Chapter 6
Bacterial Relationships in Aquaponics: New Research Directions

Alyssa Joyce, Mike Timmons, Simon Goddek, and Timea Pentz

Abstract The growth rates and welfare of fish and the quality of plant production in aquaponics system rely on the composition and health of the system's microbiota. The overall productivity depends on technical specifications for water quality and its movement amongst components of the system, including a wide range of parameters including factors such as pH and flow rates which ensure that microbial components can act effectively in nitrification and remineralization processes. In this chapter, we explore current research examining the role of microbial communities in three units of an aquaponics system: (1) the recirculating aquaculture system (RAS) for fish production which includes biofiltration systems for denitrification; (2) the hydroponics units for plant production; and (3) biofilters and bioreactors, including sludge digester systems (SDS) involved in microbial decomposition and recovery/remineralization of solid wastes. In the various sub-disciplines related to each of these components, there is existing literature about microbial communities and their importance within each system (e.g. recirculating aquaculture systems (RAS), hydroponics, biofilters and digesters), but there is currently limited work examining interactions between these components in aquaponics system, thus making it an important area for further research.

Keywords Microbiota · Aquaponics · Biofilters · Bioreactors · RAS · Hydroponics · Metagenomics

---

A. Joyce (✉)
Department of Marine Science, University of Gothenburg, Gothenburg, Sweden
e-mail: alyssa.joyce@gu.se

M. Timmons
Biological & Environmental Engineering, Cornell University, Ithaca, NY, USA
e-mail: mbt3@cornell.edu

S. Goddek
Mathematical and Statistical Methods (Biometris), Wageningen University, Wageningen, The Netherlands
e-mail: simon.goddek@wur.nl; simon@goddek.nl

T. Pentz
Eat & Shine VOF, Velp, The Netherlands
e-mail: timea.pentz@eatandshine.nl

© The Author(s) 2019

S. Goddek et al. (eds.), *Aquaponics Food Production Systems*,
https://doi.org/10.1007/978-3-030-15943-6_6

6.1 Introduction

Recirculating water in the aquaculture portion of an aquaponics system contains both particulate and dissolved organic matter (POM, DOM) which enter the system primarily via fish feed; the portion of feed that is not eaten or metabolized by fish remains as waste in the recirculating aquaculture system (RAS) water, either in dissolved form (e.g. ammonia) or as suspended or settled solids (e.g. sludge). Once the majority of sludge is removed by mechanical separation, the remaining dissolved organic matter must still be removed from a RAS system. Such processes rely on microbiota in various biofilters in order to maintain water quality for the fish and to convert inorganic/organic wastes into forms of bioavailable nutrients for the plants. Microbial communities in aquaponics system include bacteria, archaea, fungi, viruses and protists in assemblages that fluctuate in composition based on an ebb and flow of nutrients and changes in environmental conditions such as pH, light and oxygen. Microbial communities play a significant role in denitrification and mineralization processes (see Chap. 10) and thus have key roles in the overall productivity of the system, including fish welfare and plant health.

The challenges within any aquaponics system are to control inputs – water, fingerlings, feed, plantlets – and their associated microbiota to maximize the benefits of organic matter and its breakdown into bioavailable forms for target organisms. Given that optimal environmental growth parameters and nutrients differ for fish and plants (see Chap. 8), various separation and aeration systems, and biofilters containing relevant microbial assemblages, must be situated at strategic points in the water supply in order to help maintain nutrient levels, pH and dissolved oxygen (DO) levels within desired ranges for both target fish and plant species. Indeed, water quality parameters, including temperature, DO, electrical conductivity, redox potential, nutrient levels, carbon dioxide, lighting, feed and flow rates, all affect the behaviour and composition of microbial communities within an aquaponics system (Junge et al. 2017). In this regard, it is important to refine setup and operation so that each unit contributes adequate quantities of bioavailable forms of nutrients to its successor, rather than enabling proliferation of pathogens or opportunistic microbes that can consume the bulk of macronutrients needed downstream.

Various techniques for the analysis of microbial communities can yield important information about changes in community structure and function over time in different aquaponic configurations. By correlating these changes with nutrient bioavailability and operational parameters, it is possible to reduce over- or under-production of essential nutrients or the production of noxious by-products. For instance, maximizing recovery of beneficial plant nutrients from waste organic matter in the fish component depends primarily on the ability of microbiota to facilitate breakdown of nutrients within a series of biofilters and sludge digesters, whose performance is based on a range of operational parameters such as flow rates, residence time and pH (Van Rijn 2013). Since not all aquaponics system include sludge digesters, we will address this aspect in more detail in the latter half of this review whilst referring the reader to Chap. 3 for more details on solid separation techniques and Chaps. 7 and 8 for discussions on coupled vs decoupled aquaponics system. If we consider here only dissolved and suspended particulates in the water (and not sludge), all aquaponics system employ a range of different biofilters that expose the attached microorganisms to organic matter passing through the filter and provide an appropriate substrate and sufficient surface area for microbial attachment and formation of biofilms. Degradation of this organic matter provides energy to the microbial communities, which in turn release macronutrients (e.g. nitrate, orthophosphate) and micronutrients (e.g. iron, zinc, copper) back to the system in usable forms (Blancheton et al. 2013; Schreier et al. 2010; Vilbergsson et al. 2016a).

There is considerable agricultural research on the role of microbiota in plant rooting, growth and health. The preponderance of this research focuses on soil-based systems; however, research on hydroponics has also increased in recent years (Bartelme et al. 2018). The microbiota in aquaculture have also been similarly well-characterized, where the role of microbes in fish health and digestion has received considerable attention as researchers attempt to better characterize the role of gut health on nutrient assimilation. Given the importance of biofiltration in RAS systems, bacteria involved in the nitrification process for RAS have also been comparatively well-studied and thus are not be addressed here (see Chaps. 10 and 12). However, there has been comparatively limited research on microbes in aquaponics system, especially the crucial interactions of microbiota amongst various compartments of the system. This lack of research currently limits the scope and productivity of such systems, where there is considerable potential for enhancement with pre- and probiotics, as well as other opportunities to improve the health of aquaponics system through a better understanding, and thus better ability to control, the vast set of uncharacterized microbiota that affect system health and performance.

As such, this chapter focuses primarily on recent studies that reveal how and where microbial communities determine productivity within compartments, whilst also highlighting the relatively small number of studies linking those microbial communities to interactions amongst components and overall system productivity. We attempt to identify gaps where further knowledge about microbial communities could address operational challenges and provide important insights for enhancing efficiency and reliability.

6.2 Tools for Studying Microbial Communities

New technologies for studying how microbial communities change over time, and which groups of organisms predominate under particular environmental conditions, have increasingly offered opportunities to anticipate adverse outcomes within system components and thus lead to the design of better sensors and tests for the effective monitoring of microbial communities in fish or plant cultures. For instance, various 'omics' technologies – metagenomics, metatranscriptomics, community proteomics, metabolomics – are increasingly enabling researchers to study the diversity of microbiota in RAS, biofilters, hydroponics and sludge digestor systems where sampling includes whole microbial assemblages instead of a given genome. Analysis of prokaryotic diversity in particular, has been helped enormously in recent decades by metagenomic and metatranscriptomic techniques. In particular, amplification and sequence analysis of the 16S rRNA gene, based on intraspecific conservation of neutral gene sequences flanking ribosomal operons in bacterial DNA, has been considered the 'gold standard' for taxonomic classification and identification of bacterial species. Such data is also used in microbiology to track epidemics and geographical distributions and study bacterial populations and phylogenies (Bouchet et al. 2008). The methodology can be labour-intensive and expensive, but recent automated systems, whilst not necessarily discriminatory at the species and strain level, offer opportunities for application in aquaponics settings (Schmautz et al. 2017). Recent reviews summarize applications of 16S rRNA as they pertain to RAS (Martínez-Porchas and Vargas-Albores 2017; Munguia-Fragozo et al. 2015; Rurangwa and Verdegem 2015). Advances in metagenomics of microbes other than bacteria found in RAS and hydroponics rely on similar methodologies but use 18S (eukaryotes), 26S (fungi) and 16S in combination with 26S (yeasts) rRNA clone libraries to characterize these microbiota (Martínez-Porchas and Vargas-Albores 2017). Detailed rRNA libraries, for instance, have also been used in hydroponics to characterize microbial communities in the rhizosphere (Oburger and Schmidt 2016). Such libraries can be particularly useful in aquaponics, given that they can examine assemblage of microorganisms such as bacteria, archaea, protozoans and fungi and provide feedback on changes within the system.

The development of automated next-generation sequencing (NGS) has also enabled data analysis of genomes from population samples (metagenomics) that can be used to characterize microbiota, reveal temporal-spatial phylogenetic changes and trace pathogens. Applications in RAS include tracking certain bacterial strains amongst cultured fish and eliminating populations that carry virulent strains, whilst preserving carriers of other strains (review: (Bayliss et al. 2017). Metagenomic approaches can be culture- and amplification-independent, which allows previously unculturable species to become known and investigated for their possible effects (Martínez-Porchas and Vargas-Albores 2017). Next-generation sequencing techniques are commonly used in plant microbiology along with follow-up metatranscriptomics analyses. An excellent example is the first whole-plant study of microbial communities in the rhizosphere, wherein root exudates were shown to correlate with developmental stages (Knief 2014).

Proteomics is most useful when studying a particular bacterial species or strain under specific environmental conditions in order to describe its pathogenicity or possible role in symbiosis. Nevertheless, there are advances in community proteomics that build on prior metagenomic studies and use various biochemical techniques to identify, for example, secreted proteins associated with commensal or symbiotic microbial communities, and further possibilities abound as the capability of NGS technologies advance rapidly (review: (Knief et al. 2011).

Metabolomics characterizes the functions of genes, but the techniques are not organism-specific or sequence-dependent and thus can reveal the wide range of metabolites that are end-products of cellular biochemistry in organisms, tissues, cells or cell compartment (depending on which samples are analysed). Nevertheless, knowledge about the metabolome of microbial communities under particular environmental conditions (microcosms) reveals a great deal about the biogeochemical cycling of nutrients and the effects of perturbations. Such knowledge characterizes various metabolic pathways and the range of metabolites present in samples. Subsequent biochemical and statistical analyses can point to physiological states that can in turn be correlated with environmental parameters which may not be evident from genomic or proteomic approaches. Nevertheless, combining metabolomics with gene function studies has tremendous potential in furthering aquaponics research; see review (van Dam and Bouwmeester 2016).

6.3 Biosecurity Considerations for Food Safety and Pathogen Control

6.3.1 Food Safety

Good food safety and ensuring animal welfare are high priorities in gaining public support for aquaponics. One of the most frequent issues raised by food safety experts in relation to aquaponics is the potential risk of contamination with human pathogens when using fish effluent as fertilizer for plants (Chalmers 2004; Schmautz et al. 2017). A recent literature search to determine zoonotic risks in aquaponics concluded that pathogens in contaminated intake water, or pathogens in components of feeds originating with warm-blooded animals, can become associated with fish gut microbiota, which, even if not detrimental to the fish themselves, can potentially be passed up the food chain to humans (Antaki and Jay-Russell 2015). The mechanisms of introduction of pathogens to an aquaponics system are thus of concern, with the likeliest source of faecal coliforms or other pathogenic bacteria stemming from feed inputs to fish. From a biological perspective, there are potential risks of these pathogens proliferating either in biofilters, or, in one-loop systems by introducing airborne pathogens from open plant components back to the fish tanks. Although biosecurity risks are low in the relatively closed environmental space of an aquaponics system – as compared for instance to open pond aquaculture – and are even lower in decoupled aquaponics system wherein portions of the system can be isolated, there is still a perception that fish sludge could be potentially dangerous when applied to plants for human consumption. *Escherichia coli* (*E. coli*) is a human enteric pathogen causing foodborne illnesses that has been a key concern regarding the use of animal waste as fertilizer in agriculture or aquaculture, e.g. integrated pig-fish systems (Dang and Dalsgaard 2012). However, it is generally not considered to present a risk in fish-plant aquaponics. For instance, Moriarty et al. (2018)

previously demonstrated that UV-radiation treatment can successfully reduce *E. coli* but also noted that the coliforms detected in the aquaponics system were at background levels and did not proliferate in the fish raceways or in the hydroponically grown lettuce within the experimental system, and thus did not present a health risk. There is limited research on these aspects, but a few preliminary studies have found very low risks of coliform contamination, for instance, by showing no difference in coliform levels from sterilized and non-sterilized RAS water treatments applied to plants (Pantanella et al. 2015). Even though there is a potential risk of internalization of microbes within plant leaves, and thus their transmission to the consumed portions of some edible leafy plants grown in aquaponics, other studies have come to similar conclusions that the risks are minimal of introducing potentially dangerous human pathogens (Elumalai et al. 2017).

However, managing risks, or more importantly managing the perceptions of those risks, remains a high priority for government authorities and aquaponics investors. It is assumed that the quality control of feed inputs and careful handling of fish/fish wastes can limit most of these potential concerns (Fox et al. 2012). Indeed, no known human health incidents have to our knowledge currently been reported in relation to aquaponics system, and this may be a function of the fact that RAS facilities and hydroponic greenhouses typically have good biosecurity measures, including hygiene and quarantine practices that are stringently observed. Recommended microbiological practices for biosecurity have been evaluated for different aquaculture production systems and recommendations formulated into Hazard Analysis Critical Control Points guidelines, an international system for controlling food safety (Orriss and Whitehead 2000). However, there is still a need for better scientific documentation of risks for pathogen transfers to humans, and direct research into management in this area of aquaponics production.

6.3.2 Fish and Plant Pathogens

There is existing discipline-specific literature in aquaculture, hydroponics and bioengineering that can help inform and enhance microbial performance in aquaponics. For instance, microbial communities serve a wide range of important functions in fish health, including playing a key role in the digestibility and assimilation of feed, as well as immunodulation, and these functions as well as the role of probiotics in enhancing aquaculture systems are well-reviewed (Akhter et al. 2015). The role of microbes in RAS systems specifically is also well covered, including microbial management of biofilters, as well as research into pathogen control, as well as various techniques to control off-flavours deriving from RAS systems (Rurangwa and Verdegem 2015). Likewise, the microbes in the rhizosphere of plants are important for rooting and plant growth (Dessaux et al. 2016) but also for controlling the spread of pathogens in hydroponic plant production; these areas are well explored in a recent review by Bartelme et al. (2018). However, there is still a very limited understanding of linkages in the microbiome amongst the compartments of aquaponics system, knowledge that is crucial for maximizing productivity and reducing pathogen transfer.

The proliferation of opportunistic pathogens that are dangerous to fish or plant health are important considerations in the economics of aquaponics operations, given that any use of antibiotics or disinfectants can have a potentially detrimental effect on biofilter function, as well as destabilizing microbial relationships in other compartments of the system. Disinfection protocols commonly used in RAS include treating water with ultraviolet light (Elumalai et al. 2017), which, combined with ozone (and usually a combination of both), comprises a first-line abiotic approach to maintaining water quality. Fish eggs/larvae are also often quarantined before being introduced, and any intake water treated, thus reducing direct potential sources of fish pathogen entry to the system.

Incoming water to RAS is also typically allowed to 'mature' in biofilters before being fed into the recirculating system. Experiments, for instance, have shown that inoculating a pre-biofilter with a mixture of nitrifying bacteria, and 'feeding' it with organic matter until bacterial populations match the carrying capacity of the fish tanks, means that the rearing tank water is less likely to be unstable and overtaken by opportunistic bacteria (Attramadal et al. 2016; Rurangwa and Verdegem 2015). However, should pathogens become problematic, the use of high-dose UV, ozone, chemical or antibiotic treatments can sometimes be necessary, although such use is generally disruptive to other compartments of the system, especially the biofilters (Blancheton et al. 2013). Indeed, depending on the dose and location within the system, non-selective treatments for pathogens can actually favour proliferation of opportunists. For instance, high levels of ozone treatment not only kills bacteria, protists and viruses but also oxidizes DOM and affects aggregation of POM, thereby exerting selection pressure on bacterial populations (ibid.).

A detailed discussion of plant pathogens in aquaponics system and their control is included in Chap. 14 and thus is not reiterated here. However, it is worth noting that *Bacillus* species are routinely used as commercial probiotics in aquaculture, and there is growing evidence that similar *Bacillus* species are also effective for plants, that are already available in some commercial hydroponics probiotics solutions (Shafi et al. 2017). A recent study has extended such studies on *Bacillus* to include experimentation in aquaponics system (Cerozi and Fitzsimmons 2016b). The location where the probiotics are introduced – in the fish, plant or biofilters – may be important, but it is not clear from existing work whether the addition of probiotics in the fish component, with potential benefits for the fish, also has better effects on plant growth and health relative to the addition of similar levels of probiotics directly to the hydroponics compartment.

In addition to standard application probiotics, there are a variety of innovative techniques for biocontrol that may in the future become increasingly valuable for reducing the presence and proliferation of harmful microbes. In one recent study, bacterial isolates were selected from an established aquaponics system based on their ability to exert inhibitory effects on both fish and plant fungal pathogens. The goal was to culture these isolates as inocula that could subsequently act as biological controls for diseases within that aquaponics system (Sirakov et al. 2016). For instance, Sirakov et al. demonstrated that a *Pseudomonas* sp. that they isolated was effective as a biocontrol for the pathogenic fungi *Saprolegnia parasitica* of fish and *Pythium ultimum* of plants. The researchers also reported in vitro inhibition of a variety of other bacterial isolates from the different aquaponics compartments, but without testing their in vivo effects. The potential for using such isolates as biological controls is not new, but applications of NGS techniques can now reveal more about interactions of such isolates with each other and with potential pathogens, thus making it possible to optimize the effectiveness of delivery. Use of other 'omics' techniques could help reveal overall community structure and associated metabolic functions, and begin elucidating which organisms and functions are most beneficial. In future, such techniques might allow selection for 'helper strains' within microbial communities, or the identification of exudates that have anti-microbial effects (Massart et al. 2015).

6.4 Microbial Equilibrium and Enhancement in Aquaponics Units

Productivity in aquaponics system involves monitoring and managing environmental parameters in order to provide each component, whether microbial, animal or plant, with optimal growth conditions. Whilst this is not always possible given trade-offs in requirements, one of the key goals of aquaponics revolves around the concept of homeostasis, wherein maintaining stability of the system involves adjusting operational parameters to minimize unnecessary perturbations that cause stress within a unit, or detrimental effects on other components. With ever-changing microbial assemblages, homeostasis never implies a permanent state of equilibrium, but rather a goal of achieving as much stability as possible, particularly within water quality parameters.

A RAS coupled to a hydroponics system will be ever-changing, but within this configuration, the RAS component remains relatively stable, particularly in decoupled systems (Goddek and Körner 2019). The hydroponics system, on the other hand, tends to be more erratic in water quality since the plant crops are often harvested in batch modes, and rarely in synchrony with fish production.

During the initial start-up phase of any aquaponics system, water quality – particularly with regard to microbial communities in biofilters – is a concern, and in order to minimize proliferation of opportunistic bacteria, a routine practice has been to allow microbial maturation of intake water before its introduction into the RAS, adding fish only after the capacity of the biofilters matches the carrying capacity of rearing tanks at a particular stocking density (Blancheton et al. 2013). A similar practice is observed in hydroponics where at least a portion of recycled water is used to inoculate a new crop, given that mature microbial communities take time to develop and introducing all new water results in long lag times. Such practices lead to greater stability in culture conditions and greater productivity. For instance, improved performance in RAS systems has been noted when the pre-intake filter is supplied with pulverized fish food to develop microbial communities more similar to those in the rearing tanks (Attramadal et al. 2014).

6.5 Bacterial Roles in Nutrient Cycling and Bioavailability

Considerable research has been conducted to characterize heterotrophic and autotrophic bacteria in RAS systems and to better understand their roles in maintaining water quality and cycling of nutrients (for reviews, see Blancheton et al. (2013); Schreier et al. (2010). Non-pathogenic heterotrophs, typically dominated by *Alphaproteobacteria* and *Gammaproteobacteria*, tend to thrive in biofilters, and their contributions to transformations of nitrogen are fairly well understood because nitrogen cycling (NC) has been of paramount importance in developing recirculating culture systems (Timmons and Ebeling 2013). It has long been recognized that the bacterial transformation of the ammonia excreted by fish in a RAS system must be matched with excretion rates, because excess ammonia quickly becomes toxic for fish (see Chap. 9). Therefore in freshwater and marine RAS, the functional roles of microbial communities in NC dynamics – nitrification, denitrification, ammonification, anaerobic ammonium oxidation and dissimilatory nitrate reduction – have received considerable research attention and are well described in recent reviews (Rurangwa and Verdegem 2015; Schreier et al. 2010). There are far fewer studies of nitrogen transformations in aquaponics, but a recent review (Wongkiew et al. 2017) provides a summary along with discussion of nitrogen utilization efficiency, which is a prime consideration for plant growth in hydroponics.

After nitrogen, the second most essential macronutrient in aquaponics is phosphorus, which is not a limiting factor for fish that acquire it from feed, but is crucial for plants in hydroponics. However, the forms of phosphate in fish wastes are not immediately bioavailable for plants. Plants must have adequate quantities of inorganic ionic orthophosphate ($H_2PO_4^-$ and HPO_4^{2-} = Pi) (Becquer et al. 2014), as this is the only bioavailable form for uptake and assimilation. Inorganic phosphate binds to calcium above pH 7.0, so aquaponics system must be careful to maintain pH conditions near pH 7.0. As pH values rise above 7.0, various insoluble forms of calcium phosphate can end up as precipitates in sludge (Becquer et al. 2014; Siebielec et al. 2014). Hence, RAS losses of P are primarily through removal of sludge from the system (Van Rijn 2013). However, somewhere in the aquaponics system, particulate matter must be captured and allowed to mineralize in order to provide sufficient supplies of usable nutrients for crops in the hydroponics unit. The mineralization step will also release other macro- and micronutrients so that there are fewer deficiencies, thus reducing the need for supplementation in the hydroponics compartment. Given that world supplies of phosphate-rich fertilizers are dwindling and supplementation with P is increasingly costly, efforts are being made to maximize the recovery of P from RAS sludge (Goddek et al. 2016b; Monsees et al. 2017).

The bioavailability of macro and micronutrients is currently poorly understood. Previous research (Cerozi and Fitzsimmons 2016a) suggests that the availability of nutrients becomes compromised as pH is reduced below 7.0 and has resulted in coupled hydroponics system for leafy greens being operated around pH 6.0. However, recent research comparing aquaponic conditions and pH 7.0 to hydroponic conditions of pH 5.8 showed no difference in productivity (Anderson et al. 2017a, b). In these studies, hydroponic conditions at pH 7.0 reduced productivity by ~ 22% compared to hydroponic pH 5.8. Initially, the hypothesis was that the differences in productivity could be ascribed to the microbiota of the aquaponic water, but subsequent research dismissed that theory (Wielgosz et al. 2017).

In RAS where C:N ratios increase due to availability of organic matter, denitrifying bacteria, especially *Pseudomonas* sp., use carbon as an electron donor in anoxic conditions, to produce N_2 at the expense of nitrate (Schreier et al. 2010; Wongkiew et al. 2017). Biofloc systems are sometimes used to augment feed for fish (Crab et al. 2012; Martínez-Córdova et al. 2015), and biofloc is increasingly being used in aquaponics system, especially in Asia (Feng et al. 2016; Kim et al. 2017; Li et al. 2018). When biofloc is used in aquaponics (da Rocha et al. 2017; Pinho et al. 2017), nutrient cycling becomes even more complex given that DO, temperature and pH influence whether heterotrophic (carbon-utilizing) microbial communities predominate over autotrophic denitrifiers that are capable of reducing sulphide to sulphate (Schreier et al. 2010). Heterotrophs tend to have a higher growth rate than autotrophs in the presence of adequate sources of carbon (Michaud et al. 2009); therefore, manipulating feed type or regimes, or adding an organic carbon source directly, whilst monitoring dissolved oxygen levels, can help keep populations equilibrated whilst still providing hydroponics with N in a usable form (Vilbergsson et al. 2016a).

In hydroponics system, nutrient cycling has been less well-studied since inorganic compounds containing the required balance of nutrients are typically added in order to ensure proper plant growth. However, high nutrient concentrations, especially in humid warm environments such as greenhouses, easily facilitate growth of microbial communities, especially phytopathogens such as fungi (*Fusarium*) and oomycota (*Phytophthora*, *Pythium* sp.), that can quickly spread in circulating water and may result in die-offs (Lee and Lee 2015). Recent efforts to better understand hydroponic rhizobacteria and their beneficial effects in promoting plant growth (but also for inhibiting pathogen proliferation) have utilized various 'omics' techniques to analyse microbial communities and their interactions with root systems (Lee and Lee 2015).

For instance, when probiotic bacteria such as *Bacillus*, are present, they were shown to enhance P availability and also appear to have an added plant growth-promoting effect in a tilapia-lettuce system (Cerozi and Fitzsimmons 2016b). In aquaponics system, the addition of probiotics to fish feed and RAS water, as well as to the hydroponic water supply, deserves further experimentation, since microbial communities can have multiple modulatory effects on plant physiology. For example, the microbial communities (bacteria, fungi, oomycetes) of four food crops were analysed by metagenomic sequencing when maintained in a constant nutrient film hydroponics system where pH and nutrient concentrations were allowed to fluctuate naturally throughout the plants' life cycles (Sheridan et al. 2017). The authors concluded that treatment with a commercial mixture of plant growth-promoting microbes (PGPMs), in this case bacteria, mycorrhizae and fungi, appeared to confer greater stability and similarities in community composition after 12–14 weeks than in controls. They suggest that this could be attributed to root exudates, which purportedly favour and even control the development of microbial communities appropriate to successive plant developmental stages. Given the known effects of PGPMs in soil-based crop production, and the few studies that are available for soilless systems, further investigation is warranted to determine how to enhance PGPMs and to improve their effects in aquaponics system (Bartelme et al. 2018). If hydroponic cultures are more stable and plant growth is more robust with PGPMs, then the goal should be to characterize microbial communities in aquaponics via metagenomics and correlate them with optimal macro- and micronutrient availability via metabolomics and proteomics.

6.6 Suspended Solids and Sludge

The parameters for operating aquaponics at a given scale – including water volume, temperature, feed and flow rates, pH, fish and crop ages and densities – all affect the temporal and spatial distribution of the microbial communities that develop within its compartments, for reviews: RAS (Blancheton et al. 2013); hydroponics (Lee and Lee 2015).

In addition to controlling dissolved oxygen, carbon dioxide levels and pH in aquaponics, it is also essential to control the accumulation of solids in the RAS system as fine suspended particles can adhere to gills, cause abrasion and respiratory distress and increase susceptibility to disease (Yildiz et al. 2017). More relevant, the particulate organic matter (POM) must be quickly and effectively removed from RAS systems, or else excessive heterotrophic growth will cause almost all unit processes to fail. RAS feeding rates must be carefully managed to minimize solids loading on the system (e.g. avoid over-feeding and minimize feeding costs). The biophysical properties of feed – particle size, nutrient content, digestibility, sensory appeal, density and settling rate – determine ingestion and assimilation rates, which in turn have an impact on solids build-up and thus water quality. Although water quality is frequently studied in the context of nutrient cycling (see Chap. 9), it is also important to obtain a better understanding of the composition of microbial communities and changes in these based on feed composition, particulate loading and how this influences the growth of heterotrophic and autotrophic bacterial communities.

Various features of RAS system designs have been developed specifically to deal with solids (Timmons and Ebeling 2013); see also review: (Vilbergsson et al. 2016b). For instance, some biofilters function to keep substantial portions of wastes suspended in order to facilitate degradation, whilst others mechanically filter through screens or granular media. Still others rely on sedimentation to simply collect and remove sludge. However, such methods are not particularly effective at recovering nutrients within the sludge and making it bioavailable for plant use. Historically, this sludge has been handled in bioreactors for its methanogenic value or dewatered to be used as fertilizer for soil-based crops, but various newer designs have attempted to improve recovery for use in the hydroponic component. Improving recovery of this sludge is an important area of investigation given that a significant portion of the essential macro- and micronutrients required for plant growth are bound to the particulate organic matter, which, if discarded, is lost from the system. By adding an additional sludge recycling loop to aquaponics system, solid wastes can be converted into dissolved nutrients for reuse by plants rather than being discarded (Goddek et al. 2018). Digesters or remineralizing bioreactors are one way of accomplishing this, however one of the key areas that is currently under-developed includes knowledge of how microbial communities within these sludge digesters can be enhanced (e.g. through addition of microbes) or better utilized (e.g. through better engineered design of linked reactors) to recover nutrients into bioavailable forms for plants. Even though the actual microbial communities within sludge digesters have not been well researched for aquaponics, there is considerable literature on the microbiota of sludge digesters for sewage and animal wastes in agriculture, including fish effluent, that can provide further insight into ideal designs for sludge recovery in aquaponics system. Current research on the incorporation of sludge into aquaponics system involves remineralization in digesters situated between the RAS and hydroponic unit (Goddek et al. 2016a, 2018). Within aerobic or anaerobic bioreactors, environmental conditions that are favourable for waste degradation can effectively break down this sludge into bioavailable nutrients, which can subsequently be delivered to hydroponics system without the presence of soil (Monsees et al. 2017). Many one-loop aquaponics system already include aerobic (Rakocy et al. 2004) and anaerobic (Yogev et al. 2016) digesters to transform nutrients that are trapped in the fish sludge and make them bioavailable for plants. The ability to decouple these has a number of advantages that are further discussed in Chap. 8 and appears to lead to higher growth rates (Goddek and Vermeulen 2018). However, despite the many advances, the actual technology to accomplish this remains challenging. For example, some heterotrophic denitrifying bacteria cultured in anoxic or even aerobic conditions with sludge from RAS will use nitrate as an electron receptor and oxidized carbon sources for energy, while storing excess P as polyphosphate along with divalent metal ions such as Ca^{+2} or Cu^{+2}. When stressed at alkaline pH, these bacteria degrade polyphosphate and release orthophosphate, which is the necessary form for assimilation of phosphate by plants (Van Rijn et al. 2006). Inserting remineralization bioreactor units, such as those in Goddek et al. (2018), could provide a way to better recover P for hydroponics. Similar methods have, for instance, been used with trout sludge from a RAS that were treated for nitrate and P content in excess of allowable disposal limits (Goddek et al. 2015). However, the microbial communities involved in these processes are sensitive to culture conditions such as C:N ratios, oxygenation, metal ions and pH, so nitrites and other noxious intermediates can accumulate. Despite a vast literature on digesters of various organic wastes, primarily anaerobic for biogas production (Ibrahim et al. 2016), there is far less research on treating RAS wastes (Van Rijn

2013), and in the case of aquaponics system, even less available research about the relationship between nutrient bioavailability and crop growth in hydroponics system (Möller and Müller 2012). At this time, more studies of RAS sludge bioreactors could provide important insights into culture conditions for microbial populations that produce favourable results, for instance, on P recovery, and its introduction into hydroponics units.

One of the current challenges in efforts to assess the recovery of P from sludge arises when comparing trials of anaerobic and aerobic digesters for their efficacy (Goddek et al. 2016b; Monsees et al. 2017). Although both studies used similar sludge composition initially, the results were quite different. In one study (Monsees et al. 2017), measures of various soluble nutrients in aerobic treatments resulted in a 330% increase in P concentration and a 16% decrease in nitrate concentration compared to minor increases in P and a 97% decrease in nitrate in anaerobic treatments. By contrast, results from a similar study (Goddek et al. 2016b) showed that growth of lettuce plants in a hydroponic unit was superior using anaerobic supernatant, even though both anaerobic and aerobic treatments only resulted in slightly better nitrate recovery from anaerobic conditions and almost complete loss of PO_4 from both treatments (Goddek et al. 2016b). Obviously, factors such as feed composition and rates, the suspension versus settling of solids, pH (maintained at 7 ± 1 with $CaOH_2$ in the former and variable 8.2–8.65 in the latter), sampling and fish strains differed in these two studies. Nevertheless, the contrasting results for PO_4 and NO_3 indicate the need for further research to optimize nutrient recovery, with the addition of a metagenomics approach to characterize microbial communities so as to better understand their role in these processes.

6.7 Conclusions

Formerly the domain of small-scale producers, technological advances are increasingly moving aquaponics into larger-scale commercial production by focusing on improved macro- and micronutrient recovery whilst providing technical innovations to reduce water and energy requirements. However, scaling up of aquaponics to an industrial scale requires a much better understanding and maintenance of microbial assemblages, and the implementation of strong biocontrol measures that favour the health and well-being of both fish and crops, whilst still meeting food safety standards for human consumption. Further research on biocontrol of microbial pathogens in aquaponics, including potential human, fish and plant pathogens are needed, in light of the sensitivity of such systems to perturbation, and the fact that the use of chemicals and antibiotics can have profound effects on microbial populations, fish and plant physiologies, as well as overall system operation. Elucidating microbial interactions can improve the productivity of aquaponics system given the crucial roles of microbes in converting organic matter into usable forms that can allow fish and plants to thrive.

References

Akhter N, Wu B, Memon AM, Mohsin M (2015) Probiotics and prebiotics associated with aquaculture: a review. Fish Shellfish Immunol 45:733–741

Anderson T, de Villiers D, Timmons M (2017a) Growth and tissue elemental composition response of butterhead lettuce (*Lactuca sativa*, cv. Flandria) to hydroponic and aquaponic conditions. Horticulturae 3:43

Anderson TS, Martini MR, de Villiers D, Timmons MB (2017b) Growth and tissue elemental composition response of Butterhead lettuce (*Lactuca sativa*, cv. Flandria) to hydroponic conditions at different pH and alkalinity. Horticulturae 3:41

Antaki ET, Jay-Russell M (2015) Potential zoonotic risks in aquaponics. IAFP, Portland

Attramadal KJK, Truong TMH, Bakke I, Skjermo J, Olsen Y, Vadstein O (2014) RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture 432:483–490

Attramadal KJ, Minniti G, Øie G, Kjørsvik E, Østensen M-A, Bakke I, Vadstein O (2016) Microbial maturation of intake water at different carrying capacities affects microbial control in rearing tanks for marine fish larvae. Aquaculture 457:68–72

Bartelme RP, Oyserman BO, Blom JE, Sepulveda-Villet OJ, Newton RJ (2018) Stripping away the soil: plant growth promoting microbiology opportunities in aquaponics. Front Microbiol 9:8

Bayliss SC, Verner-Jeffreys DW, Bartie KL, Aanensen DM, Sheppard SK, Adams A, Feil EJ (2017) The promise of whole genome pathogen sequencing for the molecular epidemiology of emerging aquaculture pathogens. Front Microbiol 8:121

Becquer A, Trap J, Irshad U, Ali MA, Claude P (2014) From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. Front Plant Sci 5:548

Blancheton J, Attramadal K, Michaud L, d'Orbcastel ER, Vadstein O (2013) Insight into bacterial population in aquaculture systems and its implication. Aquac Eng 53:30–39

Bouchet V, Huot H, Goldstein R (2008) Molecular genetic basis of ribotyping. Clin Microbiol Rev 21:262–273

Cerozi BD, Fitzsimmons K (2016a) The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. Bioresour Technol 219:778–781

Cerozi BD, Fitzsimmons K (2016b) Use of Bacillus spp. to enhance phosphorus availability and serve as a plant growth promoter in aquaponics systems. Sci Hortic 211:277–282

Chalmers GA (2004) Aquaponics and food safety. Lethbridge, Alberta

Crab R, Defoirdt T, Bossier P, Verstraete W (2012) Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture 356:351–356

da Rocha A, Biazzetti Filho M, Stech M, Paz da Silva R (2017) Lettuce production in aquaponic and biofloc systems with silver catfish *Rhamdia quelen*. Bol Inst Pesca 43:64

Dang STT, Dalsgaard A (2012) *Escherichia coli* contamination of fish raised in integrated pig-fish aquaculture systems in Vietnam. J Food Prot 75:1317–1319

Dessaux Y, Grandclément C, Faure D (2016) Engineering the rhizosphere. Trends Plant Sci 21:266–278

Elumalai SD, Shaw AM, Pattillo DA, Currey CJ, Rosentrater KA, Xie K (2017) Influence of UV treatment on the food safety status of a model aquaponics system. Water 9:27

Feng J, Li F, Zhou X, Xu C, Fang F (2016) Nutrient removal ability and economical benefit of a rice-fish co-culture system in aquaculture pond. Ecol Eng 94:315–319

Fox BK, Tamaru CS, Hollyer J, Castro LF, Fonseca JM, Jay-Russell M, Low T (2012) A preliminary study of microbial water quality related to food safety in recirculating aquaponic fish and vegetable production systems. College of Tropical Agriculture and Human Resources, Honolulu

Goddek S, Körner O (2019) A fully integrated simulation model of multi-loop aquaponics: a case study for system sizing in different environments. Agric Syst 171:143

Goddek S, Vermeulen T (2018) Comparison of *Lactuca sativa* growth performance in conventional and RAS-based hydroponics system. Aquac Int 26:1377. https://doi.org/10.1007/s10499-018-0293-8

Goddek S, Delaide B, Mankasingh U, Ragnarsdottir KV, Jijakli H, Thorarinsdottir R (2015) Challenges of sustainable and commercial aquaponics. Sustainability 7:4199–4224

Goddek S, Espinal CA, Delaide B, Jijakli MH, Schmautz Z, Wuertz S, Keesman KJ (2016a) Navigating towards decoupled aquaponics system: a system dynamics design approach. Water 8:303

Goddek S, Schmautz Z, Scott B, Delaide B, Keesman KJ, Wuertz S, Junge R (2016b) The effect of anaerobic and aerobic fish sludge supernatant on hydroponic lettuce. Agronomy-Basel 6:37

Goddek S, Delaide BP, Joyce A, Wuertz S, Jijakli MH, Gross A, Eding EH, Bläser I, Reuter M, Keizer LP (2018) Nutrient mineralization and organic matter reduction performance of RAS-based sludge in sequential UASB-EGSB reactors. Aquac Eng 83:10–19

Ibrahim MH, Quaik S, Ismail SA (2016) An introduction to anaerobic digestion of organic wastes, prospects of organic waste management and the significance of earthworms. Springer, Cham, pp 23–44

Junge R, König B, Villarroel M, Komives T, Haïssam Jijakli M (2017) Strategic points in aquaponics. Water 9:182

Kim SK, Jang IK, Lim HJ (2017) Inland aquaponics system using biofloc technology. Google Patents

Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front Plant Sci 5:216

Knief C, Delmotte N, Vorholt JA (2011) Bacterial adaptation to life in association with plants–a proteomic perspective from culture to in situ conditions. Proteomics 11:3086–3105

Lee S, Lee J (2015) Beneficial bacteria and fungi in hydroponics system: types and characteristics of hydroponic food production methods. Sci Hortic 195:206–215

Li G, Tao L, Li X-l, Peng L, Song C-f, Dai L-l, Wu Y-z, Xie L (2018) Design and performance of a novel rice hydroponic biofilter in a pond-scale aquaponic recirculating system. Ecol Eng 125:1–10

Martínez-Córdova LR, Emerenciano M, Miranda-Baeza A, Martínez-Porchas M (2015) Microbial-based systems for aquaculture of fish and shrimp: an updated review. Rev Aquac 7:131–148

Martínez-Porchas M, Vargas-Albores F (2017) Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. Rev Aquac 9:42–56

Massart S, Martinez-Medina M, Jijakli MH (2015) Biological control in the microbiome era: challenges and opportunities. Biol Control 89:98–108

Michaud L, Lo Giudice A, Troussellier M, Smedile F, Bruni V, Blancheton J-P (2009) Phylogenetic characterization of the heterotrophic bacterial communities inhabiting a marine recirculating aquaculture system. J Appl Microbiol 107:1935–1946

Möller K, Müller T (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng Life Sci 12:242–257

Monsees H, Keitel J, Paul M, Kloas W, Wuertz S (2017) Potential of aquacultural sludge treatment for aquaponics: evaluation of nutrient mobilization under aerobic and anaerobic conditions. Aquac Environ Interact 9:9–18

Moriarty MJ, Semmens K, Bissonnette GK, Jaczynski J (2018) Inactivation with UV-radiation and internalization assessment of coliforms and *Escherichia coli* in aquaponically grown lettuce. LWT 89:624–630

Munguia-Fragozo P, Alatorre-Jacome O, Rico-Garcia E, Torres-Pacheco I, Cruz-Hernandez A, Ocampo-Velazquez RV, Garcia-Trejo JF, Guevara-Gonzalez RG (2015) Perspective for aquaponics system: “omic” technologies for microbial community analysis. Biomed Res Int 2015:480386

Oburger E, Schmidt H (2016) New methods to unravel rhizosphere processes. Trends Plant Sci 21:243–255

Orriss GD, Whitehead AJ (2000) Hazard analysis and critical control point (HACCP) as a part of an overall quality assurance system in international food trade. Food Control 11:345–351

Pantanella E, Cardarelli M, Di Mattia E, Colla G (2015) Aquaponics and food safety: effects of UV sterilization on total coliforms and lettuce production. In: Carlile WR (ed) International conference and exhibition on soilless culture, pp 71–76

Pinho SM, Molinari D, de Mello GL, Fitzsimmons KM, Coelho Emerenciano MG (2017) Effluent from a biofloc technology (BFT) tilapia culture on the aquaponics production of different lettuce varieties. Ecol Eng 103:146–153

Rakocy JE, Bailey DS, Shultz RC, Thoman ES (2004) Update on tilapia and vegetable production in the UVI aquaponics system. New dimensions on farmed tilapia. Proceedings from the 6th international symposium on tilapia in aquaculture 000, 1–15

Rurangwa E, Verdegem MC (2015) Microorganisms in recirculating aquaculture systems and their management. Rev Aquac 7:117–130

Schmautz Z, Graber A, Jaenicke S, Goesmann A, Junge R, Smits THM (2017) Microbial diversity in different compartments of an aquaponics system. Arch Microbiol 199:613–620

Schreier HJ, Mirzoyan N, Saito K (2010) Microbial diversity of biological filters in recirculating aquaculture systems. Curr Opin Biotechnol 21:318–325

Shafi J, Tian H, Ji M (2017) Bacillus species as versatile weapons for plant pathogens: a review. Biotechnol Equip 31:446–459

Sheridan C, Depuydt P, De Ro M, Petit C, Van Gysegem E, Delaere P, Dixon M, Stasiak M, Aciksöz SB, Frossard E (2017) Microbial community dynamics and response to plant growth-promoting microorganisms in the rhizosphere of four common food crops cultivated in hydroponics. Microb Ecol 73:378–393

Siebielec G, Ukalska-Jaruga A, Kidd P (2014) Bioavailability of trace elements in soils amended with high-phosphate materials. In: Selim HM (ed) Phosphate in soils: interaction with micronutrients, radionuclides and heavy metals bioavailability of trace elements in soils amended with high-phosphate materials. CRC Press/Taylor & Francis Group, Boca Raton, pp 237–268

Sirakov I, Lutz M, Graber A, Mathis A, Staykov Y, Smits TH, Junge R (2016) Potential for combined biocontrol activity against fungal fish and plant pathogens by bacterial isolates from a model aquaponics system. Water 8:518

Timmons MB, Ebeling JM (2013) Recirculating aquaculture. Ithaca Publishing Company, Ithaca. 788 p

van Dam NM, Bouwmeester HJ (2016) Metabolomics in the rhizosphere: tapping into belowground chemical communication. Trends Plant Sci 21:256–265

Van Rijn J (2013) Waste treatment in recirculating aquaculture systems. Aquac Eng 53:49–56

Van Rijn J, Tal Y, Schreier HJ (2006) Denitrification in recirculating systems: theory and applications. Aquac Eng 34:364–376

Vilbergsson B, Oddsson GV, Unnthorsson R (2016a) Taxonomy of means and ends in aquaculture production-part 3: the technical solutions of controlling n compounds, organic matter, p compounds, metals, temperature and preventing disease. Water 8:506

Vilbergsson B, Oddsson GV, Unnthorsson R (2016b) Taxonomy of means and ends in aquaculture production – Part 2: The technical solutions of controlling solids, dissolved gasses and pH. Water 8:387

Wielgosz ZJ, Anderson TS, Timmons MB (2017) Microbial effects on the production of aquaponically grown lettuce. Horticulturae 3:46

Wongkiew S, Hu Z, Chandran K, Lee JW, Khanal SK (2017) Nitrogen transformations in aquaponics system: a review. Aquac Eng 76:9–19

Yildiz HY, Robaina L, Pirhonen J, Mente E, Domínguez D, Parisi G (2017) Fish welfare in aquaponics system: its relation to water quality with an emphasis on feed and faeces-a review. Water 9:13

Yogev U, Barnes A, Gross A (2016) Nutrients and energy balance analysis for a conceptual model of a three loops off grid, aquaponics. Water 8:589

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.