Reuse of Water and Nutrients in Soilless Plant Culture

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Dissertation for the degree of *philosophiae doctor* (*ph.d.*)

University of Agder

Faculty of Engineering and Science

2023

Doctoral dissertations at the University of Agder 403

ISSN: 1504-9272
ISBN: 978-82-8427-116-3

Print: Make!Graphics
Kristiansand

Acknowledgements

I would like to thank deeply those who supported me throughout my PhD journey:

First, Prof. Helge Liltved for supervision and guidance. This work would never have been completed without his technical and scientific assistance.

My co-supervisors Randi Seljåsen and Jan Morten Homme for their suggestions and contributions.

All my colleagues, you are amazing and honest. You have been the best.

Admiration to Emma Elisabeth Horneman and Lise Askbo Fylkesnes for their help with administration.

Finally, many thanks to my family from my heart. You have been very supportive from the start.

Mars 2022

Hønefoss

Abstract

Soilless plant culture has been gaining increasing interest all over the world because soil-based agriculture is now facing several challenges, such as soil degradation, water scarcity, shrinkage of mineral reserves, population rise, climate change and expanding urbanisation. Soilless growth systems can tackle these challenges, as they are sustainable systems that provide more food with less space and resources and follow nutrient and water reuse principles. However, these systems rely on synthetic mineral fertilisers, which are mined from scarce and non-renewable resources. Therefore, finding a sustainable source of nutrients for these systems and improving the utilisation and efficiency of the nutrient solution (NS) will increase their potential for more efficient resource use.

This dissertation proposes two approaches to mitigating the dependency of soilless culture on scarce mineral fertilisers. The first approach aims to increase the lifetime of the NS used in recirculating hydroponic systems, while the second approach presents a holistic method for the treatment and use of aquacultural sludge as NS for soilless growth systems. This method includes two steps: nutrient mobilisation using aerobic digestion (AD), followed by solids precipitation using the biopolymer chitosan as the flocculant. The recovered NS was used to grow lettuce in a recirculating hydroponic system.

The outcome of the first approach showed that NS can be used for several weeks before discharge, even though many growers discard recycling NS at weekly intervals. In this study, NS was reused for 6 weeks, corresponding to a production of 1 kg lettuce per 10 litres tank volume of NS, in a closed hydroponic system without compromising the yield and apparent quality of lettuce**.**

The results from the second approach indicated that AD is an efficient method to mobilise nutrients in aquacultural waste to concentrations close to or exceeding the mineral levels recommended for soilless growth systems. In addition, the biopolymer chitosan proved to be an efficient and safe alternative for solids removal from aerobically digested aquacultural waste. The recovered NS was successfully used for lettuce production in a closed hydroponic system, with yield and quality comparable to those of lettuce grown with conventional NS. The results obtained clearly show the possibility of substituting synthetic fertilisers with recovered NS from aquaculture

waste, which can be considered an alternative and resource-efficient fertilisation strategy for soilless culture systems.

Both approaches are described in this dissertation, while detailed explanations of the materials and methods used, as well as the obtained results, can be found in the appended papers.

Abstrakt

Vannbaserte dyrkingsteknikker (hydroponi) har fått økende interesse over hele verden fordi tradisjonell grøntproduksjon nå står overfor flere utfordringer som utarming av jord, nedgang i mineralreserver, befolkningsøkning, klimaendringer og ekspanderende urbanisering. Hydroponisystemer kan være en alternativ dyrkingsform, spesielt i urbane områder, ettersom de kan gi større avlinger med mindre plass og ressurser, da gjenbruk og bedre utnyttelse av næringsstoffer og vann er mulig. Imidlertid er dagens hydroponisystemer avhengige av syntetisk mineralgjødsel som utvinnes fra knappe og ikke-fornybare ressurser. For bedre bærekraft og ressursutnyttelse er det derfor viktig å finne alternative kilder til næring ved å gjenvinne næringsstoffer fra avfallsstrømmer, samt å bedre utnyttelsen og effekten av næringsstoffene.

Denne avhandlingen foreslår to tilnærminger for å redusere forbruket og avhengigheten av knappe mineralressurser i vannbaserte dyrkingssystemer. Den første tilnærmingen tar sikte på å øke levetiden til næringsløsningen i hydroponiske resirkuleringsystemer, mens den andre tilnærmingen presenterer en helhetlig metode for behandling og bruk av slam fra akvakultur som kilde til næringsstoffer. Denne metoden inkluderer to trinn: Mobilisering av næringsstoffer som er bundet til partikler i slammet ved aerob nedbrytning, etterfulgt av koagulering ved hjelp av biopolymeren kitosan og sedimentering for å fjerne kolloider fra klarfasen.

Resultatet av den første tilnærmingen viste at næringsløsning kan brukes i hydroponisystemer med resirkulering i flere uker før løsningen må byttes, selv om mange dyrkere i dag bytter næringsløsningen med ukentlige intervaller. I denne studien ble næringsløsningen gjenbrukt i 6 uker, tilsvarende en produksjon på 1 kg salat per 10 liter næringsløsning, i et lukket hydroponisk system uten å gå på bekostning av produksjonsmengde og kvalitet.

Resultatene fra den andre tilnærmingen viste at aerob nedbrytning er en effektiv metode for å mobilisere næringsstoffer i slam fra akvakultur til konsentrasjoner nær eller over mineralnivåene anbefalt for plantedyrking i hydroponisystemer. I tillegg ble det vist at biopolymeren kitosan er en effektiv og trygg koagulant for fjerning av små partikler etter aerob nedbrytning slik at tilnærmet 90% av slamvolumet kan gjenvinnes som en klar og næringsrik løsning. Avslutningsvis ble den gjenvunnede næringsløsningen benyttet i forsøk med dyrking av salat i et resirkuleringsanlegg. Det

ble vist at denne næringsløsningen ga et utbytte og kvalitet som var sammenlignbar med salat dyrket med konvensjonell næringsløsning. Resultatene som er oppnådd viser tydelig muligheten for å erstatte syntetisk gjødsel med næring gjenvunnet fra slam fra akvakultur som kan betraktes som en alternativ og ressurseffektiv gjødslingsstrategi for vannbaserte dyrkingssystemer.

Begge tilnærmingene er beskrevet i denne avhandlingen, mens detaljerte forklaringer av materialene og metodene som er brukt, samt de oppnådde resultatene, finnes i de vedlagte artiklene.

List of Publications

The following papers included in this dissertation have been published in peer-reviewed journals and conference proceedings.

Paper A Ezziddine, M.; Liltved, H.; Seljasen, R. Nutrient solution dynamics and yield of lettuce (*Lactuca sativa*) in an EC-controlled recirculating hydroponic system. *Acta Hortic.* **2021**, *1305*, 407–414, doi:10.17660/ActaHortic.2021.1305.53.

Paper B Ezziddine, M.; Liltved, H. Quality and yield of lettuce in an open-air rooftop hydroponic system. *Agronomy* **2021**, *11*, 1–10, doi:10.3390/agronomy11122586.

Paper C Ezziddine, M.; Liltved, H. Nutrients recovery from aquaculture waste for use as fertilizer in soilless growth systems. *Acta Hortic.* **2021**, 1305, 399–406, doi:10.17660/ActaHortic.2021.1305.52.

Paper D Ezziddine, M.; Liltved, H.; Homme, J.M. A method for reclaiming nutrients from aquacultural waste for use in soilless growth systems. *Water Sci. Technol.* **2020**, *81*, 81–90, doi:10.2166/wst.2020.079.

Paper E Ezziddine, M.; Liltved, H.; Seljåsen, R. Hydroponic lettuce cultivation using organic nutrient solution from aerobic digested aquacultural sludge. *Agronomy* **2021**, 11, 1–13, doi:10.3390/agronomy11081484

Contents

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List of Abbreviations and Acronyms

AD	aerobic digestion
Al	aluminium
B	boron
Ca	calcium
Cd	cadmium
Co	cobalt
CO_2	carbon dioxide
Cu	copper
EC	electrical conductivity
DWC	deep water culture
FAO	Food and Agriculture Organization of the United Nations
Fe	iron
K	potassium
Mg	magnesium
Mo	molybdenum
Mn	manganese
N	nitrogen
Na	sodium
NFT	nutrient film technique
Ni	nickel
NS	nutrient solution
ONS	organic nutrient solution
P	phosphorus
Pb	lead
RAS	recirculating aquacultural system
S	sulphur
SDGs	sustainable Development Goals
Se	selenium
Si	silicon
Zn	zinc

Part I

Main Chapters

Chapter 1

Introduction

1.1 Context and research problem

By 2050, agriculture will need to feed a growing world population of 9.7 billion people [1]. At the same time, arable farmland, which only represents 20–30% of the world's land surface, is decreasing due to urbanisation and soil degradation, such as soil displacement by wind and water erosion, loss of nutrients, loss of organic matter, salinisation and acidification [2,3]. In addition, agriculture uses 70% of the world's freshwater supply, which will become a scarce resource in the near future for almost all countries [2]. Therefore, to provide the required food for future populations with less space and scarce resources, more investment should be made in a soilless growth system. Soilless growth systems are modified forms of farming practices based on growing plants without soil using an aerated solution of nutrient-rich water. Due to several advantages over conventional agriculture, including higher productivity and better nutrient and water utilisation, soilless growth systems can contribute to increased food production worldwide, with a lower environmental impact [4]. Nutrient solution (NS), which is composed of water and fertilisers, is a determining factor in soilless culture. Fertilisers for soilless culture should contain all the macronutrients (nitrogen, phosphorus, potassium, magnesium, calcium and sulphur) and micronutrients (manganese, iron, copper, boron, copper, molybdenum and zinc) that plants need.

Despite the growing interest in soilless culture, there are concerns about the complete dependency of soilless systems on synthetic mineral fertilisers since they require critical finite resources with associated greenhouse gas emissions for extraction, processing and transportation [5,6].

Phosphorus is mined from phosphate rock, which is processed into a variety of materials to blend or granulate into various fertiliser products. Phosphate rock is a fossil resource created by earth processes, such as magmatic occurrences, or accumulated over millions of years as sediments from the seabed [7]. After years of

mining higher quality and the most economic phosphate, the average grade has been dropping, and the remaining reserves are fraught with problems and challenges such as difficult access, higher impurities, more waste generation and higher energy requirement [8]. Estimates for the depletion of phosphate rock reserves range from 30 to 300 years [9–12].

Potassium is sourced from natural deposits, such as potash, sylvinite, granite dust and langbeinite, extracted in the form of mined salts and then transformed into nitrate and sulphate of potassium, which are fertiliser forms [13]. According to estimates published by the United States Geological Survey, potassium ores are expected to last around 400 years at the current rate of extraction. However, potassium production is strongly dominated by three countries: Canada, Russia and Belarus, which produce more than 90% of the world's potassium [14]. Current conflicts involving Russia and Belarus bring future insecurity to the supply of potassium from these countries.

In addition to phosphorus and potassium, the current literature shows that depletion of micronutrients is also a global problem [10]. Chardon and Oenema (2013) identified four micronutrients with a potential aspect of scarcity, namely boron, copper, molybdenum and zinc [15].

Accordingly, increasing the efficiency of NS used in soilless systems and finding alternative nutrient sources were the ultimate aims of this research.

Waste streams from food production sectors that cause serious environmental pollution could be an alternative nutrient source for soilless culture.

According to the Food and Agriculture Organization of the United Nations (FAO), the aquaculture industry is growing faster than other food production sectors. It is reported that by 2030, the aquacultural production of fish and shellfish is expected to reach 109 million tonnes, which represents a growth rate of 37% over production in 2016 [16]. An increasing amount of production will take place in semi-closed and closed systems, where effluent water and recirculating water are treated mechanically to remove solids waste (feed waste and faeces). Without safe disposal or use, the waste produced may cause detrimental environmental impacts, including the pollution of surface water and ground water bodies, spread of fish pathogenic microorganisms, and unpleasant odour from putrefaction [17]. However, the collected waste contains nutrients that can be utilised for fertilising purposes after appropriate treatment.

Thus, converting aquaculture waste into valuable fertilizer can not only alleviate the disposal problem but also have economic benefits and address the future scarcity of non-renewable fertilisers.

1.2 Research aims and contributions

The aim of this work was to address the scarcity of fertilisers used in hydroponics by proposing two approaches:

1- Increasing the lifetime of NS used in recirculating indoor (paper A) and outdoor (paper B) hydroponic systems by studying the dynamics of nutrient uptake and depletion and yield of lettuce.

2- Replacing chemical NS used in hydroponics with nutrients recovered from aquacultural sludge (Papers C, D, E).

The specific objectives related to the first approach included the following:

- Determining the time for growth limitation of lettuce cultivated with recycled NS.
- Investigating the effect of continuous NS recycling on the accumulation and depletion of individual nutrients.
- Develop strategies for managing NS recycling in hydroponic systems.

The specific objectives related to the second approach included the following:

- Recovery of nutrients from aquacultural sludge using AD (paper C).
- Investigating the effect of various pH values on nutrient mobilisation during AD (paper C).
- Verification of a method for nutrient mobilisation from aquacultural sludge by AD and solids separation using chitosan as a flocculant (paper D).
- Cultivation of hydroponic lettuce using organic NS recovered from aerobically digested aquacultural sludge after solids separation (paper E).

1.3 Dissertation structure

This dissertation consists of two parts.

Part I consists of five chapters, which are outlined as follows:

Chapter 1: Introduction

This chapter states the context and research problem, presents the research contribution and provides the dissertation structure.

Chapter 2: State of the art

This chapter provides a brief theoretical background by presenting an overview of aquaponics, hydroponic technologies, aquaculture, water treatment, sludge handling in recirculating aquacultural systems (RAS) and biological techniques of nutrient mobilisation in waste streams.

Chapter 3: Research strategy and methods

This chapter briefly describes the strategy, as well as the methods used in this research.

Chapter 4: Results and discussion

This chapter covers the content of the published papers.

In the first section, the content of papers A and B is summarised by discussing common intervals for discarding NS, describing nutrient imbalances due to NS recycling, presenting strategies to minimise recycling effects on plant growth and proposing the time and maximum lettuce production per unit volume of NS before discharge and refill are required, without compromising the yield and quality of plants.

In the second section, the content of paper C is covered by discussing the effect of AD on nutrient mobilisation in aquacultural sludge and investigating the effect of the pH of AD on nutrient mobilisation.

Paper D is covered in the third section by discussing the effect of chitosan on solids removal and the potential of chitosan as an efficient and safe alternative for solids removal.

The last section summarises the contributions of paper E by discussing the performance of reclaimed NS on plant growth and demonstrating that organic nutrient solution (ONS) recovered from aquacultural sludge can be used for lettuce production in hydroponic systems.

Chapter 5: Summary and outlook

This chapter summarises the major contributions of this dissertation and provides an outlook for future research directions.

The printed papers are presented in part II, with more details of contributions and results.

Chapter 2

State of the art

Soilless culture is a modern technology with roots tracing back several hundred years before BC. The first documented case of soilless culture practice was found in wall paintings in the temple of Deir el Bahari [18]. These paintings recorded that Egyptians, almost 4000 years ago, grew imported plants in containers without soil when the local soil was not suitable for that particular plant [18,19].

The oldest examples of growing plants in water are China's floating gardens. As described by Marco Polo, who travelled to China in the 13th century, these floating gardens consisted of floating rafts of reeds on which plants thrived, with roots growing through the rafts and down into the lake [20]. In addition, records from the ninth century show that Chinese farmers grew rice in paddy fields in combination with fish [21]. Similarly, the Aztecs grew crops on floating gardens made with rafts of rushes and reeds, which they called chinampas at Lake Tenochtitlan during the 10th and 11th centuries [2,19]. Modern soilless culture was developed from the findings of "water culture" experiments carried out in the 17th century to determine the requirements for plant growth [19]. Initially, soilless culture was mainly used as a research tool to study particular aspects of plant nutrition and root function [18]. German botanists, Julius von Sachs and Wilhelm Knop developed the first mineral NS for soilless culture in the nineteenth century [19]. The production of completely soluble fertilisers and the progress in plastic manufacturing have paved the way for commercial soilless culture [18].

The first commercial water culture was launched in 1929 by Professor William Frederick Gericke, who transformed his laboratory into a commercial soilless production site [19].

Modern soilless culture systems encompass a variety of non-traditional agriculture methods that can take place on lands unsuitable for cultivation, roofs, greenhouses and inside buildings. Aquaponics are an example of soilless culture systems that integrate two technologies: recirculating aquaculture with fish production and hydroponics with soilless plant culture [22]. Hydroponics is described in more detail in section 2.1, and aquaculture is detailed in sections 2.2 and 2.3.

In aquaponics, fish are fed commercial feed pellets. Uneaten feed pellets sink to the bottom of the tanks and release both finer solids and soluble compounds during decomposition. Fish also release solids (faeces) from the intestinal tract and soluble compounds (from the gills and urinary pores) based on the composition of the fish feed. Much of the solids waste is removed by sedimentation devices and mechanical filters. Finer solids and soluble waste are broken down in circulating water and biofilters into nutrients available for plant uptake by naturally occurring microorganisms. Then, the water rich in nutrients flows to the plants, which absorb these nutrients and, at the same time, clean the water. In coupled aquaponics, the clean water circulates back to the fish tank, while in decoupled aquaponics, which are currently widely adopted in Europe, the water does not return to the fish tank [2,23] (Figure 2.1).

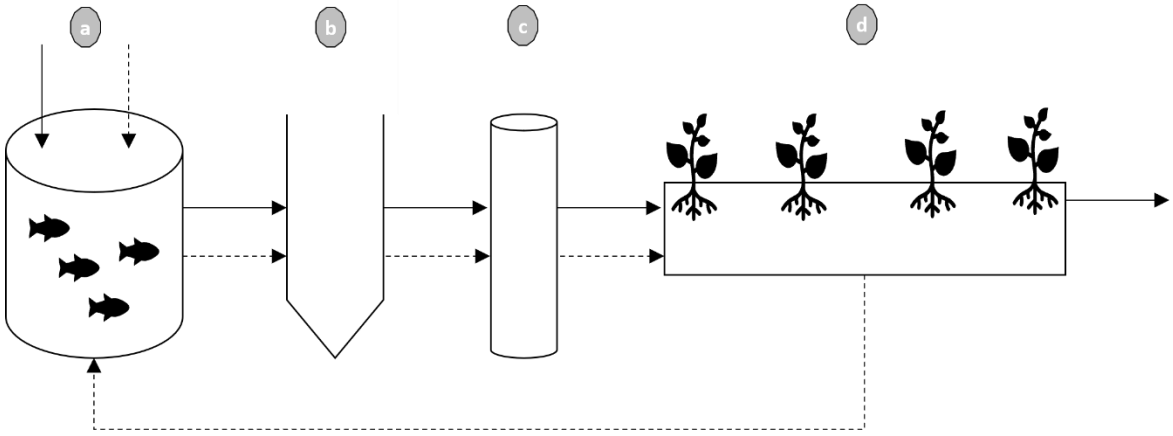

Figure 2.1: Principle of a coupled (⇢) and decoupled (⟶) aquaponic system. (a) Fish tank, (b) sedimentation devices and mechanical filters, (c) biofilters, and (d) hydroponic unit.

2.1 Hydroponic technologies

For plants to grow and be healthy, their roots need water, nutrients and oxygen. In conventional agriculture, soil supports plants' roots and provides water, nutrients and oxygen to these roots. In hydroponic systems, plants are grown without soil, with roots either supported or not by an inert medium, while water and nutrients are delivered via NS [24]. NS is a solution containing water and essential elements for plant growth. An essential element is directly involved in plant metabolism, and its absence prevents a complete plant life cycle [25]. Essential elements are divided into two main classes:

- Macronutrients: carbon (C), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).
-Micronutrients: iron (Fe), boron (B), manganese (Mn), copper (Cu), zinc (Zn) and molybdenum (Mo).

Macronutrients are required in relatively large amounts, as they contribute to more than 0.1% of plant dry matter, while micronutrients are required in small amounts [26]. There are other elements called beneficial elements that promote plant growth and can compensate for the toxic effects of other elements. Cobalt (Co), selenium (Se), silicon (Si) and sodium (Na) are the most known beneficial elements that can promote growth for specific plant species under certain environmental conditions [27]. These elements should be provided by the NS except carbon and oxygen, which are supplied by the atmosphere.

The rapid expansion of hydroponic systems worldwide is due to their advantages compared to soil-based agriculture, including resource use efficiency, minimum emission of pollutants to the outside environment, pesticide-free food, a higher production per unit area than conventional production, and a growing environment not affected by climate conditions, soil fertility or soil-borne pathogens [28].

In hydroponic systems, two types of configuration can be adopted: open-loop and closed-loop systems [2]. In open systems, the NS flows through the system only once, while in closed systems, the NS is reused via recirculation [29].

Closed systems are more attractive than open systems for several reasons. The major reason is their efficiency in water and nutrient use compared to open systems, with substantially less discharge into the environment, thereby reducing environmental and economic costs [30,31]. However, closed hydroponic systems have the potential risk of accumulation of toxic compounds and harmful plant pathogens, such as *Fusarium*,

Phytophthora and *Pythium* [32]. *Fusarium oxysporum* is responsible for root rot in lettuce, basil and tomatoes [33]. *Pythium* species are common root pathogens that are spread through water circulation systems and cause root rot in hydroponically grown cucumber, pepper and lettuce [32]

Hydroponic systems vary widely, but they can be classified according to the container used to grow plants, such as rails, buckets, beds, slabs or troughs. They can also be classified by the substrate used, water flow regime, method of root aeration or the degree of plant root submergence. The most described hydroponic technologies in the literature for growing leafy vegetables include deep-water culture (DWC), nutrient film technique (NFT) and media bed hydroponics (Figure 2.2).

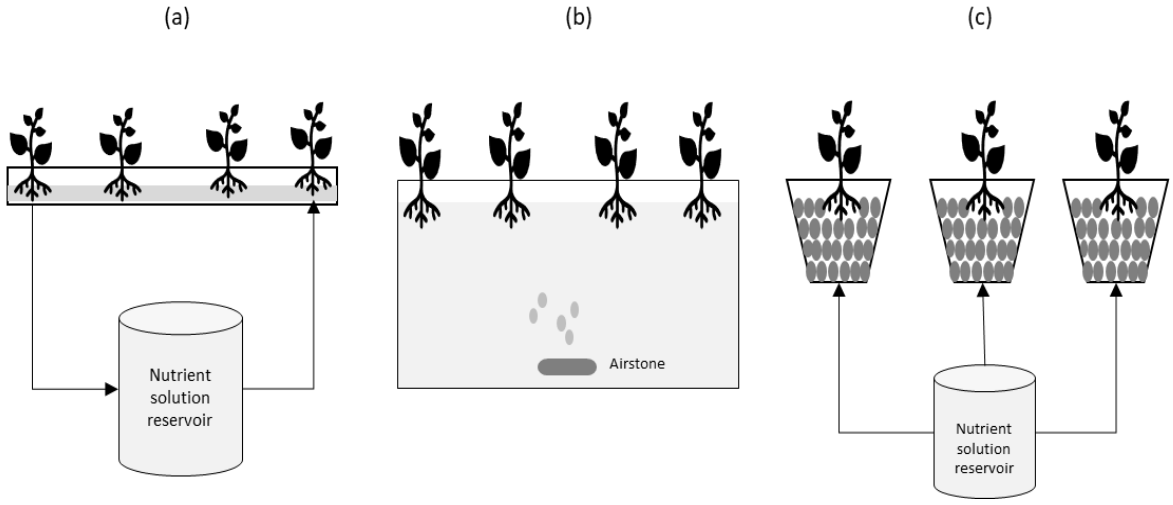

Figure 2.2: Hydroponic technologies represented by: (a) nutrient film technique, (b) deep water culture, (c) media bed hydroponics.

2.1.1 Media bed hydroponics

Media bed hydroponics consist of two main parts: a reservoir that contains the NS and a plant growing area, which can be either a single bed or several small buckets with growing media, also called the substrate. The substrate supports the plant weight and roots and holds it upright. It also provides NS and oxygen to the roots. Different substrates can be used. A good substrate should have [24]:

- A bulk density between 150 and 500 kg m^{-3}. The bulk density is the dry weight of the substrate per unit of volume, and it enables anchorage of the roots.
- At least 75% of macropores and micropores. Macropores allow good water drainage and air entry, while micropores ensure good water retention. An adequate combination of macropores and micropores is therefore essential.
- High cation exchange capacity (CEC). CEC measures how many cations can be detained on the substrate particle surface per weight unit of the substrate.
- Low EC values. The substrate should not affect the EC of the NS.
- Low cost.
- Neutral pH.

Substrates can be divided into three categories according to their origin:

- Organic substrate: They are derived from by-products of agriculture or waste from industrial activities, such as by-products of the wood industry, or from urban activities, such as sewage sludge. Organic substrates can be subjected to special processing. Peat, coconut fibre, bark, wood chips and saw dust are all examples of natural organic substrates.
- Mineral substrates: These include both natural minerals, such as sand and pumice, and products from industrial processes, such as vermiculite and perlite. Almost all mineral substrates have a low CEC and low buffering capability; therefore, they are usually mixed with other substrates.
- Synthetic substrates: These substrates can be plastic or synthetic resins. They are added to other substrates to improve their characteristics. For example, expanded polystyrene is added to improve the porosity and drainage of the substrate used.

Substrates can also be classified into two sub-categories:

- Granular substrates, such as vermiculite and perlite, have high macroporosity but low microporosity, which means good drainage but poor water retention capacity.
- Fibrous substrates, such as rockwool and coconut fibre, have high microporosity but low macroporosity [24].

According to the NS delivery to the plants, media bed hydroponics can be divided into two categories: ebb and flow system and drip system [34].

The ebb and flow method is based on periodical flooding and draining of the substrate and root zone by the NS. During flooding, a timer turns on a pump that circulates the NS from the reservoir to the media bed or buckets until it reaches an overflow tube

that maintains an appropriate NS level in the media bed or buckets. During drainage, the timer turns off and the NS drains back into the reservoir by gravity via the drainage system [35,36]. Several ebb and flow designs that have the same principle can be used, including the flooding tray design, the overflow tube height system, the surge tank ebb and flow, and the Dutch bucket hydroponics system [37,38] (figure 2.3).

In the flooding tray system, plants are grown inside plastic bags filled with substrate and placed on a shallow tray on top of a raised surface. The NS reaches the plants from one end of the tray and flows back from the other end to a reservoir located below the tray. An overflow tube controls the height of the water in the tray [39].

The overflow tube height system consists of many containers connected to one overflow tube. NS flows evenly from the reservoir to the containers at the same time. The overflow tube controls the NS level inside each container [39].

The surge tank ebb and flow system uses a surge tank instead of an overflow tube to distribute NS equally in all containers. A pump inside the reservoir helps the NS flow to the surge tank and all containers. Another pump is placed in the surge tank to push the solution back to the reservoir [39].

The drip system consists of small emitters that slowly drip the NS into the media bed or buckets. Each emitter has a mechanism for controlling the NS flow. Individual plant pots with at least one drip emitter are usually used. A pump- or gravity-based system can be used to supply NS from the reservoir to the plants. The drip system can be a recirculating drip system where the excess water left in the substrate flows back to the reservoir or a non-circulating system where the excess water runs off as a waste. The latter configuration is mostly used on a commercial scale [40].

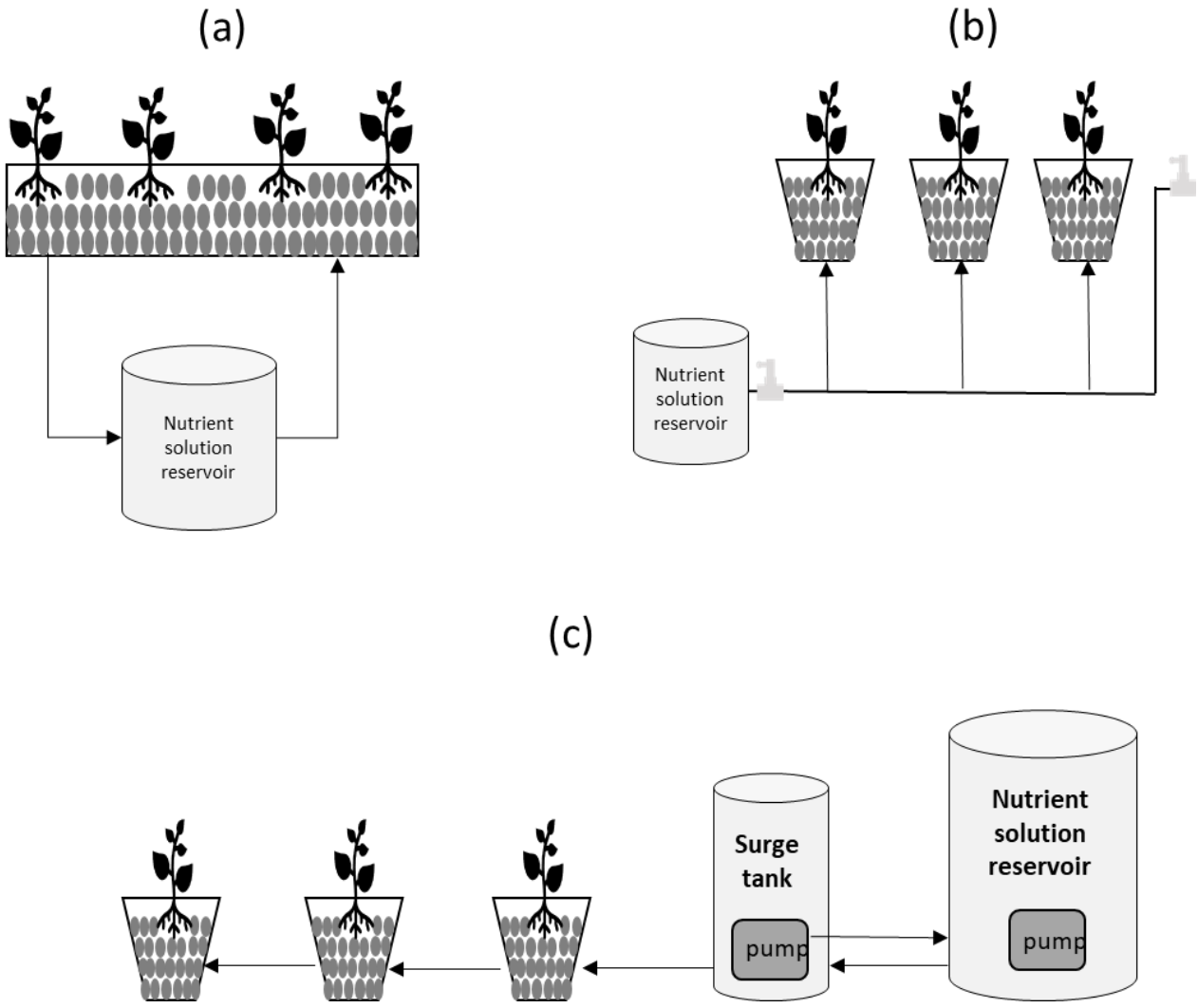

Figure 2.3: Different types of ebb and flow hydroponics: (a) flooding tray design, (b) overflow tube height design, and (c) surge tank design.

2.1.2 Deep water culture (DWC)

In DWC, as its name suggests, plant roots are submerged deeply in NS, and they are supported in net pots. Net pots allow roots to grow and spread in different directions, which promotes a healthy and strong root system. Net pots with plants are placed in holes drilled on the top of rafts, panels or boards that float above a container filled with NS. Containers are made of any inert, strong material, such as plastic, fiberglass,

wood and concrete. To avoid plant root suffocation, aeration and dissolved oxygen should be provided to the NS [40]. Two aeration methods can be used: air bubbles and falling water. Air bubbles are created by air pumps and airstones. Airstone, which is a rock-like material with small pores, is connected to the air pump by an airline. The airstone creates small bubbles that rise to the top of the NS, providing aeration to the plant roots. Falling water produces an agitated surface, which creates an aeration of the NS. The higher the water is falling and the larger the volume of water, the more dissolved oxygen will be provided for the NS until oxygen saturation is achieved.

Some variations of DWC also exist, including top-fed DWC, the Kratky Method DWC and recirculating DWC.

In top-fed DWC, a water pump is added in the NS container to improve circulation, nutrient transport to the roots and aeration. Top-fed DWC is useful when the roots are still short, as it helps the roots fully reach the NS, thus speeding up plant growth [38].

In the Kratky method, aeration is provided by leaving an air gap between the roots and the surface of the NS in such a way that part of the roots is submerged in the NS while the other part is exposed to the air [41].

In recirculating DWC, several NS containers are used. These containers are connected to a central reservoir from which aerated and calibrated NS circulates to the containers [42].

2.1.3 Nutrient film technique (NFT)

The NFT system consists of two main components: a reservoir that contains the NS and a planting volume, such as a channel, tray, gully, trough or pipe with holes for the net pots. Seedlings are normally transferred from a seedling system to the NFT system in the substrate used for sowing, e.g., rockwool plugs placed in net pots. This provides physical support for the plants. Inside the planting area, a film of 1–2 cm of NS flows along the bottom [40]. Part of the roots develop in the film of the NS, while the other part is suspended in the air above, which ensures that the roots receive sufficient oxygen and have a large enough air exchange surface.

Pipes are usually made from PVC and are white, which reflects light and avoids excessive heating. Pipes can be round or rectangular. Rectangular pipes with a width larger than their height allow for a much larger surface area for the NS, which

increases nutrient uptake and plant growth [40]. Pumps are used to circulate the NS from the reservoir to the planting area. The NS flows over plants, and once it reaches the end of the pipe, it drains back to the reservoir through the slightly downward pipes. NFT is used to grow small and fast-growing plants, including lettuce and leafy greens in general. It is also suitable for growing strawberries.

2.2 Aquaculture

Aquaculture is the rearing and production of fish, crustaceans, molluscs, aquatic plants, algae, and other aquatic animal and plant species under controlled conditions [40]. Aquaculture systems include fishponds, net-pen aquaculture, flow-through raceways and recirculation aquaculture systems (RAS).

A fishpond is a freshwater culturing method for fish in natural or artificial basins. Most fishponds use stagnant water, but running water can also be used, especially in highland sites with flowing water [43]. Fish densities in fishponds are relatively low because this method relies on the inherent treatment capacity of the earthen pond itself. As feeding rates are low (because fish densities are low) and the self-treatment of fish waste, the nutrient content in fishpond water is low.

Therefore, fishponds are an inappropriate aquaculture method to integrate with hydroponics in aquaponic systems [2].

Flow-through raceway is a land-based aquacultural method that consists of artificial channels constructed of concrete, reinforced fiberglass and plastic materials, where very large volumes of water at high turnover rates move through to maintain the required water quality. Fish waste is generally discharged into a receiving stream with little or no wastewater treatment [44]. Similarly, flow-through raceways are not appropriate for aquaponic integration because they do not allow adequate nutrient accumulation due to high water turnover rates [2].

Net-pen aquaculture, also called a sea cage or sea pen, is an offshore aquaculture method that consists of a framework made from metal, plastic material, wood or bamboo floating on the surface of a water body (open net pens), or fully underwater (submersible net pens), and supports a mesh enclosure. In net pens, residual feed and fish waste are normally discharged directly to the water body and pose a threat of environmental pollution [44].

RAS is an intensive method in which fish are raised in tanks and water is treated and reused within the system. Only a small percentage of the water is discharged daily [45]. RAS is an appropriate aquaculture method for integration within aquaponic systems because it allows fish waste accumulation, which leads to water nutrient concentrations that are applicable for hydroponic plant production [2]. RAS consists of fish tanks and various water treatment devices, normally including solid removal units, CO_2-degassing, oxygen maintenance devices, nitrifying biofilters and buffering pH facilities. More details on these units and devices are available in the next section.

2.3 RAS and water treatment

In RAS, the amount of recirculating water to be treated is huge. For example, the maximum water flow rate of a commercial Norwegian RAS with an approximate salmon smolt production rate of 1200 ton smolt/year is 16 m^3 min^{-1} [46]. The recirculating water in RAS is rich in soluble and solids compounds that need to be removed.

As previously mentioned, water treatment in RAS normally includes good particle removal before nitrifying biofilters, CO_2-degassing and oxygen supplementation, pH adjustment, and sludge handling.

2.3.1 Particle removal devices

Solids that originate from uneaten fish feed and faeces increase water turbidity, can cause unwanted sediments in the RAS system and can harm fish health. They are also a substrate for heterotrophic bacterial growth, which can lead to oxygen depletion, poor biofilter performance (by competing with nitrifying bacteria) and biofouling problems, and should be removed before biofiltration [2].

Solids are classified by their size. Solids with a diameter above 1 µm are defined as total suspended solids (TSS). Solids with a size between 1 µm and 1 nm are classified as colloids. Substances with a size lower than 1 nm are defined as dissolved matter [47].

TSS are divided into settleable solids, typically greater than 100 µm and non-settleable, also called fine solids, typically between 1 and 100 µm. Fine solids are more difficult to remove from the system and are harmful to fish because they can

accumulate in their gills, causing oxygen transfer problems and offering a suitable habitat for the proliferation of pathogenic microorganisms [47].

It is very important to remove the settable solids rapidly and efficiently before they become smaller and more difficult to remove. In RAS systems, mechanical screening or filtration systems are the most common methods for solids removal. Settleable solids can also be removed by sedimentation devices that use gravity to separate solids. Sedimentation devices are relatively cheap and do not require specialised operational skills. However, they are labour intensive and require a lot of floor space. Fine solids cannot be removed by sedimentation devices because they are too small to settle. However, they can be removed by mechanical filtration. Smaller solids can be removed by foam fractionation. More details on solids removal devices are discussed in sections 2.3.2, 2.3.3 and 2.3.4.

2.3.2 Sedimentation devices

- Swirl separators

Swirl separators, also called teacup settlers or hydrocyclones [48], consist of a round tank with a cone bottom. The water enters the tank tangentially to the tank wall, causing the water to spin around the tank's centre axis. This generates rotational forces that push large solids towards the centre where they settle via gravity into the cone-bottom [49]. Concentrated solids are flushed periodically and collected in a sludge collector tank. Treated water leaves the swirl through an outlet at the top of the unit.

- Clarifiers

Clarifiers or settling basins can have conical or rectangular designs. Both types serve to reduce the velocity of the water flow to allow large solids to settle to the bottom, where they can be removed as sludge. Conical clarifiers may have baffles to assist solids settlement. A valve at the base of conical clarifiers allows solids removal.

In rectangular settling basins, water enters one end, flows slowly through the clarifier to allow the solids to settle and discharges at the other end. Before the exit end, water flows over a weir or dam to allow the remaining solids to settle.

Obstructions inside the settling basin can be added to increase settling rates.

Sedimentation devices are also available in other configurations, such as radial flow separators.

2.3.3 Mechanical filtration

Mechanical filtration is the retention of solids in a filter, such as a microscreen, granular media or porous media filter [47].

Microscreen filters have varying pore-size openings. They allow the water to pass through, while solids are trapped by physical restriction when solids particles are bigger than the pore size openings of the screen.

Microscreens with very small pores remove a high percentage of the solids in the water, but they clog quickly and have limited hydraulic capacity. Microscreens with larger pores remove fewer solids but take longer to block. Microscreens can be static or moving screens. In commercial RAS systems, moving microscreens are used. They automatically backflush, and the backwash water is directed to a sludge-handling system or can be discharged to the domestic wastewater system [50]. Typical microscreens are the drum filter and inclined belt filter.

The drum filter consists of a moving microscreen attached to a horizontal cylinder mounted in a water tank. The water enters the inside of the drum axially and moves radially outward, so solids in the water greater than the screen pores are trapped on the screen. Typical pore size openings are 40–100 μm [47,51]. When the screen starts to clog, the water level inside the drum rises, causing the drum to rotate. As the drum rotates, a jet of water washes the collected solids off the screen into a collection trough [52].

Granular media consist of a bed of granular material through which water flows and solids either adhere to the medium or are trapped within the open spaces between the medium particles [53]. The main types of granular media filters are mono-media filter beds, which generally use sand or another granular medium; dual-media filter beds, which commonly use anthracite and sand; and multi-media filter beds [54]. In multimedia filters, the top layer of the bed consists of the lightest material, such as anthracite coal, while the bottom layer consists of the heaviest and most finely graded material, such as garnet or magnetite. The intermediate layer can be silica sand. The entire bed in multimedia filters acts as a filter rather than the top few centimetres: larger particles are removed at the top layers, and smaller ones are removed deeper in the filter media [55].

All granular media filters require backwashing to regenerate the filter capacity to remove solids [47]. A bead filter is a common granular media filter used in RAS. It

consists of floating plastic media in a sealed, pressurised container [50]. It functions as a physical filtration by removing solids while simultaneously encouraging the growth of bacteria that oxidise dissolved organics and convert ammonia to nitrate (nitrification) through biofiltration processes [56]. Water to be filtered flows into the bead filter through a media retaining screen at the bottom of the bead container. As the water flows upward, solids are captured within the beads. A screen installed at the top of the container retains the beads while the filtered water flows out. The bead filter is backwashed regularly to maintain the hydraulic capacity of the filter. For that, clean water enters backwards and solids are removed by activating a motor-driven propeller located within the bed of beads. The propeller agitates the bead bed until fluidisation occurs, expanding the bead bed and separating the solids from the beads, thereby releasing solids trapped within it. The mixing motor turns off, the beads refloat, and the solids are allowed to settle. A valve at the bottom of the container is then opened and the settled solids are removed as sludge. Bead filters require relatively little water to backwash. Bacteria growing on the beads make them sticky, allowing for even finer solids removal. The bacterial communities in bead filters include both autotrophic bacteria contributing to nitrification and heterotrophic bacteria that break down soluble organics and organic solids that are trapped within the bead bed [53].

2.3.4 Foam fractionation

In foam fractionation, air is introduced into the bottom of a closed column of water, creating fine air bubbles that collect organic material as they rise to the surface. Bubbles can collect very fine particles and some dissolved organics. Surfactant molecules with a hydrophilic end and a hydrophobic end (for example, proteins) adhere to the bubble by their hydrophobic end, leaving the hydrophilic end in the water. If this end is charged positively, it attracts negatively charged material in the water, which in turn attracts positively charged particles and so on. Bubbles with the attached particles rise to the top of the water column as foam that can be removed [47,53].

2.3.5 Nitrifying biofilters

Ammonia is a part of the soluble waste excreted by fish, which is very detrimental to fish health and therefore should be removed from recirculating water. Ammonia is

converted in nitrifying biofilters to a form that is more tolerated by fish. Different nitrifying biofilter technologies are available, but they all perform the same role.

Nitrifying biofilters consist of a vessel that holds a substrate on which nitrifying bacteria, such as *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrobacter* and *Nitrococcus*, can attach and grow. These bacteria respire oxygen and obtain energy from the oxidation of ammonia and nitrite to nitrate. Heterotrophic aerobic bacteria also grow in nitrifying biofilters, breaking down small organic solids that are trapped within the biofilters and oxidising the dissolved organic matter.

Common substrates used in nitrifying biofilters include gravel, sand, plastic beads, plastic rings and plastic plates [53]. Common nitrifying biofilter designs are trickling filters, rotating biological contactor (RBC), fluidised sand filter and moving bed bioreactors.

In trickling filters, the substrate is not completely submerged in water, which also allows aeration and CO_2-degassing, and thus assists nitrifying bacteria. The most used substrate in trickling filters is plastic media, which has a relatively low specific surface area that creates large void spaces within the substrate. The water enters the filter through the water distribution system at the top of the reactor and trickles down across the height of the reactor and through the substrate, allowing contact and therefore nitrification, to occur, and finally exists at the bottom of the filter continuously. The flow rate of the water is limited by the void space through which water can pass and the specific surface loading rate (mass of ammonia applied per hour divided by the specific surface area of the medium) [47,50,53]. High void ratios reduce the clogging of filter media, which can be a serious problem in commercial farms and must be avoided. According to the literature, the effects of the hydraulic surface load of the filter and the type of filter material are difficult to quantify. Random flow media, which are in the form of loose balls that require a special support frame, are prone to clogging. Vertical flow and cross flow, which are self-supporting blocks that can be stacked easily and taken out when necessary, are not prone to clogging, which explains why cross flow media and vertical flow have become more popular [57].

RBC consist of a substrate attached to a shaft that rotates, allowing partial submersion (approximately 40%) of the substrate in the water. Nitrifying bacteria grow on the substrate and rotate with the RBC, alternately contacting nitrogen-rich water and air. As the RBC rotates, it exchanges carbon dioxide (generated by bacteria and fish) for

oxygen from the air. The substrates used in RBC have a high specific surface area, allowing ammonia, nitrite and carbon dioxide removal in small RBC units. Plastic blocks and polyethylene tubular media are examples of substrates used in RBC [53].

In a fluidised sand filter, water flows upward through the void space of a bed of sand at a rate sufficient to lift and expand (fluidise) the bed of sand and keep the sand particles in turbulent motion, which allows good transport of dissolved oxygen, ammonia and nitrite to the nitrifying bacteria, resulting in high nitrification capacity in small units but at a high energy cost [47,53].

In a moving bed bioreactor, the substrate may consist of small plastic profiles of 10 to 50 mm diameter with a high specific surface area made of high-density polyethylene equal to 0.95 g cm^{-3} (Kaldnes K1, Norway). The substrate moves freely in the water volume and is retained within the reactor by a sieve or screen at the reactor outlet. This movement is caused by agitation set up by a bubble aeration system. Substrate filling in the reactor may be subject to preference. It can occupy up to 70% of the volume of the reactor [58].

2.3.6 Oxygen maintenance devices and CO_2-degassing

Oxygen maintenance devices provide aeration to fish. Air lift pumps can be used to move water through fish tanks, allowing both aeration and pumping. Oxygen cones can also be placed in recycled water. Aeration devices should maintain the level of dissolved oxygen above 60% saturation (5 mg/L) throughout the system [59].

In RAS, fish and microorganisms produce CO_2 which can accumulate to high concentrations in the water. If not appropriately removed, high CO_2 concentrations can negatively affect fish welfare and growth. CO_2-degassing units that can control and maintain CO2 at low concentrations include stripping columns [47,60].

Stripping columns are vertical columns filled with plastic packing media. The water is introduced at the top of the column and distributed uniformly over the packing media with a perforated plate, spray bar or spray nozzle. When the water flows downward through the packing media, it is broken up, creating a large gas–liquid interfacial area for gas transfer. As the water breaks up, large volumes of fresh air are introduced to provide a driving force for CO_2 gas transfer because fresh air has a low gas phase concentration for carbon dioxide. The degassed CO_2 is carried out of the column, with the fresh air being blown through [47].

2.3.7 pH adjustment and alkalisation

Biofilter media, such as oyster shells, can be added to the system as a source of carbonates to increase the pH and replace alkalinity loss caused by bacteria nitrification and fish metabolism. Powdered limestone and bicarbonate of soda can also be added to increase alkalinity and the pH level.

2.4 Sludge characteristics and handling

The solids removed in an RAS by the above-mentioned devices, along with the backwash water from the filter, constitute the sludge. RAS produce a high amount of sludge. The annual amount of sludge from land-based smolt production in freshwater has been estimated to be approximately 11,000 tonnes dry weight [61]. Sludge generated in RAS comes from fish feed, which contains nutrients, such as N and P, as well as other macro and microelements. In RAS, fish feed is introduced to the fish tank. Most of it is eaten by the fish and is either used for growth and metabolism or excreted as soluble waste (from the gills and the urinary pore) and as solids (faeces) from the intestinal tract and uneaten feed that sinks to the bottom of the tank. Depending on the fish species, culture conditions and feed composition, 60 to 80% of the ingested nitrogen is released as ammonia through the gills (a small amount of ammonia is also released from the urine) and as organic nitrogen from urine and faeces. Of ingested phosphorus, 17 to 30% is released in the water as soluble excretion, and 20 to 37% is released from the faeces [2,53]. Rafiee and Saad (2005) reported that 24% of the iron, 86% of the manganese, 47% of the zinc, 22% of the copper, 16% of the calcium, 89% of the magnesium, 6% of the nitrogen, 6% of the potassium and 18% of the phosphorus contained in fish feed ended up in the sludge [62].

The sludge produced by solids removal systems in RAS is characterised by a high moisture content and is relatively low in solids content (approximately 1–2% DW) [63]. Due to the large volumes of sludge produced, thickening is required before transportation and disposal. Sludge thickening can be accomplished by settling basins, belt filters, centrifuges and geotextile bags. Polymers are often used to improve the dewatering properties of sludge [45]. The intention of such treatment is to produce a low volume concentrated sludge for storage and use and to produce a good quality

clear phase, low in solids and nutrients, in compliance with discharge requirements. The concentrated sludge phase can be stabilised for land application and composting, while the clear phase can be discharged or subjected to further treatment [45].

Settling basins can concentrate the solids in the sludge up to 10% of the total solids content [64]. Settling basins function according to the same particle settling principles as previously described.

Geotextile bags are sealed porous tubular containers made of woven polyethylene material. Many studies have shown their ability to dewater sludge to over 10% of solids in less than one week and to over 30% over months [45].

Lime addition improves the settling properties of the sludge, kills sludge pathogens and increases the removal of phosphorus [17]

One of the most commonly used methods for the removal of solids in the clear phase is the use of chemicals to promote coagulation and flocculation.

Coagulation/flocculation is generally a three-step process. First, a coagulant is added to decrease the electrical charges on the surface of solids that cause the particles to repel and consequently contribute to their dispersion and stability. Using charge neutralisation and precipitate enmeshment (a process in which small particles are physically enmeshed by precipitates formed by the coagulant), microflocs are formed. Then, flocculation takes place, which brings together the microflocs to form larger flocs by slow physical mixing. In addition, organic or inorganic polymers can be added to improve flocculation [45]. Flocculation is followed by sedimentation of the gross flocs.

Several studies have been conducted using metal-based coagulants and synthetic organic polymers for flocculation and settling of solids in wastewater treatment [65]. Inorganic metal salts, such as ferric and aluminium (Al) salts, are the most commonly used flocculants due to their low cost and ease of use [66].

Cationic polymers are also used. They may carry out the dual functions of coagulation and flocculation. Chitosan is a natural cationic polymer made from shrimp and crab shells that has been shown to be an excellent turbidity remover [67].The effectiveness of chitosan as a coagulant and flocculant can be explained by its long chain structure with abundant free amino groups that are protonated in neutral and acidic media, as they impart high charge density. The positive charges neutralise the negative charges on particle surfaces and bridge the destabilised particles into aggregates [67].

2.5 Biological techniques for nutrient mobilisation in waste streams

Mobilisation, also called solubilisation or mineralisation, is the release of macro- and micronutrients from their molecules to a liquid phase in their ionic form.

Many techniques for nutrient solubilisation in waste streams have been explored, including biological treatments, chemical treatments and physical treatments or a combination of these treatments. This section will focus on biological treatments.

2.5.1 Aerobic digestion

In AD, biodegradable organic molecules can be used as carbon sources and energy sources by heterotrophic microorganisms, so they can grow and multiply. During the hydrolysis process of organic molecules by heterotrophic microorganisms, the macronutrients and micronutrients that were initially bound to the organic molecules are released in the water in their ionic forms. This phenomenon is called nutrient mobilisation. During aerobic degradation of organic molecules, carbon is oxidised into CO_2 and water to provide energy for microorganism's growth and maintenance. The released CO_2 dissolves in the water and lowers the pH, which may promote the solubilisation of precipitated minerals [2,68].

When the available organic matter runs out, microorganisms begin to die. They undergo lysis, which releases organic matter for use by other microorganisms. This process extends to the point where the available energy in active microorganisms and organic matter is sufficiently low to permit the sludge to be considered stable [69,70].

2.5.2 Anaerobic digestion

Anaerobic digestion is a four-step process carried out by different microorganisms under anaerobic conditions. These steps are hydrolysis, acidogenesis, acetogenesis and methanogenesis. During hydrolysis, organic polymers, such as polysaccharides, lipids and proteins, are broken down by extracellular enzymes into their respective monomers (simple sugars, fatty acids and amino acids). These monomers are converted into acetate, formate, hydrogen and volatile fatty acids during the next phase, which is acidogenesis. The products of acidogenesis are transformed mainly into acetic acid, carbon dioxide and hydrogen during acetogenesis. In the final phase, intermediate products of the preceding phases are further processed by methanogens

to produce biogas (methane and carbon dioxide). The final digestate is nutrient rich because a high amount of particulate-associated nutrients is mobilised during the four steps [71,72]. The organic acids produced during aerobic digestion reduce pH and elevate the solubility of P, Fe and other nutrients [73].

Chapter 3

Research strategy and methods

As mentioned previously, the aim of this work was to address the scarcity of fertilisers used in hydroponics by proposing two approaches: to show the possibilities of extending the lifetime of the NS and to demonstrate the usefulness of an alternative NS recovered from aquacultural sludge.

In the first approach, indoor and outdoor lettuce cultivation using recirculating NFT hydroponic systems were carried out.
Indoor cultivation was conducted in a growth room located at the University of Agder in Norway during a 68-day period. The growth systems consisted of a seeding system and a closed NFT system. In the seeding system, seeds of *Lactuca sativa L.* (Batavia-type, 'Partition') were seeded in rockwool cubes and inserted in net pots. The cubes in net pots were placed in trays with NS and illuminated with LED light with a wavelength peak in the blue (445 nm) spectral ranges, with a total photon flux density of 210 μmol m^{-2} s^{-1}. After 2 weeks in the seeding system, the seedlings were transplanted to the NFT system for an additional 4 weeks of growth before harvest. The NFT system included three identical parallel units designed to conduct triplicate experiments under identical conditions. Each unit hosted 12 lettuce plants in a closed loop system, which consisted of a rectangular PVC pipe and a 20 L nutrient tank. The PVC pipe was 2400 mm long and 100 mm wide and had 12 holes with diameters of 45 mm for net pots. The nutrient tank had a submerged pump, which intermittently supplied the PVC pipe (30 min on/off cycles) with NS at a flow rate of 3.5 L min^{-1}. LED lamps with wavelength peaks in the blue (445 nm) and red (660 nm) spectral ranges were suspended over the pipes, producing photosynthetically active radiation (PAR) with a flux density of 220 μmol m^{-2} s^{-1} for 18 hours per day. The temperature of the growth room was kept in the range of 22–24°C, CO_2 concentration of 410–450 ppm, and relative humidity of 35–40%. Each of the three parallel units of the NFT system was operated in continuous production mode during the experimental period. At the starting date, 9 seedlings (2 weeks old) were collected from the seedling system

and transferred to the NFT system (3 seedlings to each of the three units). After 4 weeks, the NFT system was fully stocked, and the first 3 lettuce heads of the 12 lettuce plants in each unit were harvested and replaced by 3 new seedlings. During the 68-day experimental period, there were 7 harvests, with a total production of 21 lettuce heads from each unit, which gave a total of 63 lettuce heads from the 3 parallel units of the NFT system.

For the outdoor lettuce cultivation experiments, the same seeds of *Lactuca sativa L.* were seeded in a seeding system, as described above. Seedlings were then transplanted in an open-air rooftop hydroponic system, which consisted of 3 rectangular PVC pipes, each 4000 mm long and 100 mm wide, mounted on an aluminium stand with wind shields. Each pipe had 20 holes (50 mm in diameter) to insert net pots, which allowed the cultivation of 60 lettuce heads. A nutrient container intermittently supplied the three PVC pipes with NS (30 min on/off cycles) at a flow rate of 3.5 L min^{-1}. The average PAR throughout the experimental period was 272 µmol m^{-2} s^{-1}.

To study nutrient imbalances due to NS recycling, a balanced standard NS was prepared from two commercial stock solutions and adjusted regularly to a constant pH and EC value. The volume of NS was kept constant by adding a refill solution to replace nutrient uptake and transpiration. The same NS was used for the rooftop and indoor hydroponic lettuce growth and was never discarded throughout the experimental period.

In the second approach, a holistic method was developed for the treatment and use of aquacultural sludge as NS for fertilising purposes in soilless growth systems. This method includes two steps: nutrient mobilisation using AD, followed by solids precipitation using the biopolymer chitosan as the coagulant and flocculant.

AD was used in this work for nutrient mobilisation because of its relatively simple operation and low equipment cost. In addition, it stabilises the sludge and reduces the odours and number of pathogens [70]. Moreover, under aerobic conditions, excessive nitrogen loss due to denitrification is prevented.

Chitosan was used for solids separation because it does not react with mobilised nutrients, is regarded as nontoxic, has no health concerns, and is known as a metal chelator.

The aquacultural sludge used in this study was collected from swirl separators and bead-filter backwash water at an aquaponic research facility at the Norwegian Institute

of Bioeconomy Research (Landvik, Norway). The aquaponic facility is a closed system based on RAS technology connected to tanks for vegetable production and consists of two separate identical test units. The total water volume of each unit was 8 m^3, divided into two fish tanks with a volume of 1.2 m^3 each, and two plant compartments with a volume of 3 m^3 each. The fish tanks were stocked with 530 brown trout (*Salmo trutta*), with an average weight of 97 g. The water treatment system consists of swirl separators, a combined particle biofilter, a heating/cooling unit, an aeration unit and a pH-control unit.

For AD, the sludge was distributed to 25 L polyethylene batch reactors covered with lids to minimise evaporation. Each reactor was aerated at a continuous rate of 20 L min^{-1}, provided by diffusors and a common blower. The temperature was kept constant at 22°C, and the pH was measured and monitored daily in all reactors.

After four weeks of aerobic digestion, the experiment was terminated, and chitosan precipitation was applied to remove solids. Jar tests with flocculation and sedimentation were performed. Commercial chitosan with a molecular weight of 161 kD and a degree of deacetylation of 80% was used as a coagulant. The coagulant was added during rapid mixing, and the pH was adjusted to 6. After 60 s of rapid mixing (400 rpm), followed by 10 min flocculation under slow stirring (30 rpm) and 20 min sedimentation, the nutrient-rich clear phase was reclaimed and used as an alternative organic NS for indoor hydroponic lettuce growth. Lettuce growth experiments using the recovered NS were carried out in the same growth room with the same NFT system used in the first approach.

Detailed descriptions of the materials and methods used can be found in the appended papers.

Chapter 4

Results and discussion

4.1 Reuse of nutrient solution in hydroponic systems

It is difficult to maintain a well-balanced NS in closed hydroponic systems for a long time because the individual nutrients are absorbed by plants at different rates, resulting in the accumulation or depletion of some nutrients.

To overcome problems with nutrient imbalance, several strategies have been proposed to determine and control nutrient requirements, including automated monitoring of nutrient concentrations by ion-selective electrodes [74] and development of mathematical models, software and automated systems [75]. However, these strategies can be very expensive and technically challenging for growers. Thus, many growers prefer to discard recycling NS at weekly intervals. Increasing the lifetime of NS is of utmost importance since nutrients, such as phosphorus and some micronutrients, are becoming scarce resources. The literature does not give limits for the maximum time for NS recycling, as this varies among crops, local growth conditions and the volume of NS to the amount of crop. One way to extend the lifetime of NS is to discard recirculating NS only when the negative effects on plant growth start to appear. The objective of this work was to determine the time for the growth limitation of lettuce grown in recirculating NS, indicate which nutrient was limiting, and suggest a method for the construction of a refill solution based on calculations of actual nutrient uptake rates by the plants.

4.1.1 Nutrient imbalances due to NS recycling

Usually, the concentration of nutrients in recirculating NS is controlled by electrical conductivity (EC). EC is considered a “sum parameter” since all dissolved minerals in ionic form in the solution contribute to the EC value. It is difficult to maintain a well-balanced NS in closed hydroponic systems for a long time because nutrients are absorbed by the plants at different rates.

To study nutrient imbalances due to NS recycling, a balanced standard NS to cultivate lettuce in an indoor NFT system was used in this study (paper A). Standard NS was

also used as a refill solution to compensate for transpiration and to maintain a constant NS volume. Even though a constant EC was maintained throughout the experimental period, significant decreases in the concentrations of N, P and K were observed after 68 days of NS recirculation. Compared to the initial concentrations, N, P and K were reduced by 55.7, 90.5 and 96.6%, respectively. These elements are actively absorbed by plants and may also be subjected to luxury absorption when present in high concentrations, thereby creating an imbalance in the NS [31,76]. Ca and S accumulated in the recirculated NS and were increased by factors of 2.2 and 2.9, respectively. These elements are passively absorbed by plants with slow uptake rates [25].

All micronutrients experienced an increase during the experimental period, except for Mn, with a significant decrease of 73.8%.

Adding a standard NS to maintain the EC value resulted in an imbalance in nutrient content in the recirculating NS with time.

4.1.2 Time to lettuce growth limitation

Lettuce growth during the first six weeks in the NFT system was normal and stable. After six weeks, a significant decrease in lettuce yield was observed (paper A). The weight per head declined steadily to 104 g on average at the end of the growth period, which was only 44% of the weight of the first harvest. Foliar analysis revealed a K deficiency in leaf tissue, which indicated that K depletion was the main reason for the reduced growth rate and yield of lettuce. The K content in leaf tissue was only 59% of the norm at the end of the growth period. K deficiency in leaf tissue corresponded to the low K concentration found in the recirculating NS. There was a strong linear correlation between the K concentration in the NS and the weight of the lettuces, with a coefficient of determination (R^2) of 0.91.

The reduced lettuce growth towards the end of the period implies that the recirculated NS should have been discharged and replaced by a new solution after approximately 6 weeks of operation. Alternatively, a “tailor-made” refill nutrient stock solution should have been used, with a high concentration of actively absorbed macronutrients and a low concentration of passively absorbed micronutrients, to maintain nutrient balance in the recirculating NS solution, as discussed further in section 3.1.3.

In further experiments, we cultivated lettuce in an open-air rooftop closed NFT system for 6 weeks (paper B). The same seeds and NS were used for both experiments. The NS was never discarded throughout the experimental period. The results indicated that the average fresh weight of the harvested lettuce exceeded the marked size of the commercial lettuce.

Both studies showed that NS can be reused in a closed NFT system for 6 weeks without compromising the yield and quality of lettuce. The time for nutrient exchange is system specific. A lower total NS volume implies more frequent discharge and refill. In paper A, a production of approximately 1 kg lettuce per 10-L tank volume of NS was suggested before nutrient exchange was required.

4.1.3 Strategies to maintain a balanced nutrient solution during recycling

Closed hydroponic systems, where NS is never discarded, only maintained, are the ultimate aim of hydroponics. Several strategies have been proposed to overcome problems with nutrient imbalances due to NS recycling, as mentioned previously. However, the practical implications of these strategies are somewhat limited. Quantitative management of NS is another new concept for avoiding nutrient imbalance. This implies that the refill solution used to replace nutrient uptake and transpiration is made based on calculations of actual nutrient uptake rates by the plants, which are determined by the nutrient content in leaf tissue, yield and amount of water transpired [25,76]. In this case, a tailor-made NS is needed because the uptake rate and leaf tissue content vary among crops and local growth conditions.

In our study, we proposed an alternative to NS discard and replacement with new NS after 6 weeks of recycling (paper A). This alternative was to suggest a tailor-made refill solution based on the calculation of actual nutrient absorption rates in leaf tissue and the volume of the refill solution used in the growth period. The method required the calculation of the actual absorption rates of all individual nutrients during normal growth based on leaf tissue analysis at harvest. Based on the actual nutrient absorption rates in lettuce by measuring the nutrient content in leaf tissue and the volume of the refill solution used, estimation of the required strength of the alternative refill NS was possible. The alternative solution required had higher concentrations of the nutrients that were depleted in the recirculated NS (N, P, K and Mn) and lower concentrations

of those accumulated (Ca, Mg, S, B, Cu, Fe and Mo), which will supply the lettuce with nutrient amounts based on their requirements.

4.2 Nutrient reclamation from organic waste

In recent years, large amounts of waste streams have become a problem in many countries, causing serious environmental pollution. However, some waste streams can be potential sources of mineral nutrients for plant growth. Thus, converting waste streams into valuable fertilisers can not only alleviate the disposal problem but also address the future scarcity of non-renewable fertilisers and have economic benefits. Examples of waste streams rich in nutrients include vinasse, struvite, human urine and aquacultural sludge [5] .

Vinasse is the main effluent from the distillation of fermented sugarcane used to produce fuel alcohol [77]. Approximately 10 to 18 litres of vinasse are produced for each litre of fuel alcohol produced [78]. This effluent represents an environmental pollution concern if inadequately disposed of. It has been reported that vinasse is rich in nutrients and can be utilised as a base for culture media as an alternative to vinasse disposal [77].

Struvite is a white crystalline substance made of magnesium, ammonium and phosphorus in equal molar concentrations ($MgNH_4PO_4 \cdot 6H_2O$) obtained from the precipitation of phosphate-based minerals from wastewater [5,79]. Several studies have reported the use of struvite for fertilising purposes [80–82].

Human urine contains 80% of the N comprised in urban municipal wastewater and 60% of the P, K, S and other micronutrients [5]. Many technologies have been developed for the on-site treatment of urine, including alkaline dehydration [83].

Another example of a waste stream rich in nutrients is aquacultural sludge from land-based RAS. As discussed in chapter 2.3, solids (faeces and uneaten feed) are removed from the fish tanks using several techniques. The removed solids, along with the backwash water, constitute the sludge. The sludge, which is generally discarded, can hold up to 40% of the nutrients present in fish feed [84]. Therefore, they should be considered a valuable nutrient source for plants. Appropriate treatment of the aquacultural sludge should be carried out because, as mentioned earlier, on the one hand, nutrients associated with solids are not readily available for plants; on the other hand, solids can be detrimental for plants because they can stick to plant roots,

reducing oxygen and nutrient uptake by roots [85]. Conventional wastewater treatment considers both solids and nutrient contaminants and aims to remove them. However, in hydroponics, nutrient and water recycling are of interest. By using aquacultural sludge as a nutrient source, treatment must aim to solubilise minerals trapped in solids to reduce dependency on external fertilisers and enhance the sustainability of hydroponic systems.

As discussed in chapter 2.5, many techniques for nutrient solubilisation in waste streams have been explored, including biological treatment (such as AD [68,86,87], anaerobic treatment [71,87,88] and vermicomposting [89]), chemical treatment [90,91] and physical treatment (such as ultrasound treatment [92–94], hydrothermal treatment [95,96] and freeze/thaw treatment [97,98]).

However, only a few studies have investigated nutrient solubilisation in aquacultural sludge [68,87]. Our literature search found no studies on aquacultural sludge treatments that can achieve both nutrient solubilisation and solids separation by flocculation and sedimentation. In this study, we proposed a holistic method for the treatment and use of aquacultural sludge for fertilising purposes in soilless growth systems. The method includes nutrient solubilisation by AD and polishing of the nutrient-rich solution (solids reduction) using the biopolymer chitosan as the flocculant. AD is discussed in section 4.21 and solids reduction is discussed in section 4.2.2. The effect of the reclaimed NS on plant growth is discussed in section 4.3.

4.2.1 Aerobic digestion

There are limited studies in the literature on the mobilisation of nutrients from aquacultural sludge by AD [68,87].

Monsees et al. (2017) compared nutrient mobilisation during anaerobic and AD in aquacultural sludge. They obtained higher amounts of soluble nutrients, such as phosphorus (P) and potassium (K), after AD than after anaerobic treatment, while no rise in soluble N-compounds was found. Likewise, Delaide et al. (2018) showed that AD has some ability to solubilise nutrients from aquacultural sludge by allowing an increase in soluble macro- and micronutrients of 10–60%. Their published results indicated low degrees of nutrient solubilisation. These findings contrast with our results, which showed that AD of aquacultural sludge may increase the concentrations of all soluble nutrients within 21 days of incubation to concentrations close to or

exceeding the mineral levels recommended for hydroponic vegetable growth. A high degree of solubilisation was observed, especially for N and P. Mineral analysis suggested that the resulting nutrient-rich solution could be used for soilless agriculture (paper C).

Monsees et al. (2017) attributed the increase in nutrient concentration to the pH decrease, which normally occurs during AD. To test this hypothesis, in the present study, three sets of AD experiments were conducted in batch aerated reactors for three weeks. For the first and second sets, pH was maintained at 7 and 5, respectively. For the third set, the pH was not adjusted and was in the range of 8–9. Our results indicated that AD at pH 5 and 7 for 3 weeks were both able to mobilise high amounts of nutrients from aquaculture sludge. The degree of nitrogen mobilisation was similar at pH 7 and 5, with final concentrations of 890 and 885 mg L^{-1}, respectively, which corresponds to 4.9- and 5.4-fold increases, respectively.

The present finding is much higher than in earlier investigations, which reported an increase in soluble N by a factor of 2.56 after AD at a neutral pH [87], while Monsees et al. (2017) observed a decrease in soluble N from 1 to 0.1 mg L^{-1} after AD at pH 5. The highest soluble P concentration was observed at pH 5, with a final concentration of 155 mg L^{-1} which corresponded to a 19-fold increase and to approximately 90% solubilisation of the total P, which is higher than in earlier published work by Monsees et al. (2017), who reported an increase of soluble P by a factor of 3.2 at pH 5 after AD. At a neutral pH, the concentration of soluble P was 105 mg L^{-1} at the end of the treatment, which corresponded to a 12.9-fold increase and 73% solubilisation of the total P. This degree of mobilisation was also higher than in previously published work by Delaide et al. (2018), who reported an increase of soluble P by a factor of 2.18 after AD at a neutral pH.

At pH 7, the concentrations of soluble potassium (K), calcium (Ca) and magnesium (Mg) increased by factors of 1.7, 3.2 and 2.0, respectively. At pH 5, the same concentrations increased by factors of 1.5, 5.1, 2.4, and 2.0, respectively.

For AD without pH control, the concentrations of many soluble nutrients decreased after AD, probably due to precipitation.

Potassium was the macronutrient least solubilised by AD. The observed increases in soluble K by 76% (to 110 mg L^{-1}), 50% (to 94 mg L^{-1}) and 92% (to 120 mg L^{-1}) at pH 7, pH 5 and unadjusted pH, respectively, did not raise the levels above the

recommended value of 132 mg L^{-1} for a standard hydroponic NS. Half of the dissolved micronutrients (Cu, Mo, Zn) did not increase at pH 5 and remained slightly below the recommended levels. These levels are only recommended limits since most plants grow well even at lower concentrations than the recommended levels.
Once nutrients are solubilised in the water phase of the aquacultural sludge, solids need to be removed to produce a good quality, clear phase in compliance with water quality guidelines for hydroponic systems. Solids removal from the treated aquacultural sludge will be covered in the next section.

4.2.2 Solids separation from aerobically digested aquacultural sludge

After treatment to mobilise nutrients (AD), techniques to remove solids from the treated aquacultural sludge include flocculation and sedimentation. As discussed in chapter 2.4, several studies have been conducted using metal-based coagulants and synthetic organic polymers for flocculation and settling of solids in wastewater treatment [65]. The metal salts hydrolyse and combine with suspended solids to produce settleable flocs, but they also react with dissolved phosphate to produce insoluble iron phosphate or aluminium phosphate, thereby removing valuable phosphorus from the solution [99]. Al flocculants may also leave Al residuals in the clear phase, with a potentially toxic effect on plants [67]. With the intention of using the dissolved nutrients in the sludge for plant growth, alternative flocculants that do not react with soluble phosphorus should be used. In wastewater treatment, chitosan has mainly been used as a flocculant for the removal of different types of dissolved and undissolved compounds, including suspended solids, heavy metals, humic acid, dyes, algae and bacteria [65,67]. The solids removal efficiency of various synthetic cationic polymers has been evaluated for the treatment of dilute sludge in aquaculture (backwash water from microscreen filters) [100]. However, no such studies have been found in which chitosan has been used as a flocculant in digested aquacultural sludge treatment.

To remove residual particles from the clear phase of the aquacultural sludge after AD, jar tests with flocculation and sedimentation were performed. Chitosan was used at different concentrations of chitosan (15, 25 and 40 mg L^{-1}) and compared to the

performance of a synthetic cationic polyacrylamide polymer for particle removal (paper D).

Compared to the synthetic coagulant, chitosan showed better TSS and turbidity removal at lower dosages. By using a dose of 15 mg L^{-1} chitosan, reductions in TSS and turbidity by 91% and 96%, respectively, were achieved. The same dosage of the synthetic coagulant resulted in higher TSS and turbidity concentrations. Even higher dosages of the synthetic polymer (up to 40 mg L^{-1}) did not improve the treatment efficiency beyond the results of 15 mg L^{-1} of chitosan. Increasing the chitosan dose above the threshold limit did not increase treatment efficiency. The lower dosage of chitosan required compared to the synthetic polymer compensated for part of the higher cost of chitosan. However, the cost of the flocculant will not be the limiting factor in larger-scale production of organic nutrient solution from aquacultural sludge due to the moderate volumes to be treated. With a bulk marked price of chitosan of NOK 200 per kilogram, the flocculant cost will be NOK 3 per m^3 of NS with a dosage of 15 g m^{-3}.

Chitosan also exhibited better colour removal at all dosages than the synthetic polymer. A dose of 15 mg L^{-1} of chitosan allowed for a 44.5% reduction in colour. With the same dose of the synthetic coagulant, the colour was reduced by only 13%, leaving a yellowish colour in the treated water. A yellow colour in water is normally associated with dissolved organic compounds and can be difficult to completely remove using polymers. Even the highest dosage of chitosan did not achieve a better colour removal efficiency than 49.4%. For the purpose of using the treated water in hydroponic systems, this may not be a problem since the literature indicates that the presence of dissolved organic matter in the NS at low concentrations promotes plant growth and nutrient uptake and consequently leads to higher yields [86].

Chitosan did not remove the macro- or micronutrients previously dissolved by AD, except for a 39% reduction in Zn concentration and modest declines in Cu and Mo concentrations.

Another positive effect of chitosan was the removal of potentially phytotoxic metals, including Al and Cd removals of 75% and 48%, respectively. After chitosan precipitation, the concentrations of Al and Cd were reduced from 62.5 to 15.5 μg L^{-1} and from 4.2 to 2.2 μg L^{-1}, respectively, which corresponded to 75% and 48% removal efficiencies, respectively. Al is toxic to plants, even at low concentrations. It inhibits

plant cell growth and division [101]. Cd is a heavy metal with high toxicity to crops. It is easily absorbed by plant roots and alters the cellular, molecular, biochemical and physiological mechanisms of plants, thus affecting plant growth and morphology [102]. The literature does not give limits for concentrations of Al and Cd in NS for hydroponic systems, but it is argued that Al in ionic form with a +3 charge, which is considered the soluble form, is harmful to plants even at micromolar concentrations and that Cd is phytotoxic even at lower concentrations [101,102].

Chitosan's ability to remove Cd is consistent with the results reported by Bailey et al. (1999), who found that chitosan has high specificity for heavy metals of environmental concern (e.g., lead (Pb), mercury, cadmium (Cd) and chromium). Interestingly, chitosan treatment did not reduce the concentrations of metals essential for plant growth, including Mn and Fe. Such limited removal of some metals by chitosan flocculation was explained by Guibal (2004), who argued that the interaction of metal ions with dissolved chitosan did not lead to the formation of settleable flocs. The metal–chitosan complex remained in solution. He further pointed out that due to the poor removal capacity by coagulation and flocculation, chitosan is mostly used to chelate metal ions in a variety of solid forms, such as beads, flakes and membranes [103].

Other benefits of chitosan include the absence of toxicity, which is an issue because residues of synthetic cationic polymers (polyacrylamides) may have toxic effects on plants [104].

This study demonstrated that chitosan is an efficient and safe alternative for solids removal from aerobically digested aquacultural sludge. After AD and chitosan treatment, approximately 80% of the raw sludge could be reclaimed as a nutrient-rich clear phase to be supplied to recirculating hydroponic systems. The effect of reclaimed NS on plant growth in such systems is discussed in the next chapter.

4.3 Lettuce cultivation using the recovered nutrient solution

Some studies have reported the possibility of growing vegetables using organic waste, such as pig slurry, human urine and municipal sewage [105]. However, the use of waste-derived materials for agricultural purposes is risky unless their impact on plants is carefully evaluated. Aquacultural sludge may contain heavy metals, which can limit the use of sludge as a fertiliser. Heavy metals may accumulate to levels exceeding

permissible content in crops for human consumption. In RAS, heavy metals may enter with the feed, leach from pipes and fittings, or be carried into the system with the make-up water. Many authors have documented the absorption and phytotoxic effects of heavy metals on several crops [106]. In addition, organic fertilisers may inhibit plant growth due to the high biological oxygen demand in the root zone caused by the presence of dissolved organic carbon compounds [107].

After clarifying the aerobically digested aquaculture sludge with chitosan, the performance of the recovered NS on lettuce growth was assessed in an NFT hydroponic system (paper E).

4.3.1 Lettuce fresh weight, yield and nutrient content

The lettuce grown in the recovered NS had an average shoot fresh weight of 203 g, which exceeded the marked size of the commercial lettuce of 150 g. The present findings are much higher than those in earlier investigations, which reported an average shoot fresh weight of hydroponic lettuce grown in aerobically treated RAS sludge equal to 104.8 g [86]. This low fresh weight is probably due to the presence of organic particles in the NS, as the authors did not remove the solids from the NS. In fact, it is believed that suspended solids can stick on plant roots, reducing their oxygen and nutrient uptake [85].

The average fresh weight obtained in the recovered NS was 16% lower than the average weight obtained in the conventional NS. Interestingly, there was no significant difference between the yields of lettuce grown in the recovered NS and their conventional counterparts, which means that the performance of the recovered NS regarding total yield was very close to the performance of conventional NS for hydroponic lettuce growth.

The visual quality of lettuce produced in the recovered NS was not inferior to that produced in the conventional NS.

The lettuce grown in the recovered NS had significantly higher concentrations of P, K, Ca, S and B, and significantly lower concentrations of Mg and Mo compared to the lettuce grown in the conventional NS. These differences were also reflected in the NS concentrations. The recovered NS was higher in concentration for all these elements, except for S and B. In particular, the average concentrations of K and Ca in leaves of lettuce grown in the recovered NS were 2.5 and 2.0 times higher than those

grown in the conventional solution, respectively. The low Mg content in lettuce grown in the recovered NS can also be explained by the high concentrations of K and Ca. As reported by Senbayram et al. (2015), Ca and K in excess may interfere with Mg uptake, which is known as nutrient antagonism [108]. There were no significant differences in N, Cu, Mn, Zn and Fe concentrations in lettuce leaves from the two NS treatments.

4.3.2 Heavy metal content in lettuce leaves

Heavy metal analysis (Pb, Ni, Zn, Cu and Cd) for leaf tissue samples collected from the harvested hydroponic lettuce grown with the aerobically digested sludge was carried out. The content of the heavy metals Pb, Ni, Zn and Cu in the leaves of lettuce grown in the recovered NS were all below the permissible limit for human consumption [109], except for Cd, which was slightly above the maximum concentration by 0.03 mg $kg^{-1}_{dry\ weight}$. Cd is a heavy metal with high toxicity to crops. It is easily absorbed by plant roots and alters the cellular, molecular, biochemical and physiological mechanisms of plants, thus affecting plant growth and morphology [102]. The literature does not give limits to the concentration of Cd in NS for hydroponic systems, but it is argued that Cd is phytotoxic at even low concentrations [101,102].

The contents of lead and zinc were both close to the maximum allowable concentrations for human consumption.

The concentration of heavy metals in the NS should be reduced to safely use the recovered NS for lettuce growth. In our case, it might be possible to reduce the heavy metal concentration in the NS by increasing the dose of chitosan in the solids separation step, as chitosan is considered to be a good metal chelator that did not reduce the concentrations of metals essential for plant growth, as discussed earlier. In fact, for the present study, we applied the lowest chitosan concentration (15 mg L^{-1}) tested for solids precipitation. Another strategy to reduce the heavy metal in the recovered NS is to choose fish feed with a low metal content, as in RAS, heavy metals may enter the fish feed. Additionally, different water treatment methods could be applied. In the literature, different heavy metal removal techniques have been reported, including adsorption-, membrane-, chemical-, electric- and photocatalytic-based treatments [110].

This study demonstrated that recovered NS could replace chemical fertilisers in hydroponic systems. The treated aquacultural sludge proved to be a good source of organic fertiliser that allowed the successful growth of lettuce in the NFT system with a yield comparable to the yield of lettuce grown in conventional NS.

Chapter 5

Summary and outlook

This dissertation addresses the dependence of soilless plant cultures on non-renewable fertilisers by proposing two approaches: extending the lifetime of the applied NS and using an alternative NS recovered from aquaculture sludge. Therefore, this research aimed to develop a more sustainable soilless plant culture system that directly contributes to the second development goal of the United Nations (UN SDG 2 zero hunger) and targets, in particular, UN SDG 2.4 (sustainability of food production systems), which states that "By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters" [111].

In this chapter, the major contributions of this dissertation are summarised in section 5.1. Furthermore, future research directions are discussed in section 5.2.

5.1 Conclusions

The major conclusions and contributions of this dissertation related to the first approach are summarised as follows:

- The NS was reused successfully in a closed NFT system with constant EC for six weeks, corresponding to a production of 1 kg lettuce per 10 litres tank volume of NS without compromising the yield and quality of lettuce.
- A longer period of continuous reuse of NS than six weeks resulted in lettuce growth reduction and depletion of some nutrients, including N, P, K and Mn, and accumulation of others, such as Ca, Mg, S and Cu.
- A strategy for managing the recycling of NS in hydroponic systems was proposed. This strategy included the use of a refill solution with the required concentrations of individual nutrients based on actual nutrient absorption rates in lettuce by measuring the nutrient content in leaf tissue.

In the second approach, a holistic method was developed for the treatment and use of aquacultural sludge as NS for fertilising purposes in soilless growth systems. This method includes two steps: nutrient mobilisation from aquacultural sludge using AD followed by solids precipitation using the biopolymer chitosan as the flocculant.

From the second approach, the following contributions and conclusions can be drawn:

- Macronutrients and micronutrients were mobilised by AD from aquacultural sludge. Most of the mobilised nutrients in the recovered solution were close to or exceeded the required mineral concentrations recommended for soilless growth solutions.
- AD at pH 5 and 7 for 3 weeks were both able to mobilise high amounts of nutrients from aquaculture sludge. The degrees of mobilisation of total N at pH 7 and 5 were both equal to 90%. Phosphorus mobilisation reached 90% and 73% at pH 5 and pH 7, respectively.
- The biopolymer chitosan was an efficient and safe alternative for solids removal from aerobically digested aquacultural sludge. Chitosan showed better TSS, turbidity removal and colour removal compared to a synthetic polymer. The nutrients previously dissolved by AD were not removed by chitosan, except for modest declines in Zn, Cu and Mo concentrations. Chitosan was also able to remove some phytotoxic metals, including Al and Cd.
- After AD and solids separation, approximately 80% of the aquaculture sludge was reclaimed as a clear NS.
- The recovered NS was successfully used for lettuce production in an NFT hydroponic system. The yield of lettuce grown with recovered NS was comparable to the yield of lettuce grown with conventional NS. Most lettuce heads exceeded the marked size of commercial lettuce of 150 g after the growth period.
- Foliar analysis for heavy metal content in lettuce leaves grown with the recovered NS revealed that some heavy metals were close to the maximum permissible concentrations in lettuce for human consumption. As heavy metals may enter fish feed, choosing feed with a low metal content could reduce the concentration of heavy metals in recovered NS.

5.2 Limitations and future work

- This dissertation demonstrates that AD is an efficient method to mobilise nutrients in the aquacultural sludge to concentrations close to or exceeding mineral levels recommended for soilless growth systems and shows that lettuce grown in the recovered clear phase from aerobically digested aquacultural sludge reached or exceeded the marked size of commercial lettuce. However, it should be pointed out that the extent of nutrient mobilisation and the value of lettuce yields are specific for this work, as the degree of nutrient mobilisation depends on the total amount of nutrients in the raw aquacultural sludge, which in turn vary from one facility to another depending on several factors, including fish species, fish density, fish age, fish feed, feeding management, flow regulation and water treatment (including solids separation technology and sludge handling).
- Research on optimised fish feed needs to be addressed for better nutrient mobilisation from aquacultural sludge and for the limitation of heavy metal leakage to the water phase.
- Variations and even contrasting results in nutrient mobilisation found in the literature indicate the need for further research to optimise nutrient recovery by, for example, applying a metagenomics approach to identify microbial communities and their role in mobilisation processes.
- This work does not include microbial analysis or studies of potential human pathogenic microorganisms in recovered NS. The risk of transmitting pathogenic microorganisms from the NS to the lettuce was considered very low since cold water fish are not transmitters of human pathogens.
- In this work, the performance of the recovered NS was assessed only on lettuce growth in an NFT hydroponic system. The work can be extended to the evaluation of recovered NS in other soilless culture systems, such as aquaponics, since aquaponic water may be limited to some nutrients required for optimised plant growth. Ca, K and Fe are the most limited nutrients in aquaponic water. Therefore, external nutrition is often required, especially for decoupled aquaponics, the dominant aquaponic design in Europe, which relies on up to 50% of external nutrient supplementation [2].
- Although soilless culture has improved considerably over recent years, there are still topics that require more research to exploit the maximum potential of these

systems, including, but not limited to, nutrient cycling, other nutrient mobilisation techniques and development of energy efficient systems.

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Part II

Appended papers

Paper A:

Nutrient solution dynamics and yield of lettuce (Lactuca sativa) in an EC-controlled recirculating hydroponic system

This paper has been published as:

Ezziddine, M.; Liltved, H.; Seljasen, R. Nutrient solution dynamics and yield of lettuce (*Lactuca sativa*) in an EC-controlled recirculating hydroponic system. *Acta Hortic.* **2021**, *1305*, 407–414, doi:10.17660/ActaHortic.2021.1305.53.

Nutrient solution dynamics and yield of lettuce (Lactuca sativa) in an EC-controlled recirculating hydroponic system

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Abstract

In this study, the nutrient dynamic and growth performance of lettuce in a closed recirculating hydroponic system were investigated. Lettuce was grown in three parallel nutrient film technique (NFT) units, illuminated with LED-light. A balanced standard nutrient solution (NS) was used, and the electrical conductivity (EC) and pH were adjusted regularly to constant average values of 1.16 mS cm^{-1} and 6.2 with standard deviations of ±0.12 and ±0.5, respectively. The volume of NS in each unit was kept at 20 L by adding refill solution to replace nutrient uptake and transpiration. Lettuce growth during the first six weeks in the NFT-system was normal and stable. After six weeks, a decrease in concentrations of N, P, and K was observed, with a corresponding decline in yield of lettuce. After ten weeks, lettuce weight at harvest was reduced by 56% in average compared to the control, and the concentrations of N, P and K in the NS were reduced by 54.5, 90.5 and 96.6%, respectively. Contrarily, more slowly absorbed nutrients like Ca, S, Zn, Cu, and B experienced increases by factors of 2.2, 2.9, 6.6, 4.9 and 2.5, respectively. The depletion and accumulation of nutrients in the NS were reflected in corresponding deficiency and excess levels of nutrients in leaf tissue compared to norm-values of healthy lettuce. The study showed that after six weeks, corresponding to a yield of 1 kg lettuce per 10 L tank volume of NS, the reduced growth implied that the recirculated NS should have been discharged and replaced, or a "tailor-made" refill solution should have been used to avoid depletion of some nutrients. Based on the foliar analysis and calculations of actual nutrient absorption rates, the composition of such a refill NS was suggested.

Keywords: NFT-system, recirculation, EC-control, foliar analysis, nutrient management

Introduction

Today, several leafy vegetables, including lettuce, can easily be grown in indoor and outdoor hydroponic systems. Due to several advantages over conventional farming practise, including better nutrient and water utilization, hydroponic systems can contribute to increased food production worldwide with a lower environmental impact. However, there are several possibilities to further improve the nutrient and water efficiency by increasing the lifetime of nutrient solution (NS), which is of utmost importance, since nutrients, such as phosphorus and some micronutrients, are becoming scarce resources (Chowdhury et al., 2017). Among hydroponic systems, we distinguish between open and closed cultivation systems. The closed systems utilize water and nutrient more efficiently than open systems, with substantial less discharge to the environment, thereby reduced environmental and economic costs (Bugbee, 2004). A frequently applied system is the nutrient film technique (NFT) system (Son et al., 2016).

Usually, the concentration of nutrients in the recirculating NS is controlled by electrical conductivity (EC) which is a sum parameter for all dissolved minerals in ionic form in the solution. It is difficult to keep a well-balanced NS in closed hydroponic systems for long time, because nutrients are absorbed by the plants at different rates. The actively absorbed nutrients like N, P, K, and Mn will be taken up much faster than the intermediate and passively absorbed nutrients with slower uptake rates (Tsukagoshi and Shinohara, 2016). If new NS is regularly supplied to replace transpiration, the differences in uptake rates will result in an imbalance in the recirculating NS with time, even if the EC-value is kept at a constant level. Some nutrients will accumulate, while other will be depleted. Frequently drainage and refill of new NS (one to two weeks intervals) will solve the problem, but not the cost and environmental challenges. To overcome problems with nutrient imbalance, several strategies have been proposed to determine and control nutrient requirements, including automated monitoring of nutrient concentration by ion-selective electrodes (Kim et al., 2013; Cho et al., 2018) and development of mathematical models,

software and automated systems (Domingues et al., 2012; Kozai et al., 2018). However, the practical implication of these strategies is somewhat limited. Ion-selective electrodes for monitoring are still expensive, and need skills and time for calibration and operation (Son et al., 2016). The mathematical models need a lot of input parameters and need to be adapted to the current crop in order to work (Signore et al., 2016). Another novel concept to avoid nutrient imbalance is quantitative management of the NS. This implies that refill solution is made based on calculations of actual nutrient uptake rates by the plants. Uptake rates are determined by nutrient content in leaf tissue, yield, and amount of water transpired (Signore et al., 2016; Tsukagoshi and Shinohara, 2016). However, the uptake rate and leaf tissue content will vary among crops and local growth conditions, and "tailor-made" nutrient solutions are therefore needed.

In this study, we investigated the nutrient dynamic and growth performance of lettuce (Lactuca sativa L.) during a 68 days growth period from September to December 2018 in an EC-controlled closed LED-illuminated hydroponic system, without discharge of NS. Refill solution was added regularly to maintain a constant volume, and EC- and pH-values were adjusted to constant values. The aim of the study was to highlight the accumulation and depletion of individual nutrients in the recirculating NS, determine the time for growth limitation, and determine the limiting nutrients by NS and foliar analysis. Based on the actual nutrient requirements by foliar analysis, the composition of a "tailor-made" refill solution was suggested.

Materials and methods

The growth systems

The experiment was carried out in a growth room located at the University of Agder in Norway, during a 68 days period from 27th of September to the 4th of December 2018. The growth systems consisted of a seeding system and a closed grow-out nutrient film technique (NFT) system. In the seeding system, seeds of Lactuca sativa L. (Batavia-type, 'Partition') from the seed company LOG AS, Norway, were seeded in Grodan rockwool cubes (36×36×40 mm) and inserted in net pots. The cubes in net pots were placed in trays with NS and illuminated with LED-light with a wavelength peak in the blue (445 nm) spectral ranges, with a total photon flux density of 210 μmol

m^{-2} s^{-1}. After 2 weeks in the seeding system, the seedlings were transplanted to the NFT-system for additional 4 weeks growing before harvesting.

The NFT-system included three identical parallel units for triplicate experiments. Each unit consisted of a 2400 mm long rectangular PVC-pipe with dimensions 100 mm width and 50 mm height, and a 20-L nutrient tank with a submerged pump to circulate the NS to the 12 plants in each unit. The 45 mm holes for plants in net pots were separated by 200 mm. NS was supplied intermittently by the pump and a timer (30 min on/off cycles). The flow rate was 3.5 L min^{-1}. LED-lamps with wavelength peaks in the blue (445 nm) and red (660 nm) spectral ranges were suspended over the pipes producing photosynthetically active radiation (PAR) with a flux density of 220 μmol m^{-2} s^{-1}. By illumination of 18 h day-1, the total daily illuminating dose was about 15 mol m^{-2} d^{-1}. The temperature of the growth room was kept in the range of 22-24°C, CO2 concentration at 410-450 ppm, and the relative humidity at 35-40%.

Each of the three parallel units of the NFT-system was operated in a continuous production mode during the experimental period. At the starting date (27th of September 2018), 9 seedlings (2 weeks old) were collected from the seedling system and transferred to the NFT-system (3 seedlings to each of the three units). After 4 weeks, the NFT-system was fully stocked, and the first 3 lettuce heads of the total of 12 lettuces in each unit were harvested and replaced by 3 new seedlings (2 weeks old). During the 68 days experimental period from 27th September to 4th December, there were 7 harvests, with a total production of 21 lettuce heads from each unit, which gave a total of 63 lettuce heads from the 3 parallel units of the NFT-system.

Nutrient solution

The NS used in the NFT-system was prepared from two commercial stock solutions, Nutri-A and Nutri-B, from Panponic Biosystems AS, Norway. The profile of the applied NS at the start of the experimental period is shown in Table 1. Every second day throughout the experimental period, pH- and EC-values were adjusted to maintain a pH value of approximately 6 and an EC value of approximately 1.1 mS cm^{-1} by adding new NS to compensate for nutrient uptake and transpiration losses, up to the initial volume of 20 L. A diluted nitric acid solution was used to adjust the pH. NS was never discarded throughout the experimental period.

Table 1. Soluble nutrient content of the applied NS in this study and suggestion for composition of an alternative refill-solution calculated from the actual nutrient uptake in healthy leaves in this study.

	Soluble concentration (mg L^{-1})											
	N[a]	P	K	Ca	Mg	S	Zn	B	Cu	Fe	Mn	Mo
Applied NS	111	23	140	94	23	23	0.26	0.19	0.07	1.7	0.42	0.04
Alternative refill NS	262	32	247	74	17	13	0.25	0.11	0.03	0.6	0.71	0.01

[a]N – 96% of the nitrogen in the applied NS was as NO_3-N, only 4% as NH_4-N.

Physical and chemical analysis

At every harvest, the fresh weights of all the lettuces were measured. After 40 days (the 6th of November) and after 68 days (end of the experimental period), leaf tissue from the harvested lettuces of the three parallel units were collected and analyzed for macro- and micronutrient content by the Eurofins laboratory, Norway. Leaf tissue was collected from different parts of the lettuces to avoid biases due to uneven nutrient distribution within the plants. The nutrients were dissolved by nitric acid microwave extraction and analyzed by inductively coupled plasma atomic emission spectrometry (ICP-OES) according to European Standards (DIN EN ISO 11885).

The pH value and the EC of the nutrient solution in the three units were monitored before and after adjustment every second day by using a calibrated Jenway 3150 instrument and a calibrated Hach HQ40d instrument, respectively. Samples of the NS for macro- and micronutrient analysis were collected from the three parallel units at day 0, after 34 days, and after 68 days (end of the experimental period) and sent to the LMI laboratory, Sweden, for analysis by ICP-OES according to European Standards. Before analysis, the samples were filtered through Whatman GF/C membrane filters with pore openings of 0.45 μm.

Statistics

Mean values and standard deviations of individual nutrient concentrations (n=3), leaf tissue contents (n=3) and lettuce weights (n=9) are presented on the graphs. Where standard deviation bars are not observable, they do not extend beyond the dimensions of the symbols. Statistical differences in means of individual nutrient concentrations between dates during the experimental period, and differences in lettuce weights, were tested using one-way analysis of variance (ANOVA). All tests were performed using a significance level of $P<0.05$.

Results and discussion

Nutrient dynamic of the recycled solution

The chemical composition of the applied NS at the beginning of the growth period of the NFT-system is shown in Table 1. The measured mean pH value of the NS of the three hydroponic units throughout the experimental period was 6.2 with a standard deviation of±0.5. Adjustment of the pH with diluted nitric acid was required because the pH-values tended to increase during the period. As pointed out by other researchers, the ratio of NH_4^+ to NO_3^- in the NS is an important factor regarding pH development in hydroponic systems (Libia and Gómez-Merino, 2012). pH increase will prevail where nitrate is the dominating nitrogen compound, which was the case for the NS used in this study, where 96% of the N-content was present as NO_3^--N. The EC of the recycled NS varied to some extent during the growth period as a result of nutrient uptake in plants and supplementation of refill solution. The measured mean value of the three units throughout the experimental period was 1.16 mS cm^{-1} with a standard deviation of ±0.12.

The fate of macro- and micronutrients during the growth period is shown in Figures 1 and 2. As indicated, the variations among individual nutrients were pronounced, even though the EC values in the three units were maintained at approximately constant levels (mean of 1.16 mS cm^{-1}) throughout the period. Significant decreases ($P<0.05$) in the concentration of NO_3-N, P, and K were observed to final concentrations of 47.4, 2.2 and 4.7 mg L^{-1}, respectively, after 68 days (Figure 1b). Compared to the initial concentrations, NO_3-N, P and K were reduced by 55.7, 90.5, and 96.6%, respectively. As indicated, and also pointed out by other authors, these elements are rapidly absorbed by the plants, and may also be subjected to luxury absorption when present in high concentrations, thereby creating an imbalance in the NS (Bugbee, 2004; Signore et al., 2016). The decreases in concentration of these elements were observed. On the other hand, macronutrients with passive uptake experienced accumulations in the recirculated NS, e.g. Ca and S which increased by factors of 2.2 and 2.9, to concentrations of 207.3 and 106.7 mg L^{-1}, respectively (Figure 1a).

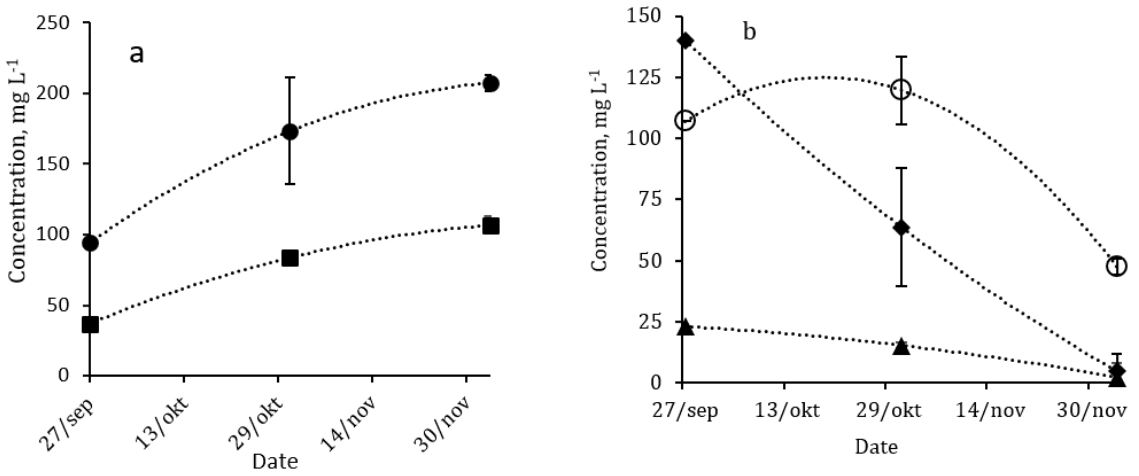

Figure 1. Concentrations of macronutrient in the recirculating solution from the start (27th of September) to the end (4th of December) of the 68 days growth period. a) ●; Ca, ■; S. b) ◯; NO3-N, ◆; K, ▲; P.

Among the micronutrients, increased NS concentrations were recorded for Si, Fe, Zn, and Cu (Figure 2). Si was the element with the highest gain, with a factor of 5.5, to a final concentration of 6.1 mg L^{-1}. Zn and Cu increased with time by factors of 2.2 and 2.9. The only micronutrient with a significant decrease was Mn which was reduced by 73.8%, to a final concentration of 0.11 mg L^{-1}. As expected, the data show that an imbalance in concentrations in the recirculated NS developed over time when refill solution was added to replace nutrient uptake and transpiration, to a constant EC-value and constant tank volume of 20 L.

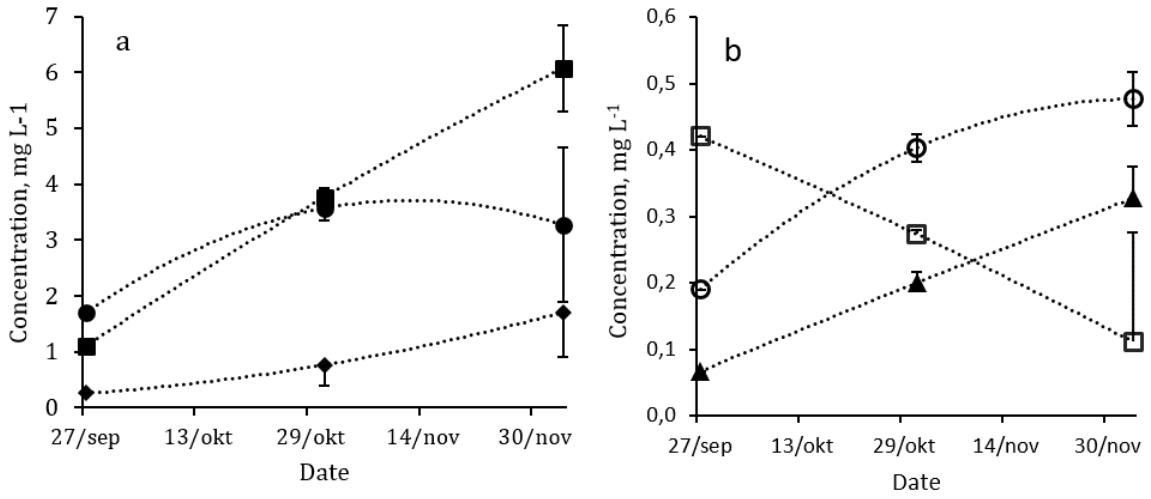

Figure 2. Concentrations of micronutrient in the recirculating solution from the start (27th of September) to the end (4th of December) of the 68 days growth period. a) ■; Si, ●; Fe, ◆; Zn. b) ◯; B, □; Mn, ▲; Cu.

Nutrients content in lettuce leaves

Leaf macro- and micronutrient concentrations in harvested lettuce after 40 days (on the 6th of November) and at the end of the growth period of 68 days (on the 4th December) compared to the norm of leaf tissue concentrations in healthy Lactuca sativa L. are shown in Figures 3 and 4 (Hartz et al., 2007). It should be noted that the leaf content of some nutrient, especially K, Ca, and Mg, may differ considerable between studies dependent on local environmental conditions (Hartz et al. 2007). The foliar analysis of the macronutrients shows that the elements with the highest accumulation in leaf tissue were N, followed by K, Ca, P, Mg and S (Figure 3), which is a composition in lettuce leaf tissue also experienced by others (Zhang, 2016), but deviates from the norm-values of Hartz et al. (2007) by a higher N-value than K-value. Generally, plants require more N and K than other elements.

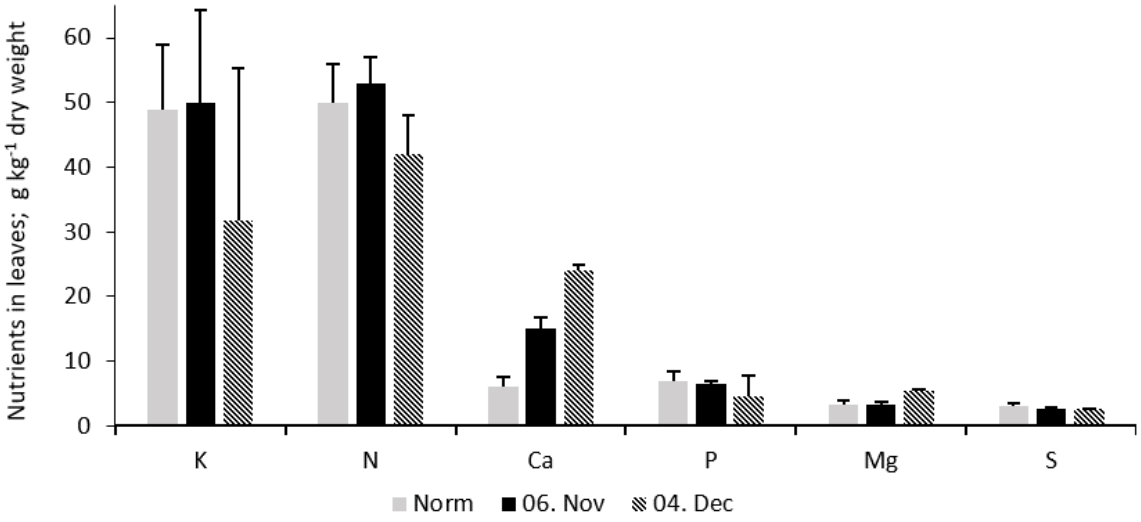

Figure 3. Leaf macronutrient concentrations in harvested lettuce on the 6th November (40 days after start-up) and on the 4th December (end of the 68 days growth period) compared to the norm of leaf tissue content in healthy *Lactuca sativa* L. (Hartz et al., 2007).

The depletion and accumulation of various elements in the NS as shown in Figures 1 and 2 were reflected in leaf tissue content (Figures 3 and 4). The two macronutrients that deviated most from the norm by Hartz et al. (2007) were K and Ca, which also were pointed out as the nutrients with highest variations between studies. The leaf tissue content of K was only 59% of the norm at the end of the growth period (4th December), while Ca content was 2.8 times higher than the normal value (Figure 3). The deficiency of K in leaf tissue corresponded to the low concentration of K found in the recirculating NS, while the elevated Ca content in the leaves corresponded to the elevated Ca concentration in the solution. This suggests that foliar analysis can be an indicator of the availability of nutrients in the recirculated NS, and that absorption to some extent is proportional to the concentration of nutrient in the recirculated NS, as also pointed out by Domingues et al. (2012). However, among the other macronutrients, there were no large deviation from the norm-values, even for

elements low in nutrient concentration in the recirculated solution, e.g. N and P. The sufficient N and P levels in leaf tissue indicate that plants may utilize low nutrient solution concentrations, here 47.4 mg L^{-1} as NO_3-N and 2.2 mg L^{-1} P at the end of the growth period, and that they also have the ability to store nutrients in roots and stems and remobilise them when required to avoid leaf deficiencies (Bugbee, 2004).

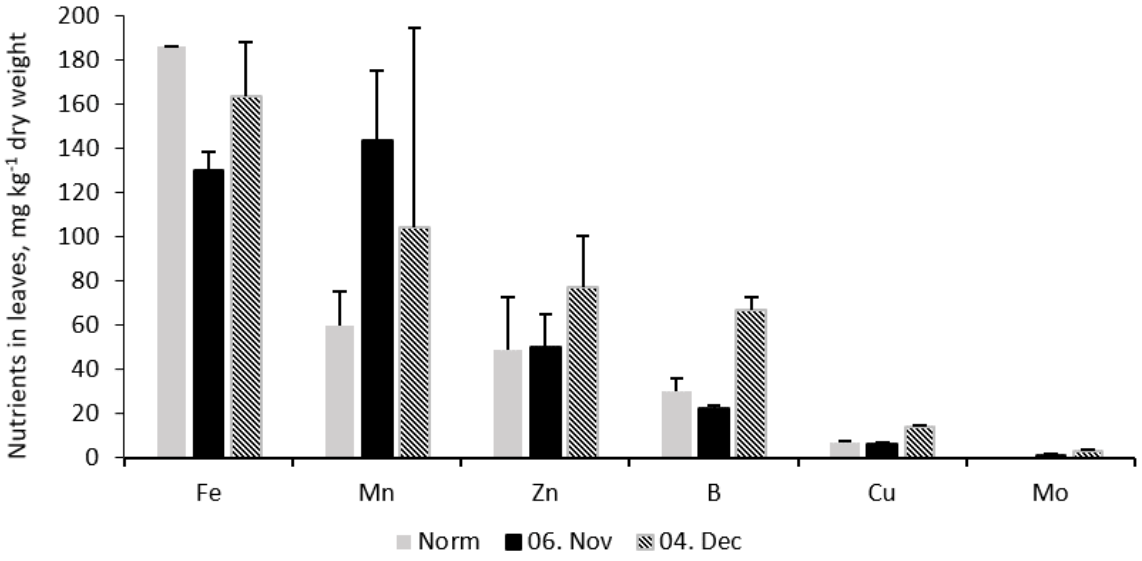

Figure 4. Leaf micronutrient concentrations in harvested lettuce on the 6th November (40 days after start-up) and on the 4th December (end of the 68 days growth period) compared to the norm of leaf tissue content in healthy *Lactuca sativa* L. (Hartz et al., 2007).

Of the micronutrient, Fe and Mn showed the highest accumulation in leaf tissue (Figure 4). Compared to the norm-value, the Fe content was low at the 6th of November, but increased toward the end of the growth period, which also corresponded to the observed increase of Fe in the NS (Figure 2a). Mn was 2.4 times higher than the norm-value on the 6th of November but was reduced to 1.7 times the norm-value after 68 days (end of the growth period), in accordance with decreased Mn concentration in solution (Figure 2b). Zn, B and Cu all accumulated in the NS, to concentrations 2.6, 2.2, and 2.1 times the norm-values after 68 days, respectively, which again was reflected in high nutrient concentrations in solution (Figure 2).

Lettuce growth and yield

The first harvest of lettuce was conducted on 25th of October, 4 weeks after the start-up of the NFT-system (27th of September). The average fresh weights of lettuce per head from the NFT-system (three parallel units) are shown in Figure 5. As indicated, the weights were more or less stable during the first three harvests (235-250 g per head). After the third harvest on the 6th of November (6 weeks after the start of the NFT-system), the weight per head declined steadily to 104 g in average at the end of the growth period (4th of December), which was only 44% of the weight of the first harvest and significantly lower ($P<0.05$). The reduced growth toward the end of the growth period corresponded to the depletion of macronutrients like K, P and N in the recirculated NS. Since there were no pronounced deficiencies of N and P in leaf tissue, the results may suggest that the deficiency of K in the NS toward the end of the growth period, with the resulting deficiency of K in leaf tissue, was the main reason for the reduced growth rate and yield of lettuce as shown in Figure 5. There was a strong linear correlation between the concentrations of K in the NS and weight of lettuces, with a coefficient of determination (R2) of 0.91. Accumulation of elements to toxic levels can be a problem in closed systems, but the elevated level of e.g. Ca in our study was probably not growth limiting. Ca is regarded as nontoxic, even at high tissue concentration (Bugbee, 2004).

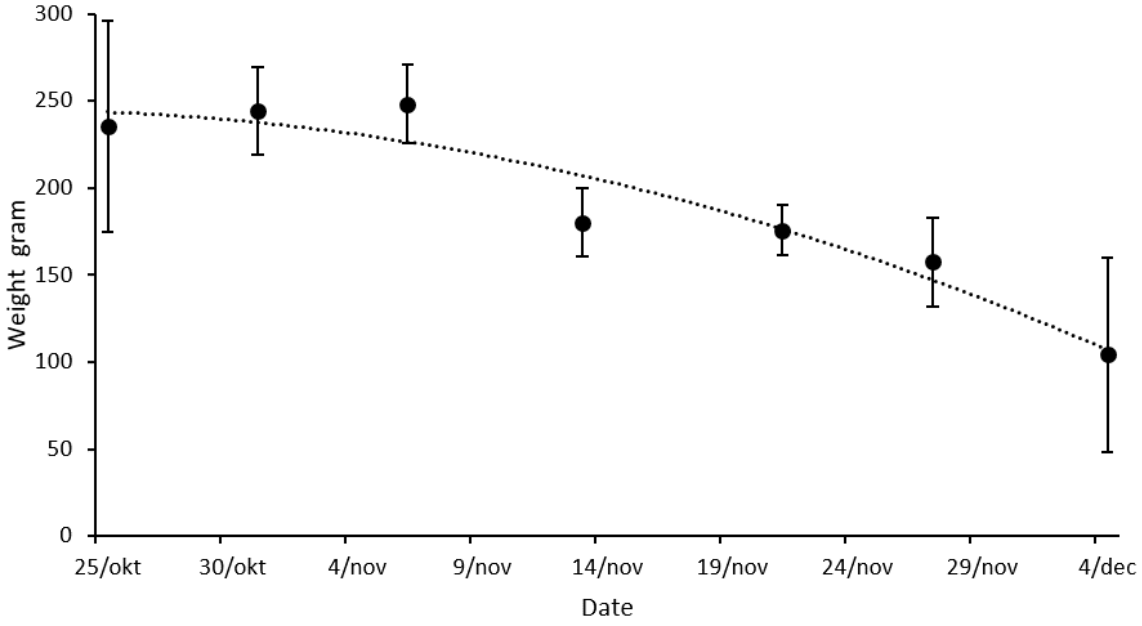

Figure 5. The average fresh weights of 6 week grown lettuces per head from the three parallel NFT-systems during the growth period from 27th September (first harvest on 25th October) to 4th of December.

The reduced lettuce growth toward the end of the period implies that the recirculated NS should have been discharged and replaced by new solution after approximately 6 weeks of operation, or a better balanced refill solution should have been applied during the growth period to avoid depletion of some elements. Bugbee (2004) suggested to use 1/3 strength of Hoagland solution for refilling. This may reduce or eliminate accumulation of elements like Ca and S but will not solve the problem with depletion of actively absorbed elements. Another strategy is to calculate the actual uptake of elements based on the leaf nutrient content per kg of plant dry weight and the amount of water used for transpiration, and then calculate the concentration of the refill solution (Bugbee, 2004; Signore et al., 2016). In our study, a total of 56 L of NS was refilled to the three parallel units of the NFT-system to replace transpiration during the period of high productivity (27th September to 6th of November). During this 6-week period with high yield, there were three harvests (9 lettuce heads per harvest, a total of 27 lettuce heads) with a total fresh weight of 6549 g with an average dry matter (DM) content of 4.2%. Based on the nutrient content in leaf tissue on the 6th of November shown in Figures 3 and 4 and the volume of refill solution applied during the period (56 L), the calculation of actual nutrient absorption rates were conducted, and the required strength of an alternative refill NS was estimated as shown in Table 1. The alternative solution shows higher concentrations of the nutrients which were depleted in the recirculated NS (N, P, K, and Mn), and lower concentrations of those accumulated (Ca, Mg, S, B, Cu, Fe, and Mo), which will supply the lettuce with nutrient amounts according to the requirements.

If discharge and refill of NS should be the strategy to avoid nutrient imbalance, the frequency will be determined by the ratio of the NS tank volume to plant growth rate. Lower volume of the recirculated NS implies more frequent discharge and refill. In our study, with a total production of 6549 g lettuce during the period of high yield (from 27th September to 6th of November), the 20 L of NS in each unit (60 L NS of the total system) should have been replaced after a fresh weight lettuce production of approximately 100 g per liters of NS, or 1 kg lettuce per 10-L tank volume of NS.

Conclusions

This study shows that NS can be reused in a closed NFT-system for several weeks without compromising yield and quality of lettuce. The only input to the system was refill of a standard nutrient solution to compensate for nutrient uptake and transpiration, while EC and pH values were kept constant. As expected, long time continuous reuse resulted in growth reduction and depletion of some nutrients and accumulation of others. After six weeks, corresponding to a yield of 1 kg lettuce per 10 L tank volume of NS, P and K concentrations in the NS were reduced to 2.2 and 4.7 mg L^{-1}, respectively. More slowly absorbed nutrients accumulated in the recirculated NS. The depletion and accumulation of nutrients in the solution were reflected in deficiency and excess levels in leaf tissue compared to norm-values of healthy lettuce. Strategies for management of recycled NS need to be implemented to avoid nutritional problems. Based on the foliar analysis and calculations of actual nutrient absorption rates in healthy lettuce, an alternative refill NS was introduced, with higher concentrations of the nutrients which were depleted (N, P, K, and Mn), and lower concentrations of those accumulated (Ca, Mg, S, B, Cu, Fe, and Mo), compared to the applied NS.

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Paper B:

Quality and Yield of Lettuce in an Open-Air Rooftop Hydroponic System

This paper has been published as:

Ezziddine, M.; Liltved, H. Quality and yield of lettuce in an open-air rooftop hydroponic system. *Agronomy* **2021**, *11*, 1-10, doi:10.3390/agronomy11122586.

Quality and Yield of Lettuce in an Open-Air Rooftop Hydroponic System

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Abstract

In this study, the yield and growth performance of lettuce in an open-air rooftop hydroponic system were investigated. Lettuce was grown in a closed recirculating nutrient film technique (NFT) unit using a standard nutrient solution (NS). Yield, fresh weight, and nutrient content in the leaf tissue of the harvested lettuce were measured. The results were compared with the results obtained in indoor hydroponic lettuce growth with artificial lightning. Despite strong winds during the growth period, 25% of the total lettuce heads weighed twice the marketable weight; however, 25% of the total lettuce heads were below the marketable weight. A more efficient nutrient uptake was indicated by the lettuces in the rooftop system compared with the uptake in the indoor system. Foliar analysis revealed a higher content of all nutrients in the leaves of rooftop hydroponic lettuce compared with indoor hydroponic lettuce. This study suggests that hydroponic rooftop-grown lettuce can be competitive with their indoor counterparts if the rooftop hydroponic system is protected from extreme weather conditions.

Keywords: rooftop hydroponic; nutrient film technique; indoor hydroponic

Introduction

Urban farming may tackle many challenges that conventional agriculture is facing, including loss of arable land, water and nutrients depletion, fast population growth, soil contamination, rapid urbanization, and climate change [1]. One type of urban farming is rooftop farming, which is the practice of growing edible or ornamental plants on top of commercial, residential, and industrial buildings [2]. The majority of the roof space in most buildings is vacant. If this space is used for agriculture, it could meet up to 77% of inhabitants' vegetable requirements [3]. Liu et al. reported that [4] they grew seven leafy vegetables in rooftop hydroponic systems and reported that rooftop hydroponic vegetables were less contaminated and were competitive in quality and cost compared with farm-grown leafy vegetables. Another study reported that 32% of the needed fresh produce in Cleveland USA could be satisfied if 62% of industrial and commercial rooftops were used to grow plants [5].

Rooftop farming could also alleviate environmental pollution by reducing the carbon dioxide in the atmosphere and helping clear the air of smog and dust [4]. Further environmental benefits of rooftop farming include water runoff management, reduction in the urban heat island effect, biodiversity conservation, and reduction in noise pollution [6]. Contrary to indoor urban farming, which relies on artificial lightning, rooftop farming can be cost effective because it uses only sunlight. Despite the advantages of rooftop farming and worldwide interest, commercial rooftop farms are very limited and most of extant roof farms are social–educational farms [7]. One reason for this could be the challenges that roof farming may face, including amplified climate conditions that can occur on rooftops, such as high wind speed, heavy rains, and extreme temperatures [6]. In addition, roofs are not designed, generally, for urban farming, which poses many obstacles that must be overcome before rooftop farming can spread more widely, such as roof weight limitations and installation and maintenance costs [8]. Another reason could be the lack of assessment studies on the practicality of rooftop farming compared with conventional or indoor urban farming. Recently, the number of articles on rooftop farming has increased [8–31]. However, our literature search found very few research articles comparing rooftop grown vegetables against their rural farm or greenhouse counterparts [4,31]. Liu et al. [4] reported that leafy vegetables grown in rooftop hydroponic systems, including lettuce, mustard, caraway, and Chinese flowering cabbage, can be as productive in yield and

quality as those sold by local farms. No comparative studies between rooftop hydroponic and indoor hydroponic vegetables were found. Therefore, the aim of this study was to investigate hydroponic lettuce grown in a rooftop system in Norway. The performance of the rooftop hydroponic system was assessed by cultivation experiments, where the fresh weight of the lettuce, the yield, and the nutrients in the leaf tissue were measured. The results were compared to results obtained in an indoor hydroponic system with artificial lightning.

Materials and methods

Indoor Hydroponic Lettuce Growth

Indoor hydroponic lettuce growth was performed in a growth room located at the University of Agder in Norway from 27 September to 4 December 2018. The growth systems consisted of a seeding system and a nutrient film technique (NFT) system. In the seeding system, seeds of Lactuca sativa L. (Batavia-type, cv. 'Partition') from LOG AS, Norway were seeded in Grodan rockwool cubes (36 × 36 × 40 mm), placed in trays with NS, and illuminated with LED light. The seedlings were inserted into net pots and transplanted to the NFT system after 2 weeks in the seeding system. The NFT system included three identical parallel units designed for conducting triplicate experiments under identical conditions. Each unit hosted 12 lettuces in a cloosed loop system, which consisted of a rectangular PVC pipe and a 20 L nutrient tank. The PVC pipe was 2400 mm long and 100 mm wide and had 12 holes with diameters of 45 mm for net pots. The nutrient tank had a submerged pump, which intermittently supplied the PVC pipe (30 min on/off cycles) with NS at a flow rate of 3.5 L min^{-1}.

LED lamps were suspended over the PVC pipes, producing photosynthetically active radiation (PAR) with a flux density of 220 µmol m^{-2} s^{-1} for 18 h per day. The temperature of the growth room was in the range of 22–24 °C, the CO2 concentration was in the range 410–450 ppm, and the relative humidity was 35–40%.

The NS was prepared from two commercial stock solutions, Nutri-A and Nutri-B, purchased from Panponic Biosystems AS, Norway. Nutrient content of the applied NS is shown in Table 1. Every second day, pH and EC values of the three parallel systems were adjusted to maintain the pH at 5.5–6.5 and the EC value at 1.1–1.2 mS cm^{-1} using a calibrated Hach HQ40d instrument with standard pH and EC sensors.

Each of the three parallel units of the NFT system was operated in a similar continuous production mode during the experimental period. At the starting date (27 September 2018), 9 seedlings (2 weeks old) were collected from the seedling system and transferred to the NFT system (3 seedlings to each of the three units). After 4 weeks, the NFT system was fully stocked, and the first 3 lettuce heads of each unit were harvested and replaced by 3 new seedlings (2 weeks old). During the 68 days experimental period from 27 September to 4 December, there were 7 harvests (on 25 and 31 October, and 6, 13, 21, and 27 November, and 4 December), with a total production of 21 lettuce heads from each unit, which gave a total of 63 lettuce heads from the 3 parallel units of the NFT system.

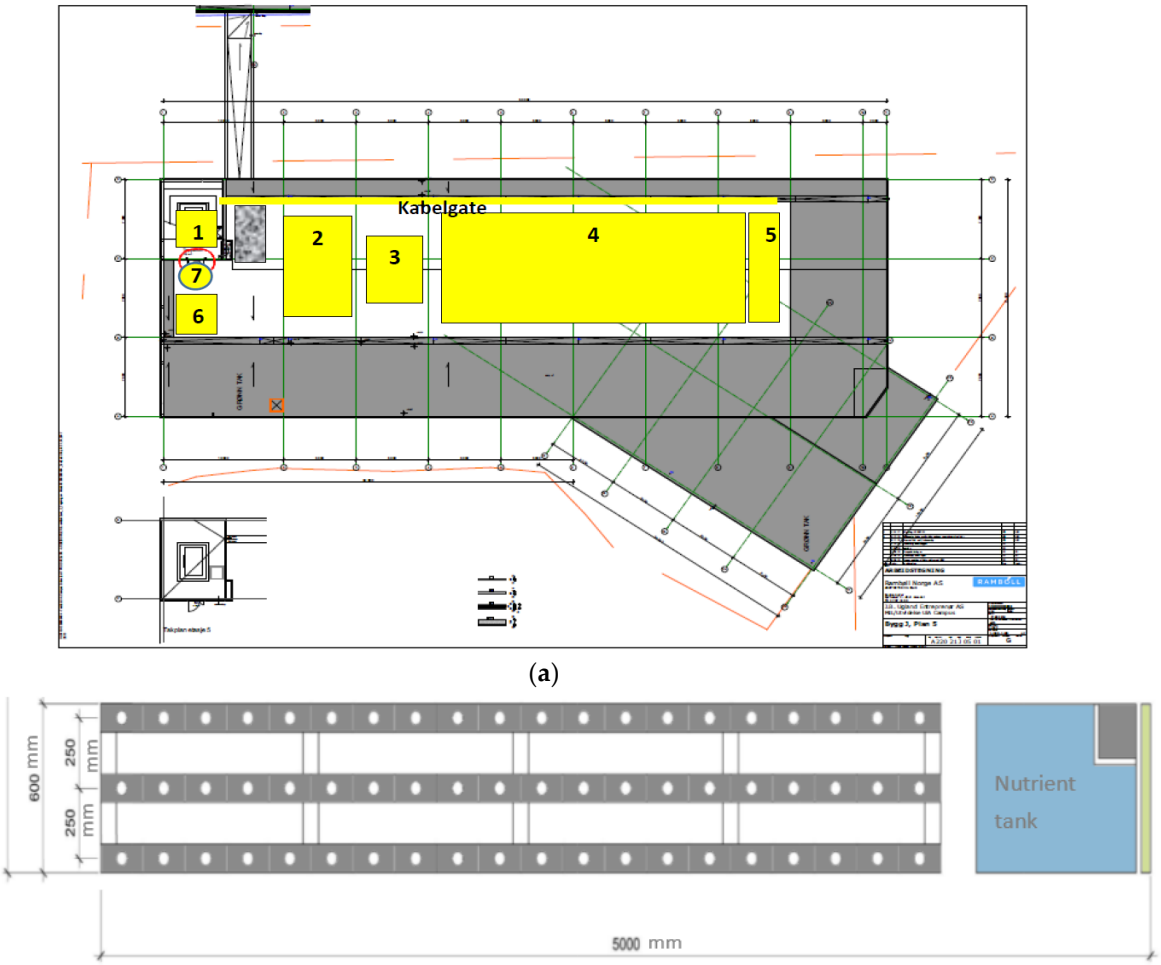
Table 1. Soluble nutrient content of the applied nutrient solution in this study.

	NO_3-N	NH_4-N	P	K	Ca	Mg	S	Zn	B	Cu	Fe	Mn	Mo
mg L^{-1}	107	4.0	23	140	94	23	23	0.26	0.19	0.07	1.7	0.42	0.04

Samples from the nutrient tank were taken throughout the growth period for nutrient analysis. Soluble nutrient analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-HSP), according to accredited standards by the Eurofins laboratory, Netherlands. Lettuce was harvested and weighed after 4 weeks in the NFT systems. Leaf tissue samples from different parts of the harvested lettuces were collected and analyzed for macro- and micro-nutrient content by inductively coupled plasma atomic emission spectrometry (ICP-OES), according to European standards (DIN EN ISO 11885).

Open-Air Rooftop Hydroponic Lettuce Growth

Seeds of Lactuca sativa L. (Batavia-type, cv. 'Partition') from LOG AS, Norway, were seeded in a seeding system as described above. Seedlings were then transplanted in an open-air rooftop hydroponic system. Open-air rooftop hydroponic lettuce growth was carried out from the 25 August to 6 October 2020 in an NFT hydroponic system, installed on the fifth floor rooftop of one building of the University of Agder in Norway, as described in Figure 1. The yellow area, no. 6, in Figure 1a is reserved for rooftop farming.



(**a**)

(**b**)

Figure 1. Principle drawing of the fifth floor rooftop of one building of the University of Agder in Norway (**a**) and drawing of the experimental rooftop hydroponic system (**b**).

High wind speed and heavy precipitation incidences at the beginning of the period gave the seedlings a hard and slow start and many of them were damaged. Due to non-optimal weather conditions, the trial lasted five weeks instead of four. The NFT system was made of 3 rectangular PVC pipes, each 4000 mm long and 100 mm wide, mounted in an aluminum stand with wind shields. Each pipe had 20 holes with 50 mm diameter for inserting net pots, which allowed the cultivation of 60 lettuce heads. A nutrient container intermittently supplied the three PVC pipes with NS (30 min on/off cycles) at a flow rate of 3.5 L min^{-1}. Same seeds and NS were used for the rooftop and the indoor hydroponic lettuce growth (Table 1). The pH and EC of the NS were

monitored and adjusted every 2nd day to the same values as in the indoor growth system (5.5–6.5 and 1.1–1.2 mS cm^{-1}), respectively. The average PAR throghout the experimental period was 272 µmol m^{-2} s^{-1}, and the daily average registred PARs (calculated from the recorded data of GHI irradiance by solar iriadiance sensor) are shown in Figure 2.

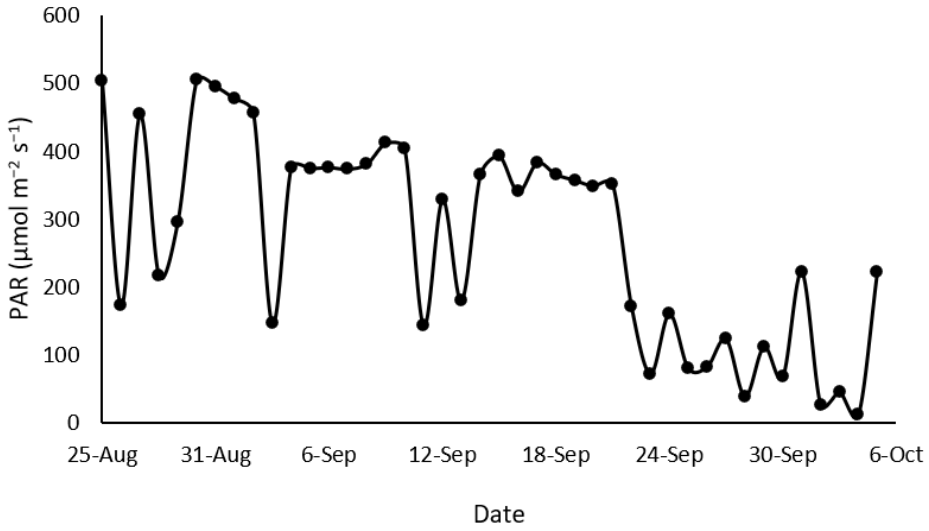


Figure 2. Daily average photosynthetically active radiation (PAR) from 25 August to 5 October 2020.

The average wind speed throughout the experimental period was 1.8 m s^{-1} and the daily average registered wind speeds, recorded by wind speed sensor, are presented in Figure 3. The registered temperature was in the range of 12–22 °C, as shown in Figure 3, with an average temperature of 16.5 °C. The relative humidity was in the range of 45–69% and the CO2 concentration was measured to 350 ppm by Elma Dt air monitoring sensor. Leaf tissue samples and NS samples (five samples) were collected and analyzed for macro- and micro-nutrient content, as described above.

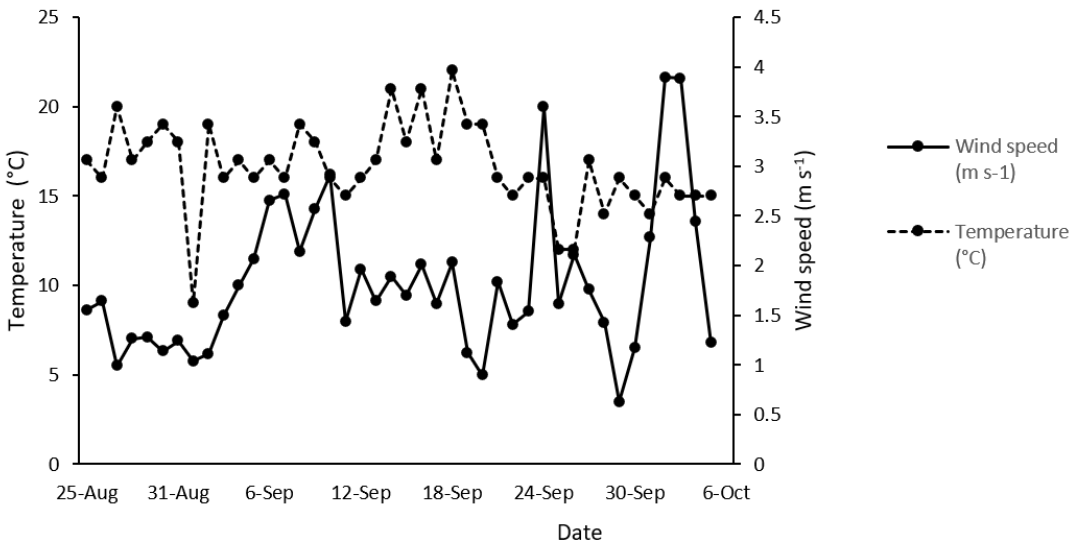


Figure 3. Daily average temperature and daily average wind speed (m s−1) from 25 August to 5 October 2020.

Data Analysis and Statistics

Data were subjected to analysis of variance (ANOVA) using SPSS Version 25. Mean differences were determined by Tukey's honestly significant difference (HSD) test at $p < 0.05$. In figures, statistically significant differences are indicated by different letters. Mean values and standard deviations are presented in the graphs.

Results and Discussion

Lettuce Growth and Yield

Shoot fresh weights and yields of the harvested rooftop and indoor hydroponic lettuce are presented in Figure 4. The lettuce grown in the indoor hydroponic system and in the rooftop hydroponic system had an average shoot weight of 243 g and 233 g, respectively. The maximum and the minimum shoot fresh weights of the indoor and rooftop hydroponic lettuce were 322 g and 196 g, and 781 g and 36 g, respectively. Although the average shoot fresh weight of the rooftop hydroponic lettuce was higher than the marketable fresh weight of 150 g, 25% of the total lettuce heads were below the marketable fresh weight. This variation was probably due to the high wind speed experienced during the beginning and at the end of the growth period, as shown in Figure 3.

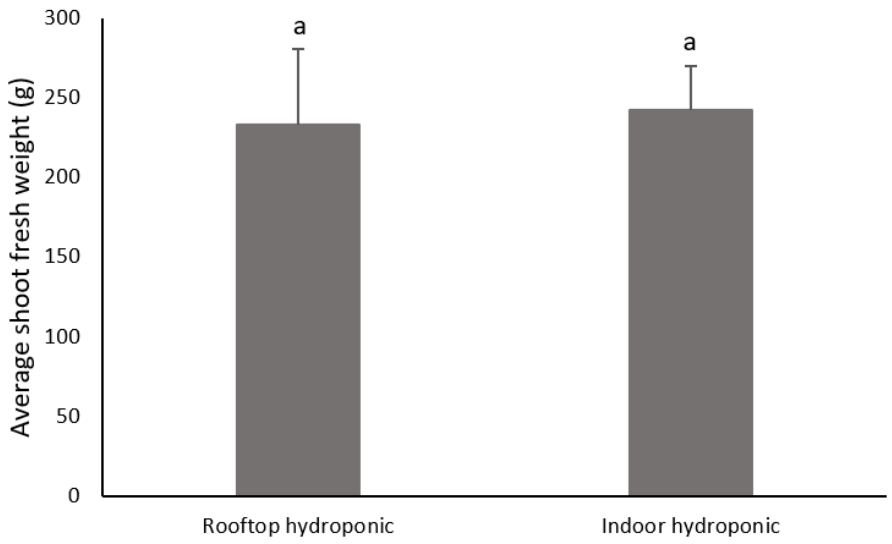


Figure 4. Average shoot fresh weight of rooftop and indoor hydroponic lettuce (bars represent standard deviation of the mean of 60 lettuce heads).

Another 25% of the total rooftop lettuce heads weighed twice the marketable fresh weight and 8% weighed more than 500 g. These lettuce were close to the fence that surrounds one side of the hydroponic system. This study suggests that rooftop hydroponic lettuce could be successfully cultivated in Norway during late season at low temperatures (12–21 °C) if the hydroponic system is protected from the wind.

Nutrient Consumption and Concentration in Lettuce Leaves

Leaf macro- and micro-nutrient concentrations in harvested rooftop hydroponic lettuce, compared to their concentrations in harvested indoor hydroponic lettuce, are shown in Figure 5. The foliar analysis of the macro-nutrients shows that the elements with highest accumulation in leaf tissue of the rooftop hydroponic lettuce were K, followed by N, Ca, P, Mg, and S, which conform to the norm-values of Hartz et al.[35]. However, macro-nutrients content in the leaves of the indoor hydroponic lettuce deviates from these norm-values by a higher N value than K value.

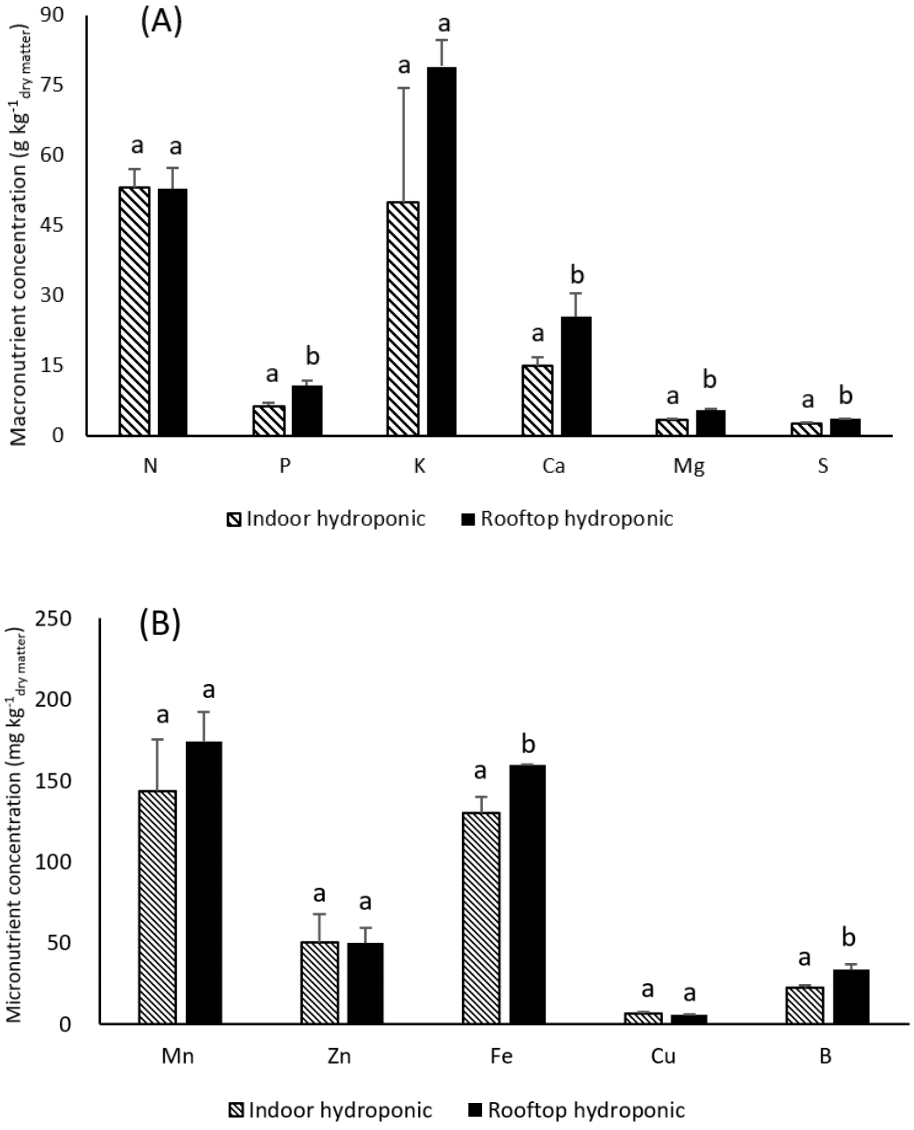


Figure 5. Leaf macro-nutrient (A) and micro-nutrient (B) concentrations in harvested rooftop and indoor hydroponic lettuce (bars represent standard deviation of the mean of three replicas).

The highest accumulation of micro-nutrient elements in the leaf tissue of both the rooftop and indoor hydroponic lettuce were Mn, Fe, Zn, B, and Cu.

As shown in Figure 5, the rooftop hydroponic lettuce accumulated more nutrients than the indoor hydroponic lettuce. The rooftop lettuce had a significantly higher concentrations of P, Ca, S, Mg, Fe, and B compared with the indoor lettuce. There were no significant differences in the other nutrient concentrations. Explanation for

this higher uptake could be the positive effect of daylight on nutrients uptake. The intensity of light causes more transpiration in plants which affects nutrients [32]. Rouphel et al. [33] reported that the total plant uptake of N, P, K, Ca, and Mg is usually enhanced by stronger natural radiation or supplemental light. Another study demonstrated that N, K, Ca, and Mg concentrations in the leafy lettuce were positively affected by light intensity [34]. Our results are in line with the findings of these previous studies as our rooftop hydroponic lettuce were exposed to stronger natural radiation (272 µmol m^{-2} s^{-1}) compared with the indoor hydroponic lettuce (220 µmol m^{-2} s^{-1}).

Generally, the nutrient content of the indoor hydroponic lettuce was closer to the norm-values than the nutrient content of the rooftop hydroponic lettuce. Ca, P, K, and Mg all accumulated in the leaves of the rooftop lecttuce, to concentrations 3, 2, 1.5, and 1.5 times the norm-values [35]. This higher absorption of nutrients did not affect lettuce yield for better, nor for worse, as shown in Figure 4.

In order to avoid a possible luxury absorption of nutrients, an alternative NS may be used to grow rooftop hydroponic lettuce exposed to the same environment conditions as those in the current study. The estimation of the required nutrient concentrations of the alternative NS should take into account that not all the available nutrients will be taken up by lettuce leaves. The nutrient consumption and the nutrient uptake in leaves are presented in Table 2, as percentages of the amount of nutrients added to the system. As indicated in Table 2, not all the consumed nutrients were used by lettuce. Some of them may be consumed by microorganism populations. Beatrix et al. [36] reported that they found 106 cfu ml^{-1} bacteria and 10 to 1000 fungi cfu ml^{-1} in a closed NFT hydroponic system.

Table 2. Nutrient consumption in rooftop hydroponic system and nutrient uptake by lettuce leaves.

	Nutrient Consumption *1 ($g/kg_{dry\ matter}$)	Nutrient Uptake *2 (%)
Macro-nutrients		
N	2.95	63.27
P	0.44	87.74
K	4.2	67.4
Mg	0.43	44.8
Ca	2	46.86
S	0.67	19
Micro-nutrients		
B	0.003	40.52
Cu	0.00043	47.5
Mn	0.012	54.57
Mo	0.00053	10.7
Zn	0.003	52.7
Fe	0.03	19.13

*1 Nutrient consumption (g/kgdry matter) is calculated by subtracting the quantity of nutrient left in the nutrient tank from the total supplied nutrient, then divided by the quantity of the produced dry matter. *2 Nutrient uptake is calculed by dividing nutrient content in lettuce leaves by nutrient consumption.

Nutrient Dynamic of the Recycled Solution

Figure 6 shows the fate of macro-nutrients during the growth period of the rooftop and indoor hydroponic lettuce. As indicated, a continous decrease in the concentration of P was observed in the NS used for indoor lettuce growth. However, the concentration of P in the NS used for rooftop lettuce growth increased gradually from 26 mg L^{-1} to 43 mg L^{-1}, despite the high accumulation of P in the rooftop lettuce leaves compared with norm-values and to the indoor lettuce (Figure 5). The N concentration in the NS used for indoor lettuce growth was reduced by 55% compared with the initial concentration, while the concentration of N in the NS used for rooftop lettuce growth remained nearly stable throughout the growth period. The N accumulation in leaf tissue was approximately equal to the accumulation in the leaves of the indoor hydroponic lettuce. The concentration of K experienced a continous decrease to final concentrations of 4.7 mg L^{-1} and 39 mg L^{-1}, in the NS used for indoor hydroponic lettuce and rooftop hydroponic lettuce, respectively, which corresponds to a reduction by 96% and 75%, respectively. Again, rooftop hydroponic leaf tissue accumulated more K than the indoor hydroponic leaf tissue.

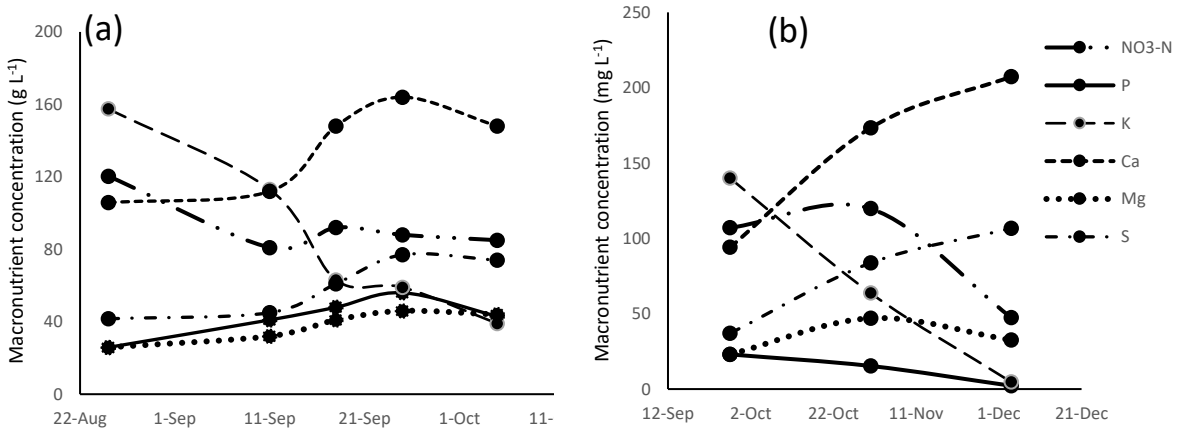


Figure 6. Concentrations of macro-nutrients in the recirculating nutrient solution in rooftop hydroponic system (a) and indoor hydroponic system (b).

To summarize, except for Ca and S, rooftop lettuce accumulated more nutrients than the indoor lettuce. The concentration of nutrients in the NS used for rooftop lettuce growth were slightly higher at the end of the cultivation period compared with the concentration in the NS used for indoor lettuce growth. Hence, we may deduce two conclusions.

First, the nutrient uptake in rooftop hydroponic lettuce were more efficient than the uptake of indoor lettuce.

Secondly, nutrients were more likely to be wasted (not used by plants) in the indoor hydroponic system. Potentially, this could be partly explained by higher micobial activity due to higher temperature in the indoor hydroponic system compared with the outdoor hydroponic system. However, our literature search found no studies supporting this hypothesis.

We demonstrated in this study that hydroponic rooftop-grown lettuce can be competitive with their indoor counterparts if the rooftop hydroponic system is protected from extreme weather conditions. This study sugggets that rooftop hydroponic systems could not only contribute to urban food production, but could also have a lower environmental footprint, since they use only sunlight and consume less nutrients compared with indoor systems and conventional agriculture, which make them more economical in the long term.

Conclusions

In this study, we successfully grew lettuce in an outdoor rooftop hydroponic system during late season in Norway. The average fresh weight of the harvested lettuce exceeded the marketed size of commercial lettuce of 150 g. However, 25% of lettuce heads were below the marketable weight, owing to strong winds during the growth period, while lettuce heads which were close to the fence that surrounds one side of the hydroponic system weighed more than 500 g. Hence, we recommend that special consideration should be taken for open-air rooftop hydroponic installation, such as wind shields. Despite the strong winds, which led to 25% of undersized lettuce, the average fresh weight of the rooftop hydroponic lettuce were statistically comparable to their indoor counterparts. Rooftop hydroponic lettuce leaves accumulated more nutrients than the indoor lettuce. This study confirmed the productive capacity of rooftop hydroponics; nevertheless, a larger scale study should be conducted.

Author Contributions: Conceptualization, M.E. and H.L.; methodology, M.E. and H.L.; software, M.E.; validation, H.L.; formal analysis, M.E.; investigation, M.E. and H.L.; resources, H.L.; data curation, M.E.; writing—original draft preparation, M.E.; writing—review and editing, H.L.; visualization, M.E.; supervision, H.L.; project administration, H.L.; funding acquisition, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Agder. Grant number: 163831-100.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting reported results are shown in the present article as tables and figures. The original data are available as Excel files, stored by the first author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Paper C:

Nutrients recovery from aquaculture waste for use as fertilizer in soilless growth systems

This paper has been published as:

Ezziddine, M.; Liltved, H. Nutrients recovery from aquaculture waste for use as fertilizer in soilless growth systems. Acta Hortic. 2021, 1305, 399-406, doi:10.17660/ActaHortic.2021.1305.52.

Nutrients recovery from aquaculture waste for use as fertilizer in soilless growth systems

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Abstract

In coming decades agriculture must produce more food with less space and resources. To overcome these challenges, new farming approaches are needed. Investments in smart soilless production systems may contribute to solve these problems. However, todays hydroponic systems rely on inorganic fertilizers which are mined from scarce and non-renewable resources. The aim of this work was to convert aquaculture waste to valuable fertilizer by aerobic digestion (AD). A mixture of drain-water from swirl separators and backwash-water from particle-filters were collected from a local land-based fish-farm with recirculation of water. Three sets of experiments were conducted in duplicate in batch aerated reactors for three weeks. For the first and second set, pH was kept at 7 and 5, respectively. For the third set, the pH was not adjusted and was in the range of 8-9. Aerobic digestion at pH 7 and 5 showed similar mobilization performance with an increase of soluble macronutrients and micronutrients to concentrations close to or exceeding mineral levels recommended for soilless growth systems. At pH 7, the concentrations of soluble phosphorus (P), nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg) increased by factors of 12.9, 4.9, 1.7, 3.2 and 2.0, respectively. At pH 5, the same concentrations increased by factors of 19.0, 5.4, 1.5, 5.1, 2.4, and 2.0, respectively. The mineral analysis suggested that the resulting nutrient-rich solution can be used for soilless agriculture. However, the ratio of NO_3-N to NH_4-N was low compared to recommended values. Further studies will be carried out to stimulate nitrification during AD to produce a better balanced

nutrient solution and to evaluate the performance of the recovered nutrient solution on plant growth.

Keywords: aerobic digestion, aquaponics, hydroponics, mobilization, nutrient solution, nutrient recovery, organic fertilizer

Introduction

By 2050 population growth will increase to reach 9 billion people (FAO, 2016). More food will be required. At the same time, arable farmland is decreasing because of urbanization and soil contamination (Kozai and Niu, 2016). Therefore, to provide the required food for the future population with less space, more investment should be made in soilless growth system. Nutrient solution which is composed of water and fertilizer is a determining factor in soilless growth systems. However, fertilizers are mined from scarce and non-renewable resources. It has been reported that nutrient depletion is currently a major problem (Kupfernagel et al., 2017). Phosphorus for example may be depleted from apatite mines within 150 years (Mehta et al., 2015). Other sources of nutrients for soilless growth system urge to be found.

In land-based aquaculture, wastewater and sludge from treatment processes contains nutrients in high amount. Approximately 70% of the phosphorus (P) and nitrogen (N) fed to fish can be found in the wastewater. The majority of P in the wastewater is associated with solids, while for N, only 15-20% is associated with solids and the remainder is dissolved (Del Campo et al., 2010). Nutrients in sludge collected from land-based aquaculture exist in three different forms: ionic or soluble form which is the only form assimilated by plants, organically associated nutrient and inorganically bound nutrient (e.g. iron phosphate). These two last forms are not readily available for plant growth. To be utilized for fertilizing purposes, the nutrients associated with particulate compounds should be mobilized into soluble forms. It has been shown that anaerobic treatment of aquaculture sludge from an aquaponic facility allowed an increase in soluble N, while soluble P remained unchanged (Monsees et al., 2017). The objective of the current study was to assess the potential of aerobic digestion (AD) to mobilize nutrients from aquacultural waste at various pH-values for subsequent usage of the recovered nutrients in soilless growth systems.

Materials and methods

Sludge-water consisting of a mixture of drain-water from swirl separators and backwash-water from particle-filters was collected from a recirculating aquaponic system located at the Norwegian Institute of Bioeconomy Research (NIBIO), Landvik, Norway. The sludge-water was distributed to six 25-L polyethylene reactors covered with a lid to prevent evaporation and equipped with diffusors to provide aeration at a rate of 20 L min^{-1} per reactor. Three sets of batch experiments were conducted in duplicate for three weeks. For the first and second set, pH was kept at 7 and 5, respectively. For the third set, the pH was not adjusted and was in the range of 8-9. The pH-values were measured daily with a calibrated pH-meter and adjusted with a diluted HCl-solution to maintain the target pH-values. Samples were collected weekly and analyzed for total suspended solids (TSS), volatile suspended solids (VSS), soluble chemical oxygen demand (SCOD), total phosphorus (TP), total nitrogen (TN) and soluble nutrients. TSS and VSS were determined according to standard method using a pre-weighed 1.6 μm Whatman glass fiber filter. TP was measured according to the NS EN ISO 1568-2 method and TN was determined according to NS 4743 method by the Eurofins laboratory, Norway. Soluble nutrient analysis for ammonium (NH_4^+-N), nitrate (NO_3^--N) and nitrite (NO_2^--N) was performed on a QuAAtro continuous segmented flow autoanalyzer while the other soluble nutrients concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) by the LMI laboratory, Sweden. All samples for soluble nutrients analysis were pre-filtered through 0.45 μm filters.

Statistics

The aerobic digestion experiments were conducted in duplicate. Mean values with standard deviations are presented. Where standard deviation bars are not observable on the graphs, they do not extend beyond the dimensions of the symbols. Data comparison was done using the statistical t-test with a statistical significance level of $p<0.01$.

Results and discussion

Characterization of the aquaculture wastewater

The concentrations of TSS, VSS, total COD, TP, TN, SCOD and nutrients of the untreated sludge-water are shown in Table 1. As indicated, the sludge-water was a dilute waste stream, with a TSS content of 0.87%. The majority of the solid was organic compounds (VSS), indicating that biological aerobic treatment will degrade organic matter and simultaneously release nutrients from the biosolids. Most of the organic compounds in the sludge-water were associated with particulate matter, with only 2.5% SCOD of the total COD. High degree of particulate association was also the case for N and P. Of the dissolved N-compounds, NH_4^+-N was the only detectable compound, comprising 26% of the TN. Fish excrete dissolved N as ammonia via the gills, and as urine (urea), which is easily hydrolysed to ammonia. As also shown in Table 1, the percentage of dissolved P was low, only 6% of TP.

Effect of aerobic digestion on nutrient mobilization at various pH-values

During the 3-week aerobic digestion period, all dissolved macronutrients increased significantly at pH 7 and 5, while no increases were observed for soluble N, Ca and Mg at unadjusted pH (Figure 1). At pH 7, the concentrations of soluble P, N, K, Ca, Mg, and sulfur (S) increased by factors of 12.9, 4.9, 1.7, 3.2, 2.0, and 2.7, respectively, and at pH 5 by factors of 19.0, 5.4, 1.5, 5.1, 2.4, and 2.0, respectively (Figure 1). By using the t-test with a statistical significance level of $p<0.01$, the differences in solubilization between pH 7 and pH 5 were significant for P, K, Ca, S, Zn, Cu, Fe and Mn. For the remaining nutrients, differences were statistically insignificant. The concentrations of dissolved N, P and Ca after 3 weeks of AD at pH 7 and 5 exceeded the recommended levels of these nutrients in hydroponic solutions for soilless growth system (Mattson and Peters, 2017) by factors from 5 to 9 (Table 2).

Table 1. Characteristics of the aquaculture sludge-water before aerobic digestion.

Parameter	Mean	Standard deviation
pH	7.9	0.01
SCOD (mg L^{-1})	147	5
COD (mg L-1)	6000	800
TSS (g L^{-1})	8.7	0.1
VSS (g L^{-1})	8.55	0.35
Total P (TP) (mg L^{-1})[1]	135	5
Total N (TN) (mg L^{-1})[1]	705	15
B (mg L^{-1})	0.14	0.01
Ca (mg L^{-1})	53.85	0.75
Cu (mg L^{-1})	0.03	0.01
K (mg L^{-1})	62.5	0.5
Mg (mg L^{-1})	21.9	0.2
Mn (mg L^{-1})	0.24	0.01
Mo (mg L^{-1})	0.01	0.003
NH_4-N (mg L^{-1})	181	2
NO_2-N (mg L^{-1})	0.03	0
NO_3-N (mg L^{-1})	0.1	0
P (mg L^{-1})	8.2	0.25
S (mg L^{-1})	26	1
Zn (mg L^{-1})	1.65	0.05

[a]TP and TN include particulate and soluble P and N compounds

Potassium was the macronutrient least solubilized by the aerobic treatment. The observed increases in soluble K by 76% (to 110 mg L^{-1}), 50% (to 94 mg L^{-1}) and 92% (to 120 mg L^{-1}) at pH 7, pH 5 and unadjusted pH, respectively, did not raise the levels above the recommended value of 132 mg L^{-1} (Table 2). At pH 7, all micronutrients rose substantially, except for Mn. In spite of lack of increase, soluble Mn was already at the concentration required for soilless growth systems (Table 2). Half of the dissolved micronutrients (Cu, Mo, Zn) did not increase at pH 5 and remained slightly below the recommended levels. Similar results were obtained for the unadjusted pH.

Nitrogen mobilization

The responses in the different nitrogen compounds under aerobic degradation at various pH values are shown in Figure 2. The TN concentration of unfiltered sludge-water (particulate N + dissolved N) experienced an increase during AD at pH 7 and 5 (Figure 2a, b). Some of this increase can be explained by a 10-15% volume decrease due to evaporation during the 3-week aeration period, even though the reactors were covered. By aerobic degradation at pH 7 and 5 (Figure2 a, b), the soluble nitrogen

(NH_4^+-N + NO_3-N) increased sharply during the first week. After that point, the rate of solubilization levelled off. At the end of the 3-week period, the soluble nitrogen values reached more than 90% of the average total nitrogen concentrations of the samples. The degree of nitrogen mobilization was similar at pH 7 and pH 5, with final concentrations of 890 mg L^{-1} and 885 mg L^{-1}, respectively, which corresponds to 4.9-fold and 5.4-fold increases, respectively. The present finding is much higher than in earlier investigations which reported an increase of soluble N by a factor of 2.56 after aerobic treatment at neutral pH (Delaide et al., 2018), while Monsees et al. (2017) observed a decrease in soluble N from 1 to 0.1 mg L^{-1} after AD at pH 5.

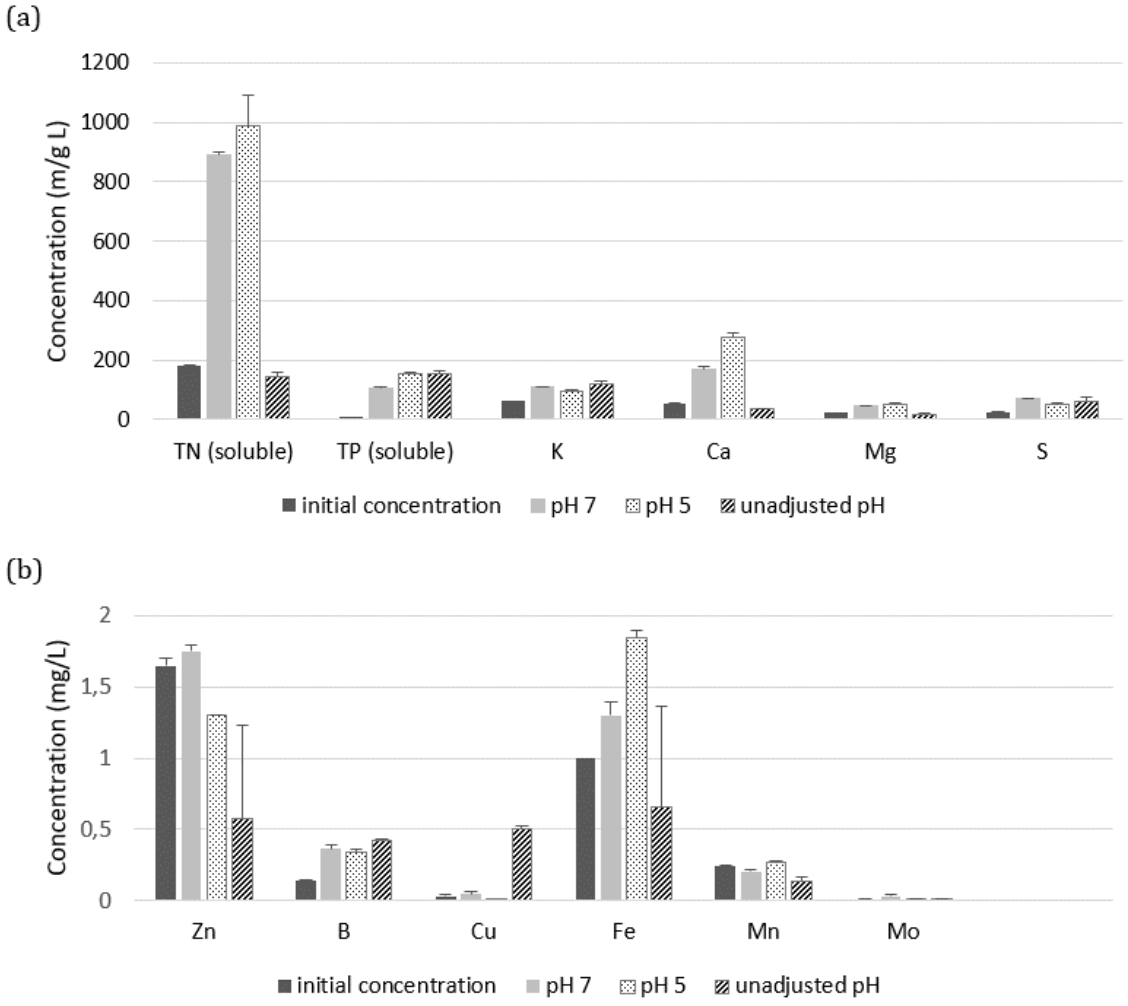

Figure 1. Soluble concentration of (a) macronutrients and (b) micronutrients in the clear-phase after 3 week of aerobic digestion.

Table 2. Soluble nutrients content in the clear-phase of the aquaculture sludge-water after 3 week of aerobic digestion at pH 7, pH 5 and uncontrolled pH, compared to a standard formulation for soilless growth system (Mattson and Peters, 2017).

	pH 7	pH 5	Uncontrolled pH	Recommended concentrations in a standard formulation for soilless growth
NH_4^+-N (mg L^{-1})[1]	862.5	987.5	146	14
NO_3^--N (mg L^{-1})[1]	27	0.2	0.1	160
P (mg L^{-1})	105	155	34	16
K (mg L^{-1})	110	94	120	132
Ca (mg L^{-1})	170	275	35.25	38
Mg (mg L^{-1})	44.5	53	19	14
S (mg L^{-1})	70.5	53	59	-
Zn (mg L^{-1})	1.8	1.3	1.3	0.1
B (mg L^{-1})	0.37	0.34	0.12	0.16
Cu (mg L^{-1})	0.05	0.006	0.03	0.11
Fe (mg L^{-1})	1.3	1.9	1.7	1
Mn (mg L^{-1})	0.2	0.2	0.2	0.2
Mo (mg L^{-1})	0.003	0.001	0.001	0.002

[a]The sum concentration of soluble NH_4^+-N and NO_3^--N was higher than the initial TN. This was partly explained by an increase in TN attributed to a volume decrease of 10-15% due to evaporation.

For AD without pH control, the total N (TN) concentration was reduced from 705 to 225 mg L^{-1} (Figure 2c). This sharp depletion of TN can be caused by dissolution of bound nitrogen to NH_4^+, with subsequent escape of NH_3-gas. At high pH-values of 8-9 as experienced in these reactors, the equilibrium between NH_4^+ and NH_3 will be shifted toward the gaseous NH_3, which may escape to the atmosphere (Glass and Silverstein, 1998). Another possibility for this reduction is that the released NH_4^+ may precipitate in the form of struvite (magnesium ammonium phosphate) at high pH. Without pH control, a modest increase in soluble N was observed during the first week (Figure 2c). Thereafter, a reduction followed to a level below the initial value of soluble N. Compared to the initial soluble N concentration, the decrease was not statistically significant.

Our data shows that the only dissolved nitrogen compound present at pH 5 and without pH-control was NH_4^+-N. At pH 7, approximately 3% of the dissolved nitrogen was in the form of NO_3^--N. This is reflected in Figure 2, which shows that the curves of soluble N and the curves of NH_4^+-N are very similar at all pH-values. These results agree with the fact that nitrification will proceed better at pH 7 than at higher or lower pH-values. The limited nitrification observed even at pH 7 can be explained by high

concentrations of organic compounds which will stimulate the growth of heterotrophic bacteria, thereby depressing the growth and activity of the nitrifying bacteria. The ratio of NO_3^--N to NH_4^+-N of the solution after aerobic digestion was low compared to what is recommended for soilless growth solutions. In future research, we will try to stimulate nitrification during AD to increase the NO_3^--N concentration and lower the NH_4^+-N concentration.

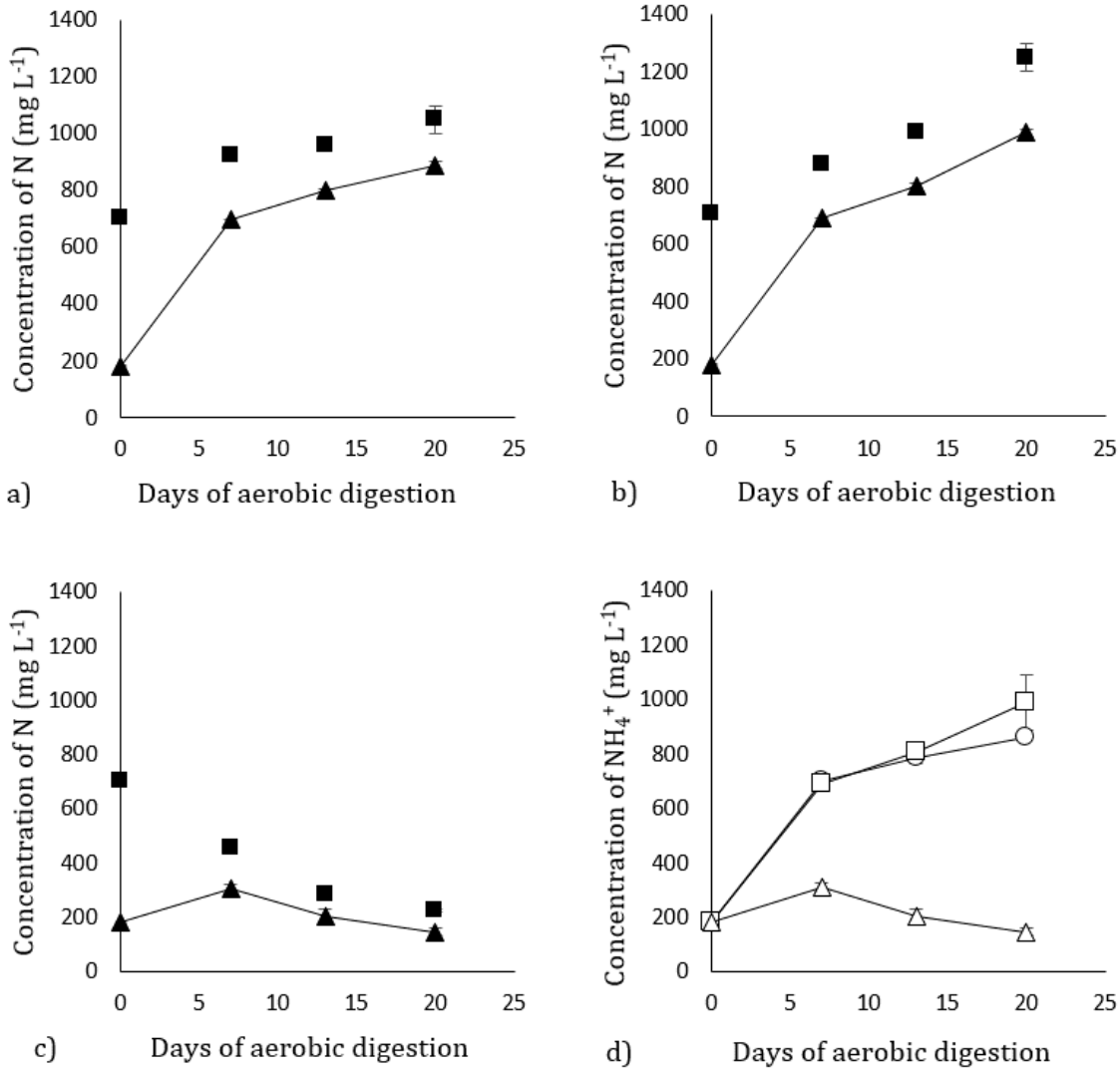

Figure 2. Mobilization of nitrogen during aerobic digestion at (a) pH 7, (b) pH 5, (c) at uncontrolled pH, and (d) NH_4^+-N at pH 7 (○), at pH 5 (□) and at uncontrolled pH (△). ■; total nitrogen (soluble + particulate), ▲; soluble nitrogen (NH_4^+-N + NO_3^--N).

Phosphorus mobilization.

Aerobic degradation of the sludge-water resulted in soluble P increases at all pH-values tested. The highest soluble P concentration was observed at pH 5 with a final concentration of 155 mg L^{-1} which corresponded to a 19-fold increase and to approximately 90% solubilization of the average TP (Figure 3). This degree of solubilization was substantial higher than in earlier published work by Monsees et al. (2017) who reported an increase of soluble P by a factor of 3.2 at pH 5 after aerobic treatment. At neutral pH, the concentration of soluble P was 105 mg L^{-1} at the end of the treatment which corresponded to a 12.9-fold increase and 73% solubilization of the average P. This degree of mobilization was also higher than in earlier published work by Delaide et al. (2018) who reported an increase of soluble P by a factor of 2.18 after AD at neutral pH. Only a modest increase to 34 mg L^{-1} was observed in the reactors without pH-control.

The dynamic of chemical and physical mechanisms related to adsorption/desorption and precipitation/solubilization are altered by pH decrease. Interestingly, the change of concentration of soluble P and Ca^{2+} during AD had similar trend at different pHs (Figure 3e). This is a strong indication that the solubility of P is ascribed to the dissolution of Ca-P compounds.

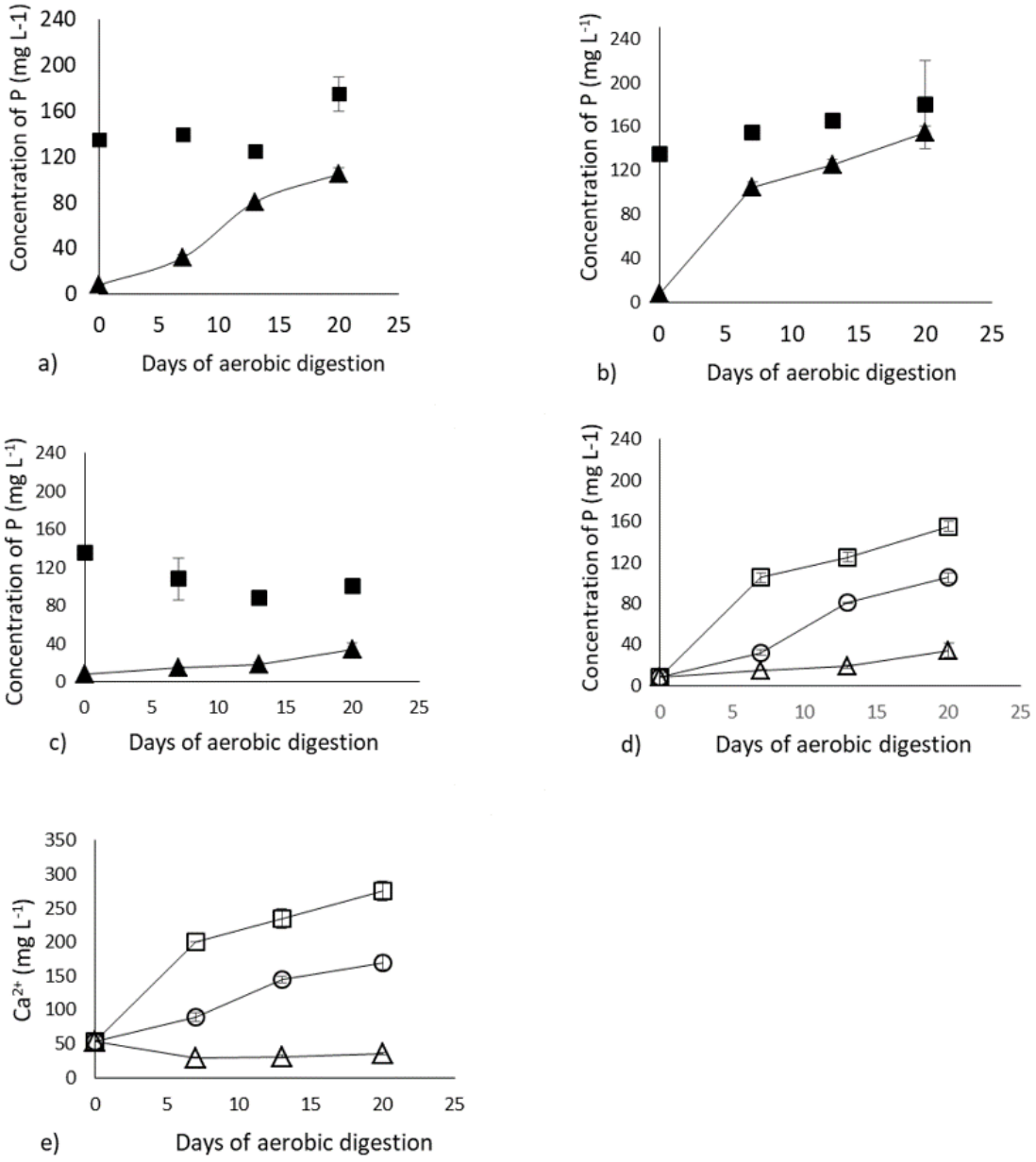

Figure 3. Mobilization of phosphorus during aerobic digestion at (a) pH 7, (b) pH 5 and (c) without pH control and d) concentration of soluble P at pH 7 (○), at pH 5 (□) and at uncontrolled pH (△), (e) soluble calcium (Ca^{2+}) at pH 7 (○), at pH 5 (□) and at uncontrolled pH (△). ■; total phosphorus (soluble + particulate), ▲; soluble phosphorus.

Conclusions

Aerobic digestion at pH 5 and 7 for 3 weeks were both able to mobilize high amounts of nutrients from aquaculture sludge-water. The concentrations of the majority of macronutrients and micronutrients in the digestate after aerobic digestion at pH 7 and 5 were close to or exceed the required mineral levels recommended for soilless growth solutions. The ratios of NO_3-N to NH_4-N of the solutions were low compared to recommended ones for hydroponic growth. Further research will be conducted to stimulate nitrification during aerobic digestion to increase the NO_3-N to NH_4-N ratio, and to verify the performance of the recovered nutrient solution on plant growth in a small-scale hydroponic system.

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Paper D:

A method for reclaiming nutrients from aquacultural waste for use in soilless growth systems

This paper has been published as:

Ezziddine, M.; Liltved, H.; Homme, J.M. A method for reclaiming nutrients from aquacultural waste for use in soilless growth systems. Water Sci. Technol. 2020, 81, 81–90, doi:10.2166/wst.2020.079.

A method for reclaiming nutrients from aquacultural waste for use in soilless growth systems

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Abstract

The aim of this work was to develop a method that allows the recovery of nutrients from aquaculture sludge, not only to alleviate the disposal problem, but also to address the future scarcity of nonrenewable fertilizers. This method includes two steps: Nutrient mobilization using aerobic digestion followed by solids precipitation using chitosan as the flocculant. The aerobic digestion experiments were conducted in aerated batch reactors, while a jar test apparatus was used to assess the capacity of chitosan to remove total suspended solids (TSS) and turbidity. During aerobic digestion, the concentration of soluble N (sum of NH_4-N, NO_2-N, NO_3-N) increased from 181 mg/L at the start to 890 mg/L after three weeks, and to 903 mg/L after four weeks and solids removal by chitosan flocculation. The corresponding concentrations for soluble P were 8.2 mg/L at start, 110 mg/L after three weeks of aerobic digestion, and 160 mg/L after four weeks of aerobic digestion and chitosan flocculation. Other macronutrients (K, Ca, Mg, S) and micronutrients (Fe, Mn, Zn, B, Cu, Mo) were mobilized to concentrations close to or higher than levels recommended for hydroponic growth of vegetables. Chitosan flocculation and precipitation using a dose of 15 mg/L resulted in a reduction of the turbidity by 96% from 156 to 6.5 FNU. After chitosan precipitation, 80% of the sludge could be reclaimed as a nutrient-rich clear phase, low in TSS and turbidity**.**

Keywords

Aerobic digestion; Aquaculture sludge; Chitosan; Fertilizer; Flocculation; Mobilization

Introduction

According to the Food and Agriculture Organization of The United Nations (FAO), aquaculture industry grows faster than other food production sectors. It is reported that by 2030, aquaculture production of fish and shellfish is expected to reach 109 million tonnes which represents a growth rate of 37 percent over the 2016 production (FAO, 2018). An increasing part of this production will take place in semi-closed and closed systems where effluent water and recirculating water is treated mechanically to remove solid waste (feed waste and feces). The collected sludge contains nutrients that may represent a pollution problem if inadequate disposal, but can also be utilized for fertilizing purposes after appropriate treatment. Thus, converting aquaculture waste into valuable fertilizer can not only alleviate the disposal problem, but also bring economic benefits and address the future scarcity of non-renewable fertilizers.

In Norway, the amount of waste from production of Atlantic salmon (Salmo salar) is large and increasing. The life cycle of the salmon requires that the hatching and early growth phase proceed in land-based freshwater farms to reach the smolt stage when the fish is ready to go into saltwater in the sea. After the freshwater phase, the smolt are transferred to floating cages in the sea where it grows to a marked weight of 4-5 kilograms. The annual amount of sludge from land-based smolt production in freshwater has been estimated to approximately 11000 tons dry weight (DW), while the amount of sludge from sea cages was estimated to 535000 tons DW (Aas & Åsgård 2017). The sludge from sea cages are usually not collected and is not directly suitable as a fertilizer for crops due to high salinity. In addition to the solid waste, fish produce dissolved waste as urine and excretion products over the gills. In land-based systems, including systems with direct flow-through of water and systems with recirculated water, the feed waste and feces should be removed as fast as possible to prevent it from being broken down and suspended by turbulence by pumping and water transport. In systems with recirculation of water, dissolution of particles into fine particles, colloids and dissolved compounds may impair water quality and pose a threat to fish health if not removed (Del Campo et al. 2010). Mechanical solids removal can be accomplished by sedimentation, mechanical filtration or swirl separation. In recirculating aquacultural systems (RAS), removal of dissolved compounds (e.g. ammonia and carbon dioxide) are also required, which demand additional treatment such as biofiltration and aeration. The sludge produced by solid

removal systems is relatively low in solid content (approximately 1-2% DW), and must be treated by dewatering and stabilization before disposal or use (Wang et al. 2013). Without safe disposal or use, the produced sludge may cause detrimental environmental impacts, including pollution of surface water and ground water bodies, spreading of fish pathogenic microorganisms, and unpleasant odour from putrefaction (Bergheim et al. 1998). After proper treatment, land application is the most common way to dispose sludge from aquaculture (Timmons et al. 2018). By land application, positive fertilizing effects on crop growth can be obtained (Goddek et al. 2016).

An alternative way to utilize the nutrients in aquacultural waste is to use the sludge from solid removal systems more directly in soilless growth systems for vegetables (hydroponic and aquaponic systems). However, nutrients associated with solids are not readily available for plant growth. Mobilizing of nutrients to soluble forms can be obtained by various treatments such as aerobic and anaerobic degradation, and then apply the nutrient rich water in soilless growth systems. Monsees et al. (2017) compared the nutrient mobilization during anaerobic and aerobic digestion of aquacultural sludge. They obtained higher increase of soluble nutrient such as P and K after aerobic than anaerobic treatment. Likewise, Delaide et al. 2018 showed that aerobic digestion has some ability to solubilize nutrients from aquacultural sludge by allowing an increase in soluble macro- and micronutrients by 10 – 60%. Delaide et al. (2018) and Monsees et al. (2017) used traditional AD for mobilization of nutrients. Recently, more advanced techniques for nutrient recovery from domestic wastewater sludge have been developed (Xu et al. 218 a, Xu et al. 2018b). Such techniques may be applied in the aquaculture industry in the future for more efficient nutrient recovery.

Once nutrients are solubilized in the water phase, solids should be removed in order to produce a good quality clear phase, in compliance with water quality guidelines for hydroponic and aquaponic systems. Techniques to remove solids from the treated aquacultural sludge include flocculation and sedimentation. Several studies have been conducted using metal-based coagulants and synthetic organic polymers for flocculation and settling of solids (Siah et al. 2014). Inorganic metal such as ferric- and aluminium salts are the most commonly used flocculants due to their low cost and ease of use (Zhang et al. 2014). The metal salts hydrolyse and combine with suspended solid to setlable flocs, but also react with dissolved phosphate to insoluble

iron- or aluminumphosphate, thereby removing the valuable phosphorus from the solution (Ge et al. 2017). Aluminium flocculants may also leave Al-residuals in the clear phase, with a potential toxic effect on plants (Yang et al. 2016). With the intention to utilize the dissolved nutrients in the sludge for plant growth, alternative flocculants, not reacting with soluble phosphorus, should be used.

Chitosan is a natural cationic polymer made from shrimp- and crab shells, shown to be an excellent turbidity remover (Yang et al. 2016). Cationic polymers may possess dual functions of coagulation and flocculation. The effectiveness of chitosan as a coagulant and flocculant can be explained by its long chain structure with abundant free amino groups that will be protonated in neutral and acidic medium which impart high charge density. The positive charges will neutralize the negative charges on particle surfaces and bridge the destabilized particles into aggregates (Yang et al. 2016). In wastewater treatment, chitosan has mainly been used as flocculant for removal of different types of dissolved and undissolved compounds, including suspended solids, heavy metals, humic acid, dyes, algae, and bacteria (Lee et al. 2014, Yang et al. 2016). Improved solid settling characteristics have been demonstrated in various effluents such as sewage, food processing wastewater, aquacultural wastewater, manure wastewater and dye wastewater (No & Meyers 2000, Yang et al. 2016). For treatment of dilute sludge in aquaculture (backwash water from microscreen filters), solid removal efficiency of various synthetic cationic polymers has been evaluated (Ebeling et al. 2005). However, no such studies have been found where chitosan has been used as flocculant. Preliminary trials with chitosan flocculation of sludge from aquaculture in our laboratory have revealed high removal of suspended solids and turbidity, but marginal removal of soluble phosphorous (data not shown). Chitosan is also known as a metal chelator (Zhang et al. 2016), and regarded as nontoxic without health concerns (Yang et al. 2016).

The objective of this study was to propose a holistic method for treatment and use of aquacultural sludge for fertilizing purposes in soilless growth systems. The method includes solubilization of nutrients by aerobic digestion and polishing of the nutrient rich solution by use of the biopolymer chitosan as flocculant.

Materials and methods

The aquaponic facility and sludge collection

Sludge was collected from swirl separators and filter backwash-water at an aquaponic research facility of the Norwegian Institute of Bioeconomy Research (Landvik, Norway). The aquaponic facility is a closed system based on RAS-technology connected to tanks for production of vegetables, consisting of two separate identical test units (unit A and unit B). Figure 1 show a flowchart of one of the two units. The total water volume of each unit was 16 m^3, divided into four fish tanks with a volume of 0.63 m^3 each, and two plant compartments with a volume of 3 m^3 each. Outside each fish tank, a swirl separator was mounted to collect uneaten fish feed and feces directly from the outgoing water. A pumping sump for each unit provided water to the fish tanks, plant compartment and water treatment system. The water treatment system consisted of aeration, heating/cooling, a combined particle- and biofilter (Polygeyser Bead Filter, New Orleans, USA), swirl separators and a pH-control unit (figure 1).

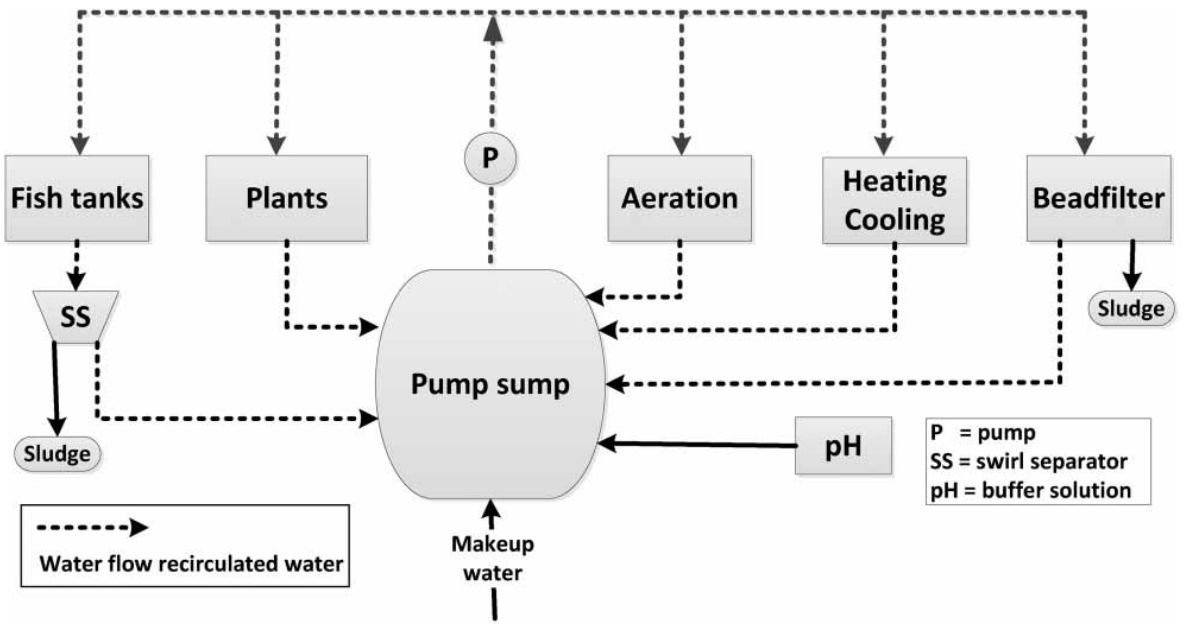

Figure 1. Flowchart of one of the two identical units of the aquaponic research facility, showing water flows, water treatment and sludge removal sites. The facility co-produced fish and vegetables with 100% recirculation of water.

During the experimental period, the fish tanks were stocked with brown trout (Salmo trutta) and salmon (Salmo salar). It was a total of 530 brown trout with an average weight of 105g in unit A, and 260 salmon with an average weight of 340g in unit B. The daily amount of feed given was 280g for each unit, distributed evenly to each tank by automatic feeders. The plant growing compartments were stocked with lettuce (Lactuca sativa L., Batavia-type, cv. 'Partition') in a deep-water floating raft system. The plant compartments were operated in a continuous production mode with weekly harvesting of lettuce heads with an average weight of approximately 150g. The harvesting took place seven weeks after seeding. The production weight ratio of fish biomass to lettuce biomass was 1:7.

The total sludge production from the aquaponic facility (from unit A and unit B) was approximately 20 liters per day. The sludge consisted of backwash-water from the two bead filters, and drainage from the eight swirl separators. The sludge from unit A and unit B was transferred and mixed in a common aerated tank, from where sludge for this study was collected. The daily amount of makeup water supplied to the system was only to compensate for evaporation and transpiration losses, and the amount of sludge taken out from filters and swirl separators.

Batch aerobic digestion

The sludge was transferred to duplicate 25 L polyethylene batch reactors covered with a lid to prevent evaporation and equipped with diffusors which provided aeration at a rate of 20 l min^{-1}. After four weeks of AD, the experiment was terminated, and chitosan precipitation was applied for removal of solids and analysis for characterization of the clear phase. During the experimental period, the pH was monitored daily and maintained at 7.0±0.3 using small amounts of a dilute NaOH- and HCl-solutions. In addition, the oxygen concentration was measured regularly. A volume of 250 ml was sampled weekly from each reactor for determination of total suspended solid (TSS), volatile suspended solid (VSS), chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD), total nitrogen (TN), total phosphorus (TP) and soluble nutrients (NH_4-N, NO_2-N, NO_3-N, P, K, Cu, Zn, S, Mn, Mg, Ca Mo, B, Fe). Samples for soluble nutrients and SCOD were centrifuged at 4500 rpm for 3 min and filtered by membrane filters with pore openings of 0.45 µm before analysis.

Solids separation

To remove residual particles from the clear-phase after AD, jar-tests with flocculation and sedimentation were performed. A commercial chitosan (Kitoflokk High from Teta Vannrensing AS, Norway) was used as coagulant at different concentrations (15, 25, and 40 mg/L), and compared to the performance of a synthetic cationic polyacrylamide polymer for sludge dewatering (Superfloc C-496 HMW from Kemira AB, Sweden). The molecular weight of the Kitoflokk High was 161 kD, and the degree of deacetylation was 80%. Chitosan stock solution (0.25%) was prepared by mixing 2.5 g chitosan powder with 100 ml distilled water, and then adding drops of a 30% HCl-solution under continuous stirring until the chitosan was dissolved. Superfloc C-496 HMW stock solution (0.25%) was prepared by adding 2.5g of powder to 100 ml distilled water under continuous stirring until the polymer was dissolved. Sludge samples were transferred to 1-litre beakers and placed in a semi-automatic jar-test device (Kemira Kemwater, Helsingborg, Sweden). Coagulant was added during rapid mixing, and pH was adjusted to 6.0±0.2 by adding drops of a dilute HCl or NaOH solution. After 60 s of rapid mixing (400 rpm), followed by 10 minutes flocculation under slow stirring (30 rpm) and 20 minutes sedimentation, water samples were carefully siphoned from the clear phase for analysis of turbidity and TSS.

Analytical methods

TSS, VSS, color, turbidity, pH and oxygen were measured in the laboratory of University of Agder according to Norwegian and European Standards. TSS and VSS were measured using pre-weighed 1.6 µm Whatman GF/A glass fiber filters. Color was determined using a calibrated Hach DR3900 spectrophotometer (Loveland, CO, USA), turbidity by using a calibrated Hach 2100Q turbidimeter (Loveland, CO, USA), pH by a calibrated Jenway 3150 instrument (Cole-Parmer, UK), and oxygen concentration by a calibrated Hach HQ40d instrument (Loveland, CO, USA). Soluble nutrients (P, K, Cu, Zn, S, Mn, Mg, Ca Mo, B, Fe) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES) according to accredited standards by the LMI laboratory (Sweden). Ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) were determined using QuAAtro continuous segmented flow autoanalyzer. For TN and TP, samples were dissolved by nitric acid microwave

extraction and analysed by inductively coupled plasma atomic emission spectrometry (ICP-OES) according to European Standards (DIN EN ISO 11885) at the Eurofins laboratory, Norway.

Statistics

The AD experiments and the polymer flocculation experiments were repeated two times. Mean values with standard deviations are presented. Where standard deviation bars are not observable on the graphs, they do not extend beyond the dimensions of the symbols.

Results and discussion

Sludge characterization

The sludge used in this study was a mixture of filter backwash-water and solids from the swirl separators of unit A and unit B in the aquaponic facility. The sludge was a dilute suspension with a solid content below 1%, mainly composed by organic matter with TSS and VSS concentrations of 8.7 g/L and 8.5 g/L, respectively. The total COD concentration was 6000 mg/l with a minor part of 147 mg/l on soluble form. Most of the essential nutrients N and P were associated with solids, even though the TSS only comprised 0.87% of the sludge (8.7 g/l). For nitrogen, the dissolved compounds (NH_4-N, NO_2-N and NO_3-N) comprised approximately 26% of the total N, with the majority on reduced form as NH_4^+. For phosphorus, only 6% of the total P was on soluble form. The suspended solids in recirculating systems are generated from feed waste, feces, and bacteria. Aquaculture solids in recirculating systems are characterized by great size variation from centimeters (cm) to microns (µm). The size variability of suspended solids is due to the ability of feed waste and feces to break down in the water column by water turbulence, fish motion and pumping (Timmons et al. 2018). Chen et al. (1993) reported that 90% of the total particles in RAS are less than 30 µm. With good feeding practice and operation, the feed waste can be reduced, and most of the waste can be taken out directly after the fish tanks by mechanical solid removal, preventing it from being broken down and suspended by turbulence and pumping.

Table 1. Characteristics of the aquacultural sludge mixture collected in duplicate from swirl separators and filter backwash-water.

Parameter	Mean values	Standard deviations
pH	7.9	±0.01
TSS (g L^{-1})	8.7	±0.1
VSS (g L^{-1})	8.5	±0.35
COD (mg L^{-1})	6000	±800
SCOD (mg L^{-1})	147	±5
Total P (mg L^{-1})	135	±5
Total N (mg L^{-1})	705	±15
Soluble nutrients		
B (mg L^{-1})	0.14	±0.01
Ca (mg L^{-1})	53.9	±0.75
Cu (mg L^{-1})	0.03	±0.01
K (mg L^{-1})	62.5	±0.5
Mg (mg L^{-1})	21.9	±0.20
Mn (mg L^{-1})	0.24	±0.01
Mo (mg L^{-1})	0.01	±0.003
NH_4-N (mg L^{-1})	181	±2
NO_2-N (mg L^{-1})	0.03	±0
NO_3-N (mg L^{-1})	0.10	±0
P (mg L^{-1})	8.2	±0.25
S (mg L^{-1})	26	±1
Zn (mg L^{-1})	1.65	±0.05

Aerobic digestion

After three weeks of AD, the TSS and VSS in the raw sludge were reduced by 31.6% and 48.5%, respectively (figure 2). The reductions in TSS and VSS were modest and in line with those reported from domestic wastewater treatment. It has been stated that solid reduction during aerobic stabilization is not expected to be higher than 40%, even after a long digestion time (Foladori et al. 2010). As shown in figure 3, the average content of total COD was reduced from 6000 mg/L to 3000 mg/l after three weeks of AD, which indicates that organic solids (VSS) were solubilized and oxidized. According to literature, 60–70% of the oxidized organic matter can be converted into new cellular biomass (Foladori et al. 2010). When all biodegradable organic matter is depleted, die-off and bacterial decay will generate new soluble matter which can be used for metabolism, generating new biomass (Schultz et al. 1999; Foladori et al. 2010). The slight observed increase in TSS and VSS on day 21 may be explained by bacterial growth induced by release of metabolites by bacterial

decay towards the end of the period (figure 2). This was supported by a pH drop after 15 days of AD (data not shown) which indicated high endogenous metabolism activity with a high alkalinity demand (Bailey 2008).

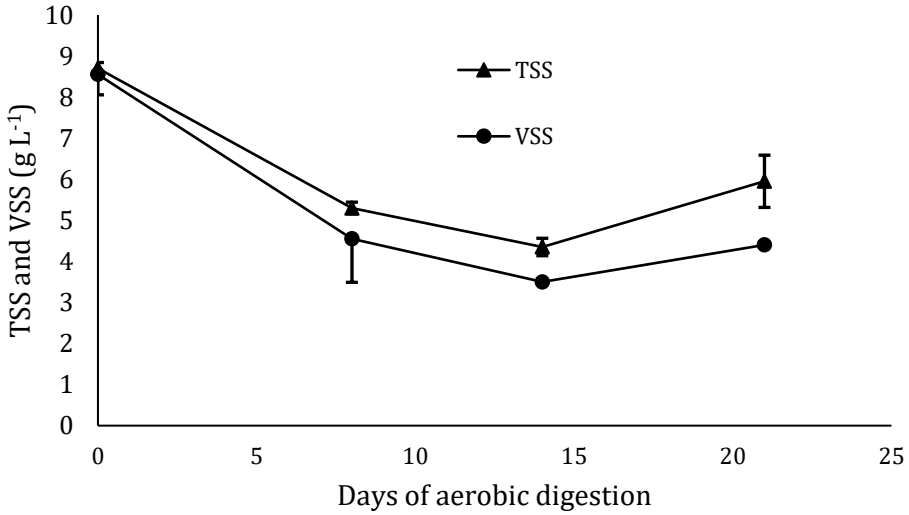

Figure 2. Concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) during aerobic digestion. Bars represent standard deviations.

The initial level of SCOD comprised only 2.5% of the total COD and increased from 147 mg/l to 288 mg/l during the three weeks of AD (figure 3). This increase can be explained by the accumulation of non-degradable soluble organic compounds in the reactors. It is known that for increasing sludge retention time, inert and endogenous residue fraction will accumulate due to biomass decay (Foladori et al. 2010). As much as 20 to 25% of cell material is composed of inert inorganic and organic compounds that are not biodegradable (Bailey 2008). It has also been reported that when most of the biomass has been broken down after aerobic stabilization, a high concentration of SCOD can remain in the sludge (Foladori et al. 2010), which support the increase in SCOD observed in our study. The data show that raw sludge from aquaculture can be digested by solubilizing some of the organic solids and oxidizing organic matter, and simultaneously release nutrients bound to solids.

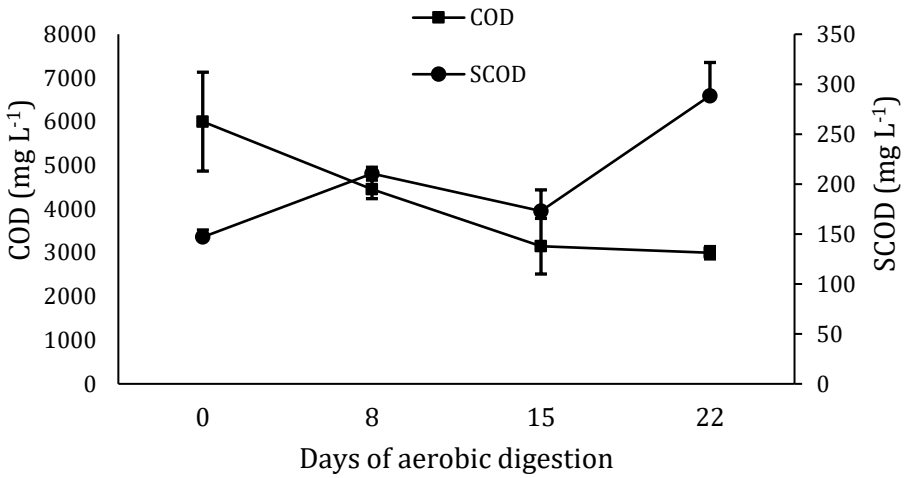

Figure 3. Concentrations of chemical oxygen demand (COD) and soluble oxygen demand (SCOD) during aerobic digestion. Bars represent standard deviations.

Solid separation after AD

For removal of particles after AD, chitosan (Kitoflokk High) was used as coagulant, and compared to the performance of a synthetic polymer (Superfloc C-496 HMW). Results of the jar-tests are shown in figure 4a and 4b. Compared to the synthetic coagulant, chitosan showed better TSS and turbidity removal at lower dosages. By using a dose of 15 mg/l chitosan, reductions of TSS and turbidity by 91% and 96%, respectively, were experienced. The same dosage of the synthetic coagulant resulted in higher TSS and turbidity concentrations. Even higher dosages of the synthetic polymer (up to 40 mg/l) did not improve the treatment efficiency beyond the results of 15 mg/l chitosan. Increasing the chitosan dose above a threshold limit did not increase the treatment efficiency. The lower required dosage of chitosan compared to the synthetic polymer will compensate for some of the higher cost of chitosan. Costs of bulk qualities obtained from suppliers indicate 4.5 Euro/kg for the polyacrylamide (Superfloc C-496 HMW) and 25.0 Euro/kg for the chitosan (Kitoflokk High). This implies that chitosan still is more expensive, even if the required dosage is 3 times lower than the dosage of the polyacrylamide.

Color removal by chitosan and the synthetic coagulant was also evaluated. As shown in figure 5, chitosan exhibited better color removal at all dosages than the synthetic polymer. A dose of 15 mg/l of chitosan allowed a reduction of color by 44.5%. With

the same dose of the synthetic coagulant, the color was only reduced by 13%, leaving a yellowish color in treated water. The yellow color in water is normally associated with dissolved organic compounds and can be difficult to remove entirely by polymers. Even the highest dosage of chitosan did not achieve better color removal efficiency than 49.4%. For the purpose of using the treated water in aquaponic systems, this may not engender a problem since it is indicated in literature that the presence of dissolved organic matter in the nutrient solution at low concentration promotes plants growth and nutrient uptake and consequently lead to higher yield (Goddek et al. 2016).

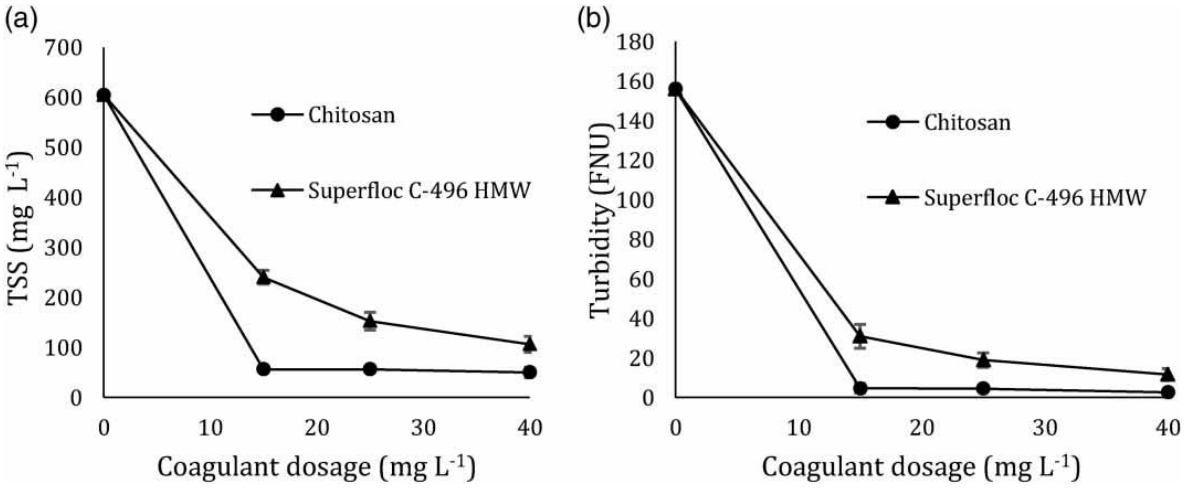

Figure 4. Concentrations of TSS (a) and turbidity (b) in the clear-phase after treatment with increasing doses of chitosan and Superfloc C-496 HMW. Bars represent standard deviations.

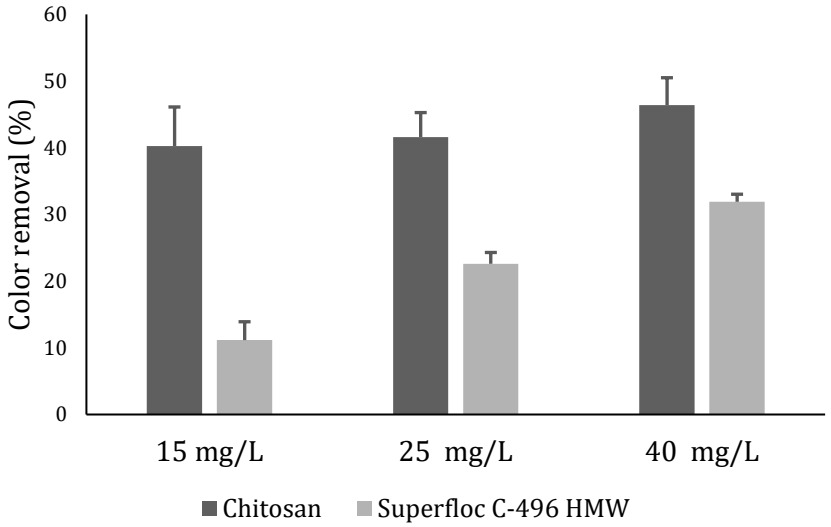

Figure 5. Color content in the clear-phase after treatment with increasing doses of chitosan and Superfloc C-496 HMW. Bars represent standard deviations.

Soluble nutrients concentrations after AD and after solid separation

The study showed that AD of the sludge was able to mobilize the majority of essential nutrient from particle associated to soluble forms (table 2). The soluble micro- and macronutrients in raw sludge before AD, after three weeks of AD, and after four weeks of AD and chitosan precipitation are shown. The concentrations of dissolved N increased from 181 mg/l before AD to 890 mg/l after three weeks of AD, and to 903 mg/l after four weeks of AD and chitosan precipitation. The corresponding numbers for dissolved P was 8.2 mg/L before AD, and 110 mg/L and 160 mg/l after three and four weeks of AD, respectively. This implied 84% mobilization of N and 63% mobilization of P after three weeks when related to the total N and total P concentrations. The corresponding mobilizations after four weeks were 79% of N and 78% of P. Increases in total concentrations of N and P (TN and TP) were experienced from start to the end of the four-week period, which was attributed to a volume decrease of approximately 15% due to evaporation and the volume decrease caused by sampling. The high percentages of mobilization indicate low content of undissolved N and P in the residual TSS and VSS. In untreated aquacultural sludge, the N content has been measured to 2.9% of TSS (Maillard et al., 2005). After three weeks, the TSS concentrations was 5950 mg/l (figure 2) which correspond to an undissolved N amounts of 172 mg/l when assuming a N-content of 2.9% of solid. This

value corresponded reasonably well to the difference between the TN concentration of 1050 mg/l and the soluble N concentration of 890 mg/l after three weeks of AD, which is 160 mg/l.

There are limited results from the literature when it comes to mobilization of nutrient from aquacultural waste by aerobic treatment. From published studies, low degree of mineral solubilization was experienced (Monsees et al. 2017; Delaide et al. 2018). Monsees et al. (2017) investigated the nutrient mobilization from aquacultural sludge collected from a clarifier during a 14 days aerobic treatment period. No total elevation in soluble N-compounds was found, while an increase of dissolved P from 9.4 to 29.7 mg/L was experienced. These findings are in contrast to our results which showed the following increases in N and P: 4.4- and 9.8-fold after two weeks of AD (data not shown), 4.9- and 13.4-fold increases in N and P after three weeks of AD, and 5.0- and 19.5-fold increases after four week AD and solid separation (chitosan flocculation) (table 2). I the study of Monsees et al. (2017), low pH may have restricted bacterial activity and thereby solubilization of nutrient. The pH was not controlled and dropped from 6.2 to 5.3 during the AD-period, while we controlled the pH to 7.0±0.3. Because N is a key component in proteins, the increase of dissolved N in the sludge during aerobic digestion is expected to be caused by the hydrolysis of fish feed proteins by extracellular enzymes excreted by microorganisms (Schultz et al. 1999).

In our study, all other dissolved macro- and micronutrients increased during AD, most of them to concentration higher than mineral levels recommended for hydroponic growth systems (table 2). Potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and boron (B) exhibited elevations in concentrations by factors of 1.7, 3.2, 2.0, 2.7, and 1.6, respectively after three weeks of AD, and by factors of 2.1, 5.0, 3.1, 3.1 and 21.8 after four weeks of AD and chitosan flocculation (table 2). The strong increase in boron concentration from week three of AD to week four of AD was unexpected. The increase may have been related to leakage from the chitosan added for flocculation. It is known that the marine environment is relatively rich in boron compared to the terrestrial environment (Carrano et al. 2009). A high concentration of boron in the final nutrient solution was regarded as positive since boron is an essential element for plant growth.

The results show that AD has a great potential to solubilize nutrients from aquacultural sludge. It has been suggested that divalent cations bound to polysaccharides are

released during aerobic digestion by destruction of flocs and solubilization of biopolymers (Novak et al. 2003). After four weeks of AD and chitosan flocculation, approximately 80% of the sludge could be reclaimed as clear-phase rich in dissolved nutrients as shown in table 2. The chitosan did not remove the macro- or micronutrients previously dissolved by AD, except of a 39% reduction in Zn concentration, and modest declines in Cu and Mo concentrations (table 2). However, the level of Zn was still 10 times higher than the recommended value for lettuce growth in hydroponic systems, while Cu concentration was slightly below recommended level (table 2). For N and P, the final concentrations were 4.8 and 4.0 higher than recommended concentrations for lettuce growth, respectively.

Table 2. Concentrations of soluble macro- and micronutrients, and metals, in raw sludge, after AD, and after AD and chitosan precipitation compared to levels in a standard nutrient solution for hydroponic growth of lettuce (Libia et al. 2012).

	Before AD		After AD (3 weeks)		After AD (4 weeks) and chitosan flocculation		Standard nutrient
	Mean	St.dev.	Mean	St.dev.	Mean	St.dev.	solution
Macronutrients							
N, mg L^{-1}	181	±2.8	890	±16.3	903	±15.5	186
P, mg L^{-1}	8.2	±0.35	110	±0	160	±0	40
K, mg L^{-1}	62.5	±0.70	110	±0	130	±0	156
Ca, mg L^{-1}	53.8	±1.06	170	±14.1	269	±3.5	168
Mg, mg L^{-1}	21.9	±0.28	44.5	±3.5	66.8	±1.13	42.0
S, mg L^{-1}	26.0	±1.4	70.5	±3.5	80.5	±0.70	60.0
Micronutrients							
Zn, µg L^{-1}	1650	±71	1750	±70	1050	±71	100
B, µg L^{-1}	140	±14	270	±35	3050	±70	450
Cu, µg L^{-1}	30	±21	49	±15	38	±4	50
Fe, µg L^{-1}	1000	±0	1300	±140	1900	±0	2000
Mn, µg L^{-1}	240	±14	205	±21	330	±0	200
Mo, µg L^{-1}	12	±4.9	31	±18	13	±1.4	40
Other metals							
Al, µg L^{-1}	<9	±0	62.5	±19	15.5	±9	-
Cd, µg L^{-1}	3	±0.10	4.2	±1.2	2.2	±1.1	-
Ni, µg L^{-1}	5.7	±1.0	12.5	±2.1	15	±5.6	-

Aluminium (Al) and cadmium (Cd) were detected in the sludge after three weeks of AD in concentrations of 62.5 µg/L and 4.2 µg/L, respectively (table 2). Al is toxic to plants in low concentrations by inhibiting plant cell growth and division (Imadi et al. 2016). Cd is a heavy metal with high toxicity to crops. It is easily absorbed by plant roots and alters the cellular, molecular, biochemical, and physiological mechanisms

of plants which affect plant growth and morphology (Shanmugaraj et al. 2019). The literature do not give limits for concentrations for Al and Cd in nutrient solution for hydroponic systems, but it is argued that Al in ionic form with +3 charge, which is considered the soluble form, is harmful to plants even at micromolar concentrations, and that Cd is phytotoxic at even lower concentrations (Imadi et al. 2016; Shanmugaraj et al. 2019). After chitosan precipitation, the concentrations of Al and Cd were reduced to 15.5 µg/L and 2.2 µg/L, respectively, which correspond to 75% and 48% removal efficiencies. Chitosan is considered as a good metal chelator (Zhang et al. 2016), and the ability to remove Cd is consistent with the results reported by Bailey et al. (1999) who found that chitosan has high specificity for heavy metals of environmental concern (e.g. Pb, Hg, Cd, Cr). Interestingly, chitosan treatment did not reduce the concentrations of metals essential for plant growth, including Mn and Fe (table 2). Such limited removal of some metals by chitosan flocculation has been explained by Guibal (2004). It was argued that the interaction of metal ions with dissolved chitosan did not lead to the formation of settleable flocs. The metal-chitosan complex remained in solution. It was further pointed out that due to the poor removal capacity by coagulation and flocculation, chitosan is mostly used to chelate metal ions in a variety of solid forms, such as beads, flakes and membranes (Guibal 2004).

Conclusions

This study shows that aerobic digestion (AD) followed by solids precipitation using chitosan as flocculant is a promising method for mobilizing and reuse of nutrients in sludge from aquaculture. The resulting nutrient solution can be used as fertilizer in soilless growth systems. During AD, the concentrations of soluble N increased from 181 mg/l at start to 890 mg/l after three weeks of AD, and to 903 mg/l after four weeks of AD and solid removal by chitosan precipitation. The corresponding concentrations for dissolved P was 8.2 mg/L at start to 110 mg/L after three weeks, and 160 mg/l after four weeks of AD and chitosan treatment. This implied approximately 80% mobilization of the total N and P content in the sludge. Other macro- and micronutrients were also mobilized to concentrations close to or higher than levels recommended for hydroponic growth of vegetables. Potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and boron (B) exhibited elevations in concentrations by factors of 1.7, 3.2, 2.0, 2.7, and 1.6, respectively after three weeks of AD, and by

factors of 2.1, 5.0, 3.1, 3.1 and 21.8 after four weeks of AD and chitosan flocculation. For removal of solids and color after AD, the biopolymer chitosan proved to be a good alternative. After coagulation, flocculation and sedimentation, the turbidity was reduced to an average level of 6.5 FNU. The chitosan did not remove the macro- or micronutrients previously dissolved by AD, except of a 39% reduction in Zn concentration, and modest declines in Cu and Mo concentrations. Another positive side effect of chitosan was removal of potential phytotoxic metals, including Al and Cd removals by 75% and 48%, respectively. After AD and chitosan treatment, approximately 80% of the raw sludge could be reclaimed as a nutrient rich clear phase to be supplied to recirculating hydroponic or aquaponic systems. The effect of the reclaimed nutrient solution on plant growth in such systems should be further studied.

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Paper E:

Hydroponic Lettuce Cultivation Using Organic Nutrient Solution from Aerobic Digested Aquacultural Sludge

This paper has been published as:

Ezziddine, M.; Liltved, H.; Seljåsen, R. Hydroponic Lettuce Cultivation Using Organic Nutrient Solution from Aerobic Digested Aquacultural Sludge. Agronomy 2021, 11, 1–13; doi:10.3390/agronomy11081484

Hydroponic Lettuce Cultivation Using Organic Nutrient Solution from Aerobic Digested Aquacultural Sludge

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Abstract

The aim of this study was to demonstrate how aquacultural sludge can be processed and utilized as an organic nutrient solution (ONS) for hydroponic lettuce production. By using a previous developed method, approximately 80% of the processed sludge was reclaimed as a clear, nutrient-rich solution. The performance of the recovered nutrient solution on lettuce growth was assessed in a nutrient film hydroponic system. The results were compared to the results obtained using a conventional nutrient solution (CNS). Yield, fresh weight, water consumption, and nutrient and heavy metal content in leaf tissue were measured. In spite of a 16% lower average fresh weight obtained in ONS compared to the weight obtained in CNS, there was no statistical difference of the yield of lettuce among the two nutrient solutions. After the cultivation period, 90% of the lettuce heads grown in ONS exceeded the marked weight of 150 g. Foliar analysis revealed a similar or higher content of all nutrients, except of magnesium and molybdenum in the leaves of lettuce grown in the ONS compared to lettuce grown in the CNS. This study shows that nutrients recovered from aquacultural sludge can be utilized as fertilizer, thereby reducing the dependency on mineral fertilizer in hydroponic and aquaponic systems.

Keywords: organic fertilizer; nutrient recovery; organic nutrient solution; nutrient film technique; aquacultural sludge; heavy metals

Introduction

In hydroponic systems, plants are grown without soil. In ordinary growth systems, soil supports plants' roots and provides water, nutrients, and oxygen to these roots. On the contrary, in hydroponic systems, plant roots are supported by an inert medium in net pots while water and nutrients are delivered via nutrient solution [1]. There are three commonly used hydroponic technologies: media bed hydroponic, nutrient film technique (NFT) and deep-water culture (DWC) technologies. NFT was the hydroponic technology used in this study. NFT systems have been widely discussed and tested since developments in hydroponics in the 1970s [2]. In NFT, seedlings are normally placed in net pots filled with substrates. Net pots are then placed in a trough, channel, gully, or pipe with holes for the net pots. This provides physical support for the plants. Inside the trough, a film of 1-2 cm of nutrient solution flows along the bottom [3]. Part of the roots develops in the film of the nutrient solution while the other part is suspended in the air above, which ensures that the roots receive sufficient quantities of oxygen and have enough air ex-change surface. Pipes are usually made from PVC (since it is inexpensive) and have a white color which reflects light and avoids excessive heating. Pipes can be round or rectangular. Rectangular pipes with a width larger than their height allow for a much larger surface area of the nutrient solution, which increases nutrient uptake and plant growth [3]. Pumps are used to circulate the nutrient solution from the reservoir to the pipes which, preferably, should be positioned on a slope to facilitate nutrient solution flow along the pipe.

Unlike soil culture, which is inefficient in water and nutrient reuse, closed hydroponic systems conserve water and nutrients through the recirculation of the nutrient solution [4]. While hydroponic systems are considered to represent a sustainable method for grow plants [5], the nutrient solution used in hydroponic systems is based on chemical fertilizers which are mined from scarce and non-renewable resources [6]. Recently, there has been an increased interest in organic hydroponics, as the market for organic food continues to grow [7]. Some studies have reported the possibility of growing vegetables using an organic nutrient solution (ONS) [4,8,9].

Several organic fertilizers have been tested in hydroponic systems such as bonito soup, rapeseed oil cake, corn oil cake, fish meal, dried brewer's yeast, fermented tomato foliage, seaweed, and vermicaste derived solutions [5,10]. Phibunwatthanawong and Riddech [9] produced a liquid organic fertilizer for

hydroponic systems from fermented molasses, distillery slop, and sugarcane leaves which had similar growth effect as chemical fertilizers.

However, some challenges in using ONS in hydroponics have been reported, such as a lower growth rate compared to conventional hydroponics and difficulties managing both pH and EC [4,5,7]. Atkin and Nichols [5] showed that lettuce grown in NFT with a conventional nutrient solution weighed 200% more than lettuce grown in ONS derived from liquid fish and liquid seaweed. Similarly, Williams and Nelson [11] reported that the fresh and dry weights of lettuce gown in NFT were lower in organically-fertilized cultivation compared to conventionally-fertilized cultivation.

The direct use of organic fertilizers in hydroponic systems may inhibit plant growth due to high biological oxygen demand in the root zone caused by the presence of dissolved organic carbon compounds [12]. Additionally, most of the nutrients in organic sources, such as waste material from the agricultural and aquacultural industry, are not in ionic forms and, hence, are not directly available for plants. For optimizing the utilization of organic waste for hydroponic plant growth, a solubilization step is required to break down organic matter and mobilize nutrients.

Aquacultural sludge contains high amounts of nutrients, the majority of which are bound to organic matter [13,14]. In order to increase the ratio of plants' available nutrients in aquacultural sludge, aerobic digestion (AD) has been used to solubilize nutrients from organic compounds while breaking them down [15–17]. The solubilization step should be followed by a solid precipitation step in order to obtain a good quality solids-free phase, in compliance with water quality guidelines for hydroponic and aquaponic systems. Chitosan, which is a natural polymer made from shrimp and crab shells, has been shown to be an effective flocculant for solids precipitation from aquacultural sludge [16]. Unlike other metal-based coagulants and synthetic polymers for the flocculation and precipitation of solids, chitosan does not combine chemically with dissolved phosphate and has no health concerns [18].

In addition to nutrients for plant growth, aquacultural sludge may also contain heavy metals which can limit the use of sludge as fertilizer. Heavy metals may accumulate to levels exceeding permissible content in crops for human consumption. In recirculating aquacultural systems (RAS), heavy metals may enter with the feed, could leach from pipes and fittings, or could be carried into the system with the make-up water [19].

The aim of this study was to demonstrate how aquacultural waste can be processed and utilized as a valuable nutrient solution for hydroponic growth. Aquacultural sludge was processed by AD, and clarified by chitosan flocculation and sedimentation, as described by Ezziddine et al., 2020 [16]. The performance of the recovered ONS solution on lettuce growth was assessed by cultivation experiments where the fresh weight of lettuce, yield, water consumption, and nutrient and heavy metal content in the leaf tissue were measured. The results were compared to the results obtained by use of a conventional mineral nutrient solution.

Materials and Methods

The Aquaponic Facility and Sludge Collection

For this study, 120 L of sludge was collected from an aquaponic research facility at the Norwegian Institute of Bioeconomy Research (NIBIO, Landvik, Norway). The aquaponic facility is a closed system based on RAS technology connected to tanks for the pro-duction of vegetables and consists of two separate identical test units (unit A and unit B). Figure 1 show a flowchart of one of the two units. The total water volume of each unit was 8 m^3, divided into two fish tanks with a volume of 1.2 m^3 each, and two plant compartments with a volume of 3 m^3 each. A swirl separator was mounted outside each fish tank to collect uneaten fish feed and feces directly from the outgoing water. A pumping sump for each unit provides water to the fish tanks, plant compartment, and water treatment system. The water treatment system consists of swirl separators, a combined particle and biofilter (Polygeyser Bead Filter, New Orleans, USA), a heating/cooling unit, an aeration unit, and a pH-control unit (Figure 1). The fish tanks were stocked with 530 brown trout (Salmo trutta) with an average weight of 97 g. The daily amount of feed given was 300 g, 75 g of which was distributed evenly to each tank by automatic feeders.

The sludge which consisted of backwash-water from the bead filters and drainage from the swirl separators was transferred and mixed in a common aerated tank from where sludge used for this study was collected.

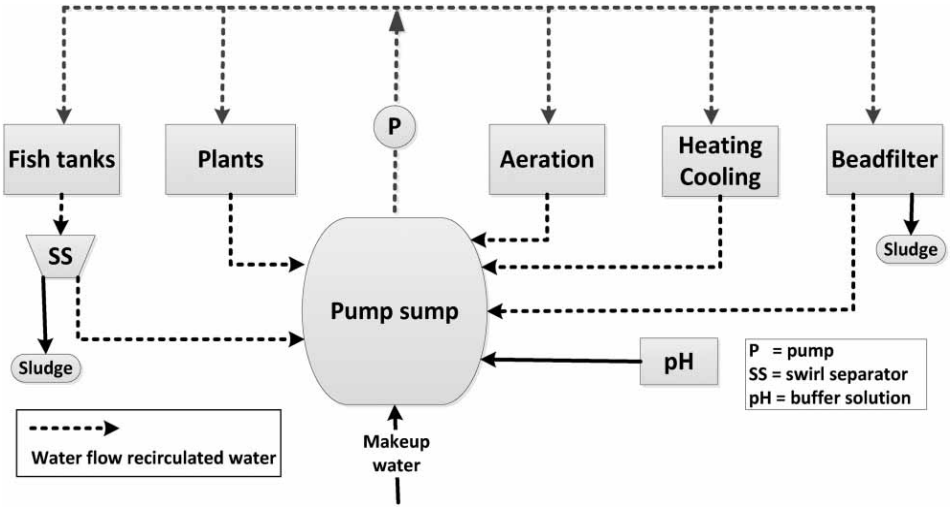

Figure 1. Flowchart of one of the two identical units of the aquaponic research facility showing water flows, water treatment, and sludge removal sites. The facility co-produced fish and vegetables with 100% recirculation of water.

Aerobic Digestion and Solid Separation

For AD, the sludge was distributed to six 25 L polyethylene batch reactors covered with lids to minimize evaporation. During the four-week digestion period, each reactor was aerated at a continuous rate of 20 L min^{-1}, provided by diffusors and a common blower. The temperature was kept constant at 22 °C. The pH was measured daily in all re-actors and maintained at approximately 7 by using solutions of HNO_3 (60%) and KOH (20%) for adjustment. The sludge was characterized before and after the four-week AD period. Representative 0.5 L grab samples were collected from each reactor and transferred to a common beaker. Well mixed duplicate composite samples for analysis were collected from the beakers. The samples were analyzed for total suspended solids (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen (TN), and total phosphorus (TP).

For removal of residual solids from the clear phase after AD, flocculation and sedimentation, using chitosan as coagulant, was conducted. According to experiences from previous research with similar sludge [16], a commercial chitosan (Kitoflokk High from Teta Vannrensing AS, Norway) at a dosage of 15 mg L−1 was applied. The chitosan had a molecular weight of 161 kD and a degree of deacetylation of 80%. Chitosan stock solution (0.25%) was prepared by mixing 0.25 g of chitosan powder

with 100 mL of distilled water, and then adding drops of a 60% HNO_3 solution under continuous stirring until the chitosan was dissolved. The chitosan solution was added to the aerobic digested sludge during rapid mixing (400 rpm) and adjustment of the coagulation pH to approximately 6. After 60 s of rapid mixing, followed by 10 min of flocculation under slow stirring (30 rpm) and 20 min sedimentation, samples were collected from the clear phase for analysis of solubilization of nutrients during the four-week AD. 0.5 L grab samples were collected from each reactor both before the four-week AD period and after AD and solid separation. From the mixture of grab samples, composite samples were collected, and membrane were filtered by using Whatman GFC 0.45 µm filters prior to analysis of the following nutrients: ammonium (NH_4-N), nitrate (NO_3-N), phosphorous (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), boron (B), and iron (Fe). A sample from the concentrated sludge (precipitated solids) was also taken for TSS, BOD, COD, TP, and TN analysis. The clear water was stocked in the fridge for use as ONS for hydroponic lettuce growth.

Lettuce Growth

The growth performance of lettuce in the nutrient solution prepared from aerobic digested aquacultural sludge was compared to growth in conventional mineral nutrient solution. The experiments were carried out in a growth room located at the University of Agder in Norway. The study with the ONS was carried out from the 7 of October to the 19 of November 2020, while the study with CNS was carried out from 27 of September to the 4 of December 2018. The studies were conducted in triplicate experimental set-ups under identical conditions. The temperature of the growth room was in the range of 21–24 °C, the CO2 concentration was in the range 450–580 ppm, and the relative humidity was 40–55%.

The growth systems consisted of a seeding system and a closed loop NFT system. In the seeding system, seeds of Lactuca sativa L. (Batavia-type, cv. “Partition”) from LOG AS, Norway, were seeded in Grodan rockwool cubes (36 × 36 × 40 mm) and placed in a tray filled with nutrient solution and illuminated with LED-light. After two weeks in the seeding system, the seedlings in the rockwool cubes were inserted into net pots and trans-planted to the NFT-system which consisted of three identical parallel units. Each unit consisted of a rectangular PVC-pipe (240 cm long, 10 cm

width and 50 cm height) with 12 holes (each with a diameter of 45 mm), and a 20 L plastic container filled with nutrient solution. From the plastic container, the nutrient solution was supplied intermittent (30 min on/off cycles) to the PVC-pipe by a submerged pump at a flow rate of 3.5 L min−1. The hydroponic system was illuminated 18 h per day with LED-light transmitting photosynthetically active radiation (PAR) of a photon flux density of 220 µmol m−2 s−1.

The pH and EC of the three parallel systems were monitored and adjusted every second day. The target values of pH and EC were 6.0 and 1200 µS cm^{-1}, respectively. Soluble N and P in the nutrient solution were measured two times per week using a Hach DR3900 spectrophotometer (Loveland, CO, USA). After four weeks in the NFT-systems, lettuce was harvested and weighed. Leaf tissue samples were collected for nutrients content and heavy metal analysis. Samples from the remaining nutrient solution were also taken for soluble nutrient analysis.

Analytical Methods

TSS, TN, TP, BOD, and COD were determined by Eurofins laboratory, Norway. For TP and TN, the nutrients were dissolved by nitric acid microwave extraction and analyzed using ICP-OES according to European standards (DIN EN ISO 11885). Soluble nutrients (P, K, Cu, Zn, S, Mn, Mg, Ca, Mo, B, Fe) were analyzed using inductively coupled plasma optical emission spectrometry (ICP-HSP) according to accredited standards by the Eurofins laboratory, Netherlands. Leaf tissue was collected from different parts of the lettuces to avoid biases due to uneven nutrient distribution within the plants. The nutrients and metals were dissolved by nitric acid microwave extraction and analyzed by inductively coupled plasma atomic emission spectrometry (ICP-OES) according to European standards (DIN EN ISO 11885). Operational parameters including turbidity, pH, EC, and TSS of the nutrient solution were measured in the laboratory of University of Agder according to Norwegian and European standards. TSS was measured using pre-weighed 0.45 µm Whatman GF/C glass microfiber filters. Turbidity was determined using a calibrated Hach 2100Q turbidimeter (Loveland, CO, USA), while pH and EC were measured using a calibrated Hach HQ40d instrument with standard pH and EC sensors. All samples were stored in a refrigerator at 4 °C and sent immediately after sampling in insulated cooler bags to the laboratory.

Data Analysis and Statistics

Data were processed in Microsoft Excel (2016) and were subjected to analysis of variance (ANOVA) using SPSS Version 25. Mean differences were determined by Tukey's honestly significant difference (HSD) test at $p < 0.05$. Mean values with standard deviations are presented. Where standard deviation bars are not shown on the graphs, they do not extend beyond the dimensions of the symbols.

Results and discussion

Sludge Characterisation Before and After AD

TSS, BOD, COD and nutrient content in aquacultural sludge can vary from one facility to another depending on several factors including fish species, fish density, fish age, fish feed, feeding management, flow regulation, and water treatment (including solid separation technology and sludge handling). The sludge used in this study had a relatively low TSS content of 1.1 g L−1 (Table 1). The suspended solids were generated from fish feces, uneaten food, and biofloc (suspended flocs formed by different microorganisms that ad-here to an organic matrix [20]). The BOD5 concentration of the sludge was 145 mg L−1 while the COD concentration was approximately twelve times higher, indicating a high share of recalcitrant organic matter which is not easily degradable by aerobic microorganisms.

During the four-week period of aerobic degradation, the sludge volume was reduced from 120 L to 101 L due to evaporation (Table 1). To account for the volume reduction of 16%, all of the following calculations were done on a mass basis. As shown in Table 1, TSS was reduced from 1.10 g L^{-1} (which corresponds to a mass of 132.0 g) to 0.46 g L−1 (which corresponds to 46.5 g), which corresponds to a reduction of 64.8% during AD. Al-so, the majority of organic matter in the sludge was oxidized, with BOD and COD reductions of 90.8% and 81.2%, respectively. There was only a small decrease in TP, while an increase in TN of 30.1% was indicated. Such an increase has also been observed during AD in other studies [16], and can partly be explained by nitrogen fixation. It has been re-ported that some free-living heterotrophs can fix significant levels of nitrogen without the direct interaction with other organisms [21]. The remaining solids in the aerobic digested sludge were precipitated using chitosan as a coagulant. After solid removal by coagulation and 15 min of

sedimentation, 96L of the 101 L was recovered as a nutrient rich clear phase for further studies, while 5 L remained as a concentrated sludge (Table 1). The solid content of the clear phase was as low as 6.7 mg L^{-1}, which corresponded to 99.0% solid removal by coagulation and sedimentation. The turbidity of the clear phase was 1.64 FNU, which is in the range of potable water quality.

Table 1. Characteristics of aquacultural sludge before and after aerobic digestion. Mean values are given.

Parameter	Before AD	After AD	Remaining Concentrated Sludge after AD and Solid Separation
Volume of sludge (L)	120	101	5 out of the 101
pH	7.1	7.0	7.0
TSS (g L^{-1})	1.10 (132.0)	0.46 (46.5)	10.00 (50.0)
BOD_5 (mg L^{-1})	145 (17.4)	16 (1.6)	-
COD (mg L-1)	1750 (210)	390 (39.4)	7900 (39.5)
Total P (TP) (mg L^{-1})	92 (11.0)	93 (9.4)	560 (2.8)
Total N (TN) (mg L^{-1})	77.5 (9.3)	120 (12.1)	170 (0.9)

Numbers in bracket represent the mass (g) of the compounds (volume of sludge multiplied by concentration).

Nutrient Mobilization during Aerobic Digestion

In Table 2, the concentrations of soluble macro and micronutrients in the sludge before AD, and in the clear phase after AD and solid separation, are shown. By comparing the masses of soluble P and N (NO_3-N) before AD (Table 2) by the masses of total P and N before AD (Table 1), it is indicated that substantial amounts of these nutrients were associated with solids initially (75.5% of P and 47.7% of N). After AD, all particulate N were mobilized and 62.8% of the total phosphorus was in soluble form. As shown in Table 2, there was a 2.6-fold elevation in the concentration of soluble P, while the corresponding increase for soluble N (NO_3-N) was 3.0-fold. Calculated increases based on mass gave 16% lower values due to the volume decrease by evaporation during AD. It was further shown that all other concentrations of dissolved macro- and micronutrients increased during AD, except Mn. Of the macronutrients, K, Ca, Mg, and S exhibited elevations in concentrations by factors of 1.8, 1.9, 1.8, and 1.4. Among the micronutrients, Fe, B, Cu, and Zn were raised by factors of 1.1, 2.0, 1.5, and 1.5, while there was a decrease in Mn-concentration.

Most of the macronutrients after AD were in concentration ranges recommended for hydroponic growth systems, except for Mg. Also, some of the micronutrients, e.g., B

and Mn, were lower in concentration than recommended. Despite these shortages in some nutrients, the results indicate that aquacultural sludge, after aerobic digestion, has the potential to fulfil the requirements of a nutrient solution for use in hydroponic systems, in line with the previous study conducted by Ezziddine et al. (2020) [16].

Table 2. Concentrations of soluble nutrients in the sludge before and after aerobic digestion. Mean values and standard deviations (STD) are given.

Parameter	Before AD		After AD and Solids Separation		Standard Recommended Range [2]
	Mean	STD	Mean	STD	
Soluble macronutrients					
NH_4-N (mg L^{-1})	<1.5		<1.5		100 to 200
NO_3-N (mg L^{-1})	40.5 (4.9)	1.5	121.7 (12.3)	3.09	
P (mg L^{-1})	22.5 (2.7)	0.5	58.0 (5.9)	3.74	15 to 90
K (mg L^{-1})	104.0	2	185.3	12.36	80 to 350
Mg (mg L^{-1})	9.7	0	17.0	0	26 to 96
Ca (mg L^{-1})	64.0	0	124.0	6.53	122 to 220
S (mg L^{-1})	13.0	0	18.0	1.41	
Soluble micronutrients					
B (mg L^{-1})	0.025	0.005	0.050		0.14 to 1.5
Cu (mg L^{-1})	0.02	0	0.03	0	0.07 to 0.1
Mn (mg L^{-1})	0.085	0.005	0.020	0	0.5 to 1
Mo (mg L^{-1})	<0.01		<0.01		0.05 to 0.06
Zn (mg L^{-1})	0.56	0	0.83	0.03	0.5 to 2.5
Fe (mg L^{-1})	1.15	0.025	1.24	0.05	4 to 10
Others					
Na (mg L^{-1})	18	0	22.3	0.94	
Cl (mg L^{-1})	26.5	1.5	41.3	4.64	

Numbers in bracket represent the mass (g) of the compounds (volume of sludge multiplied by concentration).

Comparative Lettuce Growth Studies

The chemical composition of the CNS and the ONS used in the comparative growth study are presented in Table 3. As shown, there are some differences. Among the macro-nutrients, the concentrations of N, P, K and Ca were higher in the ONS than in the CNS, while Mg and S were slightly lower. Regarding the micronutrients, the concentrations were generally lower in the ONS, except for Zn which was present at a concentration of 0.83 mg L^{-1}.

Table 3. Soluble nutrient content of the conventional mineral nutrient solution and the organic nutrient solution.

	Soluble Concentration (mg L^{-1})												
Parameter	NO_3-N	NH_4-N	P	K	Ca	Mg	S	Zn	B	Cu	Fe	Mn	Mo
Applied ONS	121.7	<1.5	58	185	124	17	18	0.83	0.05	0.03	1.2	0.02	<0.01
Applied CNS	107	4.0	23	140	94	23	23	0.26	0.19	0.07	1.7	0.42	0.04

pH and EC Values During Growth in the NFT-System

At the beginning of the growth studies with lettuce, the pH of the ONS was 6.0, while that of the CNS was 6.2, as shown in Figure 2a. The pH was adjusted every second day to the target pH-value of 6.0. The data points represent the mean pH value of the triplicate NFT-units just before pH adjustment. For the ONS, an increase in pH over time was observed, despite the pH-adjustment to 6.0 every second day. The pH increase was more severe at the end of the experimental period. Unlike the ONS, the pH of the CNS tended to decrease below 6, down to 5.5, during the early phase of the growth period. This was in spite of pH adjustment with KOH. In the literature, pH increase and decrease during growth have been explained by the NH_4-N to NO_3-N ratio of the nutrient solution [2]. By elevating the ratio (increasing NH_4-N), the pH tends to decrease in the nutrient solution. This could be some of the explanation of the observed differences in pH-behavior in our case, since NH_4-N contributed to 4% of the dissolved N in the CNS, while NH_4-N was be-low the detection limit of 1.5 mg L^{-1} in the ONS.

The recovered ONS was used undiluted with initial EC of 1383 µS cm^{-1} (Figure 2b). After 10 days, the EC value was reduced to 1206 µS cm^{-1}. Volumes of ONS was added to maintain the volume of 20 L and EC at approximately 1200 µS cm^{-1}, which was the target EC-value. However, EC continued to decrease despite nutrient additions, and was down to 912 µS cm^{-1} at the end of the experiment.

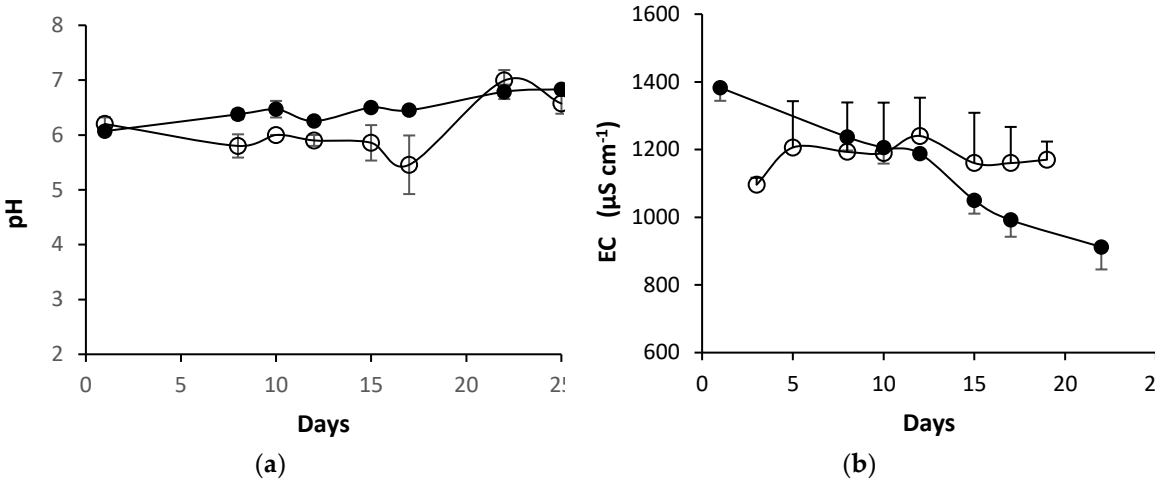

Figure 2. Variation in (a) pH and (b) EC in the organic (●) and conventional (◯) nutrient solution over the 25 days ex-perimental period.

The EC of the CNS was easier to maintain at approximately 1200 µS cm^{-1}, and remained quite stable. However, the average concentrations of the ONS and the CNS throughout the experimental periods were quite similar (1140 µS cm^{-1} and 1170 µS cm^{-1}, respectively).

PO_4-P and NO_3-N Concentrations of the ONS during Growth in the NFT-System

The concentration of NO_3-N and PO_4-P in the ONS during lettuce growth in the NFT-system are shown in Figure 3. At the beginning of the cultivation period, NO_3-N concentration decreased significantly from 120 mgL^{-1} to 63 mgL^{-1}. Then, it continued to decrease slowly until the end of the experimental period to a final concentration of 48 mgL^{-1}.

The concentration of PO_4-P was almost stable over the experimental period in the range of 53–62 mg L^{-1}. No statistically significant differences were detected between individuals PO_4-P concentrations.

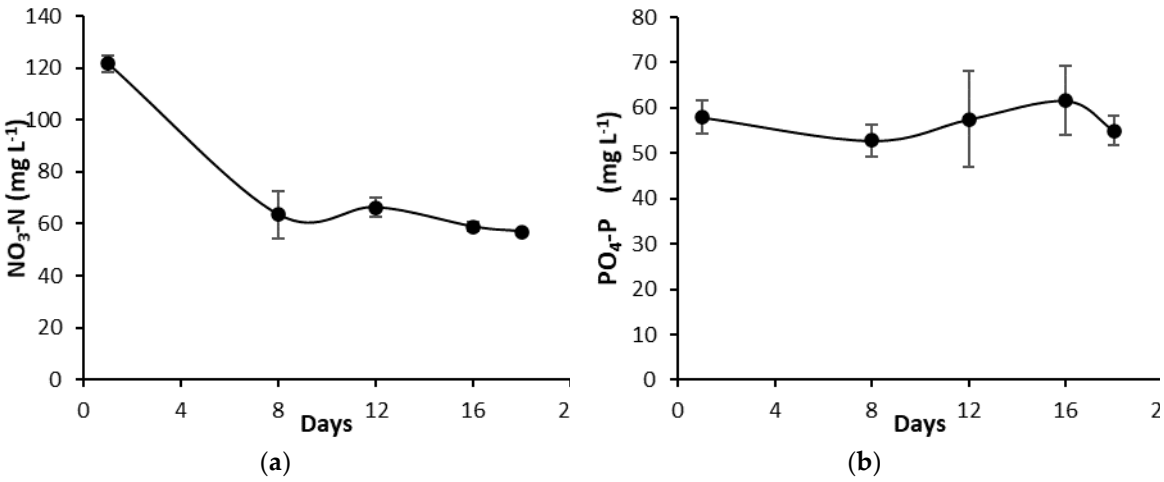

Figure 3. Concentration of (a) NO_3-N and (b) PO_4-P in the organic nutrient solution over the experimental period. Statistically significant differences are indicated by different letters.

Effect of ONS Versus CNS on Lettuce Growth and Yield

Shoot fresh weights of the harvested lettuce yields after five weeks of growth (two weeks in the seedling system and three weeks in the NFT-system) in the two different nutrient solutions are presented in the Figure 4 as box plots. The solid horizontal lines from bottom to top indicate the minimum, first quartile, median value, third quartile, and maximum values. The dots above and below represent outliers and the cross represents the average values. The lettuce grown in the ONS had equal median and average shoot fresh weights (203 g). This means there were similar numbers of plants with weights in the lower range as in the higher range. Weights between the first and the third quartile were from 165 g to 224 g. The lowest shoot fresh weight was 120 g while the highest was 270 g. One lettuce head weighing 345 g was considered to be statistically different and did not fit the fresh weight of shoots set but was still included in the statistical analysis.

The lettuces grown in the CNS had an average shoot weight of 243 g which was slightly higher than the median value of 238 g. This means that most of plants were in the lower range of the yield values. Values between the first and the third quartile were in the range from 210 g to 261 g. The minimum shoot fresh weight was 196 g and the maximum was 322 g. The shoot fresh weights of two lettuce heads (119 and 357 g) were considered as outliers but still included in the analysis.

The average fresh weight obtained in ONS is 16% lower than the average weight obtained in CNS. Nonetheless, 90% of the lettuces grown in the ONS had a shoot fresh weight substantially higher than the marked weight of commercial lettuce heads (150 g). The shoot fresh weights of the lettuces grown in ONS had larger variance than those of the CNS, as reflected by the differences in the length of the boxes (Figure 4).

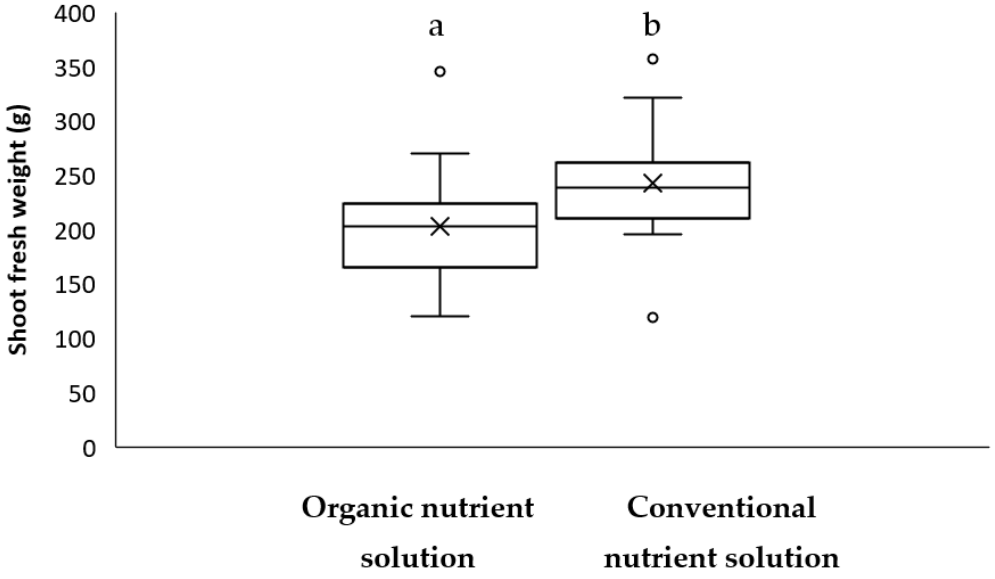

Figure 4. Shoot fresh weight of lettuce grown in organic nutrient solution and in conventional mineral nutrient solution. Different letters indicate statistically significant differences.

In Table 4, the nutrient content in leaves of lettuce grown in the ONS and in the CNS is shown. The lettuce grown in the ONS had a significantly higher concentrations of P, K, Ca, S, and B ($p < 0.05$), and significantly lower concentrations of Mg and Mo ($p < 0.05$) compared to the lettuce grown in the CNS. These differences were also reflected in the nutrient solution concentrations. The ONS was higher in concentration for all these elements, except for S and B (Table 3). In particular, the average concentrations of K and Ca in leaves of lettuce grown in ONS were 2.5 and 2.0-times higher than those grown in conventional solution, respectively. There were no significant differences in N, Cu, Mn, Zn, and Fe concentrations in leaves of lettuce

from the two nutrient solutions. The low content of Mg in lettuce grown in the ONS can also be explained by high concentration of K and Ca. As re-ported by Senbayram et al. (2015), Ca and K in excess may interfere with Mg-uptake, which is known as nutrient antagonism [22].

Table 4. Nutrients content in leaves of lettuce grown in the organic nutrient solution and in the conventional nutrient solution. Mean values and standard deviations (STD) are given. Statistically significant differences are indicated by different letters.

	Organic Nutrient Solution		Conventional Nutrien Solution		Statistical Significance
	Mean	STD	Mean	STD	
Macronutrients (g/kg Dry Matter)					
N	53 [a]	0	38.6 [a]	23.2	1
P	10.26 [a]	0.5	6.4 [b]	0.5	0.002
K	125 [a]	1.6	50 [b]	19.8	0.006
Mg	1.7 [a]	0.08	3.36 [b]	0.33	0.002
Ca	30 [a]	2.16	15 [b]	14.14	0.001
S	3.43 [a]	0.1	2.7 [b]	0.2	0.012
Micronutrients (mg/kg Dry Matter)					
B	34 [a]	2.16	22.33 [b]	1.25	0.003
Cu	5.4 [a]	1.2	6.56 [a]	0.6	0.28
Mn	119 [a]	22.5	143.3 [a]	26.25	0.37
Mo	0.6 [a]	0.04	1.43 [b]	0.4	0.046
Zn	52 [a]	21.23	50.3 [a]	14.38	0.93
Fe	102 [a]	13.95	130 [a]	8.16	0.07

Equal letters (a-a) indicate no statistical differences among mean values, while unequal letters (a-b) indicate significant differences.

The total yield of lettuce grown in the ONS was 3.87 kg m^{-2} while the CNS resulted in slightly higher yield of 5.05 kg m^{-2} (Figure 5). Interestingly, there was no significant difference between them, which means that the performance of the ONS regarding total yield was very close to the performance of CNS for hydroponic lettuce growth. Jordon et al. (2018) reported a yield of 2.18–2.58 kg m^{-2} for hydroponic lettuce grown with CNS, which was lower than the yield obtained in ONS in this study [23]. The average water consumption of the lettuce grown in the CNS was 11.9 L kg^{-1} while the lettuce grown in the ONS consumed 7.3 L kg^{-1} which corresponds to a 61.4% reduction in the water consumption compared to the conventional lettuce (Figure 5). This reduction in water consumption can be explained by higher concentration of Na and Cl in the ONS. The concentrations of Na and Cl were higher by factors of 3 and 25, respectively, in the ONS compared to the CNS. Many studies

have also reported a decrease in water consumption in plants due to salinity increase in the nutrient solution [24].

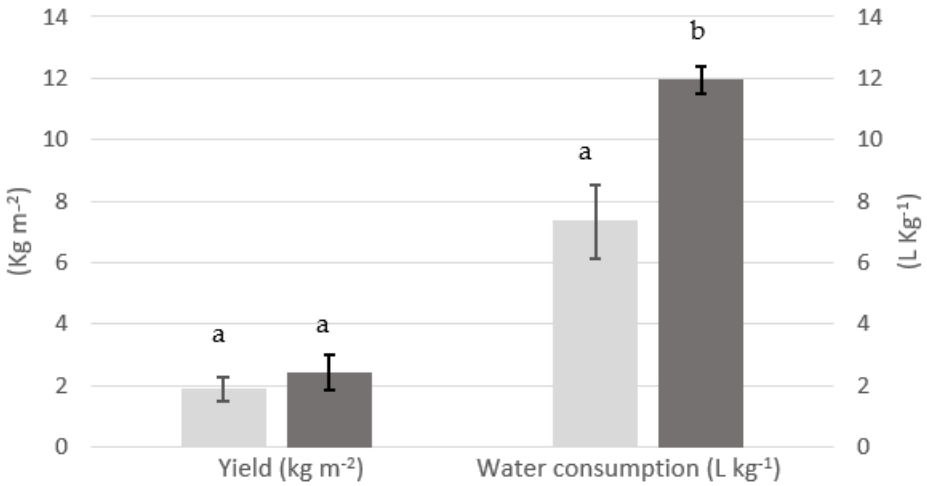

Figure 5. Yield of lettuce and water consumption by use of the organic and the conventional nu-trient solution. Different letters indicate statistically significant differences.

Heavy Metals Content in Lettuce Leaves

As we have seen so far, aquacultural sludge is a valuable source of nutrients, which can replace mineral fertilizers. However, biosolids could possess high levels of heavy metals which can be toxic to plants, animals, and humans. Many authors have documented the absorption and phytotoxic effects of heavy metals on several crops [23].

Table 5 shows the concentrations of heavy metals (Pb, Cd, Ni, Zn, Cu) in the leaves of lettuce grown in the ONS and the maximum permissible concentrations in lettuce for hu-man consumption set by the European Union and The Food and Agriculture Organization of the United Nations (FAO) [24].

As shown in Table 5, the content of the heavy metals Pb, Ni, Zn, and Cu in the leaves of lettuce grown in the ONS were all below the permissible limit for human consumption (Table 5), except for cadmium which was slightly above the maximum concentration. Eissa and Negim [256] also reported a high Cd content that exceed the permissible limit in lettuce grown on metal-contaminated soil. Zubillaga and Lavado [265] showed that lettuce grown in biosolids compost did not accumulate Cd

concentration higher than the permissible limit. The content of lead and zinc were both close to the maximum concentrations. In order to safely use the ONS for lettuce growth, the concentration of heavy metals in the nutrient solution should be reduced. Different heavy metal removal techniques have been reported in literature including adsorption, membrane, chemical, electric, and photocatalytic based treatments [27]. In our case, we can increase the concentration of chitosan in the solid separation step as chitosan is considered to be a good metal chelator [28] which did not reduce the concentrations of metals essential for plant growth such as Mn and Fe [16]. Another startegy to reduce the heavy metal in the ONS is to choose fish feed with low metal content as in RAS, heavy metals may enter with the fish feed [29].

Table 5. Heavy metals content in the leaves of lettuce grown in the organic nutrient solution and maximum permissible limit in lettuce for human consumption [256]. Mean values and standard deviations (STD) are given.

	Heavy Metal Content in Leaves of Lettuce (mg kg^{-1} Dry Weights)		Maximum Permissible Limit (mg kg^{-1} Dry Weights)
	Mean	STD	
Lead (Pb)	0.21	0.06	0.3
Cadmium (Cd)	0.23	0.05	0.2
Nickel (Ni)	<0.01	-	1.5
Zinc (Zn)	52	26.0	60–80
Copper (Cu)	5.4	1.21	40
Arsene (As)	<0,04	-	-
Chromium (Cr)	<1.0	-	-

Conclusions

The current study demonstrated that ONS recovered from aquacultural sludge can be used for lettuce production in a hydroponic system after applying measures to control some heavy metals. The aquacultural sludge proved to be a good source of organic fertilizer. After aerobic digestion, approximately, 90% of the sludge was reclaimed as a clear, nutrient-rich solution that showed good performance on hydroponic lettuce growth. About 90% of the harvested lettuce heads reached or exceeded the marked size of commercial lettuce of 150 g after the five-week growth period. The yield of lettuce (kg m^{-2}) grown in ONS was comparable to the yield of lettuce grown in CNS. Except for Mg and Mn, comparable and even higher content of nutritionally minerals were found in the leaves of lettuce grown in organic fertilizer compared to the lettuce grown in conventional fertilizer. Interestingly, the consumption of water was 7.3 L

per kg for organically-produced lettuce, and substantially lower than for conventionally-produced lettuce. In ONS grown lettuce, some heavy metals (Cd, Pb, and Zn) exceeded or were close to the maximum permissible concentrations in lettuce for human consumption, which indicates that the heavy metal content should be monitored closely when using aquacultural sludge as fertilizer for edible crop. This study suggests a method to recycle aquacultural sludge for use in organic hydroponics. It also recommends the use of aerobic degradation of sludge in aquaponic systems which can provide ONS to be reinserted into the water loop in coupled aquaponics or into the hydroponic system in decoupled aquaponics.

Author Contributions: Conceptualization, M.E. and H.L.; methodology, M.E. and H.L.; software, M.E.; validation, H.L.; formal analysis, M.E.; investigation, M.E. and H.L.; resources, H.L., and R.S.; data curation, M.E.; writing—original draft preparation, M.E.; writing—review and editing, H.L., and R.S.; visualization, M.E.; supervision, H.L.; project administration, H.L.; funding acquisition, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Agder.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting reported results are shown in the present article as tables and figures. The original data are available as Excel files, stored by the first author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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