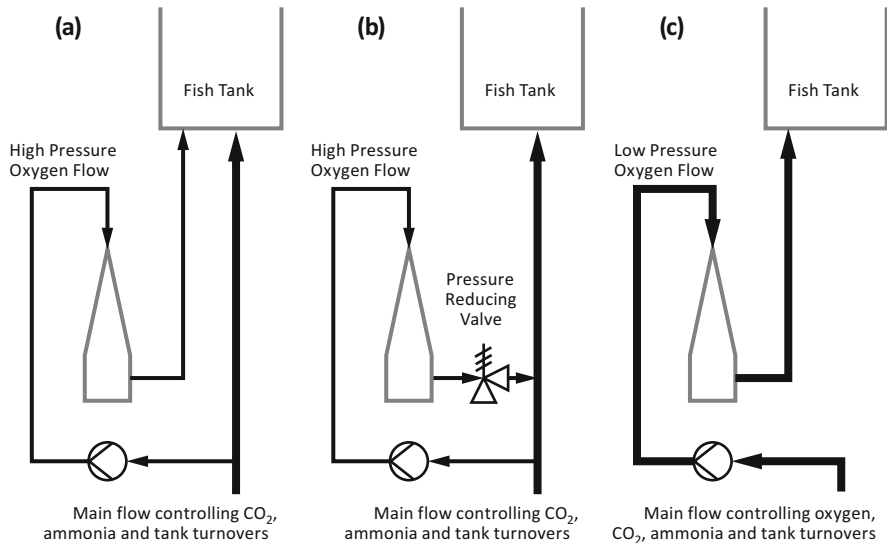
Fig. 3.5 Gas transfer alternatives for recirculating water returning into fish tanks. If the gas contacting vessel allows for pressurization, oxygen can be transferred in high concentrations in relatively small, high-pressure streams (**a**, **b**). However, oxygen at lower concentrations can be transferred into the main recirculation loop, but for this, the oxygen transfer device must be much larger to handle full flow of the system (**c**)

replace or work in parallel with nitrifying biofilters in order to reduce inherent limitations. These include large reactor sizes, susceptibility to crashing, long start-up times and poorer performance in both cold water and marine systems.

Anammox-based Processes

An alternative biological ammonia removal pathway considered for RAS is the anammox process (Tal et al. 2006), which occurs under anaerobic conditions. Anaerobic ammonia oxidation is a process which eliminates nitrogen by combining ammonia and nitrite to produce nitrogen gas (van Rijn et al. 2006). The anammox process is of interest to RAS because it allows for complete autotrophic nitrogen removal, in contrast to traditional combinations of nitrifying biofilters with hetero-trophic denitrification systems requiring organic carbon addition (van Rijn et al. 2006). Moreover, in the anammox pathway, only half of the ammonia released by the fish is aerobically oxidized to nitrite (requiring oxygen), while the other half is anaerobically converted to nitrogen gas along with the nitrite produced. This may provide savings in oxygen and energy use in RAS (van Rijn et al. 2006).

Anammox reactor prototypes have been demonstrated successfully (Tal et al. 2006, 2009), while anammox activity has been suspected to occur in marine denitrification systems (Klas et al. 2006). The European FP7 project DEAMNRECIRC was also successful in creating anammox reactor prototypes for

cold water and seawater aquaculture applications. However, commercial applications of the technology have as yet not been identified by the authors.

Chemical Removal of Ammonia

Ammonia removal systems based on ion exchange and electrochemical oxidation processes are being proposed as alternatives to nitrifying biofilters. Ion exchange processes rely on using adsorptive materials such as zeolites or ion-selective resins to extract dissolved ammonia from the water (Lekang 2013), while electrochemical oxidation processes convert ammonia to nitrogen gas through a number of complex oxidation reactions (Lahav et al. 2015). By comparison, ion exchange processes are suitable for waters with low concentrations of ions (i.e. freshwater), while electrochemical oxidation processes take advantage of the chloride ions present in the water to produce active chlorine species which readily react with ammonia (Lahav et al. 2015) and are thus suitable for waters with higher concentrations of chloride ions (i.e. brackish and marine waters).

Although ion exchange processes are not new, their application into RAS has been limited by their capacity to maintain performance over time: the filtering material eventually becomes 'saturated', losing its adsorptive capacity and, thus, must be regenerated. Gendel and Lahav (2013), proposed a novel approach to an ion exchange-based ammonia process in tandem with an innovative adsorbent regeneration process using electrochemical oxidation. Electrochemical oxidation of ammonia is a process which has received greater attention in recent years, and several concepts have been investigated and have been launched commercially, for example, EloxiRAS in Spain.

Factors limiting the application of these technologies into commercial RAS include, in the case of ion exchange processes, poor economic performance, difficulty to regenerate large amounts of adsorbent materials on demand (Lekang 2013), system complexity requiring the addition of chemical reagents, high electricity consumption and a high degree of suspended solids removal (Lahav et al. 2015), which is often impractical in large-scale RAS. In the case of ammonia electrooxidation processes, the production of toxic reactive species requiring active removal is their most important limitation, although their high solids control requirement, often possible only with pressurized mechanical filters, is also a challenge in RAS operating with large flows and low pressure.

3.3.3 Fine Solids Control

Fine solids are the dominant solids fraction in RAS with particles <30 μm forming more than 90% of the total suspended solids in the culture water. Recent investigations have found that more than 94% of the solids present in the culture water of a RAS are <20 μm in size or 'fine' (Fernandes et al. 2015). The accumulation of fine solids mainly occur as larger solids bypass the mechanical filters (which are not 100% efficient) and are eventually broken down by pumps, friction with surfaces

and bacterial activity. Once solids sizes are reduced, traditional mechanical filtration techniques are rendered useless.

In recent years, the production, control, fish welfare effects and system performance effects of fine solids continue to be explored. The effects of fine solids on fish welfare were initially investigated through fisheries research (Chen et al. 1994). However, the direct effects of fine solids in RAS on fish welfare have not been thoroughly investigated until recently. Surprisingly, separate work on rainbow trout by Becke et al. (2016) and Fernandes et al. (2015) showed no negative welfare effects in systems with suspended solids concentrations of up to 30 mg/l in exposure trials lasting 4 and 6 weeks, respectively. Despite these findings, the indirect effects of fine solids accumulation in RAS are known (Pedersen et al. 2017) and are reported to be mostly linked to the proliferation of opportunistic microorganisms (Vadstein et al. 2004; Attramadal et al. 2014; Pedersen et al. 2017) since fine solids provide a high-surface area substrate for bacteria to colonize. Another important negative effect of fine solids accumulation is the increase in turbidity, which makes visual inspection of fish difficult and may hamper photoperiod control strategies which require light penetration in the water column to occur. Fine solids control strategies used in modern RAS include ozonation, protein skimming, floatation, cartridge filtration and membrane filtration (Couturier et al. 2009; Cripps and Bergheim 2000; Summerfelt and Hochheimer 1997; Wold et al. 2014). Protein skimmers, also known as foam fractionators, are also relatively popular fine solids control devices, especially in marine systems (Badiola et al. 2012).

3.3.4 Ozonation

Knowledge of ozone (O_3) application in RAS has existed since the 1970s and 1980s (Summerfelt and Hochheimer 1997). However, its application has not been as widespread as other processes such as nitrifying biofilters or mechanical filters (Badiola et al. 2012). Aside from fine solids treatment, ozone, as a powerful oxidizer, can be used in RAS to eliminate microorganisms, nitrite and humic substances (Gonçalves and Gagnon 2011). Recent years have seen an increase in knowledge about the potentials and limitations of ozone applied in both freshwater and marine RAS. Importantly, the ozone doses that can be safely achieved to improve water quality in both freshwater and seawater systems have been confirmed in several publications (Li et al. 2015; Park et al. 2013, 2015; Schroeder et al. 2011; Summerfelt 2003; Timmons and Ebeling 2010), with the conclusion that ozone doses over recommended limits (1) do not improve water quality further and (2) may cause negative welfare effects, especially in seawater systems where excessive ozonation will cause the formation of toxic residual oxidants. In coldwater RAS, ozonation requirements to achieve complete disinfection of the process flow have been determined (Summerfelt et al. 2009).

Ozonation improves microscreen filter performance and minimizes the accumulation of dissolved matter affecting the water colour (Summerfelt et al. 2009).

However, excessive ozonation may severely impact farmed fish by causing adverse effects including histopathologic tissue damage (Richardson et al. 1983; Reiser et al. 2010) and alterations in feeding behaviour (Reiser et al. 2010) as well as oxidative stress (Ritola et al. 2000, 2002; Livingstone 2003). Additionally, ozonation by-products may be harmful. Bromate is one of these and is potentially toxic. Tango and Gagnon (2003) showed that ozonated marine RAS have concentrations of bromate that are likely to impair fish health. Chronic, sublethal ozone-produced oxidants (OPO) toxicity was investigated in juvenile turbot by Reiser et al. (2011), while rainbow trout health and welfare were assessed in ozonated and non-ozonated RAS by Good et al. (2011). Raising rainbow trout to market size in ozonated RAS improved fish performance without significantly impacting their health and welfare while high OPO doses affect welfare of juvenile turbot.

3.3.5   Denitrification

In most recirculating aquaculture systems, nitrate, the end product of nitrification, tends to accumulate. Such accumulation is commonly controlled by dilution (introducing new water in the system). The control of nitrate by dilution may be a limiting factor to a RAS operation due to environmental regulations, poor availability of new water, the cost of treating the incoming and effluent water streams or the costs associated with chilling or heating the new water.

Biological nitrate removal in RAS can be achieved by facultative anaerobic bacteria using a dissimilatory pathway to convert nitrate to nitrogen gas in the presence of carbon and nitrate as electron donors (van Rijn et al. 2006). Denitrification reactors are thus biological reactors which are typically operated in anaerobic conditions and generally dosed with some type of carbon source such as ethanol, methanol, glucose, molasses, etc. Denitrification technology has been under development since the 1990s (van Rijn and Riviera 1990), but its popularity among the recirculating aquaculture industry has only increased over the past years, offering innovative denitrification reactor solutions.

One of the most notable applications of denitrification systems in aquaculture is the 'zero exchange' RAS (Yogev et al. 2016), which employ anaerobic digestion of biosolids produced in the system to produce volatile fatty acids (VFA) which are then used by denitrifiers as a carbon source. Klas at al. (2006) developed a 'single-sludge' denitrification system, where production of VFA from biosolids and denitrification occur in a single, mixed reactor. Suhr et al. (2014) developed the single-sludge concept further, adapting it for end-of-pipe treatment of fish farming effluents and adding an extra step which separates VFA production from the denitrification reactor in a hydrolysis tank. These works have provided valuable information on the possibilities of using aquacultural biosolids instead of expensive inorganic carbon sources for denitrification. Furthermore, Christianson et al. (2015) studied the effectiveness of autotrophic, sulphur-based denitrification reactors as an alternative to conventional heterotrophic denitrification reactors. Autotrophic reactors produce

less biomass (solids) and can be supplied with sulphur particles, which are cheaper than conventional inorganic carbon sources.

VFAs are also the precursor component in the production of biopolymers such as Polyhydroalkanoates (PHAs), used to produce biodegradable plastics (Pittmann and Steinmetz 2013). This could hold potential for fish farms employing anaerobic activated sludge processes to be part of the 'biorefinery' concept applied to wastewater treatment plants.

3.3.6 Microbial Control

Microbial communities are important constituents of the aquatic ecosystem. In aquaculture production systems, they play significant roles in nutrient recycling, degradation of organic matter and treatment and control of disease (Zeng et al. 2017). Developing efficient, productive, biologically secure and disease-free RAS requires a thorough understanding of all life support processes from physical and chemical (gas transfer, thermal treatment, ozonation, UV irradiation, pH and salinity adjustments) to biological processes (nitrification, denitrification and aerobic heterotrophic activity). While physical and chemical processes can be controlled, biological filtration systems rely on the interaction of microbial communities with each other and their environment as a consequence of nutrient input (fish waste output) and, as such, are not as easily controlled (Schreier et al. 2010). Recent studies using molecular tools have not only allowed for evaluating microbial diversity in RAS but have also provided some insight into their activities that should lead to a better understanding of microbial community interactions. These approaches are certain to provide novel RAS process arrangements as well as insight into new processes and tools to enhance and monitor these systems (Schreier et al. 2010). Current understanding of RAS biofilter microbial diversity in both freshwater and marine systems is based on studies using 16S rRNA and functional gene-specific probes or 16S rRNA gene libraries rather than culture-based techniques (Table 3.1).

Insights into the temporal and spatial dynamics of microbiota in RAS are also still limited (Schreier et al. 2010), and potential solutions to maintain or restore beneficial microbial communities in RAS are lacking (Rurangwa and Verdegem 2015). Besides a microbial community that purifies the water, microbiota in RAS can also harbour pathogens or produce off-flavour-causing compounds (Guttman and van Rijn 2008). Given the difficulty to treat disease during operation without negatively affecting beneficial microbiota, microbial management in RAS is rather a necessity from the start-up through the whole production process. Microorganisms are introduced into RAS through different pathways: make-up water, air, animal vectors, feed, fish stocking, dirty equipment and via staff or visitors (Sharrer et al. 2005; Blancheton et al. 2013). Specific microbes can also, on the other hand, be applied intentionally to steer microbial colonization to improve system performance or animal health (Rurangwa and Verdegem 2015).

Table 3.1 Primary activities associated with RAS biofiltration units and participating microorganisms. (From Schreier et al. 2010)

Process	Reaction	Microorganism	
		Freshwater	Marine
Nitrification			
Ammonium oxidation	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$	*Nitrosomonas oligotropha*	*Nitrosomonas sp.*
			Nitrosomonas cryotolerans
			Nitrosomonas europaea
			Nitrosomonas cinnybus/ nitrosa
			Nitrosococcus mobilis
Nitrite oxidation	$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$	*Nitrospira spp.*	
		Nitrospira marina[a]	*Nitrospira marina*[a]
		Nitrospira moscoviensis[a]	*Nitrospira moscoviensis*[a]
Denitrification			
Autotrophic	$S_2^- + 1.6NO_3^- + 1.6H^+ \rightarrow$		*Thiomicrosporia denitrificans*
(sulfide-dependent)	$SO_4^{2-} + 0.8N_2(g) + 0.8H_2O$		*Thiothrix disciformis*[a]
			Rhodobacter litoralis[a]
			Hydrogenophaga sp.
Heterotrophic	$5CH_3COO^- + 8NO_3^- + 3H^+ \rightarrow$		*Pseudomonas fluorescens*
	$10HCO_3^- + 4N_2(g) + 4H_2O$	*Pseudomonas sp.*	*Pseudomonas stutzeri*
		Comamonas sp.	*Pseudomonas sp.*
			Paracoccus denitrificans
Dissimilatory nitrate Reduction to ammonia (DNRA)	$NO_3^- + 2H^+ + 4H_2 \rightarrow NH_4^+ + 3H_2O$		Various *Proteobacteria* and *Firmicutes*
Anaerobic ammonium oxidation (Anammox)	$NH_4^+ + NO_2^- \rightarrow N_2(g) + 2H_2O$		*Planctomycetes spp.*
			Brocadia sp.[a]
Sulfate reduction	$SO_4^{2-} + CH_3COO^- + 3H^+ \rightarrow$		*Desulfovibrio sp.*,
	$HS^- + 2HCO_3^- + 3H^+$		*Dethiosulfovibrio sp.*,
			Fusibacter sp., *Bacteroides sp.*
Sulfide oxidation	$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$		*Thiomicrospira sp.*

Table 3.1 (continued)

Process	Reaction	Microorganism	
		Freshwater	Marine
Methanogenesis	$4H_2 + H^+ + HCO_3^- \rightarrow$ $CH_4(g) + 3H_2O$		Methanogenic Archaea [Mirzoyan and Gross, unpublished]

[a]Microorganisms identified solely on the basis of partial 16S rRNA gene or functional gene sequences

One of the approaches for inhibiting pathogen colonization is the use of probiotic bacteria that may compete for nutrients, produce growth inhibitors, or, quench cell-to-cell communication (quorum sensing) that allows for settling within biofilms (Defoirdt et al. 2007, 2008; Kesarcodi-Watson et al. 2008). Probiotic bacteria include *Bacillus*, *Pseudomonas* (Kesarcodi-Watson et al. 2008) and *Roseobacter* spp. (Bruhn et al. 2005), and bacteria related to these have also been identified in RAS biofilters (Schreier et al. 2010) (Table 3.1). To obtain the information needed to manage microbial stability in RAS, Rojas-Tirado et al. (2017) have identified the factors affecting changes in the bacterial dynamics in terms of their abundance and activity. Their studies show that bacterial activity was not a straightforward predictable parameter in the water phase as nitrate-N levels in identical RAS showed unexpected sudden changes/fluctuations within one of the systems. Suspended particles in RAS provide surface area that can be colonized by bacteria. More particles accumulate as the intensity of recirculation increases, thus potentially increasing the bacterial carrying capacity of the systems. Pedersen et al. (2017) explored the relationship between total particle surface area (TSA) and bacterial activity in freshwater RAS. They indicated a strong, positive, linear correlation between TSA and bacterial activity in all systems with low to moderate recirculation intensity. However, the relationship apparently ceased to exist in the systems with the highest recirculation intensity. This is likely due to the accumulation of dissolved nutrients sustaining free-living bacterial populations, and/or accumulation of suspended colloids and fine particles less than 5 μm in diameter, which were not characterized in their study but may provide significant surface area.

In RAS, various chemical compounds (mainly nitrates and organic carbon) accumulate in the rearing water. These chemical substrata regulate the ecophysiology of the bacterial communities on the biofilter and have an impact on its nitrification efficiency and reliability. Michaud et al. (2014) investigated the shift of the bacterial community structure and major taxa relative abundance in two different biological filters and concluded that the dynamics and flexibility of the bacterial community to adapt to influent water changes seemed to be linked with the biofilter performance. One of the key aspects for improving the reliability and sustainability of RAS is the appropriate management of the biofilter bacterial populations, which is directly linked to the C (carbon) availability (Avnimelech 1999). It should be noted that RAS have properties that may actually contribute to microbial stabilization, including long water retention time and a large surface area of biofilters for bacterial

growth, which could potentially limit the chances of proliferation of opportunistic microbes in the rearing water (Attramadal et al. 2012a).

Attramadal et al. (2012a) compared the development of the microbial community in a RAS with moderate ozonation (to 350 mV) to that of a conventional flow-through system (FTS) for the same group of Atlantic cod, *Gadus morhua*. They found less variability in bacterial composition between replicate fish tanks of the RAS than between tanks of the FTS. The RAS had a more even microbial community structure with higher species diversity and periodically a lower fraction of opportunists. The fish in RAS performed better than their control in the FTS, despite being exposed to an apparent inferior physico-chemical water quality. While researching the effects of moderate ozonation or high-intensity UV irradiation on the microbial environment in RAS for marine fish larvae, Attramadal et al. (2012b) emphasized that a RAS for such larvae should probably not include strong disinfection because it leads to a reduction in bacterial numbers, which is likely to result in a destabilization of the microbial community. Furthermore, their results support the hypothesis of RAS as a microbial control strategy during the first feeding of fish larvae.

RAS and microbial maturation as tools for K-selection of microbial communities was the subject of the study by Attramadal et al. (2014) in which they hypothesized that fish larvae that are reared in water dominated by K-strategists (mature microbial communities) will perform better, because they are less likely to encounter opportunistic (R-selected) microbes and develop detrimental host–microbe interactions. The results of their experiment showed a high potential for increasing fish survival by using K-selection of bacteria, which is a cheap and easy method that can be used in all kinds of new or existing aquaculture systems. Small changes in the management (organic load and maturation of water) of water treatment give significantly different microbiota in fish tanks (Attramadal et al. 2016). On the other hand, humic substances (HS) are natural organic compounds, comprising a wide array of pigmented polymers of high organic weight. They are end products in the degradation of complex organic compounds and, when abundant, produce a typical brown to dark-brownish colour of the soil and water (Stevenson 1994). In a zero-discharge aquaculture system, HS-like substances were detected in the culture water as well as in the fish blood (Yamin et al. 2017a). A protective effect of HS was reported in fish exposed to toxic metal (Peuranen et al. 1994; Hammock et al. 2003) and toxic ammonia and nitrite concentrations (Meinelt et al. 2010). Furthermore, evidence was provided for their fungistatic effect against the fish pathogen, *Saprolengia parasitica* (Meinelt et al. 2007). In common carp (*Cyprinus carpio*) exposed to (a) humic-rich water and sludge from a recirculating system, (b) a synthetic humic acid and (c) a Leonardite-derived humic-rich extract, infection rates were reduced to 14.9%, 17.0% and 18.8%, respectively, as compared to a 46.8% infection rate in the control treatment (Yamin et al. 2017b). Likewise, the exposure of guppy fish (*Poecilia reticulata*), infected with the monogenea *Gyrodactylus turnbulli* and *Dactylogyrus* sp. to humic-rich culture water and feed, reduced both the infection prevalence (% of infected fish) and the infection intensity (parasites per fish) of the two parasites (Yamin et al. 2017c).

It is believed that the fundamental research in the area of microbial ecology of the nitrification/denitrification reactor systems in RAS may provide innovations which may alter and/or improve the reactor performance in RAS drastically. Up until now, the microbial community in reactors is still difficult to control (Leonard et al. 2000, 2002; Michaud et al. 2006, 2009; Schreier et al. 2010; Rojas-Tirado et al. 2017) and many of the inefficiencies of the system originate from this (Martins et al. 2010b).

3.3.7 Energy Efficiency

Economic viability of fish production in a recirculating aquaculture system depends, in part, on minimizing the energy requirements of operating such facilities. RAS require a higher technical infrastructure than open systems, thus energy costs in RAS have already been rated as major constraints which may prevent this technology from widespread application (Singh and Marsh 1996). Of all the costs associated with the electricity use in RAS, ventilation and water cooling are generally the most important. In indoor RAS, building ventilation is important to control humidity and carbon dioxide levels. Poor humidity control may result in a rapid deterioration of building structures, while atmospheric carbon dioxide accumulation will affect carbon dioxide stripping processes operating in the RAS and cause dizziness in workers. In order to keep an acceptable atmosphere inside the facilities, ventilation or air conditioning plants are widely in operation (Gehlert et al. 2018). These ventilation systems may be fitted with measures to reduce energy use. Furthermore, in order to develop an environmentally sustainable RAS, energy may be assumed as a key driving parameter, and in particular, energy can be considered an important indicator. Energetic performance analysis of the RAS has been performed by Kucuk et al. (2010) to contribute to the energy management in the RAS. In order to improve the energetic performance of the RAS, they recommended that operating conditions of the components, particularly, the pumps should be optimized and improved based on the fish production capacity of the system.

To increase the efficiency, RAS managers need guidelines and tools to optimize production. Energy audits can provide real data that can be used for decision-making. Badiola et al. (2014) investigated the total energy consumption (kWh) of a RAS cod system continuously for 14 months and identified the heat pump as a top energy consumer of rearing fish requiring high water thermal treatment. Gehlert et al. (2018) concluded that ventilation units offered a significant potential for energy savings in the RAS. Most of the time, when climate parameters in the facility stay within a desired range, air flow rates can be kept at low levels for saving energy. Additionally, energy saving measures in the RAS may include: software with energy performance data, alternative energy sources to heat the water and the use of frequency converters (Badiola et al. 2014).

3.4 Animal Welfare Issues

3.4.1 Introduction

During the last decade, fish welfare has attracted a lot of attention, and this has led to the aquaculture industry incorporating a number of husbandry practices and technologies specifically developed to improve this aspect. The neocortex, which in humans is an important part of the neural mechanism that generates the subjective experience of suffering, is lacking in fish and non-mammalian animals, and it has been argued that its absence in fish indicates that fish cannot suffer. A strong alternative view, however, is that complex animals with sophisticated behaviours, such as fish, probably have the capacity for suffering, though this may be different in degree and kind from the human experience of this state (Huntingford et al. 2006).

The UK government's Farm and Animal Welfare Committee (FAWC) has based their guidelines on the 'Five Freedoms' framework, which defines ideal states rather than specific levels of acceptable welfare (FAWC 2014). Freedom from hunger and thirst, discomfort, pain, injury, disease, fear and distress, as well as the freedom to express normal behaviour, provides us with a defined framework with which to assess welfare issues. Physical health is the most universally accepted measure of welfare and is undoubtedly a necessary requirement for good welfare. In a competitive, expanding and emerging industry, aquaculturists who incorporate welfare considerations into their daily husbandry practices can gain a competitive advantage and added price premium (Olesen et al. 2010) through improved consumer perception and confidence in their products. Grimsrud et al. (2013) provided evidence that there is a high willingness to pay, among all Norwegian households, to improve the welfare of farmed Atlantic salmon through increased resistance to diseases and salmon lice, which may imply less use of medicines and chemicals in the production process.

In intensive RAS, animal welfare is tightly connected to the performance of the systems. Over the past few years, animal welfare in the RAS has been mostly studied from the perspective of water quality and fish crowding effects on growth performance, stress bioindicators or the development of health disorders. The main goal of animal welfare research in the RAS has been to build and operate systems that maximize productivity and minimize stress and mortalities. Topics of interest have been stocking density limits (Calabrese et al. 2017), concentration limits of nitrogenous compounds in the rearing water (Davidson et al. 2014), concentration limits for dissolved carbon dioxide (Good et al. 2018), the effects of ozonation (Good et al. 2011; Reiser et al. 2011) and to a lesser extent, the accumulation of recalcitrant compounds in the RAS (van Rijn and Nussinovitch 1997) with limited water exchanges and noise (Martins et al. 2012; Davidson et al. 2017).
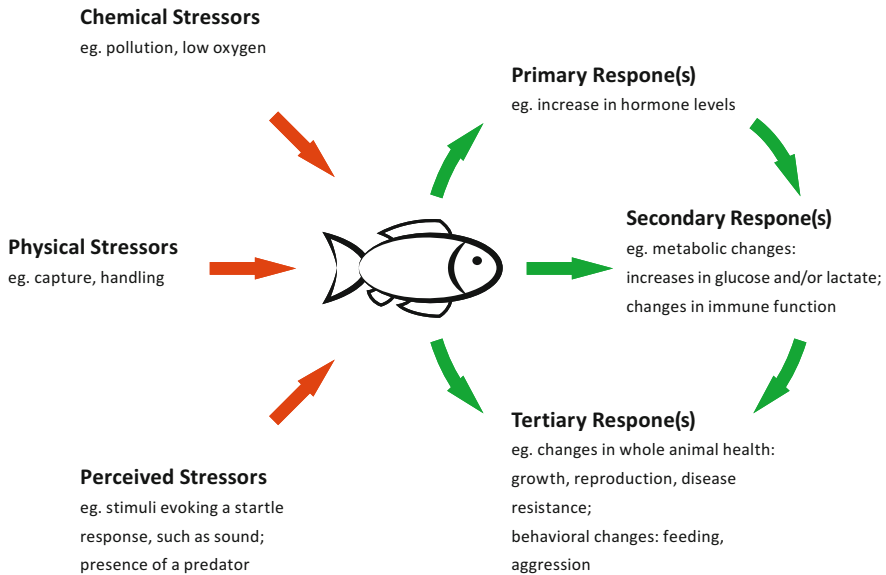
Chemical Stressors
eg. pollution, low oxygen

Physical Stressors
eg. capture, handling

Perceived Stressors
eg. stimuli evoking a startle
response, such as sound;
presence of a predator

Primary Respone(s)
eg. increase in hormone levels

Secondary Respone(s)
eg. metabolic changes:
increases in glucose and/or lactate;
changes in immune function

Tertiary Respone(s)
eg. changes in whole animal health:
growth, reproduction, disease
resistance;
behavioral changes: feeding,
aggression

Fig. 3.6 Physical, chemical and other perceived stressors can affect fish and cause primary, secondary and/or whole-body responses. (After Barton 2002)

3.4.2 Stress

The stress response in fish is an adaptive function in the face of a perceived threat to homeostasis and stress physiology does not necessarily equate to suffering and diminished welfare (Ashley 2007) (Fig. 3.6). Stress responses serve a very important function to preserve the individual. Welfare measures in aquaculture are, therefore, largely associated with the tertiary effects of stress response that are generally indicative of prolonged, repeated or unavoidable stress (Conte 2004).

Stocking density is a pivotal factor affecting fish welfare in the aquaculture industry, especially RAS where high densities in confined environments are aimed at high productivity. Although rarely defined, stocking density is the term normally used to refer to the weight of fish per unit volume or per unit volume in unit time of water flow through the holding environment (Ellis et al. 2001). The concept of minimum space for a fish is more complex than for terrestrial species as fish utilize a three-dimensional medium (Conte 2004).

Beyond providing for the physiological needs, the FAWC (2014) recommends that fish 'need sufficient space to show most normal behaviour with minimal pain, stress and fear'. Stocking density is, therefore, an area that illustrates both the significance of species differences and the existence of a complex web of interacting factors that affect fish welfare. Calabrese et al. (2017) have researched stocking density limits for post-smolt Atlantic salmon (*Salmosalar* L.) with emphasis on production performance and welfare wherein fin damage and cataracts were

observed in stocking densities of 100 kg m^{-3} and above. However, the effect of stocking density on measures of welfare varies between species. For instance, sea bass (*Dicentrarchus labrax*) showed higher stress levels at high densities, as indicated by cortisol, innate immune response and expression of stress-related genes (Vazzana et al. 2002; Gornati et al. 2004). High stocking densities in juvenile gilthead sea bream (*S. aurata*) also produce a chronic stress situation, reflected by high cortisol levels, immunosuppression and altered metabolism (Montero et al. 1999). In contrast, Arctic charr (*Salvelinus alpinus*) feed and grow well when stocked at high densities while showing a depressed food intake and growth rates at low densities (Jorgensen et al. 1993).

Diet may also play an important role in stress sensitivity. African catfish (*Clarias gariepinus*) receiving a diet with a high supplementation of ascorbic acid (vitamin C) during early development showed a lower stress sensitivity (Merchie et al. 1997). On the other hand, common carp (*Cyprinus carpio*), fed large doses of vitamin C, showed a more pronounced cortisol (a steroid hormone released with stress) increase in response to stress when compared to fish fed recommended levels of the vitamin (Dabrowska et al. 1991). Tort et al. (2004) have shown that a modified diet providing a supplementary dosage of vitamins and trace minerals to assist the immune system may help to co-reduce some of the effects of winter disease syndrome. Other common aquaculture diseases regarding animal welfare and stress are reviewed in Ashley (2007).

3.4.3 Accumulation of Substances in the Process Water

Intensive and 'zero-discharge' RAS offer significant environmental advantages. However, culturing of fish in continuously recycled water raises the question of whether substances released by the fish into the water may accumulate, resulting in decreased growth rates and impaired welfare. The existence of growth retardation in Nile tilapia (*Oreochromis niloticus*) by comparing the growth, feeding behaviour and stress response of fish cultured in the RAS with different levels of substances accumulated (TAN, NO_2-N and NO_3-N, orthophosphate-P) was investigated by Martins et al. (2010a). Results showed that the large individuals had a trend towards growth retardation in the highest accumulation RAS while small individuals, on the contrary, seem to grow better in such systems based on high levels of blood glucose as a stress indicator. A similar study done by the same author on carp embryos and larvae (Martins et al. 2011) found results that suggest that the concentration of substances (orthophosphate-P, nitrate, arsenic and copper) were likely to affect the development. Despite these findings, the authors claim that overall, the percentage of mortalities and deformities recorded in the study were relatively low compared to other studies. In both studies, the authors used systems with very limited water exchange rates with the aid of denitrification reactors (30 litres of new water per Kg of feed per day). Similarly the accumulation of hormones in coldwater salmonid RAS has been studied by Good et al. 2014, 2017). Their research in 2014 found

neither a relationship between water exchange rate and hormone accumulation (except for testosterone) nor a link between hormone accumulation and precocious maturation in Atlantic salmon, but further study was suggested. Their study in 2017 focused on the use of ozonation for the reduction of hormones in the same RAS, with inconclusive results regarding the steroid hormone accumulation, but with a positive reduction of oestradiol by ozone.

On the other hand, the accumulation of humic substances in 'zero exchange' RAS has shown to have a protective effect against bacterial infections (Yamin et al. 2017a) and ectoparasites (Yamin et al. 2017b). Humic acids have also been shown to reduce ammonia and nitrite toxicity (Meinelt et al. 2010). This has implications for RAS operated with ozonation, as ozone may improve water quality while sacrificing the apparently beneficial effects of humic substances.

3.4.4 Health and Behaviour

The fundamental characteristics of good welfare are good health and absence of disease and, with respect to aquaculture, good productivity (Turnbull and Kadri 2007; Volpato et al. 2007). While the physical health of an animal is fundamental for good welfare (Ashley 2007; Duncan 2005), the fact that an animal is healthy does not necessarily mean that its welfare status is adequate. Thus, welfare is a broader and more overarching concept than the concept of health. Physiological and behavioural measures are intrinsically linked and are dependent on one another for a correct interpretation with regard to welfare (Dawkins 1998).

The behaviour of animals and in our case fish, represents a reaction to the environment as fish perceive it and behaviour is therefore a key element of fish welfare. Changes in foraging behaviour, gill ventilation activity, aggression, individual and group swimming behaviour, stereotypic and abnormal behaviours have been linked with acute and chronic stressors in aquaculture and can therefore be regarded as likely indicators of poor welfare (Martins et al. 2011). Behavioural welfare indicators have the advantage of being fast and easy to observe and therefore are good candidates for use 'on-farm'. Examples of behaviour that are commonly used as an indicators of welfare are changes in food-anticipatory behaviour, feed intake, swimming activity and ventilation rates (Huntingford et al. 2006). However, Barreto and Volpato (2004) caution the use of ventilation frequency as an indicator of stress in fish because although ventilation frequency is a very sensitivity response to disturbance, it is of limited use because it does not reflect the severity of the stimulus.

3.4.5   Noise

Farmed fish are cultured over long periods of time in the same tanks of the same colour(s) and the same shape and exposed to the same, potentially harmful, background noises (Martins et al. 2012). Intensive aquaculture systems and particularly recirculating systems utilize equipment such as aerators, air and water pumps, blowers and filtration systems that inadvertently increase noise levels in fish culture tanks. Sound levels and frequencies measured within intensive aquaculture systems are within the range of fish hearing, but species-specific effects of aquaculture production noise are not well defined (Davidson et al. 2009).

Bart et al. (2001) found that mean broadband sound pressure levels (SPL) differed across various intensive aquaculture systems. In his study, a sound level of 135 dB re 1μPa was measured in an earthen pond near an operating aerator, whereas large fiberglass tanks (14 m diameter) within a recirculating system had the highest SPLs of 153 dB re 1μPa.

Field and laboratory studies have shown that fish behaviour and physiology can be negatively impacted by intense sound. Terhune et al. (1990) have observed decreased growth and smoltification rates of Atlantic salmon, *Salmo salar*, in fiberglass tanks that had underwater sound levels 2–10 dB re 1μPa higher at 100–500 Hz than in concrete tanks. Therefore, chronic exposure to aquaculture production noise could cause increased stress, reduced growth rates and feed conversion efficiency and decreased survival. However, Davidson et al. (2009) found that after 5 months of noise exposure, no significant differences were identified between treatments for mean weight, length, specific growth rates, condition factor, feed conversion or survival of rainbow trout, *Oncorhynchus mykiss*. Similar findings are described by Wvysocki et al. (2007). However, these findings should not be generalized across all cultured fish species, because many species, including catfish and cyprinids, have much greater hearing sensitivity than rainbow trout and could be affected differently by noise. For instance, Papoutsoglou et al. (2008) provided initial evidence that music transmission under specific rearing conditions could have enhancing effects on *S. aurata* growth performance, at least at specific fish sizes. Moreover, the observed music effects on several aspects of fish physiology (e.g. digestive enzymes, fatty acid composition and brain neurotransmitters) imply that certain music could possibly provide even further enhancement in growth, quality, welfare and production.

3.5   Scalability Challenges in RAS

RAS are capital-intensive operations, requiring high funding expenditure on equipment, infrastructure, influent and effluent treatment systems, engineering, construction and management. Once the RAS farm is built, working capital is also needed until harvests and successful sales are achieved. Operational expenditures are also

substantial and are mostly comprised of fixed costs such as rent, interest on loans, depreciation and variable costs such as fish feed, seed (fingerlings or eggs), labour, electricity, technical oxygen, pH buffers, electricity, sales/marketing, maintenance costs, etc.

When comparing productivity and economics, RAS will invariably compete with other forms of fish production and even other sources of protein production for human consumption. This competition is likely to exert a downwards pressure on the sale price of fish, which in turn must be high enough to make a RAS business profitable. As in other forms of aquaculture production, reaching higher economies of scale is generally a way to reduce the cost of production and thus obtain access to markets. Some examples of reduction in costs of production that can be achieved with larger facilities are:

1. Reduced transportation costs on bulk orders of feed, chemicals, oxygen.
2. Discounts on the purchase of larger quantities of equipment.
3. Access to industrial electricity rates.
4. Automation of farm processes such as process monitoring and control, feeding, harvesting, slaughter and processing.
5. Maximization of the use of labour: The same manpower was needed to take care of 10 tons of fish as was needed to take care of 100 tons of fish or more.

Following the increase of economies of scale in the net pen aquaculture sector, larger RAS are being developed on scales not considered a decade ago. The last decade has seen the construction of facilities with production capacities of thousands of tons per year, and this sheer size increase of RAS facilities is bringing new technical challenges, which are explored in the next section.

3.5.1 Hydrodynamics and Water Transport

Proper control of the hydrodynamic conditions in fish tanks is essential to ensure uniform water quality and adequate solids transport towards the tank outlets (Masaló 2008; Oca and Masalo 2012). Tanks which are not capable of flushing metabolites quickly enough will have less carrying capacity. Ensuring proper hydrodynamic performance in fish tanks is an important aquacultural engineering research topic which has helped the industry design and operate tanks of different shapes and sizes. However, increasing tank sizes used in commercial RAS are posing new engineering challenges to designers and operators. Recent investigations are underway to optimize the hydrodynamic characteristics in large octagonal tanks used for salmon smolt production (Gorle et al. 2018), by studying the effect of fish biomass, geometry and inlet and outlet structures in large tanks used in Norwegian smolt facilities. Similarly, Summerfelt et al. (2016) found a trend towards a decreasing feed loading rate per unit flow in modern tanks compared to tanks built more than a decade ago in Norwegian smolt facilities. A lowered feed burden effectively results in improved tank water quality as the recirculating water is treated at a faster rate,

preventing the accumulation of metabolites and the depletion of oxygen in the tank even further compared to older tanks which operated at higher feed burdens. Future work will likely provide more information on the hydrodynamics of tanks with more than 1000 m^3 in volume. Other examples of enormous tanks which are currently being used are the tanks used in the RAS 2020 systems (Kruger, Denmark) or the Niri concept (Niri, Norway). Adoption of these new concepts using larger tanks will play a vital role in their profitability, as long as proper hydrodynamic conditions are achieved.

3.5.2 Stock Loss Risk

In RAS, the intensive rearing conditions can lead to sudden and catastrophic loss of fish if the system fails. Sources of system failure may include mechanical failure of pumping systems and RAS equipment, power outages, loss of oxygenation/aeration systems, hydrogen sulphide build-up and release, operational accidents and more. These risks and solutions to them need to be identified and incorporated into operational procedures.

The increasing size of RAS operations may also mean an increased risk of financial loss if catastrophic loss of fish occurs. On the other hand, risk-mitigation measures and system redundancy may also increase the cost of a RAS project and thus, designers and engineers must strike a balance between these elements.

Aside from industry and media reports, little academic research has been done on the risk of commercial RAS ventures. Badiola et al. (2012) surveyed RAS farms and analysed the main technical issues, finding that poor system design, water quality problems and mechanical problems were the main risk elements affecting the viability of RAS.

3.5.3 Economics

Debate on the economic viability of RAS focuses mostly on the high capital start-up costs of recirculating aquaculture farms and the long lead time before fish are ready to be marketed, as well as the perception that RAS farms have high operating costs. De Ionno et al. (2007) studied the commercial performance of RAS farms, concluding that economic viability increases with the scale of the operation. According to this study, farms smaller than 100 tons per year of production capacity are only marginally profitable in the Australian context where the study took place. Timmons and Ebeling (2010) also provide a case for achieving large economies of scale (in the order of magnitude of thousands of tons of production per year) which allow for the production cost reductions through vertical integration projects such as the inclusion of processing facilities, hatcheries or feed mills. Liu et al. (2016) studied the economic performance of a theoretical RAS farm with a capacity of 3300 tons per

year, compared to a traditional net pen farm of the same capacity. At this scale the RAS operation reaches similar production costs compared to the net pen farm, but the higher capital investment doubles the payback period in comparison, even when the fish from the RAS farm are sold at a premium price. In the future, costly and strict licensing requiring good environmental performance may increase the viability of RAS as competitive option for the production of Atlantic salmon.

3.5.4   Fish Handling

On land-based farms, fish handling is often required for various reasons: to separate fish into weight classes, to reduce stocking densities, to transport fish across growing departments (i.e. from a nursery to an on-growing department) or to harvest fish when they are market ready. According to Lekang (2013), fish are handled most effectively with active methods such as fish pumps and also with passive methods such as the use of visual or chemical signals that allow the fish to move themselves from one place on the farm to the next.

   Summerfeltet al. (2009) studied several means to crowd and harvest salmonids from large circular tanks using Cornell-type dual drains. Strategies included crowding fish with purse seines, clam-shell bar crowders and herding fish between tanks taking advantage to their innate avoidance response to carbon dioxide. Harvesting techniques included extracting the fish through the sidewall discharge port of a Cornell-type dual-drain tank or using an airlift to lift the crowded fish to a dewatering box. AquaMAOF (Israel) employs swimways and tanks sharing a common wall to passively transfer the fish through the farm, with harvesting taking place using a pescalator (Archimedes screw pump) at the end of a swimway. The RAS2020 concept from Kruger (Denmark) uses bar graders/crowders permanently installed in a donut-shaped or circular raceway tank to move and crowd the fish without the need for fish pumps.

   Despite continuing developments on this topic, the increasing size of RAS farms will keep challenging designers and operators on how to handle fish safely, economically and without stress. The expanding range of designs, species under production and operation intensity of RAS farms may result in various and novel fish transport and harvest technologies.

3.6   RAS and Aquaponics

Aquaponic systems are a branch of recirculating aquaculture technology in which plant crops are included to either diversify the production of a business, to provide extra water filtration capacity, or a combination of the two.

   As a branch of RAS, aquaponic systems are bound to the same physical, chemical and biological phenomena that occur in RAS. Therefore, the same fundamentals of

water ecology, fluid mechanics, gas transfer, water depuration etc. apply in more or less equal terms to aquaponics with the exception of water quality control, as plants and fish may have specific and different requirements.

The fundamental economic realities of RAS and aquaponics are also related. Both technologies, are capital intensive and highly technical and are affected by economies of scale, appropriate design of the components, reliance on market conditions and the expertise of operators.

3.6.1   Welfare

In aquaponic systems, the uptake of nutrients should be maximized for the healthy production of plant biomass but without neglecting the best welfare conditions for the fish in terms of water quality (Yildiz et al. 2017). Measures to reduce the risks of the introduction or spread of diseases or infection and to increase biosecurity in aquaponics are also important. The possible impacts of allelochemicals, i.e. chemicals released by the plants, should be also taken into account. Moreover, the effect of diet digestibility, faeces particle size and settling ratio on water quality should be carefully considered. There is still a lack of knowledge regarding the relationship between the appropriate levels of minerals needed by plants, and fish metabolism, health and welfare (Yildiz et al. 2017) which requires further research.

3.6.2   Microbial Diversity and Control

As mentioned earlier in the chapter, aquaponics combines a recirculating aquaculture system with a hydroponic unit. One of its most important features is the reliance on bacteria and their metabolic products. Also, Sect. 3.2.6.discussed the importance of microbial communities and its control in RAS. Bacteria serve as the bridge that connects fish excrements, which are high in ammonium concentration, to plant fertilizer, which should be a combination of low ammonium and high nitrate (Somerville et al. 2014). As aquaponic systems can have different subunits, i.e. fish tanks, biofilter, drum filter, settler tanks and hydroponic units, each having different possible designs and different optimal conditions, the microbial communities in these components may differ considerably. This provides an interesting topic of research with the ultimate goal of improving system management processes. Schmautz et al. (2017) attempted to characterize the microbial community in different areas of aquaponic systems. They concluded that fish faeces contained a separate community dominated by bacteria of the genus *Cetobacterium*, whereas the samples from plant roots, biofilter and periphyton were more similar to each other, with more diverse bacterial communities. The biofilter samples contained large numbers of *Nitrospira* (3.9% of total community) that were found only in low numbers in the periphyton or the plant roots. On the other hand, only small

percentages of *Nitrosomonadales* (0.64%) and *Nitrobacter* (0.11%) were found in the same samples. This second group of organisms are commonly tested for their presence in aquaponics systems as they are mainly held responsible for nitrification (Rurangwa and Verdegem 2015; Zou et al. 2016); *Nitrospira* has only recently been described as a total nitrifier (Daims et al. 2015), being able to directly convert ammonium to nitrate in the system. The dominance of *Nitrospira* is thus a novelty in such systems and might be correlated with a difference in the basic setup (Graber et al. 2014).

Schmautz et al. (2017) also emphasized that the increased presence of *Nitrospira* does not necessarily correlate to larger activity of these organisms in the system, as its metabolic activities were not measured. In addition, many species of bacteria and coliforms are inherently present in aquaponic recirculating biofilters carrying out transformations of organic matter and fish waste. This implies the presence of many microorganisms that can be pathogens for plants and fish, as well as for people. For this purpose, some microorganisms have been considered safety indicators for products and water quality in the system (Fox et al. 2012). Some of these safety indicators are *Escherichia coli* and *Salmonella* spp. Much needed research has thus recently been carried out in order to ascertain microbial safety of aquaponic products (Fox et al. 2012; Sirsat and Neal 2013). One future direction for the analysis of microbial activity in aquaponics has been identified by Munguia-Fragozo et al. (2015), who reviewed the *Omic* technologies for microbial community analysis. They concluded that metagenomics and metatranscriptomics analysis will be crucial in future studies of microbial diversity in aquaponic biosystems.

- From a period of technological consolidation to a new era of industrial implementation, RAS technology has considerably developed over the past two decades. The last few years have seen an increase in the number and scale of recirculating aquaculture farms. With the increase in acceptance of the technology, improvements over traditional engineering approaches, innovations and new technical challenges keep emerging.
- Aquaponics combines a recirculating aquaculture system with a hydroponic unit. RAS are complex aquatic production systems that involve a range of physical, chemical and biological interactions.
- Dissolved oxygen (DO) is generally the most important water quality parameter in intensive aquatic systems. However, addition of sufficient oxygen to the rearing water can be achieved relatively simply and thus, the control of other water parameters become more challenging.
- High dissolved carbon dioxide (CO_2) concentrations have a negative effect in fish growth. The removal of CO_2 from water to concentrations below 15 mg/L is challenging due to its high solubility and the limited efficiency of degassing equipment.
- Ammonia has been traditionally treated in recirculation systems with nitrifying biofilters. Some emerging technologies are being explored as alternatives to ammonia removal.

- Biosolids in RAS originate from fish feed, faeces and biofilms and are one of the most critical and difficult water quality parameters to control. A multi-step treatment system where solids of different sizes and removed via different mechanisms, is the most common approach.
- Ozone, as a powerful oxidizer, can be used in RAS to eliminate microorganisms, nitrite and humic substances. Ozonation improves microscreen filter performance and minimizes the accumulation of dissolved matter affecting the water colour.
- Denitrification reactors are biological reactors which are typically operated under anaerobic conditions and generally dosed with some type of carbon source such as ethanol, methanol, glucose and molasses. One of the most notable applications of denitrification systems in aquaculture is the 'zero exchange' RAS.
- In aquaculture production systems microbial communities play significant roles in nutrient recycling, degradation of organic matter and treatment and control of disease. The role of water disinfection in RAS is being challenged by the idea of using microbially mature water to control opportunistic pathogens.
- In intensive RAS, animal welfare is tightly connected to the performance of the systems. The main goal of animal welfare research in RAS has been to build and operate systems that maximize productivity and minimize stress and mortalities.

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