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Iron supplementation and management in aquaponic systems: A review

Nasser Kasozi[a,b], Roman Tandlich[c], Martin Fick[d], Horst Kaiser[e], Brendan Wilhelmi[a,*]

[a] Department of Biochemistry and Microbiology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa
[b] Animal Resources Research Programme, Abi Zonal Agricultural Research and Development Institute, National Agricultural Research Organisation, P.O. Box 219, Arua, Uganda
[c] Faculty of Pharmacy, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa
[d] Practical Aquaponics, Salem, Grahamstown, 6140, South Africa
[e] Department of Ichthyology and Fisheries Science, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

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ABSTRACT

Iron is one of the essential micronutrients for plant development and fish growth in aquaponic systems. Iron is an essential element for photosynthesis, DNA synthesis, and many other cellular functions for plants. With regard to fish, it is an integral component of proteins involved in cellular respiration and oxygen transfer. Aquaponic systems are often iron deficient due to low amounts of iron in commercial fish feeds. Therefore, iron needs to be supplemented to ensure optimal plant performance. Adding these supplements requires close management of the system, and careful selection of chelating substances as the Fe-chelate bioavailability is environment dependent. Reports of iron supplementation and management in aquaponic systems are limited. This review critically examines iron supplementation strategies, different sources of iron and factors influencing iron uptake for optimal biomass production. The effectiveness of different iron chelates is also discussed. Furthermore, optimum ranges of dissolved iron concentrations for different components in an aquaponic system are reported. This review aims to provide a better understanding of iron optimisation strategies to minimise iron deficiency and enhance biomass growth.

1. Introduction

The global human population size currently stands at 7.6 billion and it is expected to reach 9.8 billion in 2050 (United Nations, 2017). With this rise, the demand for soil and land for crop production is likely to increase (Tyson et al., 2011; Wortman, 2015; Saha et al., 2016). The current traditional food production systems are challenged by declining resources resulting from climate change and a growing population. Alternative agricultural practices such as aquaponics, which includes plant and aquatic species, and hydroponics (plants only) have the potential to provide consumers with high-quality, safe and nutritious food using limited land, water, and no soil (Rakocy et al., 2006; Love et al., 2015; Knaus and Palm, 2017; Wongkiew et al., 2017). In aquaponics systems, plants grow rapidly using dissolved nutrients that are excreted directly by fish or generated from the microbial breakdown of fish excretions (Rakocy et al., 2006; Love et al., 2014). This approach to farming has been considered a possible sustainable solution to the inadequacies of traditional fish and crop production, as well as a means to reduce the unemployment rate in many underdeveloped and developing countries (Bosma et al., 2017).

Several authors have reported on various factors needed for optimum production in aquaponics systems (Rakocy et al., 2006; Roosta and Hamidpour, 2013; Ghasemi et al., 2014). While optimising a system, the combination of fish and plants should be compatible with the characteristics of each production type to balance nutrient production from fish and nutrient uptake by plants (Love et al., 2015; Yildiz et al., 2017). In aquaponics, concentrations of nutrients available to plants are driven by many factors, including fish species, growth stage, stocking density, feeding rate and feed composition, and the rate of microbial nitrification (Tyson et al., 2004; Rakocy et al., 2006; Tyson et al., 2011; Yildiz et al., 2017).

Iron (Fe) is regularly added as iron salts to fish feed in aquaculture (Bury and Grosell, 2003). However, in aquaponic systems that exclusively depend on fish waste to supply nutrients for plants, Fe levels are too low to sustain hydroponic vegetable production (Seawright et al., 1998; Rakocy et al., 2006; Tyson et al., 2011; Roosta and Mohsenian, 2012; Sallenave, 2016). Therefore, optimising plant production may require Fe supplementation. However, supplementation requires close management of the system in order to balance the Fe requirements of fish, plants and microbes.

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* Corresponding author.
E-mail address: b.wilhelmi@ru.ac.za (B. Wilhelmi).

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Fe is essential for many vital processes in plants, including chlorophyll biosynthesis, DNA synthesis, nitrogen reduction, and photosynthetic electron transfer. In aquaponics, Fe is commonly available in one of two states, *i.e.*, as soluble ferrous iron (Fe^{2+}) and as insoluble oxidised ferric iron (Fe^{3+}) (Andrews et al., 2003; Bartelme et al., 2018). Fe^{2+} is available to plants, however, in aerobic environments and at circumneutral pH, it is often oxidised to Fe^{3+} (De Kreij et al., 1999; Bury and Grosell, 2003). Fe^{3+} is not readily utilizable by plants and microbes because it regularly forms insoluble oxides or hydroxides which limit bioavailability (Zuo and Zhang, 2011; Radzki et al., 2013; Chatzistathis, 2014). Fe management in aquaponics systems is a crucial process as its deficiency impacts on the economics of this type of farming. In order to promote the expansion of aquaponic systems and to minimise Fe deficiency, it is vital to identify suitable remedies to solubilise Fe in soilless systems. While different synthetic chelators have been adopted and applied by aquaponic practitioners, most of these organic Fe-complexes are photoreactive and their degradation rates depend mainly on temperature and the pH of the solution (Svenson et al., 1989; Albano and Miller, 2001). This paper critically reviews published knowledge on the different sources of Fe and its application and management in aquaponic systems in order to advance an optimised aquaponic system.

2. Types of aquaponic systems

A typical aquaponic system consists of a fish tank, a biofilter for nitrification and denitrification and other processes that detoxify ammonia and compounds excreted by the fish, a sump as a reservoir and a grow bed (hydroponics) (Rakocy et al., 2006; Love et al., 2015). Aquaponics combines hydroponics and fish culture in a controlled environment to create a balanced ecosystem that benefits both crops and fish (Somerville et al., 2014). Conventional hydroponics requires mineral fertilizers in order to supply the plants with necessary nutrients but aquaponics systems use the available effluent water from the fish that is rich in nutrients for plant growth (Rakocy et al., 2006). Thus, ammonia nitrogen excreted by fish provides a nitrogen source for plant growth. Aquaponics can provide a harvest of crop as well as fish, all without the need for soil. There is no waste involved because of the continuous recycling of nutrients. Aquaponics systems function on less water as well, because additional water is only required to replace water lost to evaporation. The system thus uses fish, microorganisms and plants, and encourages sustainable use of water and nutrients, including recycling. There are mainly three types of aquaponic systems that are classified based on the type of grow bed (Somerville et al., 2014; Engle, 2015; Zou et al., 2016; Delaide et al., 2017). These are the nutrient film technique, deep water culture and media based culture.

2.1. Nutrient film technique

In the nutrient film technique (NFT), the plant roots are exposed to a thin layer of nutrient-rich water that runs through horizontal pipes. The holes drilled into the aquaponic pipes should be at least 7–9 cm in diameter, and should match with the size of the net cups (Somerville et al., 2014). Although this technique has a very low evaporation rate, it is more complicated and expensive than the media bed culture (Somerville et al., 2014; Wongkiew et al., 2017). Channel slope, length, and flow rate must be calculated to ensure that the plants receive sufficient water flow, oxygen and nutrients (Somerville et al., 2014). Maintaining a shallow stream allows the roots to have a larger air exchange surface. A slope of about 1 cm/m of pipe length is needed to allow easy flow of water through the pipe. NFT offers high oxygen levels to the plant roots that eventually facilitates high yield of vegetables (Wongkiew et al., 2017). However, NFT is only suitable for small vegetable species because the grow bed cannot support tall plants (Engle, 2015; Wongkiew et al., 2017).

2.2. Deep water culture

The deep-water culture (DWC) or raft method involves suspending plants in polystyrene sheets with their roots hanging into the water. This method is the most common for large commercial aquaponics and more cost-effective than large-scale media beds (Somerville et al., 2014; Engle, 2015). The DWC method allows the plant roots to absorb nutrients in the water without clogging the water channel, although aeration for DWC units is vital (Somerville et al., 2014).

2.3. Media based culture

Media based culture systems are prominent due to their simplicity, reliability and ease of use (Somerville et al., 2014; Zou et al., 2016; Wongkiew et al., 2017). In media bed units, the media (*e.g.*, pumice stones, sand, gravel or expanded clay pebbles) perform a number of functions, such as provision of support to the plant roots as well as becoming a substrate for mechanical and biological filtration (Somerville et al., 2014; Zou et al., 2016). Thus, this type of aquaponic system does not require a dedicated biological filter (Love et al., 2014; Zou et al., 2016). However, evaporation is high in media beds as much of the surface area is exposed to the sun. Media beds are either designed to flood- and- drain, or as constant flow systems (Somerville et al., 2014). In flood-and- drain systems, plant roots are temporarily exposed to a static nutrient solution before the solution is drained away through a bell siphon. In constant flow systems, water flows through the bed, or can be distributed through a drip irrigation array (Somerville et al., 2014). The media bed provides a niche for diverse populations of micro and macro-organisms, which require and remove Fe from the system.

3. Sources of nutrients in aquaponics system

Aquaponics systems derive nutrients predominantly from the waste products of fish (Roosta and Hamidpour, 2013; Wongkiew et al., 2017; Cerozi and Fitzsimmons, 2017). Aquacultural effluents are rich in dissolved and suspended solids that contain high levels of nitrogen and phosphorus produced from fish excretion via the gills, faeces and from uneaten feed (Roosta and Hamidpour, 2013; Cerozi and Fitzsimmons, 2017). For instance, ammonia nitrogen of which 80–90% may be excreted by fish via the gills provides a readily available nitrogen source for plants (Wongkiew et al., 2017). As the aquaculture effluent flows into the hydroponic component, nutrients are transformed by nitrification and mineralisation processes. Both macronutrients and micronutrients are essential for plants, but in differing amounts (Rakocy et al., 2006). There are six macronutrients (nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorous (P) and sulphur (S)) and micronutrients (iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo)), respectively (Rakocy et al., 2006; Somerville et al., 2014). Although all of these nutrients exist in solid fish waste, some nutrients, especially Ca, K, and Fe may be limited in aquaponics and may result in deficiencies for plant growth (Rakocy et al., 2006; Cerozi and Fitzsimmons, 2017). However, the optimal Fe levels for leafy and fruity vegetables in aquaponics systems are not yet well established. In addition, the basic composition of Fe solutions is crop-specific (Table 1). One challenge is to find the right fish feed composition for aquaponics in order to attain a Fe concentration that meets both the requirements of fish and plants.

4. The role of iron

Fe plays a role in a wide range of functions in aquaponics systems. In plants, Fe is important for photosynthesis, enzyme activation, protein synthesis and osmotic potential (Scandalios, 1990; Kosegarten et al., 1998; Brand et al., 2000; Fernández et al., 2006). It is an important cofactor of many enzymes, including those involved in the biosynthetic pathway of chlorophylls (Kosegarten et al., 1998; Vigani, 2012).

Table 1
Fe concentrations in hydroponic and aquaponic solutions for different plant species.

Plant name	pH range	System	Fe concentration mg/L	References
Lettuce (*Lactuca sativa*)	5–6.2	Hydroponic	2.2	Sonneveld and Voogt (2009)
Basil (*Ocimum basilicum*)	7.0–7.6	Aquaponic	2.5	Rakocy et al. (2004a)
Tomato (*Solanum lycopersicum*)	5–6.2	Hydroponic	0.8	De Kreij et al. (1999); Sonneveld and Voogt (2009)
Okra (*Abelmoschus esculentus*)	6.8–7.4	Aquaponic	1.3	Rakocy et al. (2004b)
Tomato (*Solanum lycopersicum*)	7.0–7.7	Aquaponic	0.2	Roosta and Mohsen Hamidpour (2013)
Tomato (*Solanum lycopersicum*)	7.0–7.7	Hydroponic	1.1	Roosta and Mohsen Hamidpour (2013)
Sweet pepper (*Capsicum annuum*)	5.5–6.5	Hydroponic	0.8	De Kreij et al. (1999)

Besides being a co-factor for various proteins, Fe can also influence other components of the cell. For example, under Fe-deficient conditions, *Mycobacterium smegmatis* shows decreased DNA and RNA levels while DNA synthesis was shown to be blocked in Bacillus *subtilis* (Messenger and Barclay, 1983). Fe also plays a significant role in the activation of certain antioxidant enzymes that are vital in alleviating salt-stress oxidative damage to plant cells. For instance, Fe is an essential component of ribonucleotide reductase, an essential enzyme for meristematic growth (Kosegarten et al., 1998).

Insufficient Fe uptake leads to Fe-deficiency symptoms such as interveinal chlorosis in leaves with necrotic patches and distorted leaf margins and as a result, crop quality and yields can be reduced (Kosegarten et al., 1998; Somerville et al., 2014). Similarly, the efficiency of ribulose-1,5-bisphosphate carboxylase is down-regulated under Fe-deficient conditions (Larbi et al., 2006). The lower photosynthetic rate of severely Fe-chlorotic leaves results in lower stomatal conductance and thus a decreased transpiration rate (Larbi et al., 2006). Thus, a decrease in photosystem II efficiency is considered an important factor that influences photosynthetic rates under Fe deficiency in plants (Larbi et al., 2006).

In fish, Fe is involved in oxygen transfer, electron transport and cellular respiration, with a special importance for the function of haemoglobin (Watanabe et al., 1997). There is limited information on the absorption and metabolism of iron in fish, but the process is usually the same as in other vertebrates (Watanabe et al., 1997; Bartelme et al., 2018), and often Fe requirements of the fish are met or frequently exceeded with the use of commercial feeds (Watanabe et al., 1997). The concentration of Fe in the diet that is required to prevent signs of iron deficiency for different fish species ranges from 30 to 170 mg Fe / kg (Watanabe et al., 1997).

Aquaponic systems are reliant on bacteria for nutrient conversion, and these microorganisms require Fe for growth. In addition, Fe in bacterial cells also influences cell composition, nitrogen fixation, amino acid and pyrimidine biosynthesis, regulation of metabolic products, and enzyme activity (Vasil and Ochsner, 1999; Messenger and Barclay, 1983). Although Vasil and Ochsner (1999) reported the typical Fe concentration needed for optimal bacterial growth to be in the range of 0.3–1.8 μM, there is limited information on microbial iron transport and demand in aquaponic systems. Bacteria control their Fe requirements in response to Fe availability by down-regulating the expression of Fe-containing proteins during Fe-restricted growth (Messenger and Barclay, 1983; Vasil and Ochsner, 1999; Andrews et al., 2003) and multiple parallel pathways for both Fe^{3+} and Fe^{2+} forms have been reported (Sandy and Butler, 2009; Lau et al., 2016). For instance, bacteria growing under aerobic conditions often produce siderophores to solubilize and capture $Fe^{3+.}$ Thus, in aquaponic systems, which are predominantly aerobic, bacteria can acquire Fe^{3+} to maintain Fe homeostasis through siderophore-mediated iron uptake. Fe^{2+} uptake systems have also been identified in bacteria which grow in anaerobic environments (Andrews et al., 2003; Lau et al., 2016) which are reported to diffuse freely through outer membrane porins, and are then transported into the cytoplasm through ATP-binding cassette transport (Lau et al., 2016).

In aquaponics system, fish acquire Fe predominantly from the diet, however, the Fe uptake pathway is influenced by the chemical form of Fe (Fe^{3+}, Fe^{2+}, Fe -inorganic or -organic complexes) which the transport epithelium encounters (Bury and Grosell, 2003). Fe can be partially absorbed via the gills, but the majority is absorbed in the intestines (Bury and Grosell, 2003). Therefore, the form in which Fe is presented in the feed has a profound effect on bioavailability and molecular evidence suggests that the small fraction of Fe^{3+} present in the gut lumen is reduced to Fe^{2+} prior to importation into the intestinal absorptive cells of fish (Naser, 2000; Bury and Grosell, 2003). Although the information on the functions of Fe regulation in fish is limited, transferrin and ferritin are reported to be involved in tissue distribution and storage of Fe (Naser, 2000). Fe can be directly excreted via the faeces without absorbing it through the gut wall if the fish is fed on higher dietary Fe levels. Excess Fe can also be transferred to the gills through the blood by transferrin and later released into water which probably can also be utilised by plants in aquaponics system.

Fe deficiency causes anaemia, low haematocrit values and a reduced Fe concentration in blood plasma, whereas excess uptake of Fe causes reduced growth, poor feed utilisation and mortality (Bury and Grosell, 2003; Phippen et al., 2008).

Fe toxicity results mainly from the formation of iron flocs on the gill epithelium, causing gill clogging and epithelial damage (Dalzell and MacFarlane, 1999). The effects of Fe toxicity in fish depends on the species, the size of the fish, type and concentration of Fe, the duration of exposure and the environmental conditions (Phippen et al., 2008; Mashifane and Moyo, 2014). The early life stages of freshwater fish are sensitive to Fe and the lethal concentration (LC) is dependent on water pH (Table 2). Difficulties with measuring Fe toxicity have been documented (Havas and Hutchinson, 1982; Phippen et al., 2008; Mashifane and Moyo, 2014).

5. Main classes of Fe fertilisers

Fe fertilisers are grouped into three main classes: inorganic Fe compounds such as iron salts, synthetic Fe chelates (such as Fe-EDTA, Fe-EDDHA) and natural Fe-complexes (humates and amino acids) (Chen et al., 2004; Fernández et al., 2005; Roosta and Mohsenian, 2012; Chatzistathis, 2014). Overuse of these forms of iron may have negative environmental impacts (Radzki et al., 2013). The effectiveness of various synthetic and natural chelates has been investigated (Table 3). In aquaponic systems, synthetic chelates are used to maintain suitable concentrations of Fe for plants (Lucena et al., 1990; Ghasemi et al., 2014).

5.1. Inorganic sources

Three inorganic iron salts [$FeSO_4.7\ H_2O$], [$FeCl_3.6\ H_2O$] and [$Fe(NO_3)_3.9\ H_2O$] are typically used in foliar fertilisation (Borowski and Michalek, 2011; Álvarez-Fernández et al., 2004; Roosta and Mohsenian, 2012). Although the research was not done in an aquaponics system, Borowski and Michalek (2011) reported that foliar fertilisation of beans with inorganic iron salts significantly increased chlorophyll and carotenoid content in the leaves and their stomatal conductance and photosynthesis and transpiration rate as compared to Fe-EDTA. The

Table 2
Fe toxicity values in fish species commonly reared in aquaponic systems.

Fish species	Fe concentration (mg/L)	Form of iron	Test Duration	pH	References
Brown trout *(Salmo trutta) (15–30 g)*	28	Iron (III) sulphate liquor	96 hour LC_{50}	7·62	Dalzell and Macfarlane (1999)
Salmo trutta (15–30 g) Banded Tilapia	47	Analytical grade of iron Sulphate	96 hour LC_{50}	7·62	Dalzell and Macfarlane (1999)
(Tilapia sparrmanii)	1.57	Ferric chloride	96 hour LC_{90}	5.0	Wepener et al. (1992)
	1.57	Ferric chloride	96 hour LC_{30}	7.4	Wepener et al. (1992)
Mozambique tilapia (*Oreochromis mossambicus*) (fry)	8.3	Ferric chloride	96 hour LC_{50}	7.4	Mashifane and Moyo (2014)
Oreochromis mossambicus (fingerlings)	9.0	Ferric chloride	96 hour LC_{50}	7.4	Mashifane and Moyo (2014)
Brown trout (*Salmo trutta*)	2.0	Ferric chloride and Iron sulphate	3 days - gill damage	5.0–6.0	Peuranen et al. (1994)
Common carp *(Cyprinus carpio)* (3.5 cm length)	0.56–1.36	N/A	96 hour LC_{50}	7.1	Alam and Maughan (1995)
Cyprinus carpio (6.0 cm length)	1.22–2.25	N/A	96 hour LC_{50}	7.1	Alam and Maughan (1995)

leaves treated with $Fe(NO_3)_3$ showed the highest content of photosynthetic pigments and the most intense gas exchange. Álvarez-Fernández et al. (2004) reported that foliar applications of $FeSO_4$ at an application rate of 500 mg Fe /L caused increased leaf chlorophyll concentrations of Fe-deficient pear trees. Similar findings were reported by Roosta and Mohsenian (2012), who concluded that pepper plants in an aquaponic system treated with $FeSO_4$ at a rate of 0.5 g Fe/ L showed the highest values of vegetative and reproductive growth when compared to the application of iron chelates. The effectiveness of these inorganic salts could be either due to the functioning of the reductase activity or direct uptake of Fe (II) through an iron-regulated transporter (Roosta and Mohsenian, 2012).

5.2. Synthetic Fe chelates

Chelates are compounds that stabilise metal ions and protect them from oxidation and precipitation. The efficacy of a chelate mainly depends on both the chelate stability and its reactions in the medium and the ability of the plant to take up the metal (Lucena et al., 1990). Synthetic Fe (III) chelates are widely used to maintain Fe solubility in hydroponic solutions (De Kreij et al., 1999; Sallenave, 2016; López-Rayo et al., 2019). Several aquaponic practitioners have adopted an application rate of 2 mg/L of chelated iron every 2–3 weeks as recommended by Sallenave (2016). However, the type of chelate to apply depends on the solution pH since pH affects the stability constant of Fe chelates.

Fe chelates are sensitive to light so that hydroponic systems containing chelates should be protected from exposure to daylight (Svenson et al., 1989). Fe-EDDHA and Fe-HBED are the strongest chelates of any of the commonly used complexing materials and they maintain iron availability to plants above pH 8.5 (Fig. 1). An overview of the main Fe chelates used in aquaponics is given below.

a Iron ethylendiaminetetraacetic acid (EDTA): Although Fe-EDTA has been the most commonly used chelating agent, it is a slightly toxic form of chelated iron to plants and may thus present challenges in aquaponic systems (Rakocy et al., 2006; Vadas et al., 2007; Ghasemi et al., 2014). It has the ability to form complexes with free metal cations (such as Zn, Cu, and Mn), thereby decreasing their availability for plant uptake (Albano and Miller, 2001; Vadas et al., 2007). Furthermore, EDTA is sensitive to photodegradation with a half-life under severe sunlight conditions of 11 min at pH 7.0 upon illumination in a Xenotest 1200 apparatus derived from the annual maximum of a solar spectrum (Svenson et al., 1989; Albano and Miller, 2001). It is stable in a pH range between pH 4.0–6.3 (Albano and Miller, 2001). Considering the requirements of fish and microbes in aquaponics, Fe-EDTA is effective in the narrow pH range of 6.2–6.3. Using this chelate necessitates regular replenishment (Rakocy et al., 2006).

b Iron N-hydroxyethylethylenediaminetriacetic acid (HEDTA): It is bioavailable to a pH of 7.0 but it is not an effective chelate because of its rapid degradation (Chen et al., 1995).

c Iron (S, S)-N,*N*'-ethylenediamine disuccinic acid (EDDS): It is a biodegradable chelator (López-Rayo et al., 2019), however, it is not an effective chelate for hydroponic systems due to rapid degradation. Vadas et al. (2007) reported that Fe-EDDS concentration decreased rapidly within seven days during a one-month experiment in a hydroponic system.

d Iron diethylenetriaminepentaacetic acid (DTPA): It is stable over a pH range from 4.0 to 7.0 (Albano and Miller, 2001). Therefore, in aquaponics, it is recommended for systems at pH of between 6.0 and 7.0. Several authors have recommended this type of chelate in comparison with Fe-EDTA and Fe-EDDS for aquaponic systems mainly because of its low biological degradation (Vadas et al., 2007; Sallenave, 2016). With respect to its performance in hydroponics systems, Vadas et al. (2007) found that Fe-DTPA concentrations remained stable at approximately 17 μM during a one-month experiment compared to Fe-EDDS concentration in the nutrient solution that decreased rapidly within the first week from 40 μM to below 3 μM. However, Svenson et al. (1989) reported that the half-life of Fe-DTPA under high sunlight conditions was eight minutes, indicating a rapid photolytic degradation in the presence of blue and UV light.

e Iron ethylenediamine-*N,*N'-bis (2-hydroxyphenylacetic acid) (EDDHA): It is stable between pH 4.0 and 9.0 (Albano and Miller, 2001). Because of its effectiveness over a broad pH range, it is a good iron chelate, especially when starting up systems. It is available for plant uptake up to a pH of 9 and is stable in a recirculating aerobic system. Based on a study under hydroponic culture (Lucena et al., 1990), Fe-EDDHA maintained more Fe in solution than either Fe-EDTA or Fe-polyflavonoid for a period of 11 weeks. It is considered to have a longer lasting effect than Fe-EDTA (Lucena et al., 1990; Nadal et al., 2012).

f Iron N,N′-bis (2-hydroxybenzyl)ethylenediamine-*N,*N′-diacetic acid (HBED): It has traditionally been used as an iron-chelating agent for humans (Brittenham, 1992), however very limited information is available about its application in aquaponic systems. It is currently considered as the strongest chelate with the highest stability (Nadal et al., 2012). It is stable over a wide pH range of 3.5–12.0 (De Kreij et al., 1999). Based on a soil-based study, HBED could be an alternative to EDDHA for correcting the iron chlorosis of plants grown on calcareous soils because of its high stability and low reactivity with the soil mineral phases (De Kreij et al., 1999; Nadal et al., 2012).

5.3. Amino acid chelates

A few investigations have indicated that Fe(II) amino acid chelates are stable in hydroponic nutrient solutions (Ghasemi et al., 2012, 2014) although their uptake mechanism is not clear. The Fe(II)–amino chelates used as complexing agents include arginine, glycine and histidine.

Table 3
Effects of forms of Fe on plants in aquaponic and hydroponic systems.

Plant name/cultivar	Form(s) of Fe	Effect	Conclusion	System	References
Pepper (*Capsicum annum*)	$FeSO_4$, Fe-EDTA, Fe-EDDHA	Vegetative and reproductive growth parameters were highest in plants treated with $FeSO_4$.	Foliar fertilisation of pepper plants with different Fe sources had a beneficial effect on the essential nutrient uptake and transport in plants.	Aquaponic	Roosta and Mohsenian (2012)
Tomato (*Lycopersicon esculentum* var. Marglobe)	Hoagland solution, two bacterial strains supplemented with Fe	Bacterial siderophore treatments significantly increased plant yield, chlorophyll and iron content.	Siderophores from the strain *Chryseobacterium* C138 were effective in supplying Fe to iron-starved tomato plants with or without the presence of bacteria.	Hydroponic	Radzki et al. (2013)
Tomato (*Solanum lycopersicum*)	Fe- EDDHA	Significant increment of Fe concentrations in the leaves.	The concentrations of Fe were higher in the leaves of aquaponic-grown plants as compared to those from hydroponic systems.	Aquaponic and hydroponic	Roosta and Hamidpour (2013)
Melon (*Cucumismelo*) soybean(*Glycine max*) and ryegrass (*Lolium perenne*)	Humic acid	Enhanced the maintenance in solution of Fe, in all tested solutions.	Humic acid alone without addition of Fe resulted in partial growth enhancement.	Hydroponic	Chen et al. (2004)
Lettuce (*Lactuca sativa*)	Fe-DTPA, Fe-EDDS and Fe-EDTA	The final shoot concentrations of Fe were similar among chelator treatments.	EDDS concentration decreased rapidly within seven days of the experiment.	Hydroponic	Vadas et al. (2007)
Tomato (*Lycopersicon esculentum* Mill Cvs. Rani and Sarika)	Iron (II)–amino acid chelates	Increased the supply of nutrients and improved growth.	Fe-amino acid chelates can be used as an alternative to Fe-EDTA to supply Fe.	Hydroponic	Ghasemi et al. (2014)
Strawberry (*Fragaria vesca*)	Fe-EDTA, Fe-EDDHA and Fe-polyflavonoid	Fe-polyflavonoid produced chlorosis in plants due to its low stability in solution.	Fe-EDDHA was the best chelate to supply Fe to the strawberry plants.	Hydroponic	Lucena et al. (1990)

These amino acids have a high ability to form complexes with metal nutrient elements, and as a result, they increase phytoavailability of metal nutrients (Zhou et al., 2007). They are not very sensitive to photodegradation and their degradation is biological (Ghasemi et al., 2012). Ghasemi et al. (2014) suggested that tomato plants supplied with Fe(II)–amino in the form of $Fe(arginine)_2$, $Fe(glycine)_2$ and $Fe(histidine)_2$ accumulated higher levels of potassium in their shoots and had an increased tolerance to salt-stress and improved growth compared with those supplied with Fe–EDTA. The beneficial role of Fe (II)–amino acid chelates in alleviating salt-induced damages could be related to their direct effect on protein synthesis and the integrity of cell membranes (Ghasemi et al., 2014).

6. Factors influencing Fe absorption in aquaponics system

In soil-based environments, a number of factors (soil, plant and anthropogenic) influence Fe uptake by plants (Chatzistathis, 2014). Here, we concentrate on these major factors influencing Fe uptake by plants, bacteria, and fish in aquaponic systems. Although microbial Fe use is an important factor in all environments with photosynthetic activity, the extent of Fe demand by microbes in aquaponic systems is not fully understood.

6.1. pH

The major factor influencing Fe availability in aquaponics and uptake by plants is pH (Somerville et al., 2014; Zou et al., 2016; Wongkiew et al., 2017). pH affects the stability of Fe chelates, which in turn influences the bioavailability of the Fe. It also controls fish metabolism, the toxicity of ammonia and heavy metals to fish, microbial activities and biological oxidation of ammonium to nitrite and nitrate (van Rijn et al., 2006; Zou et al., 2016). It is therefore crucial to stabilise pH in the aquaponic system since it is critical to all living organisms within a recirculating system, *i.e.*, fish, plants and microbes (Zou et al., 2016; Yildiz et al., 2017). Nitrifying bacteria function adequately within a pH range of 6.0–8.5 (Somerville et al., 2014). In addition, pH is an important factor influencing denitrification. Zou et al. (2016) reported that nitrous oxide (N_2O) emissions in aquaponic systems decreased as the pH was increased to 9.0, indicating that a low pH (6.0) significantly inhibited ammonia oxidising bacteria.

Roosta (2011) investigated the effect of the nutrient solution pH on Fe concentration in the leaves of lettuce plants at pH 5, 6, 7, and 8. The plants were slightly less green at pH 7, and a visible chlorosis appeared at pH 8. Fe concentration in leaves also decreased at pH 8 of the nutrient solutions leading to a reduction in shoot growth. Rhizosphere pH has also an important ecological impact on the mobilization of Fe and the activity of microorganisms. Exposure of plants to suboptimal Fe availability is associated with several changes in root morphology and a decrease in lateral root length (Schmidt, 1999).

A mechanism for Fe uptake is present in teleost fish. The physiological characterisation of intestinal Fe absorption has been mainly described in marine fish (Bury et al., 2001; Cooper et al., 2006). Bury et al. (2001) demonstrated that the posterior intestine is the primary site of Fe absorption in European flounder (*Platichthys flesus*). In addition, Kwong and Niyogi (2008) reported that Fe uptake rate in rainbow trout (*Oncorhynchus mykiss)* is significantly higher in the anterior intestine than in the mid and posterior intestine. On the increase in mucosal pH from 7.4 to 8.2, there was a significant reduction in Fe absorption in both mid and posterior intestine, implying the involvement of a Fe^{2+}/H^+ symporter (Kwong and Niyogi, 2008). Both ferric and ferrous iron uptake rates were significantly inhibited when luminal pH was increased to pH 8 suggesting a proton gradient is essential for the absorption of Fe which resembles the characteristics of a mammalian Fe^{2+}/H^+ symporter (DMT1). Taking into consideration the importance of pH as indicated above, it is therefore important to balance pH in an aquaponic system although it is not possible to achieve an

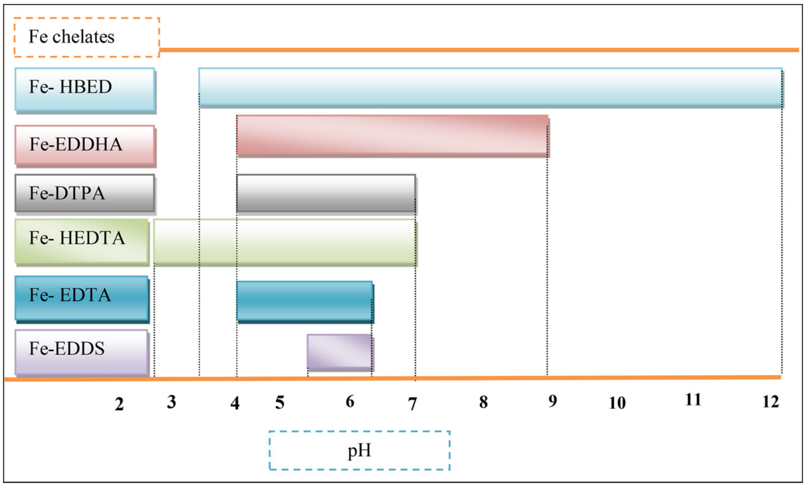

Fig. 1. Summary of pH ranges over which Fe chelates are stable.

optimal pH for every component (Tyson et al., 2011; Zou et al., 2016). Tyson et al. (2011) proposed that sustainable production in aquaponics can be improved by maintaining water pH in the range of 7.5–8.0 rather than the optimum pH for plant production of pH 5.5–6.5. In practice, pH can be increased using potassium hydroxide and calcium hydroxide (Rakocy et al., 2006; Sallenave, 2016).

6.2. Plant- microbial interactions

Plants and microorganisms have evolved active strategies of iron uptake based on acidification, chelation, and/or reduction processes (Römheld and Marschner, 1986; de Santiago et al., 2009). Plants have developed two main strategies to acquire iron (Johnson et al., 2002; Conte and Walker, 2011; Radzki et al., 2013). The majority of plants, dicotyledons and non-graminaceous monocotyledons use an acidification/reduction strategy (Strategy I). Strategy I is primarily based on acidification of the rhizosphere followed by reduction of Fe^{3+} ions by membrane-bound Fe(III)-chelate reductase and subsequent uptake of Fe^{2+} into the root cells (Conte and Walker, 2011; Radzki et al., 2013). With Strategy II, strong Fe chelators called phytosiderophores are synthesized by the plant and secreted into the rhizosphere, where they chelate and help to solubilise Fe^{3+} (Conte and Walker, 2011; Vigani, 2012; Radzki et al., 2013). In Strategy I species, such as tomato which are commonly grown in aquaponics, the response to Fe deficiency has various components. Fe uptake is increased by secretion of chelators, proton extrusion, ferric reduction and enhanced activity of a ferrous transporter in the root plasma membrane (Schmidt, 1999). Subsequently, the available ferric Fe^{3+} is reduced to the more soluble ferrous Fe^{2+} by ferric reduction oxidases at the apoplast. The reduced Fe^{2+} is imported into root cells by Fe^{2+} regulated transporters such as the iron-regulated transporter (Schmidt, 1999).

Bacteria have evolved several strategies to compete for Fe from extremely insoluble ferric oxyhydroxide polymers in the natural environment or from iron-protein complexes such as transferrin and lactoferrin in the host tissues (Andrews et al., 2003; Song et al., 2018). In addition, a number of other strategies used by bacteria in the management of Fe as suggested by Andrews et al. (2003) are outlined below.

a) High-affinity Fe transport enabling iron to be scavenged, in various forms from the environment.
b) Deposition of intracellular Fe stores to provide a source of Fe that can be drawn upon when external supplies are limited.
c) Employment of redox stress resistance systems (*e.g.* degradation of iron-induced reactive oxygen species and repair of redox stress-induced damage).
d) Control of Fe consumption by down-regulating the expression of iron-containing proteins under Fe -restricted conditions.
e) An over-arching Fe-responsive regulatory system that coordinates the expression of the above iron homeostatic machinery according to iron availability.

In aquaponics, most bacteria, and some plants respond to Fe stress by the induction of high-affinity Fe transport systems that utilize biosynthetic chelates called siderophores. To competitively acquire Fe, some microbes have transport systems that enable them to use other siderophore types in addition to their own. In aquaponic systems, production of siderophores occurs in various microbes of the genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Streptomyces, Gliocladium*, and *Trichoderma* (Lee and Lee, 2015; da Silva Cerozi and Fitzsimmons, 2016; Schmautz et al., 2017). In addition, Raaijmakers et al. (2010) reported that using *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces griseoviridis* may prevent or diminish the effect of plant pathogens. For instance, siderophore production by *Chryseobacterium* spp. was effective in supplying Fe to iron-starved tomato plants in hydroponics culture (Radzki et al., 2013).

Bacterial iron metabolism is best understood in *Escherichia coli*. This organism is able to use a combination of separate siderophore receptors and transporters, whereas other microbial species, such as *Streptomyces pilosus*, use a low specificity, high-affinity transport system that recognizes more than one siderophore type (Andrews et al., 2003). By either strategy, such versatility may provide an advantage under Fe-limiting conditions; allowing the use of siderophores produced at another organism's expense, or Fe acquisition from siderophores that could otherwise sequester Fe in an unavailable form. Possibly such competition has been a factor in the evolution of broad transport capabilities for different siderophores by microorganisms and plants.

An important group of bacteria reported in aquaponics is *Pseudomonas* spp. These have an assortment of iron acquisition and regulatory genes and they play a vital role in stimulating plant growth and in controlling several plant diseases (Schmautz et al., 2017). They function as a biocontrol agent by depriving the pathogen from iron nutrition, thus resulting in increased crop yield. For instance, Lee and Lee (2015) reported that *Pseudomonas* spp. show antagonistic and antifungal activity against *Fusarium graminearum* and prevent root rot.

Similarly, Schmautz et al. (2017) reported a relatively high proportion of reads from the plant roots of lettuce plants grown in aquaponic systems as being assigned to *Pseudomonas* spp. These bacteria have the capacity to take up exogenous xenosiderophores via different TonB-dependent receptors (Cornelis et al., 2009). The uptake of Fe is controlled by a ferric uptake regulator (Fur) which directly controls genes involved in iron uptake, or indirectly, via extracytoplasmic sigma factors or via other regulators (Vasil and Ochsner, 1999; Cornelis et al., 2009).

Another important group of bacteria reported in aquaponics is *Bacillus* spp. These have several iron-regulated uptake systems for the utilization of exogenous siderophores of varying structural classes (Miethke et al., 2013). However, the mechanism of these plant growth-promoting rhizobacteria in promoting plant growth and prevention of diseases is still poorly understood in aquaponics. Niazi et al. (2014) showed that *Bacillus* genomes have a capability for promoting plant growth and suppressing plant diseases by producing phytohormones and antibacterial/antifungal compounds. García et al. (2004) reported that *Bacillus licheniformis* increased the diameter and weight of tomatoes and peppers, and promoted higher yields of each crop.

Emerging evidence has also linked the important roles of Fe in influencing disease among aquatic organisms. *Vibrio* spp., like most other organisms, have an absolute requirement for Fe (Payne et al., 2016; Song et al., 2018). The *Vibrios* are a unique group of bacteria inhabiting a vast array of aquatic environments (Zhang et al., 2019). Transporters for ferric and ferrous iron not complexed to siderophores are also common to *Vibrio* species. Although not yet reported in aquaponics, Fe has been shown to be a critical component in different *Vibrio* species (Song et al., 2018). For example, *Vibrio splendidus* is an opportunistic pathogen that can infect a broad range of hosts including cod larvae, turbot and shellfish (Song et al., 2018). In particular, *V. splendidus* can heavily infect the sea cucumber, *Apostichopus japonicus* and cause skin ulceration syndrome thereby hindering the aquaculture of *A. japonicus* and resulting into substantial economic losses (Song et al., 2018). It has also been reported that *Vibrio anguillarum* requires its naturally produced siderophore, anguibactin, to cause infection in fish (Naka and Crosa, 2011). The amount of Fe required by *Vibrios* varies depending on the cell's physiology and metabolism, but concentrations in the medium range of 0.1 μM–5.0 μM provide sufficient iron for optimal growth of bacteria under laboratory conditions (Payne et al., 2016).

6.3. Root environment

Root health is crucial for plant survival irrespective of the agricultural system. A vast array of different compounds such as sugars, amino acids, organic acids, fatty acids, aromatic acids, aliphatic acids, sterols, hormones, vitamins, enzymes, phenolics and nucleotides are found in the rhizosphere (Rovira, 1959). The type and quantity of compounds released depend on plant species, cultivar, age and environmental conditions. For instance, reduction of Fe^{3+} to Fe^{2+} is a prerequisite for Fe uptake by tomato roots (Holden et al., 1991). The tomato root plasma membrane Fe-chelate reductase is capable of reduction of various Fe^{3+} chelate complexes both *in vivo* and *in vitro* (Holden et al., 1991). Fe deficiency is also responsible for excretion of organic compounds by roots, and therefore impacts rhizodeposition and the associated microbial abundance, diversity and activity. The rhizosphere is also a place of high microbial activity (Schmautz et al., 2017). However, in aquaponic systems, research related to the role of microbes in the rhizosphere is limited. Bacterial populations in the rhizosphere can convert nutrients into different forms that are more accessible for uptake by roots including micronutrients such as iron (Bartelme et al., 2018).

6.4. Feed and fish related factors

The major factors influencing iron absorption in fish include; the quantity of organic and inorganic components of the diet, the quantity ingested and the health of the fish (Watanabe et al., 1997; Yildiz et al., 2017). In addition, the management of feeding has a very important effect on feed utilisation by fish. The feeding time and the location of feeders can have an influence on the quantity of solids produced and their distribution within a fish tank (Watanabe et al., 1997). Therefore, solid management and microbial nitrification is essential for Fe distribution in the aquaponics system. In addition, the ingredients utilised in feed formulation could influence the quantity of Fe produced. For example, feeds of animal origin such as fish meal and meat meal are rich sources of Fe containing about 400–800 mg/kg. Oil seeds contain 100–200 mg Fe/kg, while cereals contain 30–60 mg Fe/kg (Watanabe et al., 1997). It is reported that the concentration of Fe in the diet required to prevent iron deficiency syndromes in fish is in the range of 30–170 mg Fe/ kg (Watanabe et al., 1997).

7. Methods of Fe application in aquaponics

7.1. Foliar application

The option to deliver small amounts of Fe to plants under aquaponics conditions through foliar sprays could be a target-oriented, cheaper strategy to overcome Fe deficiency, although variable responses to Fe sprays have been reported (Borowski and Michalek, 2011; Roosta and Mohsenian, 2012). These variations are plant-related, environmental, and physico-chemical factors (Fernández et al., 2008). Based on the few studies that have reported on foliar Fe application in aquaponics systems (Liu, 2004; Schonherr et al., 2005; Roosta and Mohsenian, 2012), the following conclusions can be drawn.

a Fe needs to be supplemented regularly via this method.
b Fe supplementation through this method is time consuming and ultimately less effective when using Fe chelates.
c Plants treated with inorganic iron salts (such as $FeSO_4$) via this method grow better compared to those receiving either Fe-EDTA or Fe-EDDHA.
d Fe chelates penetrate the leaves slowly and 100% humidity is essential for significant penetration rates.

7.2. Addition to water

At present, there is a paucity of information on the effect of adding Fe to water in an aquaponic system on plant yield as well as fish growth and health. However, in fish, the addition of Fe in water as ferrous sulphate improved growth of swordtail (*Xiphophorous helleri*) and platy fish (*X. maculatus*), which suggests a nutritional benefit from Fe dissolved in water (Roeder and Roeder, 1966). This method is now practiced and preferred by many aquaponic practitioners in South Africa using Fe-EDDHA at an application rate of 2 mg/L to prevent chlorosis and it is applied every three weeks as recommended by Rakocy et al. (2004b) and Sallenave (2016). Considering the limitations of foliar applications, Fe supplementation in aquaponics via water could effectively treat iron deficiency.

The behaviour of Fe in aquatic ecosystems is dynamic and influenced by a complex set of environmental conditions, including light, dissolved oxygen content and pH (Randall et al., 1999; Phippen et al., 2008). However, there is general agreement that continuous addition of Fe without considering its correct concentration in aquaponic solution is dangerous, because iron can cause toxicity to fish (Dalzell and Macfarlane, 1999; Bury and Grosell, 2003; Phippen et al., 2008). Randall et al. (1999) suggested a safe level of dissolved Fe concentration of 1.69 mg/L for aquatic organisms. In a subsequent study, Phippen et al. (2008) recommended an optimum range of 0.35–1.0 mg/L as the total iron concentration. Therefore, to avoid iron toxicity to fish in an aquaponic system, the Fe concentration in solution should be below 1.7 mg/L (Fig. 2).

In summary, fish and feed waste provide most of the Fe required by the plants in aquaponics systems however Fe concentrations supplied by the fish are significantly lower than what is required by the hydroponic system. Since hydroponics systems do not include fish, a higher Fe concentration than in aquaponics may be suitable to maintain plant growth although this is influenced by a number of factors as indicated in Fig. 2. Thus, dissolved Fe concentration for hydroponics in the range

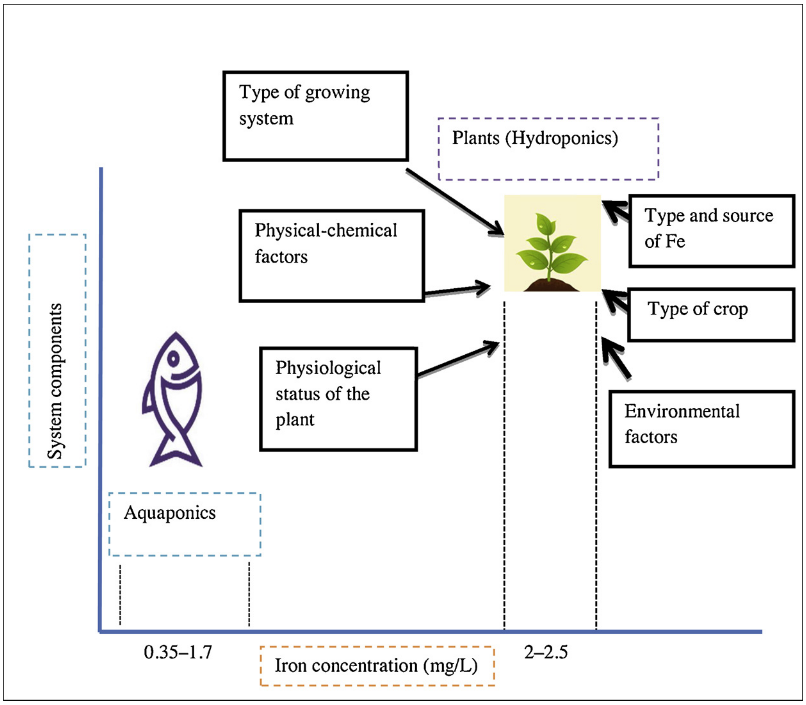

Fig. 2. Optimum ranges of dissolved iron concentrations (mg/L) for different components of aquaponic system.

of 2–2.5 mg/l is recommended (Sonneveld and Voogt, 2009). Roosta and Hamidpour (2011) reported that foliar application of Fe increased vegetative growth of tomato plants in aquaponic and hydroponic systems. On the other hand, cluster number per plant in aquaponics was lower than in hydroponics treatments, but it increased with foliar Fe application.

8. Conclusion and future prospects

Fe is a key element required for sustainable production of fish, plants, and growth of bacteria in aquaponic systems. Because this system combines plants with fish production, it has unique nutritional requirements that include an optimum Fe concentration for a healthy, balanced and functioning system. In aquaponic systems, fish metabolic wastes contain nutrients including Fe for the plants but the Fe concentrations supplied by the fish in such systems are significantly lower and unbalanced compared to hydroponic systems. Therefore, given the importance of Fe in aquaponic systems, practitioners must carefully replenish Fe in order to meet the requirements of the complex ecosystem. While the use of chelates to supply Fe is recommended, careful selection of chelates is important since their effectiveness is often affected by changes in pH. If the pH rises to above 6.5, the application of Fe-EDDHA or Fe-HBED chelates is recommended. In addition, system design and management are crucial for effective Fe optimisation as the chelates are sensitive to light, thus, nutrient solutions containing chelates should be protected from exposure to daylight. Additionally, further comparisons are needed to evaluate the methods of Fe application, *i.e.*, either as a foliar spray or as addition to water.

Although Fe supplementation in aquaponics may counteract iron deficiency, future research should also test whether this may enhance population growth of bacterial fish pathogens. However, it has been suggested that pathogenic bacterial loads in aquaponics systems were below the level that could be considered harmful to fish (Sirsat and Neal, 2013; Elumalai et al., 2017; Schmautz et al., 2017). Future work needs to show the possible interactions between Fe supplementation and fish welfare as additional Fe is added to a system.

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