Tampere University

Thao Nguyen

BIOELECTROCHEMICAL NUTRIENT REMOVAL FROM RECIRCULATING AQUACULTURE SYSTEM WATERS

Master's thesis
Faculty of Engineering and Natural Sciences
Associate Professor (tenure track) Marika Kokko
MSc Veera Koskue
May 2022

ABSTRACT

Nguyen Phuong Thao: Bioelectrochemical nutrient removal from recirculating aquaculture system waters
Master's thesis
Tampere University
Master of Science (Technology) Degree Programme in Environmental Engineering
May 2022

Recirculating aquaculture system (RAS) is a highly engineered aquaculture system which can operate independently of the local climate while maintaining high production capacity. However, fish cultured in RAS may accumulate distasteful off-flavor compounds (OFCs), which are removed by cleaning the fish with high volumes of water. OFCs have been found to be highly abundant in the aerobic biological filters employed in RAS for nitrification of ammonia. Therefore, utilizing alternative methods such as bioelectrochemical system (BES) for ammonium removal under anaerobic condition may mitigate the production of OFCs in RAS.

The main objective of the thesis was to determine the maximum total ammonia nitrogen (TAN) removal efficiency achievable with a BES configuration referred to as bioelectroconcentration cell (BEC) and evaluate its potential for application in RAS facilities. Additionally, the thesis contributed to the growing area of research on bioelectrochemical technologies, more specifically toward the capability of BES for nutrient removal from low strength wastewater streams.

In the experiment, anodic electroactive microorganisms were first enriched in the triplicate BEC reactor set-ups fed with synthetic RAS water containing more organics and buffers as found in typical freshwater RAS waters. The content of organics and buffers were gradually decreased over the course of enrichment period. As the microbial community had matured, the experiment progressed towards continuous phase, during which decreasing hydraulic retention times (HRTs) of 18.7 h, 13.6 h, and 9.8 h were tested to determine the optimal operational condition for TAN removal with the system.

During enrichment phase, the reactors progressed in a predictable manner and demonstrated capability for nitrogen removal under batch mode operation with maximum TAN removal efficiency of 81.7 ± 17.7%. During continuous phase, maximum TAN removal efficiency (33.5 ± 26.5 %) and TAN removal rate (5.7 ± 4.9 g_N m^{-3} d^{-1}) were achieved with the BEC set-ups at HRT of 18.7 h, while lower removal efficiencies were obtained with HRTs of 13.6 h (13.3 ± 4.2%) and 9.8 h (11.3 ± 5.9%), which suggested inverse relationship between TAN removal efficiency and HRT. The TAN removal rate was independent of the HRT as minimum TAN removal rate was achieved at HRT of 13.6 h. Although there was potential for TAN removal, the system requires further optimization to be comparable to the treatment efficiency demanded in RAS facilities. Additionally, due to the low electrical conductivity of RAS water, the TAN removal rates and efficiencies obtained in the experiment were also insignificant compared to most BEC set-ups previously described. Overall, the thesis served as a preliminary investigation of bioelectrochemical treatment of RAS waters.

Keywords: Recirculating aquaculture system, nitrogen, total ammonia nitrogen, nutrient removal, water treatment, bioelectrochemical system, bioelectroconcentration cell

PREFACE

This thesis was conducted as part of FLAVOUR, which is a collaborative project between academic researchers and RAS industry partners in Sweden, Denmark, and Finland. I would like to thank FLAVOUR partners for entrusting me with this part of the project. I also want to thank the researchers from Natural Resource Institute Finland (Luke) in Laukaa for granting us a visit to their research facility and contributing their insights to my experiment.

As a young researcher, I would not be able to complete this thesis without the immense (emotional) support from my supervisor Marika Kokko, whose words to me on my first day “I think you have a lot of potentials” has helped me overcome my self-doubt many times. I want to thank my secondary supervisor Veera Koskue for enthusiastically answering to all my questions and fostering my reactors when I am gone. I would also like to express my gratitude to my mentor Johannes Jermakka, who have laid the early-stage foundation for the experiment sand taught me everything he knows.

My time in the lab was accompanied by amazing labmates who I look up to every day. I wish everyone great success in their journey and hope one day we can walk the same hall together once more.

This thesis concludes my academic journey in Tampere University as an international master’s student. I wouldn’t achieve what I have today without the support of my lecturer, training supervisor and teacher tutor Hannele Auvinen. Thank you for all the opportunities you have given me from the moment I got accepted to this degree programme.

Finally, I want to dedicate my deepest gratitude to family for supporting me in any way possible. Thank you, Ume, for shadowing my thesis writing process while chewing on my laptop cables. And thank you, my lifelong partner and bubble tea sponsor The, for believing in our future.

Tampere, May 2022

Nguyen Phuong Thao

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

AEM	Anion exchange membrane
BEC	Bioelectroconcentration cell
BES	Bioelectrochemical system
CEM	Cation exchange membrane
DOM	Dissolved organic matter
EC	Electrical conductivity
HRT	Hydraulic retention time
MEC	Microbial electrolysis cell
MES	Microbial electrosynthesis cell
MFC	Microbial fuel cell
MIB	2-methylisoborneol
NHE	Normal hydrogen electrode
OFCs	Off-flavor compounds
RAS	Recirculating aquaculture system
RE	Reference electrode
SHE	Standard hydrogen electrode
TAN	Total ammonia nitrogen
TOC	Total organic carbon
UV	Ultraviolet
WE	Working electrode
WWTP	Wastewater treatment plant

A_m	Membrane area (m^2)
C	TAN concentration (mg L^{-1})
$C_{anolyte,TAN}$	Molarity of TAN in the anolyte inflow (mol m^{-3})
CE	Coulombic efficiency (%)
$c_{substrate}$	Molarity of the substrate (mol L^{-1})
E^0	Standard reaction potential (V)
$E^{0\prime}$	Reaction potential at neutral pH condition
E_{emf}	Theoretical electrode potential (V)
E_{eq}	Total cell potential (V)
F	Faraday constant (96845 C mol^{-1})
I	Current (A)
j	Current density (A m^{-2})
L_N	TAN load ratio
m_{TAN}	Mass of TAN transferred per day (mg d^{-1})
n	Number of electrons transferred in the reactions
Q_{anode}	Flowrate to the anodic chamber (m^3 s^{-1})
R	Universal gas constant (8.31447 J mol^{-1} K^{-1})
T	Absolute temperature (K)
V	Liquid volume at the anode (L)
V_R	Hydraulic volume of the reactor
ΔG_r^0	Gibbs free energy of the reaction (J mol^{-1})
Π	Reaction quotient

1. INTRODUCTION

Stimulated by population growth, together with dietary preference and technological advancement, aquaculture is projected to reach 53% of the global fish production by 2030 (FAO, 2020, pp. 164–165). The industry provides employment for approximately 20.5 million people worldwide, over 90% of whom are located in Asia (FAO, 2020, p. 37). However, aquaculture production has damaging effects on the environment, such as eutrophication, chemical pollution, and loss of biodiversity (Ahmad et al., 2021). Aquaculture is also vulnerable against the impacts of climate change e.g., drought and hurricanes (Ahmed & Turchini, 2021). Therefore, the industry should extensively implement sustainable production technologies, such as recirculating aquaculture systems (RAS).

In addition to high product yield and water recirculation rate, RAS can be considered when local climate, water supply, seasonality and environmental regulations are limiting factors for aquaculture site selection (Ahmed & Turchini, 2021). However, a major challenge of RAS is the production of off-flavor compounds (OFCs), which create unpleasant muddy smell and taste to the fish (Tucker, 2000). Consequently, these compounds discourage customers from consuming fish and cause economic loss to the aquaculture industry. The existing depuration practice for removal of OFCs involves keeping fish off-fed in clean water for days to weeks (Burr et al., 2012). Thus, depuration consumes a large volume of water, increases production cost, and contradicts the environmental benefits of RAS.

To support the economic and environmental sustainability of RAS, OFCs must be effectively managed. Direct OFCs removal methods, including depuration optimization (Schram et al., 2016), chemical (Schrader et al., 2010) and physical treatment (Cook et al., 2001) of RAS water, have been proposed. On the other hand, OFCs have been observed to be highly abundant in aerobic biological filter units necessary for ammonium oxidation (Guttman & van Rijn, 2008). The biofilters maintain RAS water quality by nitrifying ammonia into nitrate (Tidwell, 2012, p. 255). Therefore, alternative methods for ammonium removal under anaerobic condition could be considered as a viable OFCs mitigation strategy.

Bioelectrochemical system (BES) is a technology which utilizes electroactive microorganisms as biocatalyst for energy and chemical production as well as wastewater treatment (Naradasu et al., 2020). However, very few studies to date have tested BES for RAS water treatment (Pous et al., 2021; Sander et al., 2018). Compared to biofilters, nitrogen removal from RAS water with BES may be beneficial, as many BES configurations operate in anaerobic condition (Kokko et al., 2016), which may suppress the production of OFCs in RAS.

The primary objective of the thesis is to determine the maximum TAN removal efficiency achievable with a BES set-up referred to as bioelectroconcentration cell (BEC) and evaluate its potential for application in RAS facilities. Subsequently, the optimal hydraulic retention time for TAN removal will be proposed.

The remaining research objective of the thesis is to compare the TAN removal efficiency achieved in the thesis to existing studies which utilized BEC for treatment of other wastewater streams. As BEC is a relatively new configuration, its application is still limited to several concentrated streams (Koskue et al., 2021; Ledezma et al., 2017). Treatment of low conductivity streams with BEC has been proven to be challenging (Monetti et al., 2019). Therefore, the thesis will evaluate the treatment potential of BEC for a novel wastewater stream and contribute to existing research in bioelectrochemical nutrient removal.

The following chapter will concern the structure of RAS and how OFCs are produced and removed. Next, a brief overview of BES and its applications for nitrogen removal will be described. The experimental methodology is introduced, after which the results are analyzed and discussed.

2. OVERVIEW OF RECIRCULATING AQUACULTURE SYSTEMS

The Food and Agriculture Organization of the United Nations defines aquaculture as the farming of aquatic species including fish, crustaceans, mollusks and aquatic vegetation (FAO, 2008, p. 15). In the last two decades, aquaculture has grown from contributing to 25.7% of global fish production in 2000 to 46% in 2016–2018 (FAO, 2020, p. 23). This is primarily explained by the growing population, whose demand for aquaculture products only increases due to perceived nutritional benefits of fish and dietary habits (Carlucci et al., 2015). Due to the growth of the industry as well as global trade, aquaculture products export has become a vital sector of many countries' economy. In addition to providing employment to approximately 20.5 million people worldwide (FAO, 2020, p. 37), aquaculture also strengthens local economy by providing foundation for livelihoods in gastronomy and tourism (Kim et al., 2017). Therefore, it is reasonable to state that aquaculture is crucial to global health, economy, and social stability.

2.1 Categories of aquaculture systems

While economic and social values of aquaculture have widely been recognized, environmental value of aquaculture has not received equal attention, especially in developing countries where technology or monetary resources are limited. The most discernible environmental impacts of aquaculture are discharge of organics, nutrients, pharmaceuticals, and disinfectants into water bodies (Ahmad et al., 2021). Local biodiversity is damaged by land use change as well as introduction of non-native species (Boyd & McNevin, 2015, p. 14). Although it was noted that the impacts level of aquaculture is minuscule compared to agriculture (Boyd & McNevin, 2015, p. 324), developments in sustainable aquaculture practices are required to protect ecosystems and natural resources. The impacts of aquaculture on the local environment vary by the aquaculture production system, which can be categorized based on cultivation intensity i.e., the extent of anthropogenic interventions and inputs to the production system (Tidwell, 2012, p. 64).

On the lowest level, low intensity or extensive aquaculture systems rely fully on natural biological processes to function. As water-suspended cages and nets are the most used techniques in extensive aquaculture system, it can also be termed open system. Water is passively exchanged by tides or currents, while the main mechanism for oxygenation is diffusion and photosynthesis by aquatic plants and algae (Tidwell, 2012, p. 65). Due to low management requirement, open system is easy to start up and requires little

maintenance. However, reliance on natural processes minimizes farmers' ability to regulate the rearing quality and efficiency.

An aquaculture system with less reliance on environment can be referred to as semi-intensive or semi-closed. Clean water from rivers, streams and rainfall are supplied into constructed enclosures, such as ponds and flow-through raceways, and is either discharged into water bodies or is treated and reused. Aerators or constant flow-through of water are main methods for ensuring sufficient oxygen level in the ponds (Tidwell, 2012, p. 68). With more man-made elements incorporated, farmers have more control over water quality parameters and can effectively monitor fish conditions. Thus, the output of semi-intensive system can double that of extensive system (Tidwell, 2012, p. 68). The investment and maintenance cost of semi-intensive systems will respectively be higher.

At maximum intensity level, intensive aquaculture system or closed system is the most engineered and regulated production system. While the original water source also originates from rivers and streams, the total water consumption of intensive systems is minimized with internal water reuse. Closed system consists of serial unit processes, which were designed to create optimal rearing condition regardless of location or season (Tidwell, 2012, p. 74). Therefore, intensive system has the maximum rearing capacity and production efficiency compared to other systems. However, intensive system demands high investment cost and meticulous monitoring; additionally, due to dependency between unit processes, the failure of one unit could jeopardize the entire system. The characteristics of aquaculture systems and their most significant environmental impacts are summarized in Table 1.

Table 1. *Aquaculture systems characteristics (Ghamkhar et al., 2020; Tidwell, 2012)*

Categories of aquaculture systems	**Examples of farming structure**	**Annual production (kg ha^{-1})**	**Environmental impacts**
Extensive/Open	Cages and net pens in rivers and sea Unfertilized ponds	≤100	High risks of eutrophication Damages to local ecosystem
Semi-intensive/ Semi-closed	Aerated, fed ponds Flow-through raceways	~10000	Eutrophication High water consumption
Intensive/Closed	Recirculating aquaculture system	≥100000	High cumulative energy demand and greenhouse gas emission

From a life cycle assessment perspective, most environmental impacts exerted by extensive and semi-intensive systems are local while intensive system has higher global impacts. Within the intensive aquaculture category, recirculating aquaculture system (RAS) is the most well-developed and prevalent technology in the recent decades. RAS

is an indoor and controlled system where fish are kept in tanks or raceways with continuous water recirculation of up to 95% (European Commissions & Directorate-General for Maritime Affairs and Fisheries, 2020, p. 11). According to Ahmed & Turchini (2021), the land-based advantage of RAS allows it to adapt to climate change, considering global effects such as drought and hurricanes can be major threats to fish production. In addition to independence from local climate variations, high production efficiency of RAS ensures the ability of aquaculture industry to sustain global food demand, which only increases annually. Therefore, RAS could be considered a viable long-term solution for the economic sustainability of aquaculture.

2.2 Global application of recirculating aquaculture system

RAS was originally developed in Japan in the 1950s and first applied commercially in 1980s (Ahmed & Turchini, 2021). Since then, RAS has been applied for production of various aquatic organisms with at least 360 facilities (Badiola et al., 2018) around the world, most of which are located in North America and Northern Europe (Figure 1).

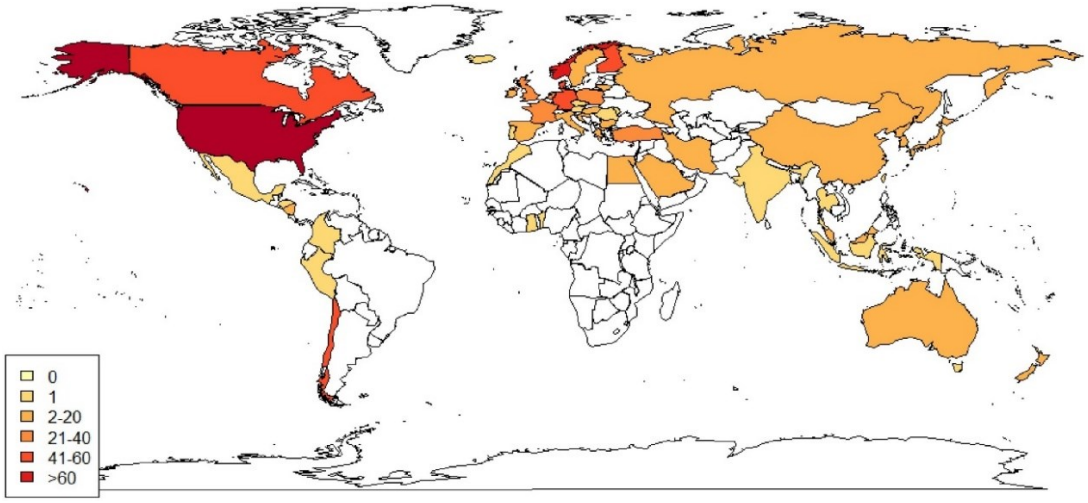

Figure 1. *Global distribution of RAS facilities by country (reprinted from Aquacultural Engineering, 81, Badiola et al., Energy use in Recirculating Aquaculture Systems (RAS): A review, 58, Copyright 2018, with permission from Elsevier)*

Multiple factors contribute to viable long-term applications of RAS. First, due to the ability to fully manage water quality and culture environment, RAS can be considered when local climate, water supply, seasonality and environmental regulations are limiting factors for aquaculture site selection (Ahmed & Turchini, 2021). Consequently, RAS can be designed for culturing freshwater, brackish and marine water aquatic species. Although Atlantic salmon is the most lucrative product to be cultured in RAS (O'Shea et al., 2019, p. 86), other successful species includes turbot, trout, and eel (Badiola et al., 2018). Additionally, RAS shortens the production cycle by providing ideal condition for juvenile

growth and reduce the mortality rate before transfer to net pens for full-size growth. Moreover, due to high stocking density ranging between 70–120 kg m^{-3} (Ahmed & Turchini, 2021), RAS lowers the total production cost for the same amount harvested. This suggest RAS to be a high financial return aquaculture system with corresponding environmental benefits.

2.3 Structure of recirculating aquaculture system

Compared to other aquaculture systems, RAS has the most comprehensive and efficient water treatment system of all. The basic structure of RAS is presented in Figure 2.

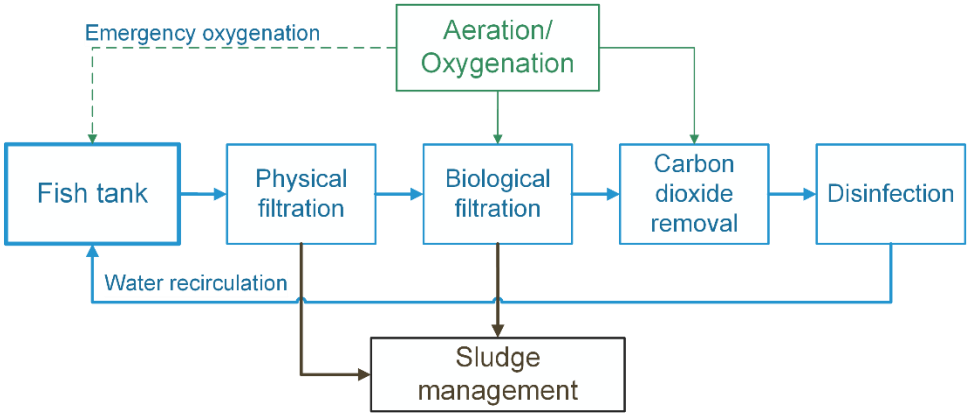

Figure 2. *System diagram of RAS with direction of air and oxygen (thin green arrows), water (blue arrows), and sludge (brown arrows) in the system (adapted from Lindholm-Lehto et al., 2020; Pulkkinen et al., 2018)*

Although the structure of RAS can be engineered specifically to individual aquaculture processes, all systems aim to remove excess feed and feces, suspended particles, ammonia, carbon dioxide and harmful bacteria, thus ensuring high water recirculation capability of RAS. Remote data recording and control is achievable with probes and sensors installed in each unit process (European Commissions & Directorate-General for Maritime Affairs and Fisheries, 2020).

2.3.1 Physical filtration

Due to high stocking density, solids i.e., residual feeds, fecal matters and inorganic particles also accumulate in RAS fish tank at high level. Suspended solids can directly affect fish wellbeing by clogging the gill, degrading into ammonia which reduces dissolved oxygen level, as well as decreasing the treatment efficiency of biological filters (Xiao et al., 2019). Therefore, physical filtration is introduced as preliminary treatment unit to efficiently eliminate the majority of solids from the system and alleviate the organic load on

subsequent biological filtration process. For settleable solids and large diameter suspended solids, solid-liquid separators such as swirl separator and hydrocyclone are capable of removing approximately 80% total particulate matter in the water (Xiao et al., 2019). To remove the remaining fraction of particulate matters, other physical filtration techniques can be considered. Microscreen drum filters reject particles larger than 60 µm, while sand filters exclude particles ranging between 30–75 µm (Xiao et al., 2019). Overall, the choice and combination of physical filtration equipment depends on the budget, available facility area and treatment target. For example, the research facility presented in Pulkkinen et al. (2018) utilized both swirl separator and microscreen drum filter for each rearing unit with 500 L capacity, while for a smaller size system, Lindholm-Lehto et al. (2020) only employed drum filters for their 450 L rearing units.

2.3.2 Biological filtration

Over 35% of the total nitrogen in the feed is converted into ammonium nitrogen by the fish and bacteria (Yamamoto, 2017, p. 29). The concentration of ammonia, which is toxic to the fish, must be kept under 3 mg L^{-1} total ammonia nitrogen (TAN) and 1 mg L^{-1} TAN for warm water and cold water fish, respectively (Tidwell, 2012, p. 266). Therefore, to effectively manage the concentration of ammonia in the water, biological filtration is employed.

After physical filtration, water is directed to biological filtration units or biofilters, where TAN removal is achieved with nitrifying bacteria in aerobic condition as indicated in equation (1) (Tidwell, 2012, p. 256). Nitrification consumes alkalinity, which can be maintained with addition of sodium bicarbonate or slaked lime.

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O \quad (1)$$

While there are various biofilter configurations, only a few which efficiently remove TAN at reasonable costs are applied RAS, such as submerged biofilter, trickling filter and moving-bed biofilter (Figure 3). The efficiency of a biofilter depends on the specific surface area; high specific area enables more space for biofilm growth, thus increasing the biomass load on the filter (Xiao et al., 2019).

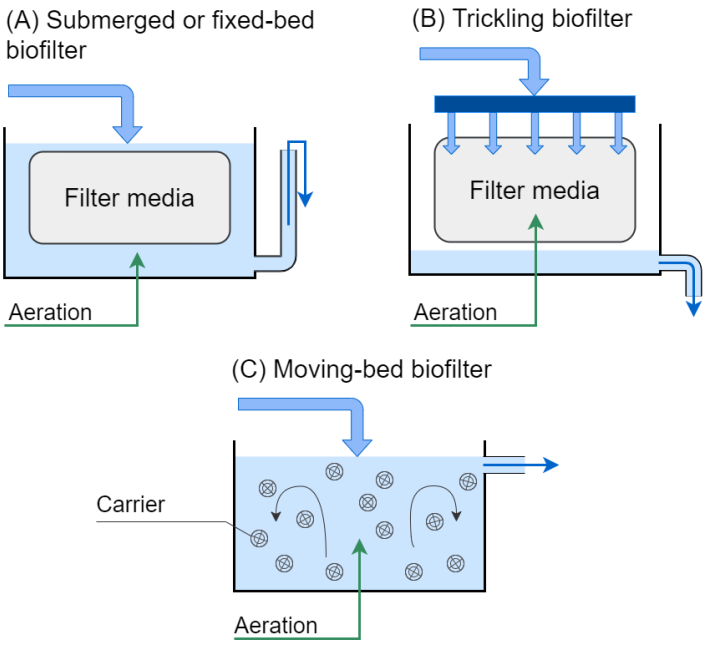

Figure 3. *Common biofilters in RAS: (A) Submerged or fixed-bed biofilter, (B) trickling biofilter, and (C) moving bed biofilter (adapted from Yamamoto, 2017 with permission from Springer Nature)*

In submerged or fixed-bed biofilter process (Figure 3a), the water is treated by the biofilm layer on the surface of filter medium, which remains fixed and fully submerged in the water. The unit is supplied with constant aeration at the bottom of the tank to ensure sufficient nitrification. Submerged biofilter has clear advantages in terms of simple initial start-up and resilient to changes in water quality (Pedersen et al., 2015). However, over long-term operation, biomass and particulate matters accumulation can clog the media, creating dead zones which lack sufficient O_2 for nitrification. Thus, backwashing at least once a week is required to ensure optimal filter efficiency. As a result, the maintenance of submerged filters is laborious and costly (Yamamoto, 2017, p. 32).

Trickling filter in RAS also employs a fixed media, although unlike submerged biofilter, the media fully resides on top of water level (Figure 3b). RAS water is distributed on top of the filter and drips on top of the media made of polymers or light weight clay aggregates (Xiao et al., 2019). Air is forced from underneath the porous media to ensure sufficient oxygen level across the filter and maintain constant treatment efficiency. Water treated by the biofilm, which inhabits the media surface, is collected at the bottom of the tank. Trickling filter can tolerate a wide range of hydraulic and organic loading rate, has lower operational cost compared to submerged filters (Xiao et al., 2019) and can also be employed for CO_2 removal (Pulkkinen et al., 2018). However, these filters' large floor space requirement and high media cost makes them the least considered biofilters (Dalsgaard et al., 2013).

In moving-bed filters or moving-bed bioreactor (MBBR), biofilm is enriched on polymer carriers, which freely move in the treatment tank by aeration or agitation (Figure 3c). Constant movement of carriers minimizes formation of anaerobic zones and ensures high and stable treatment efficiency. Additionally, mixing of carriers induces shear force on the outer layer of the biofilm and keep the biomass at controlled level. Thus, MBBR does not require backwash, and results in lower operational and maintenance cost. MBBR can be installed in series or combined with other biofilter techniques as described in Pulkkinen et al. (2018). Overall, these advantages undoubtedly allow moving-bed biofilter to be a preferred choice over other biological filter alternatives. In rare cases, MBBRs are not considered if exceptional water quality is required for production of luxurious species such as Arctic char or caviar-oriented sturgeons (Dalsgaard et al., 2013).

Lastly, other biofilter technologies are available for limited applications, due to their challenging operation or high investment cost. For example, fluidized sand biofilter is a novel technology which uses sand as biofilm carrier, which is maintained at fluidized state by upward water flow. Thus, the specific area of the filter is significantly magnified, allowing for highly efficient nitrification and organic degradation at minimal floor space (Xiao et al., 2019). However, high energy consumption and challenging start-up phase are the main barriers for fluidized sand biofilter to be applied in large scale RAS facilities. Therefore, it is mainly considered for treatment of aquarium recirculation systems (Kato & Kawamata, 2017) and production of luxurious fish (Dalsgaard et al., 2013). Similarly, rotating biological contactor is a less conventional biofilter with great treatment efficiency. It is composed of series of lightweight polymer disks attached on a horizontal shaft, which rotate at slow speed while partially submerged in a half-cylinder treatment tank (Yamamoto, 2017, p. 34). Like trickling filter, the biofilm is frequently exposed to air. In contrast to other biofilters, no additional aeration is required in rotating biological contactor, yet high ammonia removal efficiency of up to 80% is frequently achieved (Xiao et al., 2019). However, due to the design, operational problems such as rupture of rotary shaft and media deterioration commonly occur (Brazil, 2006). In conclusion, these biofilter alternatives would require further modification and optimization to have equal competitiveness to their common counterparts. A summary of the discussed RAS biological filters is available in Table 2.

Table 2. *Biological filtration alternatives for RAS water treatment and their advantages and disadvantages*

Biofilters	Specific TAN removal rate (g m^{-3} d^{-1})	Advantages	Disadvantages	References
Fixed-bed biofilter	56–183	Simple start up, low investment cost, robust	Prone to clogging, high operational cost	(Dalsgaard et al., 2013) (Suhr & Pedersen, 2010)
Moving-bed biofilter	150–240	Good treatment efficiency, self-cleaning, low overall cost	Unfit for stringent water quality requirement, low hydraulic capacity	(Dalsgaard et al., 2013) (Rusten et al., 2006)
Trickling filter	24–640	Can tolerate various hydraulic and organic loads	High media cost, occupy large facility space	(Crab et al., 2007) (Xiao et al., 2019)
Fluidized sand biofilter	1920–2880	Excellent treatment efficiency	High energy cost, challenging initialization	(Crab et al., 2007) (Dalsgaard et al., 2013)
Rotating biological contactor	33–138	Low energy consumption, high treatment efficiency	Unstable operation, high overall cost	(Brazil, 2006) (Crab et al., 2007)

Biological filtration is the core of RAS water treatment and should be evaluated extensively prior to application. Additionally, many factors can affect ammonia removal in the system, such as temperature, ammonia loading and hydraulic retention time (HRT) (Xiao et al., 2019). Thus, biofiltration unit should work harmoniously with other treatment unit in RAS. Moreover, combination of biofilters can negate their individual disadvantages and ensure reliable ammonia removal. In some cases, denitrification can be considered to remove excess nitrate from nitrification (Xiao et al., 2019). Continuous developments in biofilter designs and materials not only enhance water treatment efficiency but also enable extensive application of RAS for aquaculture production.

2.3.3 Carbon dioxide removal unit

Carbon dioxide accumulation in densely stocked fish tanks can negatively affect fish wellbeing and growth rate. The tolerance limit of most fish species is 20 $mgCO_2$ L^{-1}, while for certain species the limit can be twice as high (Dalsgaard et al., 2013). Additionally, high CO_2 level lowers the pH, which not only consumes alkalinity but also corrodes equipment and induces dissolution of metals such as aluminum, copper, and zinc into the water (Aslam et al., 2019). Therefore, CO_2 removal units are implemented in most RAS designs, although a small fraction of CO_2 can be removed in moving-bed biological filters

due to intensive aeration (Pulkkinen et al., 2019). Based on current literature on RAS set-ups in Nordic countries (Mota et al., 2019; Pulkkinen et al., 2018), the most frequently applied method for CO_2 removal is with degasser column. The operating mechanism of degassing columns resembles trickling filters, in which water is distributed on top of a porous solid column while air is forced upward through the column. The aeration rate can also be controlled based on CO_2 level of treated water (Pulkkinen et al., 2018) to minimize energy consumption. However, degassing column also has high floor space requirement, which should be a key design consideration.

2.3.4 Disinfection unit

Disinfection is a critical unit in RAS which prohibit entry, growth, spread and release of pathogens (Lazado & Good, 2021). Effective pathogen inactivation can be achieved both chemically and physically. Chemical disinfection in RAS primarily employs ozone, which can be dosed directly into the water (Lazado & Good, 2021). Its main mechanism for pathogen inactivation is denaturation of cell membrane, enzyme and nucleic acids (Davis, 2013). At concentration as low as 0.2–1 mg L^{-1}, ozone is an effective measure for disinfection of recirculating water due to its strong oxidation capability (Xiao et al., 2019). Additionally, it is capable of oxidizing suspended and organic matters, thus ozone treatment can also be referred to as 'water polishing' due to higher clarity and low organic content in treated water (European Commissions & Directorate-General for Maritime Affairs and Fisheries, 2020, p. 14). However, ozone should be applied cautiously due to residual disinfectant and by-products, which can harm fish health and lower production rate. Ozone poses high occupational risk with inhalation exposure limit of 0.1 ppm (Davis, 2013), thus frequent staff training, sufficient ventilation and personal protective equipment are necessary.

Physical disinfection in RAS is confined to ultraviolet (UV) irradiation, which destroys DNA or RNA of bacteria and virus at UV wavelength between 200–300 nm, thus inhibiting pathogens' ability to replicate (Davis, 2013). UV treatment unit can be constructed as open channels or as pressurized tube-and-shell systems. It is considered more favorable than ozone treatment, as it is reliable, free of disinfection by-products and safe for workers in RAS facilities. UV disinfection is interfered by water turbidity and requires frequent cleaning of lamp casing to maintain optimal transmittance. UV lamps also have limited lifetime, after which the disinfection capability is compromised. These factors make UV disinfection a sensitive, expensive and high maintenance treatment unit. (Xiao et al., 2019)

2.3.5 Aeration and oxygenation

Due to high stocking density and production rate, oxygenation is employed in RAS to provide optimal dissolved oxygen level between 6–11 $mgO_2\,L^{-1}$ (Dalsgaard et al., 2013). Oxygenation can be integrated with CO_2 degassing by injection of oxygen into the column before recirculation back to fish tanks (Lindholm-Lehto et al., 2020), or can take place in a separated unit using various configurations (Figure 4) such as U-tube, oxygenation cone or low-head oxygenators (Summerfelt et al., 2000).

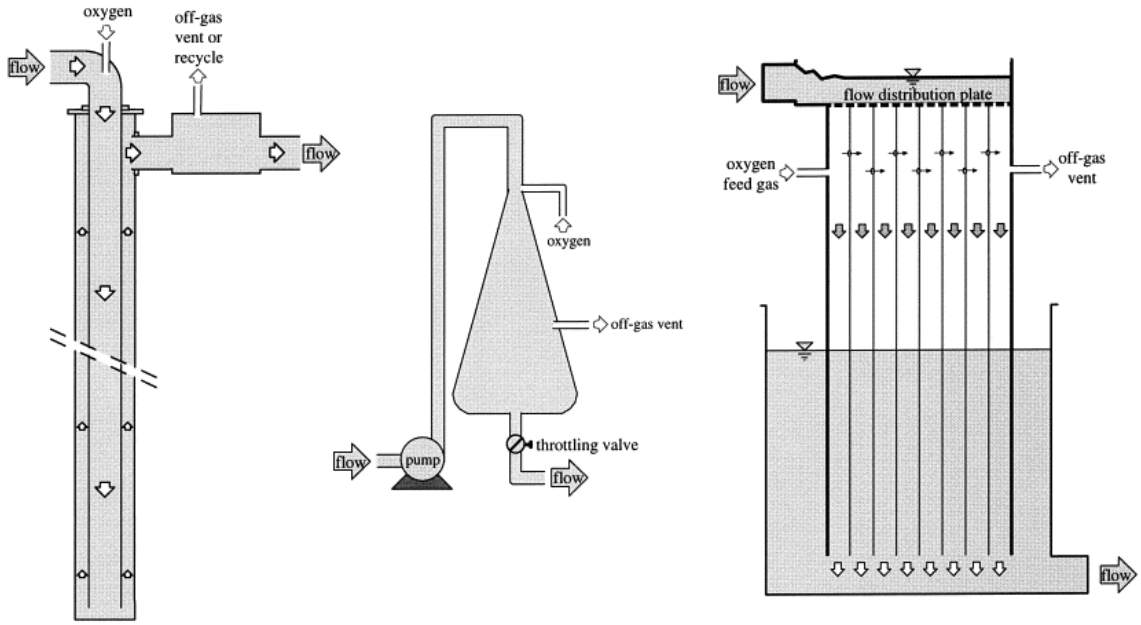

Figure 4. *U-tube oxygenator (left), oxygenation cone (center), and multi-stage low head oxygenators (right) employed for oxygen distribution in RAS by Summerfelt et al. (2000) licensed under CC BY-NC-ND 4.0.*

Nearly all RAS systems described in Dalsgaard et al. (2013) have automatic monitoring and control of oxygenation units. As a safety measure, direct oxygenation lines are installed into fish tanks to sustain viable dissolved oxygen level in case of system failure. In conclusion, aeration and oxygenation units should be designed to match economic feasibility and operational scale of RAS facility.

2.3.6 Sludge management

Sludge is collected from excess feed and feces separated during physical filtration and from backwashing and exfoliation of biofilters. Previous mass balance analyses have shown that sludge mass accounts for 11–38% of the total system feed input (Hopkins et al., 1994) and 23% of the input nitrogen mass (Hall et al., 1992). Due to high quantity and nutrient content, sludge is a significant environmental burden of RAS facilities. Although sludge from RAS can be disposed by land application or composted (Tidwell,

2012, p. 254), it can be a source for carbon and nutrient recovery. For example, anaerobic digestion is an attractive alternative for reuse of sludge from RAS, as it lowers long-term sludge treatment cost and can contribute up to 5% of facility's energy demand (Mirzoyan et al., 2010). In addition, the sludge can be fed to polychaetes, a class of marine worms, which can be used as a nutritious fish feed (Gómez et al., 2019).

2.4 Production and removal of off-flavor compounds in recirculating aquaculture system

A major problem of RAS is the production and accumulation of off-flavor compounds (OFCs) in the system. OFCs are associated with unpleasant taste and odor in fish and aquatic organisms (Davidson, Schrader, et al., 2014). According to Carlucci et al. (2015), such undesirable taste and smell and the presence of bones are the main reasons that discourage consumers from eating fish. Although non-toxic, these compounds deter consumers' attraction toward fish consumption and affect future purchase, therefore cause continuous economic loss to the aquaculture industry (Davidson, Schrader, et al., 2014).

2.4.1 Production of off-flavor compounds

Off-flavor compounds research mainly concerns two semi-volatile compounds, geosmin and 2-methylisoborneol (MIB). The odor of geosmin is described as earthy or muddy, while MIB's odor is characterized as musty-medicinal (Tucker, 2000). The human detection threshold of geosmin and MIB in fish fillet can be as low as 0.4 µg kg^{-1} and 0.7 µg kg^{-1}, respectively (Burr et al., 2012; Robertson et al., 2005).

In aquatic environment, several producers of OFCs have been recognized, namely actinomycetes and cyanobacteria (Robertson et al., 2005). In extensive and semi-intensive aquaculture, off-flavor periods are observed during the warmest months especially in nutrient-enriched ponds due to accelerated growth of cyanobacteria in such environment (Tucker, 2000). However, in the case of RAS, OFCs are mainly associated with the persistent presence of actinomycetes as well as other off-flavor producing species in the system's biofilters and RAS water. The content of geosmin in RAS water varies significantly, ranging from 1–3 ng L^{-1} to 10–75 ng L^{-1} depending on the facility (Azaria & Van Rijn, 2018).

Multiple operational parameters in RAS accelerate OFCs production and aggravate their negative impacts. The aerobic and organic-rich environment in RAS biofilters has been proven to be viable for off-flavor producers, which are classified as facultative anaerobic and heterotrophic bacteria (Guttman & van Rijn, 2008). However, Lukassen et al. (2021) suggested weak negative correlation between geosmin producing *Sorangium sp.* and

dissolved oxygen level, suggesting aerobic condition is not the sole promoter of OFCs production. Heat exchangers and drum filters were also observed to have high concentration of OFCs (Schrader & Summerfelt, 2010), thus warm temperature appears to stimulate off-flavor producers in RAS similar to cyanobacteria in semi-extensive ponds. High stocking density and feeding load in RAS also raise the organic load to the biofilters producers. In addition, fish species and fat content contribute to the accumulation of OFCs in fish tissue (Lindholm-Lehto & Vielma, 2019) while high phosphate fish diet may stimulate geosmin production (Azaria & Van Rijn, 2018). In conclusion, although there are discrepancies between studies regarding the generation of OFCs in RAS, it is generally agreed that OFCs producers are most active in biological filters and nutrient-rich zones in the system.

2.4.2 Off-flavors removal in recirculating aquaculture systems

The presence of OFCs in fish can significantly reduce the selling price or cause the product to be unmarketable (Azaria & Van Rijn, 2018). Considering the high investment cost and production density of RAS, OFCs are substantial economic risks to aquaculture facility owners, who are continuously pursuing solutions for this challenge. In commercial scale RAS, depuration is the only method capable of consistently removing OFCs from fish (Davidson et al., 2020).

The main mechanism for depuration is the reversible diffusion of OFCs through fish gills into the water (Lindholm-Lehto et al., 2019). Depuration is only conducted prior to harvesting, during which the fish are kept unfed in a clean, odor-less water environment from 2 to 16 days. The water is partially reused in a recirculation system without biofilter to minimize bacterial growth (Davidson et al., 2014). The removal efficiency often depends on the fish species and duration. Depuration is associated with economic loss to business owner as well as diminishing the environmental benefits of RAS. The fish can lose up to half of their fatty tissue mass due to the famine condition (Lindholm-Lehto et al., 2019), which decreases the product quality as well as total revenue of the batch. Additionally, large volume of water is spent during depuration to maintain minimal level of OFCs in the water. The HRT of the depuration recirculation system may range between 2 to 11 hours (Davidson et al., 2020). This practice thus contradicts the initial objective of RAS, which is to conserve water by maximizing water reuse in the system. To reduce economic and environmental impacts of depuration, some methods were suggested to accelerate this process, including pre-disinfection of depuration tank without aeration media (Davidson et al., 2014) and forced exercise (Schram et al., 2016).

Aside from OFCs removal from fish tissues, research also aims to manage OFCs concentration in the RAS water, thus lowering the degree of accumulation in fish tissue. Physical removal of off-flavors using activated carbon has proven successful for drinking water treatment, but when applied to RAS water, the efficiency was not consistent due to the interference of other organic compounds (Cook et al., 2001). Chemical treatment of OFCs using ozone and advanced oxidation processes (O_3/H_2O_2, O_3/UV) were also suggested due to their applicability in drinking water treatment. However, at tolerable concentration of 1 μg L^{-1}, ozone has negligible impacts toward concentration of OFCs in the water (Schrader et al., 2010). Finally, promising results have been achieved with biodegradation and adsorption of OFCs using microorganisms isolated from RAS trickling filter and sludge (Azaria & Van Rijn, 2018). However, biodegradation of OFCs often takes days to achieve desirable efficiency and can be reversed, thus further optimization is demanded prior to commercial application (Lindholm-Lehto & Vielma, 2019). Therefore, despite of its cost, depuration remains the only commercially viable option for OFCs removal. From a business owner's perspective, the greatest loss is the declination of product quality and loss of customers.

In conclusion, OFCs challenge sustainable operation of RAS and pose substantial economic burden to aquaculture farmers. Identifying solutions for OFCs monitoring and removal depend on the joint expertise and partnerships between aquaculture industry, scholars as well as governmental bodies. Efficient OFCs management not only benefits the prosperity and social perception of aquaculture industry but also supports and protects water bodies around the world.

3. BIOELECTROCHEMICAL SYSTEMS FOR NUTRIENT REMOVAL AND RECOVERY

3.1 Overview and principles of bioelectrochemical technologies

Bioelectrochemical system (BES) can be described as an electrical circuit which utilizes electron exchange between bacteria and electrodes. The first instance of the electrical effect of organics biodegradation was observed by Potter (1911), which laid the foundation for BES research. After this, the topic received several revisions between 1970–2000 until gaining tremendous popularity during the early 2000s (Scopus, 2022) when the demand for renewable and non-fossil energy sources rapidly increased (Kim et al., 2000).

3.1.1 General structure of bioelectrochemical systems

BES consists of at least an anode, which acts as an electron acceptor, and a cathode as an electron donor. These components are connected in an external electrical circuit while maintaining ionic contact via the electrolyte. They are commonly separated into their own compartments with the use of a separator that allows ions to pass through, although in single-chamber BES separators are not used. The simplified structure of a BES is represented in Figure 5.

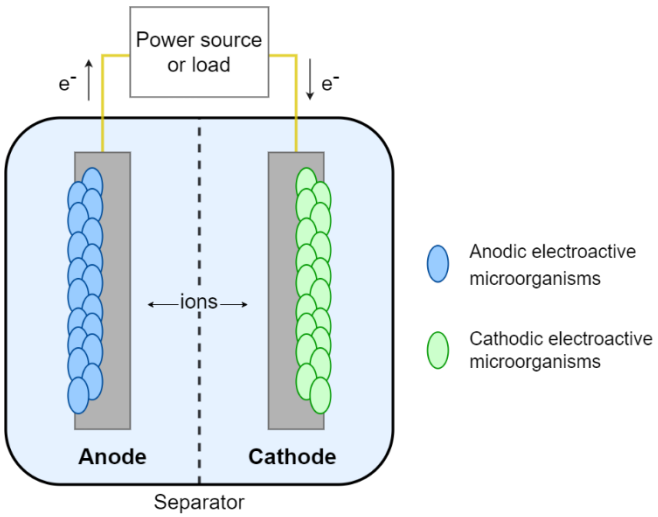

Figure 5. *A bioelectrochemical system includes at least two compartments, both of which can accommodate electroactive microorganisms (adapted from Hamelers et al., 2010 with permission from Springer Nature)*

BESs can be further categorized based on the method of electron utilization. For instance, a system that produces energy from organic biodegradation are referred to as microbial fuel cell (MFC). On the other hand, microbial electrolysis cell (MEC) and microbial electrosynthesis cell (MES) utilize additional energy along with the presence of biocatalysts to support an otherwise nonspontaneous reaction (Hamelers et al., 2010; Kokko et al., 2018). Other innovative applications of BES, such as hydrogen production and environmental remediation, are comprehensively reviewed in Kumar & Kuppam (2020).

3.1.2 Anodic organisms in bioelectrochemical systems

BES depends on a marginal group of bacteria, known as electroactive microorganisms, to generate energy and catalyze electrochemical reactions. The most prominent families of electroactive bacteria are *Geobacter* and *Shewanella*, which have been the main research subjects for studies dedicated to characterizing microorganisms in BES (Koch & Harnisch, 2016). They are classified as exoelectrogens, anodic or anode-respiring bacteria, capable of transferring electrons from their internal electron transport chain to an external anode (Logan, 2009; Naradasu et al., 2020). The natural habitat of these organisms varies substantially; *Geobacter* are highly abundant in anoxic environments such as freshwater and aquifer sediment and wastewater (Koch & Harnisch, 2016), while *Shewanella* thrive in freshwater, brackish and marine habitats (Hau & Gralnick, 2007). For optimal start-up and operation of BES, selection of electroactive inoculum should rely on the target reaction, operational environment and desired performance (Freguia et al., 2012).

3.1.3 Electrode and separator materials

Electrode materials are one of the most critical abiotic elements which ensure optimal performance in BES. The material should be selected based on conductivity, biocompatibility, specific surface area, chemical stability, durability, and cost (Kokko et al., 2016, p. 282). The most common and cost-effective electrodes can be made of carbon materials (graphite, activated carbon) and metals (stainless steel, titanium, copper, nickel) (Kerzenmacher, 2019, p. 142). Carbon-based materials are favorable in BES due to their wide usage range, good biocompatibility, high conductivity, and specific surface area. Currently, the most prevalent metal-based electrode material is stainless steel due to its high conductivity and durability (Kerzenmacher, 2019, pp. 143–144).

The role of the separator in BES is to physically separate the electrode compartments, reduce oxygen diffusion to the anode and reduce distance between electrodes (Kokko

et al., 2016, p. 282). Only in single chamber BES would the separator be absent. The most dominant separator utilized in BES are membranes, which sufficiently separate the anolyte and catholyte while allowing ions to pass through. Different membrane types can be considered depending on their permeation target, such as anion or cation. However, using membranes also has drawbacks in terms of increased material cost, aggravated internal resistance and susceptibility to biological and chemical fouling (Kokko et al., 2016, p. 282). Most notably, a phenomenon known as pH splitting, in which metal ions (Na^+, K^+) compete with H^+ to permeate the cathodic chamber, may occur with the usage of cation exchange membrane (CEM). This results in a highly acidic anolyte, which inhibits microbial activity while the basic catholyte affects soluble state of metal ions in cathodic chamber (Ho et al., 2017).

3.2 Nitrogen removal and recovery with bioelectrochemical systems

3.2.1 Bioelectrochemical configurations for nitrogen removal

Conventional wastewater treatment plants (WWTPs) have been designed to remove organics and nutrients from wastewater. However, the current treatment practices employed in WWTPs are energy and resource intensive. For example, the dependency of activated sludge process on aeration is a major cost factor in wastewater treatment and can account for 60% of operational cost in WWTPs (Nancharaiah et al., 2016, p. 175). Therefore, to provide an alternative to conventional processes, BES have been proposed as a nutrient removal alternative from various wastewater streams due to several advantages. First, most nutrients in wastewater, such as ionic ammonium and orthophosphate can be removed in BES by directional ion transport induced by the system's electrical circuit. This enables simultaneous nitrogen and phosphorus removal, which is often challenging in conventional treatment processes (Chen et al., 2016). In addition, the biomass yield of exoelectrogens is less than a third of activated sludge's biomass yield (Perazzoli et al., 2018). Thus, BES has low sludge production, handling, and disposal costs. Finally, the organics available in wastewater can be utilized as an energy source for electroactive microorganisms in BES. By simultaneously degrading the organics and generating electricity, MFC integration into existing WWTPs could provide an economical and long-term approach for wastewater treatment (Naradasu et al., 2020, p. 13). On the other hand, electrons generated by exoelectrogens can be used in MEC for production of value-added products such as hydrogen or hydrogen peroxide (Logan & Rabaey,

2012). Therefore, BES goes beyond nutrient removal and provides opportunities for nutrient recovery, energy, and chemical production. Overall, it is important to recognize the primary goal of BES to engineer the system and processes accordingly.

With respect to the scope of the thesis, only studies which concern nitrogen removal or recovery will be discussed in this section. Municipal wastewater is one of the target streams studied for simultaneous nutrient removal and energy production with BES. To treat this stream, Zhang et al. (2013) designed a U-shaped MFC for secondary treatment of municipal wastewater and achieved nitrification in the cathodic compartment, which allowed for approximately 80% ammonium concentration reduction in the cathodic effluent. Therefore, to facilitate total nitrogen removal, an additional denitrification MFC set-up was used in the study. With denitrification, the total nitrogen (as sum of total Kjeldahl nitrogen, nitrate, and nitrite) reduction achieved was 76.2% while only 27.1% reduction was observed in the set-up without denitrification (F. Zhang et al., 2013).

When energy production is not a priority in BES, other system configurations could be considered for nitrogen removal and recovery. The performance of several BESs dedicated towards nitrogen removal/recovery are summarized in Table 3. Using a bioelectroconcentration cell (BEC) configuration, Ledezma et al. (2017) succeeded in removing up to 57.7 ± 2.47% of NH_4-N from synthetic urine. However, using the same configuration, Monetti et al. (2019) was only able to remove an average of 15 ± 2.7% NH_4-N from untreated domestic wastewater.

Table 3. *Performance of BESs for nitrogen removal from different wastewater streams*

Types	**Waste streams**	**Conductivity (mS cm^{-1})**	**Maximum TAN removal %**	**References**
MFC	Municipal wastewater	Not reported	80% ammonium 76.2% total nitrogen	(F. Zhang et al., 2013)
BEC	Municipal wastewater	1.3 ± 0.1	15 ± 2.7%	(Monetti et al., 2019)
BEC	Synthetic reject water Real reject water	7.8 ± 0.5 5.7 ± 0.2	75.5 ± 2.7 53.2 ± 4.9	(Koskue et al., 2021)
BEC	Synthetic urine	19.5 ± 0.5	59.7 ± 2.47	(Ledezma et al., 2017)
MEC	Synthetic urine Human urine	40.5 ± 1 30.6 ± 1.9	60.9 47	(Arredondo et al., 2019)

Monetti et al. (2019) emphasized existing limitations of BEC for nutrient removal, such as the low conductivity and buffer capacity of waste streams utilized. Therefore, concentrated waste streams with high ionic conductivity were considered more ideal for electrolytes for BES (Koskue et al., 2021, p. 2). For example, utilizing a similar BEC set-up (Figure 6) and reject water from anaerobically digested sludge as the feed, Koskue et al. (2021) achieved up to 53.2 ± 4.9% TAN removal from real digestate reject water.

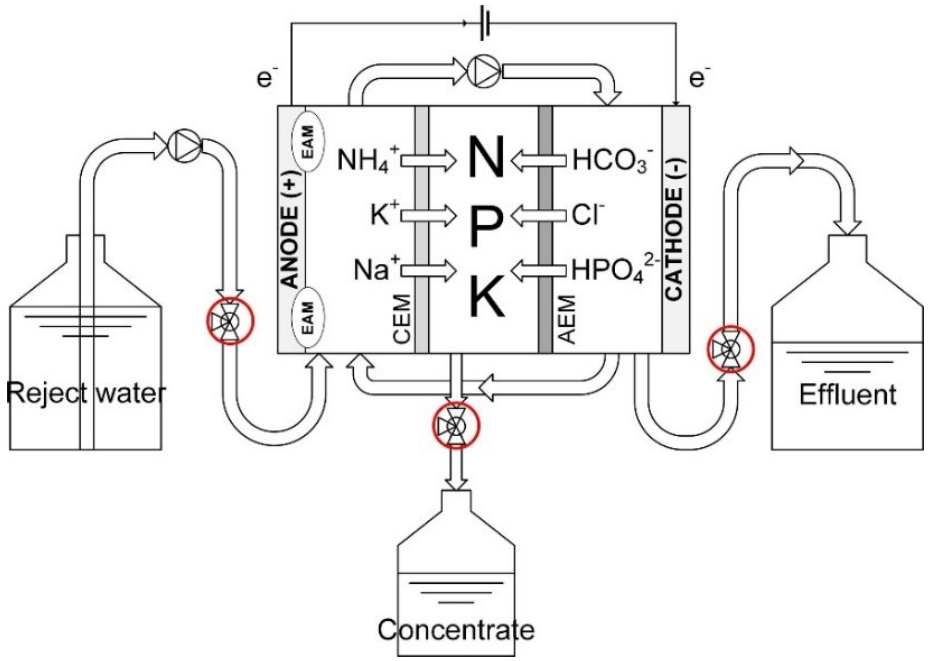

Figure 6. *Bioelectroconcentration cell (BEC) configuration for nutrient removal and recovery from digestate reject water by Koskue et al. (2021) licensed under CC BY-NC-ND 4.0.*

An equivalent TAN removal efficiency was observed in the study by Arredondo et al. (2019), in which diluted human urine was utilized as the feed. Their MEC set-up also included a recovery unit referred to as transmembrane chemisorption to enhance ammonium recovery. Using urine as the feed, the results achieved by Arredondo et al. (2019) was comparable to the NH_4-N removal efficiency discussed in Ledezma et al. (2017). These results imply the suitability of BES for nutrient removal of highly concentrated waste streams, although decent nutrient removal of low conductivity streams remains achievable.

3.3 Evaluation of bioelectrochemical system's performance

In BES, cell potential or voltage is used as indicator of the microbial community's enrichment state and their capability for electricity production (Naradasu et al., 2020). The total cell potential or voltage can be determined with formula (2) (Kokko et al., 2016, pp. 267–

268), in which E_{eq} is the total cell potential (V) and E_{emf} indicates the theoretical electrode potential (V).

$$E_{eq} = E_{emf}^{cathode} - E_{emf}^{anode} \quad (2)$$

A positive cell potential in MFC implies that electricity is produced, while systems such as MEC with a negative cell potential requires additional energy to facilitate chemical reactions taking place (Rozendal et al., 2008). It should be noted that these values are calculated without taking losses such as ohmic and mass transport losses (Kokko et al., 2016, p. 269) into considerations. In realistic conditions, the cell voltage achieved by MFC is often lower than the calculated voltage, while excess electricity is provided to MEC to ensure that the desired chemical reactions will proceed. (Rozendal et al., 2008)

The theoretical electrode potential of the anode or cathode can be calculated based on formula (3) (Logan, 2008, p. 30), where E^0 is the standard reaction potential at temperature of 298 K and pressure of 1 bar (V), R is universal gas constant (8.31447 J mol^{-1} K^{-1}), T is the absolute temperature (K), n is the number of electrons transferred in the reactions, F is the Faraday's constant (96485 C mol^{-1}), and Π the reaction quotient calculated in (4) as the molar concentrations of reactants and products raised by their respective stochiometric coefficients.

$$E_{emf} = E^0 - \frac{R \cdot T}{n \cdot F} \ln(\Pi) \quad (3)$$

$$\Pi = \frac{[products]^p}{[reactants]^r} \quad (4)$$

At standard condition, E^0 is calculated using equation (5) (Logan, 2008, p. 32), where ΔG_r^0 is the Gibbs free energy of the reaction (J mol^{-1}). An extensive list of Gibbs free energy constant for the formation of various compounds are available in Heijnen & Kleerebezem (2010), in which a negative ΔG_r^0 value indicates that energy can be obtained from the reaction and vice versa.

$$E^0 = -\frac{\Delta G_r^0}{n \cdot F} \quad (5)$$

Values of standard reaction potential can be reported using standard hydrogen electrode (SHE) as a reference since E^0 of H_2 is 0 V under standard condition. Additionally, it can be reported at normal condition based on the assumption that the microbial cell's pH is constant at 7. In this case, the pH-adjusted $E^{0\prime}$ value uses normal hydrogen electrode

(NHE) for reference and $E^{0\prime}(H_2)$ equals to –0.414 V (Logan, 2008, p. 30). All potential values can be reported versus SHE or NHE, as well as versus silver/silver chloride (Ag/AgCl) reference electrode, since it is commonly used in experimental BES set-ups (Logan, 2008, p. 32; Naradasu et al., 2020, p. 12).

In addition to calculation of energy generated or required by the system, the current performance is also assessed. First, current density is often used for direct comparison of current generation in BES experiments regardless of their sizes and configurations. It is calculated using formula (6), in which j is the current density (A m^{-2}), I denotes the current generated by the cell (A) and A_m is the system membrane area (m^2).

$$j = \frac{I}{A_m} \tag{6}$$

Another crucial indicator of BES performance which can be derived from the current is the electrons recovery efficiency. The efficiency of substrate conversion into electricity is evaluated using Coulombic efficiency by integrating the current over time and dividing it by the total charges available in the organics fed to the cell, as calculated in equation (7) (Kokko et al., 2016, pp. 268), where CE stands for Coulombic efficiency, c is the molarity of the substrate (mol L^{-1}) and V is the volume of anodic compartment (L).

$$CE = \frac{\int I \, \mathrm{d}t}{n \cdot \mathrm{F} \cdot c \cdot V} \cdot 100\% \tag{7}$$

To allow for better comparison across BES set-ups, TAN load ratio (L_N) has recently been suggested as an indicator of TAN removal and recovery. It is based on the hypothesis that for each mole of electrons recovered from organics oxidation, one mole of NH_4^+ is removed from the system under the acidic condition of the anode (Arredondo et al., 2019, p. 2056). Load ratio above 1 indicates surplus in current to transport all TAN across membrane area (Kuntke et al., 2018). L_N is calculated using formula (8), where $C_{anolyte,TAN}$ is the molarity of TAN in the anolyte inflow (mol m^{-3}) and Q_{anode} is the flowrate to the anodic chamber (m^3 s^{-1}). (Rodríguez Arredondo et al., 2017)

$$L_N = \frac{j}{C_{anolyte,TAN} \cdot Q_{anode} \cdot \frac{F}{A_m}} \tag{8}$$

Increasing load ratio in BES may prove challenging (Arredondo et al., 2019) as current density is often restricted by the type of waste stream and system configuration. Nevertheless, TAN load ratio is a useful tool for predicting the upper limit of removal and identifying constrains in BES. For example, to achieve equivalent TAN removal from a weak

stream such as domestic wastewater, greater current density needs to be applied compared to a stream with more concentrated TAN level e.g., urine. Additionally, a threshold point load ratio has been observed, after which the removal efficiency achieved is no longer cost-efficient (Rodríguez Arredondo et al., 2017). Therefore, an optimized load ratio should be established based on experimental data of individual BES set-ups.

3.3.1 Application of bioelectrochemical systems for treatment of recirculating aquaculture system's water

Although previously recognized as a suitable method for simultaneous organics and nutrient treatment of low strength wastewater streams such as domestic wastewater (Monetti et al., 2019; San-Martín et al., 2018), few studies to date have investigated the applicability of BES in aquaculture. Previously, Algar et al. (2020) has demonstrated bioelectrochemical treatment of aquaculture net pens' sediment layer using a set-up referred to as sediment microbial fuel cell. Nitrogen removal has also been addressed by Jiaqi et al. (2020) using MFC set-up enriched with algae and photo-electro-catalyzed cathode. The system achieved up to 94% TAN removal from mariculture wastewater, while algae, predominantly cyanobacteria, was recognized to have critical role in the system (Jiaqi et al., 2020).

The applicability of BES for treating a sensitive and low strength stream such as RAS water remains limited. As previously described, denitrification may be employed in RAS to remove excess nitrate produced by biofilters and reduce the total nitrogen level in the water. To provide an alternative to existing denitrification set-ups, Sander et al. (2018) provided a proof of concept for bioelectrochemical treatment of marine RAS water using a lab-scale BES operated at different inflows and buffering capacity levels. Autotrophic denitrification took place in the cathodic compartment, while oxygen was being produced in the anode. The denitrification rate achieved was within the range previously reported in bioelectrochemical denitrification set-ups, thus the concept was proposed as a viable alternative for nitrate removal in marine RAS (Sander et al., 2018), although the treatment efficiency may vary in freshwater RAS due to low salinity.

Aquaponics is a hybrid system which combines recirculating aquaculture with hydroponic plants cultivation (FAO, 2008). To treat this stream, Pous et al. (2021) proposed a BES set-up which resembles a trickling filter. In their biofilter set-up, nitrification proceeded as normally in the aerobic upper portion while bioelectrochemical denitrification took part in the polarized lower section, which is submerged in the water. The reactor achieved maximum TAN removal rate of 94 $g_N\ m^{-3}\ d^{-1}$, which is comparable to the TAN removal rate of a trickling filter, while simultaneously providing nitrate removal (Pous et al., 2021).

In summary, the early development stage of bioelectrochemical RAS water treatment focuses on experimenting various reactor designs for organics and nitrogen removal. Additionally, little is known about the evolution of off-flavor compounds (OFCs) in these systems. Thus, for bioelectrochemical technology to be applicable on commercial scale, research on this topic should center on various practical matters, including:

a) Is the treatment efficiency of bioelectrochemical set-up comparable or superior to existing biological filtration units?

b) How do OFCs behave and evolve in bioelectrochemical set-ups?

c) What operational parameter in BES can be optimized to enhance nutrient removal or reduce OFC generation and accumulation in RAS water?

The aim of the thesis was to explore the potential of BES for nitrogen removal from RAS water. Thus, the thesis provides an insight on questions (a) and (c) stated above while contributing to the growing area of research on bioelectrochemical technology, more specifically toward the capability of BES for nutrient removal from low strength wastewater streams.

4. MATERIALS AND METHODS

In the following subchapters, the experimental and analytical procedure of the thesis will be described and justified. As the thesis was conducted during the preliminary investigation of bioelectrochemical treatment of RAS water, a synthetic RAS water medium was developed based on existing literature and data on RAS water characteristics. Next, the configuration of the BES employed for nutrient removal of RAS water will be described. The study was conducted in two major stages, enrichment and continuous, whose procedures will be separately reported. The final sections of the chapter will justify the sample preparation and analytical methods.

4.1 Synthetic medium composition

Utilizing a synthetic feed offers several advantages from operational perspective. First, the precise formula of synthetic medium enables accurate quantification of system's intake. Additionally, the medium can be adjusted, prepared and stored for a longer period, thus ensuring consistent feed quality. Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*), which are cultivated in freshwater RASs, are the most produced species in the Nordic countries (Dalsgaard et al., 2013). Therefore, freshwater RAS was chosen as the reference for determining the synthetic medium composition. The medium composed of four parts: inorganics salts, organics, micronutrients, and buffers. All parts were prepared with analytical or biochemical grade chemicals and Milli-Q water.

With respect to the inorganic fraction, several papers concerning rainbow trout production in RAS, namely Davidson et al. (2011, 2019) were used as the basis for determining the nitrogenous species (NH_4^+, NO_3^-) composition. The concentration of remaining inorganic ions was determined based on data on quality of Finnish lake water (Tampereen Vesi, 2020), which is the main water source of RAS facilities in Finland as described in the study by Pulkkinen et al. (2018). As the result, the inorganic fraction consisted of 5.9 mg L^{-1} NH_4Cl, 13.8 mg L^{-1} $CaCl_2$, 3.2 mg L^{-1} $MgCl_2$, 5.8 mg L^{-1} $MgSO_4$, 5.7 mg L^{-1} KCl, 11.5 mg L^{-1} NaCl, 16.2 mg L^{-1} $NaNO_3$ and 22.4 mg L^{-1} Na_2HPO_4. The inorganics were prepared in a concentrated solution and stored at 5 ^oC.

Organic matters in RAS derived from intake water, uneaten feed, excrements as well as fish biomass. Although it is acknowledged that organic matters consist of both suspended and dissolved organic matters (DOM), only the DOM fraction was replicated in the synthetic feed formula. The decision was based on the assumption that the BES

studied in the experiment will replace or operate in parallel to the biofilters in RAS, thus the inflow would be free of most suspended solids. The composition of organic compounds in the synthetic feed was modified based on the article by Aguilar-Alarcón et al. (2020), although some biochemical classes were eliminated from the estimation as they were at the time challenging to analyze or offered no benefits to anodic bacteria employed in BES. The final RAS recipe consisted of volatile fatty acid, protein, and carbohydrate. Next, the simplest compound of each organic class was utilized for the synthetic feed formula, in which sodium acetate (CH_3COONa) was used to replicate volatile fatty acids, glycine (NH_2CH_2COOH) represented proteins, and D(+)-Glucose ($C_6H_{12}O_6$) simulated carbohydrates. The following equations describe the biochemical degradation of chosen organics by anodic microorganisms.

$$CH_3COO^- + 2\ H_2O \rightarrow 2\ CO_2 + 7\ H^+ + 8\ e^- \quad (9)$$

$$C_2H_5NO_2 + 4\ H_2O \rightarrow 2\ HCO_3^- + 7\ H^+ + NH_4^+ + 6\ e^- \quad (10)$$

$$C_6H_{12}O_6 + 12\ H_2O \rightarrow 6\ HCO_3^- + 30\ H^+ + 24\ e^- \quad (11)$$

The share of each compound was determined by their contribution to the synthetic medium's total organic carbon (TOC), in which 24% of the medium's TOC originated from sodium acetate, 19% was contributed by glycine and the remaining 57% derived from glucose. The ratio of these compounds remains unchanged throughout the experiment, although the TOC level of synthetic feed was adjusted on several occasions, which will be explained in subchapter 4.3. The organic component of the medium was prepared in a concentrated form, sterile filtered and stored in autoclaved vials at 5 °C.

Buffers and micronutrients were components in the feed which were not necessarily present in RAS water but were beneficial to the operation of BES. To provide metal ions that are needed in the metabolism pathways of anodic microorganisms in BES, 1 mL of concentrated micronutrient solution formulated according to Rabaey et al. (2005) was added to every liter of synthetic feed prepared. During BES operation, the anodic pH tends to decrease, which may become unfavorable for anodic microorganism (Kokko et al., 2016, p. 279). Therefore, buffers were added to maintain stable and optimal pH level for BES operation. For enrichment phase, phosphate buffer consisting of Na_2HPO_4 and NaH_2PO_4 were used, as it is the most employed buffer in microbial growth media (Merck, n.d.). In practice, bicarbonate buffer system is the primary buffer method of RAS water, thus phosphate buffer was replaced by sodium bicarbonate ($NaHCO_3$) during the continuous phase. The complete synthetic medium composition during enrichment and continuous phase are described in Appendix A and Appendix B, respectively.

4.2 Experimental set-up

Bioelectrochemical nitrogen removal from RAS water was conducted using bioelectro-concentration cell (BEC) set-up previously employed for nutrient removal from digestate reject water, urine and domestic wastewater (Koskue et al., 2021; Ledezma et al., 2017; Monetti et al., 2019). In BEC, due to the potential difference between the electrodes and the use of cation exchange membrane (CEM) and anion exchange membrane (AEM), ions in the electrolyte are forced to migrate through the membranes and collected in a concentrated solution (Ledezma et al., 2017, p. 120). Throughout this thesis, the abbreviation BEC will be used to distinguish the experimental set-up from its broader definition as a BES. The experiment was conducted in triplicate reactors, whose structure is displayed in Figure 7.

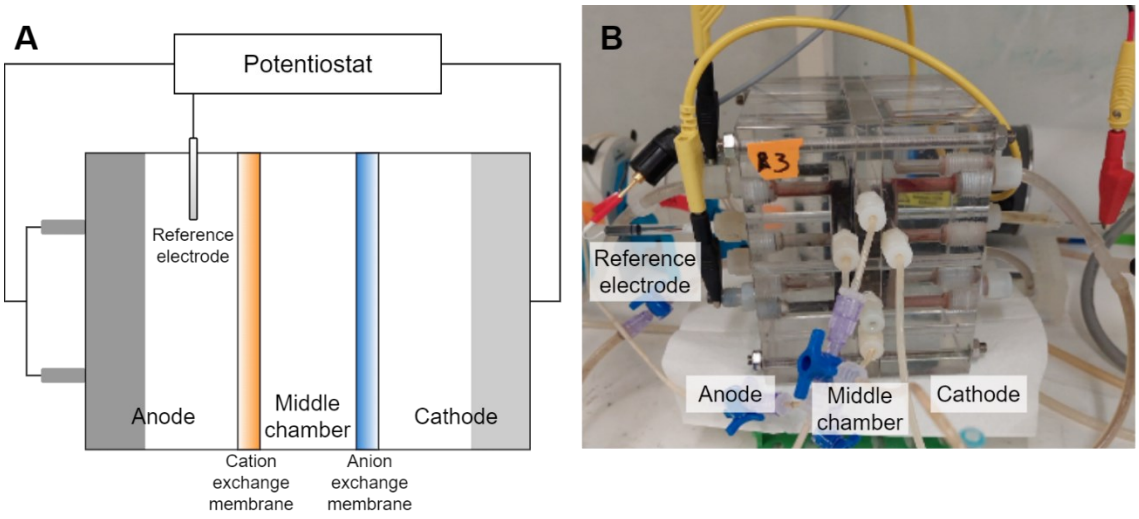

Figure 7. *(A) Structure of BEC, and (B) photo of BEC reactor with corresponding annotations*

The reactors were assembled from acrylic plastic frames, which formed 3 chambers of the BEC, each has the dimension of 50 mm x 70 mm x 10 mm. The working electrode (WE), in this case the anode, was prepared by filling the anodic chamber with graphite granules that were acid-base washed. Two graphite rods inserted into the anodic chamber via Swagelok fittings functioned as conduits between granules and electrical wires. To establish the working potential for the WE, an Ag/AgCl in 3 M NaCl (BASi, USA) reference electrode (RE) was introduced to the anodic chamber. The counter electrode or the cathode composed of a 70 mm x 50 mm stainless-steel mesh connected to a titanium wire, which was sealed in place with silicone gel. The middle or the concentrate chamber was separated from the anodic and cathodic chamber respectively by a 70 mm x 50 mm CEM (CMI-7000, Membranes Internationals, USA) and an AEM (AMI-7100, Membranes Internationals, USA) of similar dimension. Glass beads were added to the

middle chamber to prevent membrane deformities. The volume of middle chambers was 20 ± 2 mL after addition of glass beads. The measured reactors' hydraulic volume i.e., the volume of anodic and cathodic chambers after completion was 65 ± 6 mL. In this experiment, chronoamperometry was used as the main method for assessing the current generated by the reactors. A multi-channel potentiostat (Bank Elektronik, Germany) was employed to impose a working potential of 0 V vs Ag/AgCl in 3M NaCl, corresponding to 0.209 V vs SHE.

4.3 Experimental stages

The experiment was conducted in two major stages each with different aim. The main objective of enrichment phase, which lasted around 120 days, was to enrich the anodic microbial community in the anode of the triplicate BECs. As the microbial community had matured, the experiment shifted to continuous phase to assess the applicability of BEC in RAS water treatment. During continuous phase which lasted about 70 days, different HRTs were tested to determine the optimal operational condition for TAN removal with the system.

4.3.1 Enrichment phase

Enrichment phase was conducted in batch mode under anaerobic conditions. The enrichment medium was recirculated from the cathode to the anode at a constant rate of 20 mL min^{-1} with peristaltic pump (Masterflex L/S, USA). A gas bag was fixed to the recirculation bottle to collect gaseous compounds produced in the reactor. The concentrate was collected from an overflow port into a 100 mL glass bottle, which was kept anaerobic. To complete the internal electrical circuit of BEC, 0.01 M NaCl was added to the concentrate chamber at the beginning of the experiment. The operational configuration of the enrichment phase is illustrated in Figure 8a.

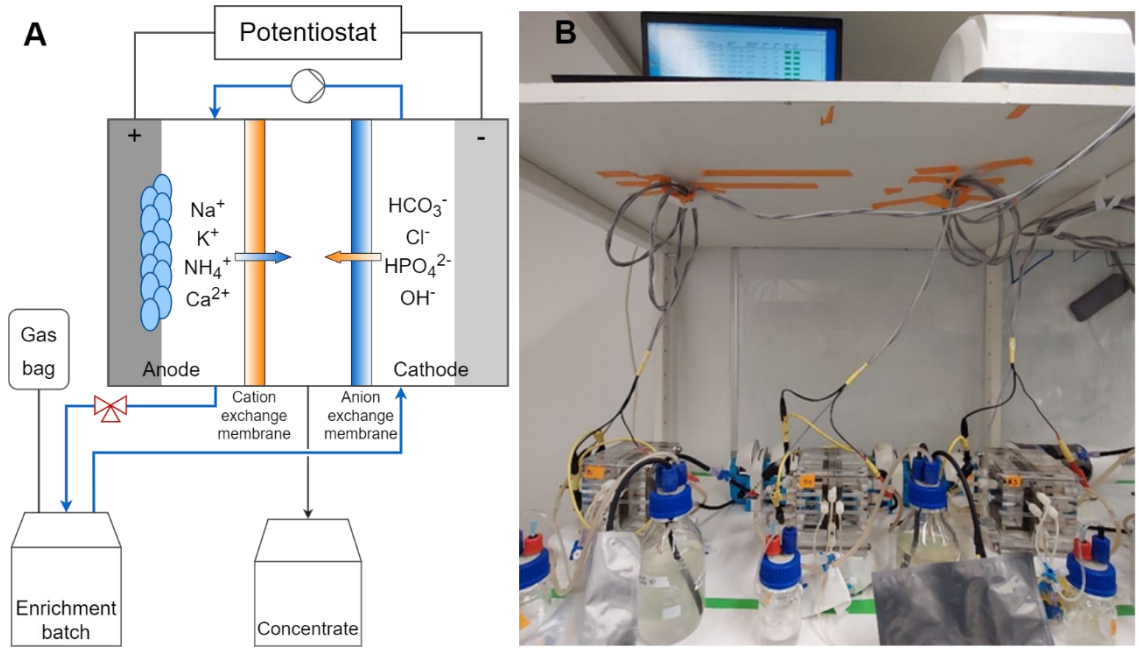

Figure 8. *(A) Experimental configuration of enrichment phase, and (B) overview of triplicate enrichment laboratory set-up*

The inoculum for enrichment of anodic microorganisms was acquired from mixed culture in anaerobic digested sewage sludge from Viinikanlahti WWTP (Tampere, Finland) and organic compost from Tarastenjärvi waste management plant (Tampere). The sewage sludge was thoroughly mixed before filtering through a thinly woven fabric cloth to extract the liquid fraction, which was used as part of the inoculum. To obtain exoelectrogens from solid organic compost, roughly 50 mL of the compost was diluted into 100 mL of MQ water and filtered through the fabric cloth. Approximately 1.5 mL of each inoculum, preserved at 5 ºC, was injected into filled anodic chamber for inoculation, which was conducted at start-up of the experiment and repeated once after 3 weeks.

To gradually develop and acclimatize the anodic microbial community to RAS water, the enrichment phase was conducted in several smaller steps with different buffer and organic composition while the inorganics and micronutrient components remained unchanged. Once a week, half of the medium was replaced with freshly prepared synthetic feed with doubled organic strength to reach the target concentration of organics. After changing the medium, NaOH was added to bring the pH level close to neutral value. Table 4 summarizes the steps within enrichment phase and the respective buffer and organics concentration.

Table 4. *Buffer and organics composition during enrichment steps*

Steps	Days	Buffer concentration (g L^{-1})		Organics concentration (mg L^{-1})		
		Na_2HPO_4	NaH_2PO_4	Sodium acetate	Glycine	Glucose
ST	0 – 25	4.1	2.5	17.8	7.7	13.0
E100	25 – 74	4.1	2.5	250.0	108.5	183.0
E50	74 – 103	2.7	1.7	125.0	54.3	91.5
E25	103 – 117	1.4	0.8	63.0	27.1	45.8

Abbreviation: ST, start-up

During the start-up step (ST), the feed began with 50 mM phosphate buffer at pH 7.0. The strong buffer level also helped maintaining high electrical conductivity in the cell and reduce ohmic losses. The synthetic feed's theoretical TOC level was set at 15 mg L^{-1} based on the average concentration in RAS water (Davidson et al., 2011). In the next step, referred to as E100, the content of all organic components was increased to accelerate the enrichment period, while the medium's buffer capacity was kept constant. The reactors were enriched with this medium composition until stable current density signal and conductivity were observed across reactors. In the subsequent E50 and E25 steps, the concentration of organics was decreased by half of the previous steps, while the buffer concentration was reduced to 17 mM at the final step.

4.3.2 Continuous phase

A multichannel peristaltic pump (ISM834C, Ismatec) was used to deliver the influent at varying flowrates. Unlike enrichment configuration, the influent was immediately fed to the anodic chamber to deliver the organics to anodic bacteria. Next, the anolyte was transported to the cathodic chamber before being recirculated back to the anode. The overflow exerted by the inlet pump was collected as the system's effluent. Concentrate was collected identically to the enrichment phase with the addition of internal concentrate recirculation to promote ions migration. All recirculation flowrates were kept constant at 20 mL min^{-1} by peristaltic pumps (Masterflex L/S, USA). Gas bag filled with N_2 was inserted to the influent bottle to displace the liquid volume drawn by the inlet pump, while an empty gas bag was installed to effluent bottle to collect generated gaseous compounds and maintain anaerobic condition in the reactors. The operational configuration of continuous phase is displayed in Figure 9.

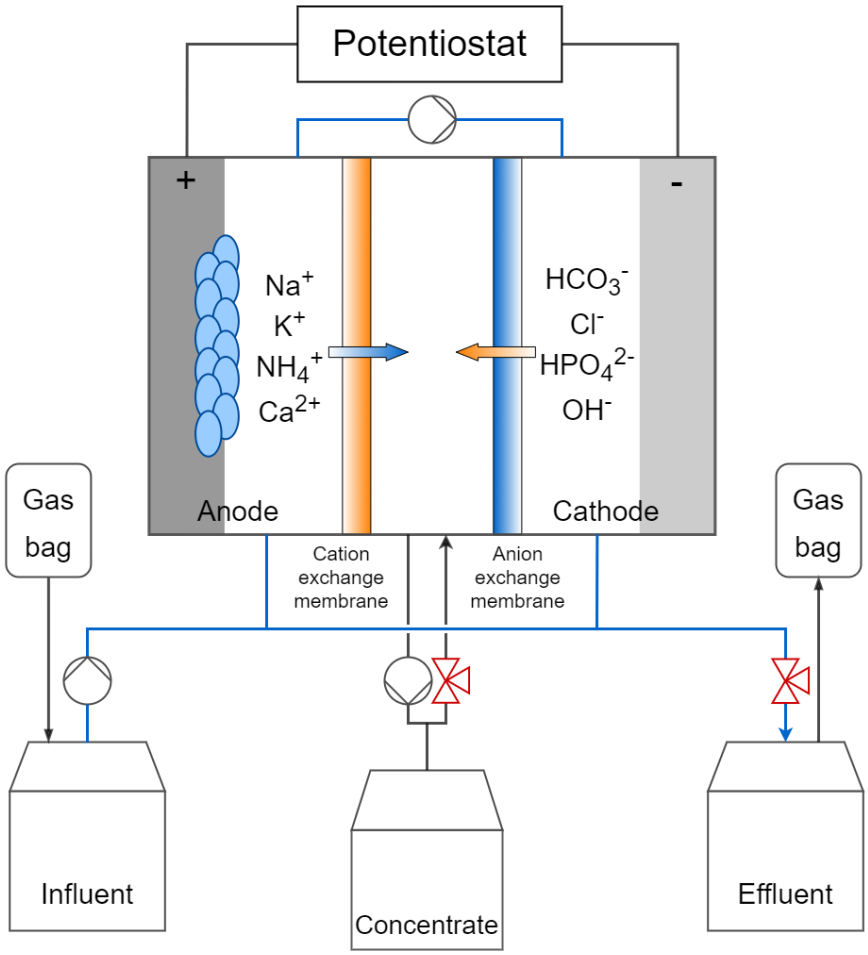

Figure 9. *Experimental configuration of BEC reactor in continuous phase*

While the inorganics and micronutrient components remained unchanged, the influent of continuous phase used 4.4 mM $NaHCO_3$, equivalent to 369.6 mg L^{-1} to simulate bicarbonate buffer system in RAS water. This concentration was twice as strong as the alkalinity level of RAS water samples acquired from a research facility (Laukaa, Finland). On the other hand, the organics concentration was reverted to 125 mg L^{-1} sodium acetate, 54.2 mg L^{-1} glycine and 91.5 mg L^{-1} glucose. This decision was taken to ensure that the anodic microbial community received sufficient chemical energy from the substrates for TAN removal. Thus, the medium's TOC level was stronger than real RAS water, and with this composition the influent's pH and conductivity was 8.3 and 0.7 mS cm^{-1}, respectively. The influent was prepared into autoclaved bottles and replaced every 2 to 3 days.

Continuous operation of reactors lasted in total 75 days, during which 4 different HRTs were experimented for TAN removal. A summary of the steps within continuous phase and their parameters are given in Table 5.

Table 5. *Hydraulic retention times and average influent flow rates during continuous phase*

Steps	Days	HRT (h)	Average inflow rate ($mL\ h^{-1}$)	Theoretical TAN loading rate ($mg\ d^{-1}$)
C1	117 – 138	23.5	3.0	Not measured
C2	145 – 158	18.7	3.7	1.0
C3	158 – 176	13.6	5.0	1.4
C4	176 – 196	9.8	6.8	1.9

During the initial step C1, the HRT set for the system presented problems with low electrolyte conductivity and rusting in the cathode of R2. Therefore, no samples were taken, and the HRT was decreased to 18.7 h, equivalent to a flowrate of 3.7 mL h^{-1}. After the reactors entered steady-state condition as constant conductivity was recorded in the concentrate samples, all reactors were sampled. In the subsequent steps, the HRTs were adjusted stepwise to 13.6 h and 9.8 h, corresponding to an average inflow rate of 5 mL h^{-1} and 6.8 mL h^{-1} respectively. Overall, sampling of reactors was conducted mainly during steps C2, C3 and C4.

4.4 Sampling and analyses

4.4.1 Sample collection and preparation procedure

To assess the performance of enrichment phase, samples were collected from the outlet tube of anodic chamber after the recirculation medium was replaced, in the middle and at the end of the feeding cycle. Concentrate samples were retrieved directly from the collection bottles once a week. During continuous operation, samples were obtained from the effluent outlet tube and middle chamber's recirculation loop. Reactors were allowed to stabilize for approximately 2 weeks at the beginning of each HRT before intensive sampling were conducted, which lasted for 4 to 6 days. During stabilization period, sampling took place once a week, whereas 4 sets of samples were taken during intensive sampling period.

Immediately after collection, 1 mL of unfiltered sample was transferred to 1.5 mL microcentrifuge tube for pH and electric conductivity (EC) measurements. The remaining sample volume was filtered through 0.2 µm syringe filter (Macherey-Nagel, Germany) into 15 mL tube, which was stored at 5 °C for a maximum of 20 days prior to analysis and further preservation. During continuous phase, in addition to collection of samples, the mass of effluent and concentrate collection bottles were recorded for calculation of mass balances.

4.4.2 Measurements and analyses

With each set of samples collected from the experiments, routine measurements on samples' pH, EC, and reactors' cell potential were performed. The pH of unfiltered samples was measured using SlimTrode pH electrode (Hamilton, USA) connected to 330i pH meter (WTW, Germany). EC measurement was conducted using an Inlab 752 probe connected to a SevenMulti conductivity meter (Metter Toledo, USA). Cell voltage and electrode potential were measured using a multimeter (CEM Instruments, China) twice a week during enrichment period and once per day during intensive sampling period.

Filtered samples were analyzed for TOC and TAN to evaluate organics and TAN removal in the system, respectively. TOC-V_{CHP} analyzer (Shimadzu, Japan) coupled with ASI-V autosampler unit were used for TOC analysis. During enrichment phase, TAN was measured using LCK304 test kits and DR 2800 spectrophotometer (HACH, USA). In continuous stage, samples were analyzed with DX-120 ion chromatograph (Dionex, USA) equipped with IonPac CS12A cation-exchange column, which was eluted with 0.02 M methanesulfonic acid at 1 mL min^{-1}. Spiking technique was performed to distinguish ammonium peak from sodium peak in the chromatogram by adding 1 mL of 100 mg L^{-1} NH_4^+ solution, equivalent to 296.6 mg L^{-1} NH_4Cl, and 2 mL of sample into 7 mL of MQ water. The NH_4^+ concentration in the sample can be determined by subtracting 10 mg L^{-1} from the total NH_4^+ concentration measured by the instrument.

4.4.3 Calculations and data processing

With respect to nutrient removal performance in BES, several parameters are collectively used to evaluate the performance of the systems based on the mass of TAN in the system during continuous phase. The mass balance was evaluated using the following equations, in which C is the TAN concentration (mg L^{-1}), V (L d^{-1}) and m_{TAN} (mg d^{-1}) is the volume produced and mass of TAN transferred per day, respectively.

$$m_{TAN} = C \cdot V \tag{12}$$

$$C_{influent} \cdot V_{influent} = C_{effluent} \cdot V_{effluent} + C_{removed} \cdot V_{concentrate} \tag{13}$$

From the mass of TAN, the TAN removal efficiency (%) could be calculated using equation (14). The mass of TAN in the influent was calculated using the theoretical TAN level available in the synthetic feed, which composed of TAN derived from glycine degradation described in equation (10) and from NH_4Cl in the inorganics fraction.

$$\text{TAN removal efficiency} = \frac{m_{TAN\ influent} - m_{TAN\ effluent}}{m_{TAN\ influent}} \cdot 100\% \quad (14)$$

In addition to the removal efficiency, TAN removal rate ($g_N\ m^{-3}\ d^{-1}$) can also be used to indicate the removal rate of the system while taking into consideration system's volume. The removal rate can be calculated using equation (15), in which V_R is the hydraulic volume of the reactor (m^3).

$$\text{TAN removal rate} = \frac{m_{TAN\ influent} - m_{TAN\ effluent}}{V_R} \quad (15)$$

Finally, from the current density data and TAN loading, TAN load ratios of the systems at steps C2, C3 and C4 were calculated using equation 8. Recorded data was processed using Microsoft Excel and imported to Veusz 3.4, licensed under GNU General Public License version 2 or later, for graphical illustrations.

5. RESULTS

5.1 Enrichment of anodic biofilm

The pH and EC of the enrichment medium were used as indications of reactor performance during enrichment phase. Previously described in equations 9–11, the biodegradation of organics produces protons which reduce the pH of electrolyte. Thus, pH drop indicates microbial activity, while the energy produced by exoelectrogens facilitates ions transport into the middle chamber and reduces the medium's EC. During the enrichment period which lasted approximately 120 days, these parameters evolved consistently. Figure 10 demonstrates the pH and EC of the triplicate reactors' recirculation medium under enrichment stage.

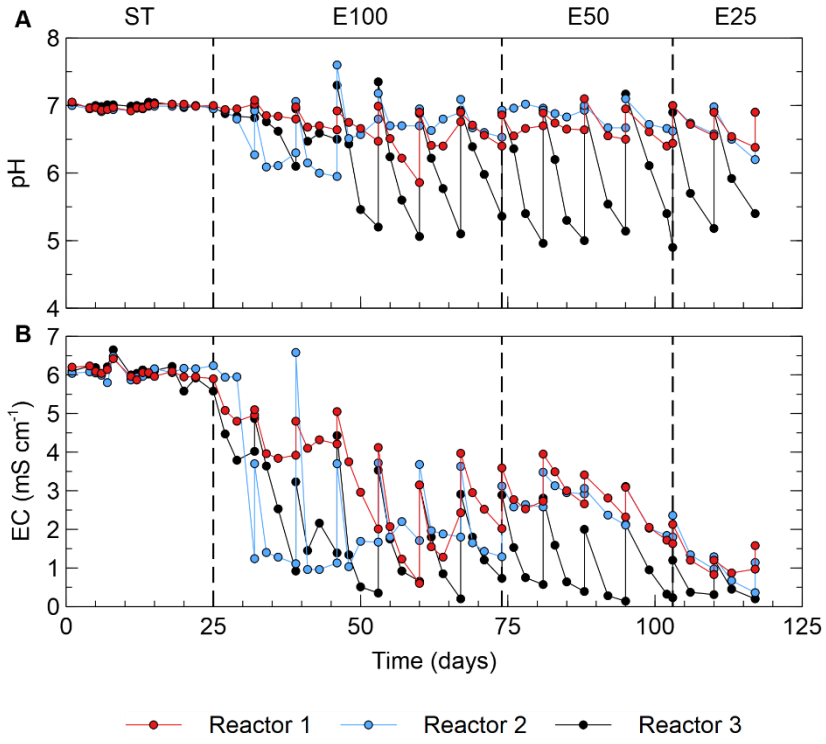

Figure 10. *(A) pH and (B) electrical conductivity (EC) of the recirculation medium over enrichment period*

During the initial enrichment step, the pH of all reactors' medium was stable around 7 while EC values ranged between 5.5–6.5 mS cm^{-1}. Compared to the pH and EC of 7 and 6.1 mS cm^{-1} in freshly prepared medium, respectively, these results indicated minimal anodic microbial activity during ST step due to low organics concentration available in the medium. In subsequent steps, the addition of organics had discernible influences on the reactors. During each feeding cycle of one week, both pH and EC dropped sharply

during the first 4 days following medium replacement and stalled toward the end of the feeding cycle. The reduction magnitude of EC could be as high as 5.6 mS cm^{-1}.

An overall trend could be observed in the pH following step E100, during which rapid pH decline was immediately observed in reactors 2 and 3 following medium composition modification. On the other hand, the same phenomenon was only observed from day 53 in reactor 1, indicated slower enrichment progress in this reactor. The reduction of both organics and buffers in the medium during steps E50 and E25 helped maintaining a stable operational pH range for reactors 1 and 2, whose pH were consistently above 6. However, the pH level of reactor 3 repeatedly dropped to 5 at the end of feeding cycle independent of the varying organics level in the medium. The minimum pH value of enrichment period was observed in reactor 3 at 4.9, while the minimum pH in reactors 1 and 2 was 5.9 and 6, respectively.

The effects of various medium compositions across enrichment stages were clearly demonstrated by the EC of the recirculation medium. Similar to the changes in pH at the beginning of E100, immediate EC decline was observed in reactors 2 and 3, while the decrement in reactor 1 occurred more gradually. This emphasizes the correlation of pH and EC parameters as indicators of reactor performance. The minimum EC recorded was 0.14 mS cm^{-1} in reactor 3 in step E50, while the minimum EC recorded in reactor 2 was in E25 at lowest buffer concentration. Extended operation of reactors under low conductivity was observed to have negative effects on the cathode (Figure 11), which required replacements.

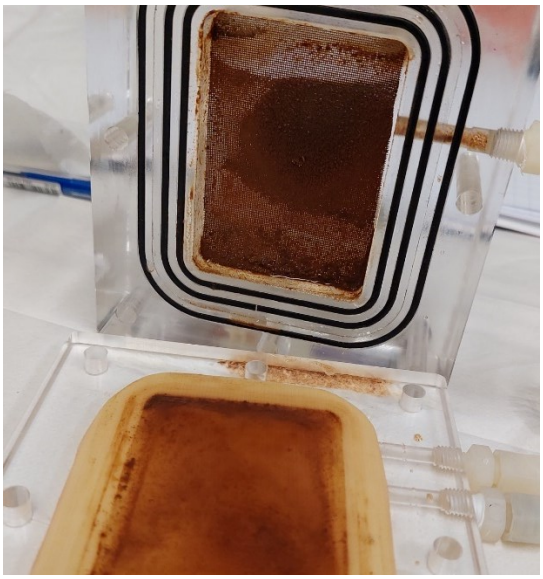

Figure 11. *The cathode was damaged extensively under low EC condition*

Similar to the recirculation medium, the pH and EC of the concentrate were evaluated and are displayed in Figure 12. No concentrate was generated by the reactors until the first week of step E100. The pH values of the concentrate throughout enrichment phase consistently ranged between 6.5 to 7.5 with reactor 3 situated in the lower range. This could be explained by increased protons produced in the anode of reactor 3 which was transported through the membrane to the middle chamber. No clear discrepancy in terms of pH could be observed between enrichment steps. On the other hand, the EC of concentrate directly reflected the medium composition. The maximum EC observed was 30.2 mS cm^{-1} during step E100, while the highest EC detected in steps E50 and E25 was 20.5 mS cm^{-1} and 16.5 mS cm^{-1}, respectively. The decline aligned with corresponding the buffer ratio and suggested that the majority of ions available in the concentrate derived from buffer component of the recirculation medium.

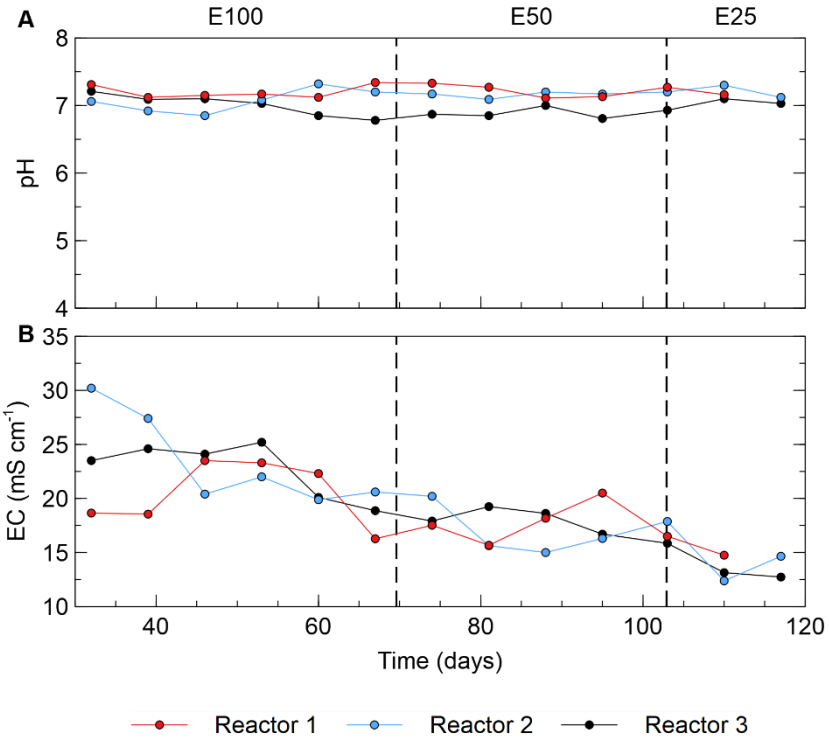

Figure 12. *(A) pH and (B) EC of concentrate over enrichment period*

Along with the medium pH and EC, TOC was used as a direct indicator of microbial organics degradation. Figure 13 indicates the medium TOC level of all reactors.

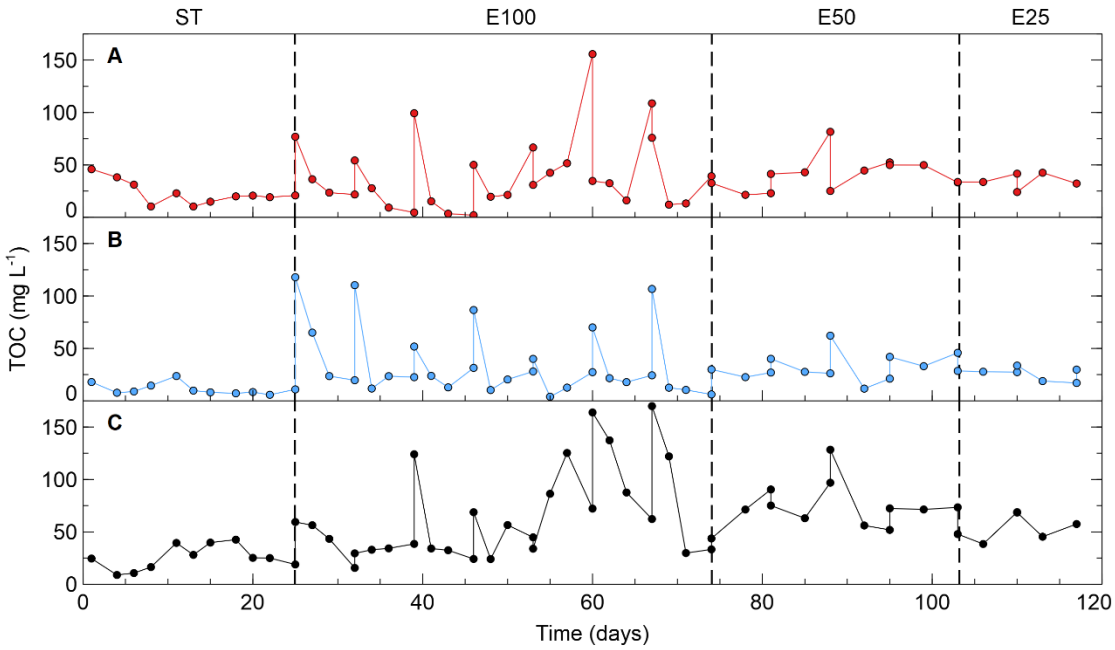

Figure 13. *(A-C) TOC level in the recirculation medium of reactors 1-3, respectively*

In reactors 1 and 2, the TOC decline rapidly at the beginning of feeding cycle and stabilized towards the end. The decrement was not prevalent in ST step but could be observed in E100 stage, during which TOC level fell to the minimum concentration of 2 mg L^{-1} in reactor 1. In addition, the reduction of organics in different enrichment steps could be discerned from the figure; for instance, the maximum TOC of E50 stage was 88 mg L^{-1}, approximately half the maximum value of 160 mg L^{-1} observed in E100. Towards the end of enrichment period, TOC level in both reactors decreased to an average of 30 mg L^{-1}. On the other hand, the TOC level recorded in reactor 3 progressed differently compared to reactors 1 and 2. During various enrichment cycles, the TOC appeared to increase after medium change, for example in the feeding cycles of day 53–60 and day 74–81. This result contradicts the behavior in reactors 1 and 2, in which the TOC decreased in a predictable manner. In step E25, the average TOC concentration in reactor 3 was 57 mg L^{-1}, double the level measured in other reactors. Because TOC is an aggregate measurement method, which is unable to indicate the degradation of specific organic components, alternative analytical techniques are required to accurately reflect the biodegradation rate.

As the enrichment period was conducted over an extended timespan, only a portion of current data will be described in the thesis. Figure 14 displays the current density recorded in reactor 1 during the transition from enrichment step E50 to step E25. The current density was normalized by the area of membranes, which was 0.0035 m^2. During the first two days of feeding cycle, the current density peaked within two hours after medium

replacement in both steps before decreasing and stabilized toward the end of the batch. The maximum current density recorded in step E50 was 5.2 A m^{-2}, while in E25 the peak current density was 4.7 A m^{-2}. In addition, the decline rate of current density in step E50 was more gradual compared to the rate observed in E25. Thus, the results validated the dependence of current density on the availability of organics in the medium. However, towards the end of the enrichment cycle, the average current density in both steps were comparable.

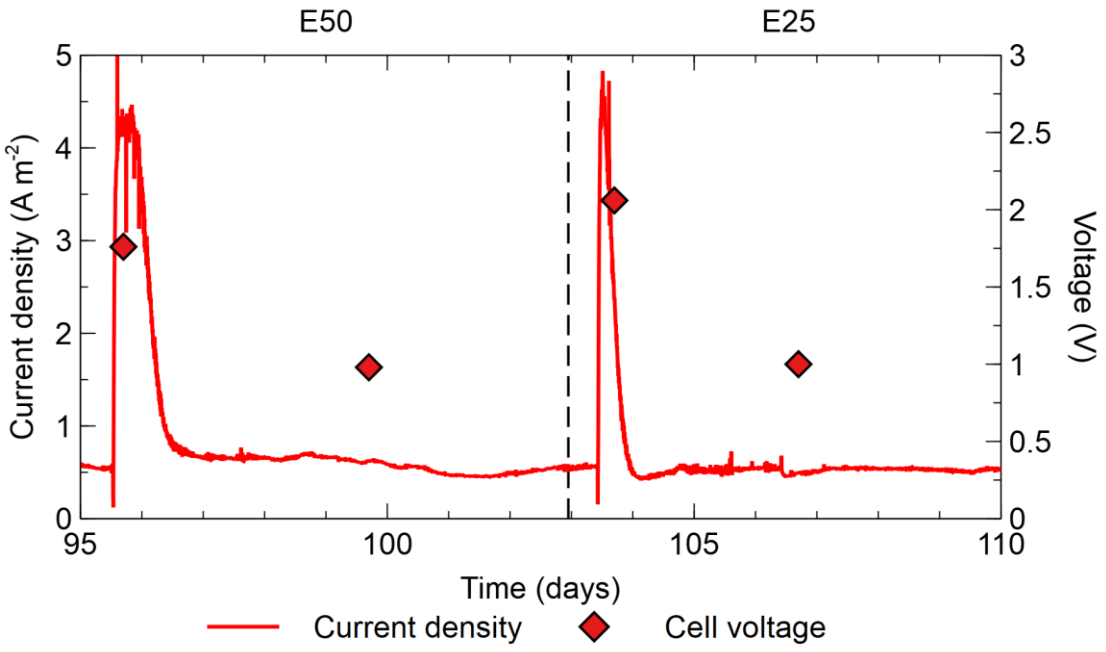

Figure 14. *Current density and measured cell voltage of reactor 1 from day 95 to day 100 of enrichment period*

Cell voltage, which is a conventional indicator of cell performance, is also displayed in Figure 14. After medium replacement, the cell voltage increased to 1.8 V in step E50 and 2.1 V in E25. As the substrate availability decreased, the voltage also declined to approximately 1 V at the end of the feeding cycle in both steps. Previously described in section 3.2.2, total cell voltage increases due to the current produced by exoelectrogens from organic substrate. Thus, the results achieved align with bioelectrochemistry theory previously described.

During enrichment period, TAN removal efficiency was also estimated to preliminary investigate the capability of the set-up for nitrogen removal, although batch mode configuration was not the primary evaluation basis of the study. The results from E100 to E25 enrichment batches indicate increased TAN removal efficiency as enrichment period progressed, ranging from 35.5 ± 19.4 % in E100 to 81.7 ± 25 % in E25. The increased efficiency could be explained by the maturity of the anodic biofilm that facilitated NH_4^+

migration across cation exchange membrane (CEM). In contrast, higher TAN removal rate was obtained during step E100 at 7.5 ± 5.4 g_N m^{-3} d^{-1}, compared to the rate of 5.8 ± 1.2 g_N m^{-3} d^{-1} achieved during step E25. This was likely due to the TAN level derived from glycine, which was most abundant in step E100. Towards the end of E100, the removal rate was particularly high in all reactors, which could be explained by the optimal maturity of anodic biofilm as well as high organics level during this step. Table 6 provides a summary of TAN removal rates and efficiencies of triplicate reactors over the enrichment period.

Table 6. *TAN removal efficiencies and removal rates obtained over enrichment period*

Step	Number of batches	TAN removal efficiency (%)	TAN removal rate (g_N m^{-3} d^{-1})
E100	9	35.5 ± 19.4	7.5 ± 5.4
E50	9	60.2 ± 22.5	5.4 ± 2.4
E25	2	81.7 ± 17.7	5.8 ± 1.2

Overall, the results acquired from enrichment period suggested consistent operation across reactors in terms of pH, EC, TOC, TAN removal rate and efficiency. Although there were discrepancies across different reactors, clear trends could be identified from the results. Thus, the reactors were considered stable and adequate for transition towards continuous operation.

5.2 Continuous operation

In between intensive sampling periods of continuous phase were stabilization periods, during which the reactors were given time to adjust to the modified organics and TAN loading rate. Thus, data collected during stabilization periods were utilized for evaluating the maturity of reactors for intensive sampling and will not be described in this study. Additionally, as step C1 was considered unsuitable for reactors operation, its data was not analyzed.

Figure 15 displays the pH and EC of the effluent during intensive sampling periods at varying HRTs. During C2, the average pH was 5% lower than the pH level of 5.9 measured in step C3. The pH progressively increased to 6.1 ± 0.6 in step C4. Although the concentration of buffer and organics in the influent was constant, the influent flow rate determines their loadings to the system. In this case, ratio of buffer to organics was sufficient to maintain a suitable environment for anodic microorganisms, although compared to the influent pH level of 8.3, the pH level measured from the effluents were substantially lower. Conversely, the adjustment of inflow rate had minimal effect on the recorded EC

level. In C2, the EC ranged between 0.7 to 0.2 mS cm^{-1} and averaged at 0.3 mS cm^{-1} across reactors. In the following steps, EC increased to 0.4 ± 0.2 mS cm^{-1} before declining to 0.3 ± 0.1 mS cm^{-1} in C4. Compared to the influent's EC of 0.7 mS cm^{-1}, the reduction was the most significant in step C3.

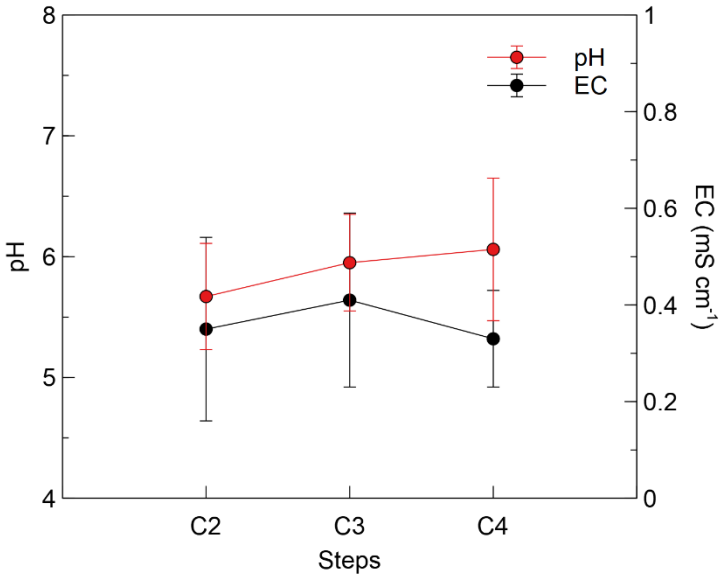

Figure 15. *pH and EC of effluent during intensive sampling periods*

In contrast to the effluent, effects of varying HRT could be observed from the pH and EC of the collected concentrate, indicated in Figure 16. During C3 and C4, concentrate's pH was measured at 7.3 ± 0.3 and 7.2 ± 0.6, respectively. The increment was substantial compared to the pH of 6.3 ± 0.5 obtained in C2. The result may suggest bicarbonate buffer to be the primary component in the concentrate due to its alkalinity. However, precise analysis is needed to determine the fraction of buffer entering the middle chamber. With respect to the EC of concentrate, a clear trend could be speculated from Figure 16, in which the average EC measured was 8.3 ± 1.1 mS cm^{-1} in C2. The EC increased by 1.3 mS cm^{-1} in step C3 (9.6 ± 1.1 mS cm^{-1}) and raised to the maximum level in C4 (10.4 ± 0.9 mS cm^{-1}). In conclusion, the decrease of HRT, which corresponded to increased organics and buffer loading rate to the system, had elevated both the pH and EC of the concentrate.

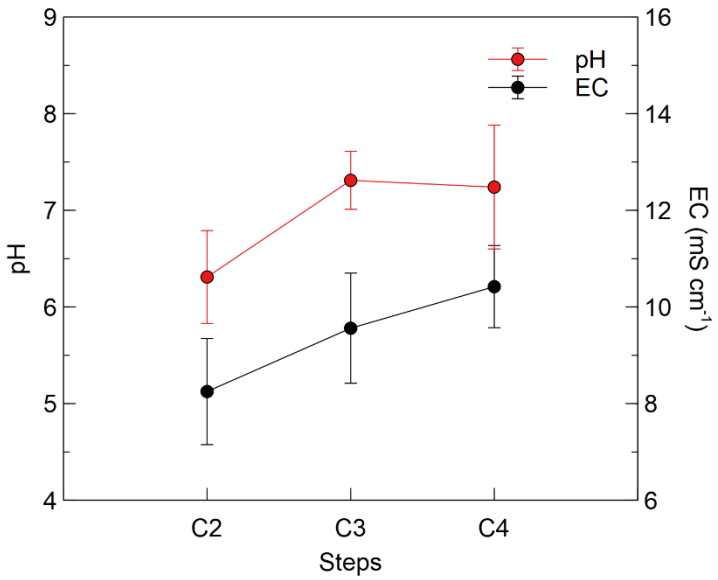

Figure 16. *pH and EC of concentrate during intensive sampling periods*

Throughout continuous operation, the current density data obtained from reactor 1 became extremely noisy, which could be explained by gas bubbles in the system or poor reference electrode connection; therefore, only current data from reactors 2 and 3 were evaluated. Figure 17 displays the current density obtained from reactor 2 during intensive sampling periods (C2–C4).

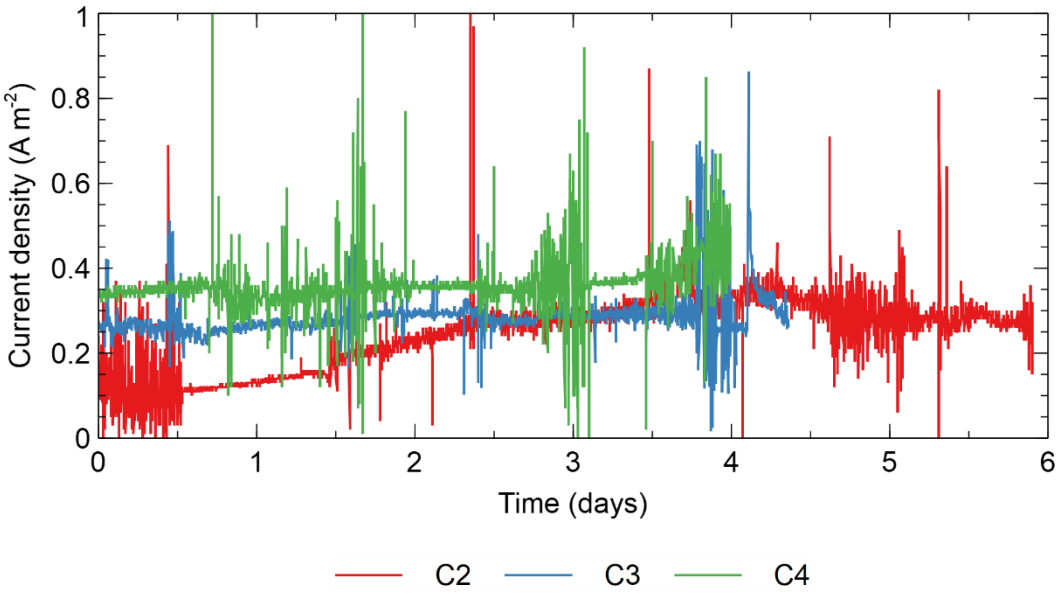

Figure 17. *Current density of reactor 2 during intensive sampling periods*

Unlike the trend observed during enrichment, the current density was stable during the majority of the intensive sampling period with exception of noise. Thus, constant organic load to the system ensures stable current production. In both reactors 2 and 3, the maximum current density of 0.4 ± 0.1 A m^{-2} was reached during step C4, followed by 0.3 ±

0.1 A m^{-2} achieved in both steps C3 and C2. Therefore, an inverse relationship could be identified between HRT and current density. As current density is determined by the organics loading to the system, this result was expected. The obtained current densities were used for computing the TAN load ratio, which will be described in chapter 5.3.

The cell voltage of the reactors was also evaluated during intensive sampling period. During C2, the cell voltage measured was 2.1 ± 0.9 V for the triplicate reactors. The voltage increased to 2.5 ± 1.2 V during C3, indicating enhanced cell voltage with higher organic loading. However, a similar trend was not observed during C4, during which measured voltage was 2.3 ± 0.9 V. Thus, no strong correlation between cell voltage and inflow rate was found.

From the measured pH, EC of effluent and concentrate as well as current density, a clear trend could be speculated. The elevated organics and buffer loading as a result of shorter HRT collectively increased the pH of effluent and concentrate, while only the EC of the concentrate was affected by the HRT. Consequently, the current density was 7–16% higher after each step, indicating the impacts of HRT towards current production by anodic microorganisms.

5.3 Removal of total ammonia nitrogen with continuous bioelectroconcentration cell set-up

The theoretical inlet TAN concentration of 11.7 mg L^{-1} was used as the basis for calculation of TAN removal efficiency and removal rate. This level was determined based on the inorganic NH_4^+ available in the synthetic medium, as well as the theoretical maximum NH_4^+ that could be generated from glycine degradation. Therefore, the removal efficiency and rate described in this chapter were considered the minimum efficiency achievable with the set-up.

Throughout the experiment, the maximum TAN removal efficiency achieved was in step C2 with 56.9 ± 12.6 % in reactor 1. During this step, the overall TAN removal efficiency was 33.5 ± 26.6 % while the removal rate was 5.7 ± 4.9 g_N m^{-3} d^{-1}, which were the highest compared to other steps, indicated by Figure 18. Based on the figure, increased TAN loading to the system had negative effects towards TAN removal efficiency. In C3, the overall TAN removal efficiency decreased to 13.27 ± 4.23%, approximately half efficiency obtained during the previous step. The decline was not as significant in step C4, during which TAN removal efficiency reached the minimum of 11.3 ± 5.6%.

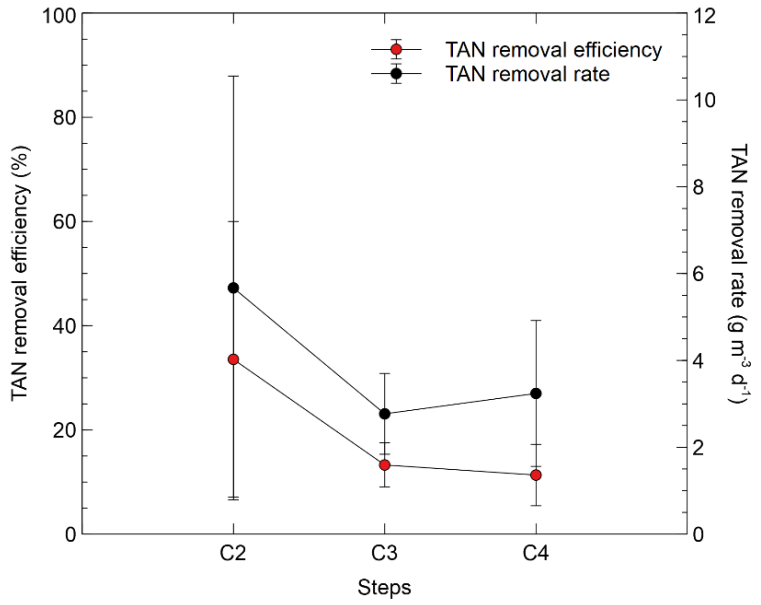

Figure 18. *TAN removal efficiencies and rates of continuous operation steps*

A decline in TAN removal rate in C3 (2.8 ± 0.9 g_N m^{-3} d^{-1}) was observed with decreased HRT from 18.7 h 13.6 h. However, a similar trend was not observed in C4, during which TAN removal rate measured increased to 3.2 ± 1.7 g_N m^{-3} d^{-1}. This could be caused by the elevated TAN loading rate in this step, which increases the overall TAN removal rate. In addition, due to negligible TAN removal achieved in reactors 1 and 2, only results from reactor 3 was considered in C4. Therefore, TAN removal rate appeared to be independent of the HRT. The detailed result of TAN removal efficiency and rate obtained during continuous operation could be obtained from Appendix C.

Based on the current density obtained in intensive sampling periods, TAN load ratio was calculated for each period to evaluate the parameter's ability to illustrate TAN removal efficiency. Figure 19 presents the TAN removal efficiency of each step with respect to the average TAN load ratio calculated. During C2, the average load ratio was 11.9. In the following step, the load ratio decreased to approximately 9.2 and 9.3 in steps C3 and C4, respectively. Based on the fitting analysis, strong correlation (R^2 = 0.988) between TAN load ratio and TAN removal rate was established. However, as the efficiency obtained in C3 and C4 was comparable to each other, the results presented may have been an over estimation of the correlation between these parameters.

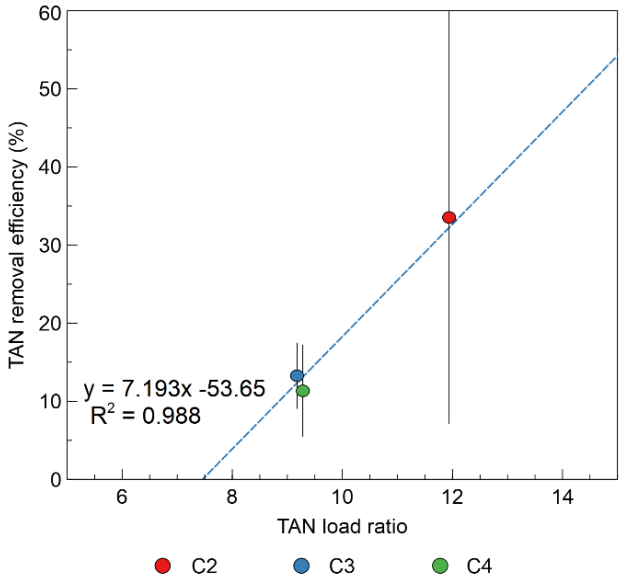

Figure 19. *Correlation between TAN removal efficiency and TAN load ratio of continuous operation steps*

In conclusion, TAN removal efficiency and removal rate was the most optimal in step C2, during which the system operated with an average HRT of 18.7 h. In C2, the set-up was able to remove 33.5 ± 26.5% of the theoretical inlet TAN loading, while a removal rate of 5.7 ± 4.9 g_N m^{-3} d^{-1} was achieved during this step. In the following steps C3 and C4, the removal efficiency achieved was comparable to each other at 13.3 ± 4.2% and 11.3 ± 5.9%, respectively. The increase in flowrate may have inverse effects on TAN removal rate, although this was not observed in step C4. Overall, the results suggest direct effects of influent flowrate on TAN removal capability of the system.

6. DISCUSSION

6.1 Effects of medium composition on reactor performance

During batch mode operation of enrichment period, changes in organics and buffer composition in the recirculation medium had direct impacts on the pH, EC, current density, and TOC level in the reactors. Compared to previously described batch mode BESs, the development of these parameters behaved in a predictable manner. The reduction of pH within each feeding cycle was a common phenomenon observed in BES due to the degradation of organics by exoelectrogens (Rozendal et al., 2008). Towards the end of the batch, both the pH and EC of the recirculation medium collapsed, which was found to have detrimental effects on the BES cell structure. The cathodes showed clear signs of rusting and were replaced several times throughout the experiment along with fouled membranes. A possible explanation for this phenomenon might be the acidic condition of the recirculation medium's pH which induced corrosion. Previously, operation under low pH and EC condition was proven to be unfavorable for BES performance. At pH 5, anodic microorganisms have been shown to perform inadequately (Biffinger et al., 2008), while low EC increases the internal resistance and hinders ion transportation in the cell (Zhang et al., 2019). Therefore, to mitigate this problem, the duration of enrichment batch could be shortened in future experiments employing low EC feed such as RAS water.

In terms of current production, peak current density was reached within two hours after starting a new feeding cycle, after which it decreased as organics in the medium depleted. Similar trend was observed in Cerrillo et al. (2016), in which the current density immediately raised after new medium was fed to the cell. The fact that the peak current density was not reached instantly after feeding could be attributed to the partial medium replacement procedure conducted. Additionally, the composition of the organics in the medium may have effects on the current density. The majority of carbon available in the recirculation medium was provided by glucose, while glycine and acetate contributed about 19% and 23% of the medium's TOC, respectively. Glucose must be degraded for example into acetate and formate before it could be used by anodic microorganisms for energy production (Zhao et al., 2020). While the presence of glucose may provide diverse anodic microbial community capable of utilizing a wide variety of substrates, the Coulombic efficiency of the system may be dampened due to competing metabolism pathways such as fermentation (Chae et al., 2009).

During enrichment, the set-up demonstrated its capability for nitrogen removal. The removal efficiency of TAN in batch mode reached up to 81.7 ± 17.7% in step E25 of enrichment period, which was considerably high compared to other systems operated in batch mode. For example, using pig slurry as the feed for a MFC, Cerrillo et al. (2016) obtained maximum TAN removal efficiency of 40%. In another batch mode set-up which coupled MEC with forward osmosis, Qin et al. (2016) effectively removed 63.7 ± 6.6% of ammonium from landfill leachate. Therefore, batch mode remains an attractive configuration for TAN removal and recovery. However, continuous mode should be explored for nutrient removal to diversify the treatment alternatives, as well as to resolve the challenges of batch mode operation, such as extremely low pH and EC at the end of the batch.

For the case of RAS water treatment, batch mode operation would be inadequate to treat the immense volume of water recirculated in the system. Additionally, there are risks of overestimating the TAN removal efficiency obtained in enrichment period. Both the organics concentration and the TAN level recorded in the experiment was higher than real RAS water, which allowed for more ions to be removed through the membranes into the concentrate chamber. Thus, the efficiency may not be guaranteed when real RAS water is fed into the system. Moreover, the overestimation of TAN removal efficiency could be amplified due to the inaccuracy of ammonium test kits employed for TAN analysis. Thus, the values obtained should only be used as a suggestion of the system's maximum capability for TAN removal.

6.2 Continuous bioelectrochemical nitrogen removal with bioelectroconcentration cell

During continuous operation, the system demonstrated improved TAN removal efficiency with longer HRT. The maximum TAN removal efficiency and removal rate was obtained in step C2, during which the reactors operated with an average HRT of 18.7 h. However, longer HRT does not necessarily guarantee higher TAN removal efficiency, as demonstrated in the study by Koskue et al. (2021). In their research, optimal TAN removal efficiency was obtained with HRT of 9.8 h, while at HRT of 20 h the TAN removal efficiency was 10% lower. Such discrepancy between these results and those obtained in this thesis could be a result of varied feed composition, as the feedstock concerned in Koskue et al. (2021) was reject water from anaerobically digested sludge with the EC of 7.8 ± 0.5 mS cm^{-1}. RAS water, on the other hand, had very low EC of 1.2 mS cm^{-1} (Davidson et al., 2014). With low conductivity streams such as domestic wastewater (1.3 ± 0.1 mS cm^{-1}), Monetti et al. (2019) achieved maximum TAN removal efficiency (15 ± 2.7%) with

longer HRT in their BEC set-up. However, at lower feed's conductivity, the efficiency obtained in this thesis (33.5 ± 26.5%) was higher than results published in Monetti et al. (2019). Thus, although EC is not the only factor contributing to TAN removal efficiency, high conductivity feed may have improved TAN removal rate (Table 7).

Compared to other BEC systems other than Monetti et al. (2019), the TAN removal efficiency and removal rate obtained in this thesis was substantially lower. The experiment also demonstrated the challenges concerning TAN removal from RAS water with BEC, which was RAS water's low EC. Additionally, while not directly addressed in this study, permeation of NH_4^+ from the middle chamber via anion exchange membrane to the cathode could decrease system's TAN removal efficiency (Ledezma et al., 2017). When compared against biofilters utilized in RAS, the TAN removal rate of the BEC in this thesis did not match the rate of neither moving-bed biofilter (240 g_N m^{-3} d^{-1}) nor trickling filter (640 g_N m^{-3} d^{-1}). Thus, further research should be undertaken to optimize the reactor performance and enhance the operational condition for BEC treating RAS water. Table 7 recaps the maximum TAN removal efficiency and removal rate achieved in the thesis' experiment and compares them with the TAN removal efficiencies and rates previously achieved in BEC set-ups, as well as common biofilters in RAS.

Table 7. *Maximum TAN removal efficiencies and rates of BEC set-ups and RAS biofilters*

Configurations	**Feed**	**EC (mS cm^{-1})**	**TAN removal efficiency (%)**	**TAN removal rate (g_N m^{-3} d^{-1})**	**References**
BEC	Synthetic reject water	7.8 ± 0.5	75.5 ± 2.7	551 ± 67	(Koskue et al., 2021)
	Domestic wastewater	1.3 ± 0.1	15 ± 2.7	29.9 ± 5.0	(Monetti et al., 2019)
	Synthetic urine	19.5 ± 0.5	59.7 ± 2.47	8670	(Ledezma et al., 2017)
	Synthetic RAS water	0.7	33.5 ± 26.5	5.7 ± 4.9	This thesis
Moving-bed biofilter	RAS water	1.2	95.3	240	(Abubakar Tadda et al., 2021)
Trickling filter			40–100	640	(Lekang & Kleppe, 2000) (Davidson et al., 2014)

Finally, TAN load ratio was analyzed in this study to evaluate the suitability of this parameter as an indicator of TAN removal efficiency. The load ratio calculated in all steps of the thesis were above 9, indicating that there was surplus current for ammonium migration and removal. Compared to the maximum load ratio of 1.26 for BEC fed with synthetic reject water (Koskue et al., 2021), the load ratio obtained in this study was considerably higher. Therefore, for future research, the organics concentration in the synthetic RAS water could be reduced. During step C2, with the HRT of 18.7 h, the set-up achieved both maximum TAN load ratio and removal efficiency. Although regression analysis has demonstrated strong correlation (R^2 = 0.988) between TAN load ratio and TAN removal efficiency, the sample size was relatively small (n = 3). Therefore, further analysis is needed to confirm the correlation specifically for BECs fed with RAS water.

The thesis has demonstrated the applicability of a novel BEC set-up for treatment of RAS water and proposed an optimal HRT for TAN removal. The development of the system, as well as challenges regarding system operation were also described in detail. As the experiment was conducted as the initial investigation of BES applicability for RAS water treatment, real RAS water was not used, which limited the practical aspect of the experiment. Consequently, the behavior of OFCs in the system was outside of the scope of the thesis and should be evaluated in future studies. Despite these limitations, the thesis has fulfilled its role as an exploratory step towards bioelectrochemical treatment of RAS water and contributed to the numerous applications of BESs.

7. CONCLUSIONS

In this thesis, the applicability of bioelectrochemical nitrogen removal from RAS water was investigated using a triplicate BEC set-up. The anodic microbial community was first enriched in reactors fed with synthetic RAS water operated under batch mode configuration. The system was subsequently updated to continuous operation, during which varying HRTs were tested to determine the optimal operational HRT for TAN removal.

During enrichment period, anodic microorganisms were supported by the organics available in the synthetic recirculation medium. The pH of the recirculation medium in all enrichment steps was maintained between 5 to 7.5, while EC reduced to as low as 0.14 mS cm^{-1}, which had damaging effects on the cathode material. Shortly after feeding, maximum current densities of 5.2 A m^{-2} and 4.7 A m^{-2} were obtained in steps E50 and E25, respectively.

During continuous experiment, the reactors operated with decreasing HRTs of 18.7 h, 13.6 h, and 9.8 h. The decline in HRT elevated the pH and EC of effluent and concentrate collected from the reactors. The pH of the effluent was consistently between 5.5 to 6.5, while EC was maintained above 0.2 mS cm^{-1}. Maximum current density (0.39 ± 0.1 A m^{-2}) was achieved with the lowest HRT of 9.8 h.

With respect to the system's TAN removal capability, maximum TAN removal efficiency (33.5 ± 26.5 %) and TAN removal rate (5.67 ± 4.88 g_N m^{-3} d^{-1}) were achieved at HRT of 18.7 h, which indicated inverse relationship between TAN removal efficiency and HRT. Consequently, peak TAN load ratio of 11.94 was achieved with this HRT. Strong correlation (R^2 = 0.988) between TAN load ratio and TAN removal efficiency was demonstrated. However, due to small sample size (n = 3), further analysis is needed to confirm the accuracy of TAN load ratio as an indicator of TAN removal efficiency in BECs fed with RAS water.

Based on the demonstrated TAN removal rate, further research should be conducted to enhance the system to match the TAN removal rate (24–640 g_N m^{-3} d^{-1}) required in RAS facilities. In addition, the mechanisms of OFCs production and degradation in the BEC system should also be addressed by feeding the system with real RAS waters containing off-flavor compounds. In conclusion, the thesis has fulfilled its role as a preliminary investigation for bioelectrochemical nitrogen removal from RAS water.

REFERENCES

Abubakar Tadda, M., Li, C., Gouda, M., El-Fatah Abomohra, A., Shitu, A., Ahsan, A., … Liu, D. (2021). Enhancement of nitrite/ammonia removal from saline recirculating aquaculture wastewater system using moving bed bioreactor. *Journal of Environmental Chemical Engineering 9, 105947*.

Aguilar-Alarcón, P., Gonzalez, S. V, Simonsen, M. A., Borrero-Santiago, A. R., Sanchís, J., Meriac, A., … Mikkelsen, Ø. (2020). Characterizing changes of dissolved organic matter composition with the use of distinct feeds in recirculating aquaculture systems via high-resolution mass spectrometry. *Science of the Total Environment 749, 142326*.

Ahmad, A., Rozaimah Sheikh Abdullah, S., Abu Hasan, H., Razi Othman, A., & Ismail, I. (2021). Aquaculture industry: Supply and demand, best practices, effluent and its current issues and treatment technology. *Journal of Environmental Management 287*, *112271*.

Ahmed, N., & Turchini, G. M. (2021). Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *Journal of Cleaner Production 297, 126604*.

Algar, C. K., Howard, A., Ward, C., & Wanger, G. (2020). Sediment microbial fuel cells as a barrier to sulfide accumulation and their potential for sediment remediation beneath aquaculture pens. *Scientific Reports 10, 13087* .

Arredondo, M. R., Kuntke, P., Ter Heijne, A., & Jn Buisman, C. (2019). The concept of load ratio applied to bioelectrochemical systems for ammonia recovery. *Journal of Chemical Technology & Biotechnology 94*, 2055–2061.

Aslam, S. N., Navada, S., Bye, G. R., Mota, V. C., Terjesen, B. F., & Mikkelsen, Ø. (2019). Effect of CO2 on elemental concentrations in recirculating aquaculture system tanks. *Aquaculture 511, 734254*.

Azaria, S., & Van Rijn, J. (2018). Off-flavor compounds in recirculating aquaculture systems (RAS): Production and removal processes. *Aquacultural Engineering Journal 83*, 57–64.

Badiola, M., Basurko, O. C., Piedrahita, R., Hundley, P., & Mendiola, D. (2018). *Energy use in Recirculating Aquaculture Systems (RAS): A review*. *Aquacultural Engineering 81*, 57–70.

Biffinger, J. C., Pietron, J., Bretschger, O., Nadeau, L. J., Johnson, G. R., Williams, C. C., … Ringeisen, B. R. (2008). The influence of acidity on microbial fuel cells containing

Shewanella oneidensis. *Biosensors and Bioelectronics 24*, 900–905.

Boyd, C. E., & McNevin, A. A. (2015). *Aquaculture : resource use, and the environment* (A. McNevin, Ed.). Hoboken, New Jersey: John Wiley & Sons Inc.

Brazil, B. L. (2006). Performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. *Aquacultural Engineering 34*, 261–274.

Burr, G. S., Wolters, W. R., Schrader, K. K., & Summerfelt, S. T. (2012). Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, Salmo salar, cultured in a recirculating aquaculture system. *Aquacultural Engineering 50*, 28–36.

Carlucci, D., Nocella, G., De Devitiis, B., Viscecchia, R., Bimbo, F., & Nardone, G. (2015). Consumer purchasing behaviour towards fish and seafood products. Patterns and insights from a sample of international studies. *Appetite 84*, 212–227.

Cerrillo, M., Oliveras, J., Viñas, M., & Bonmatí, A. (2016). Comparative assessment of raw and digested pig slurry treatment in bioelectrochemical systems. *Bioelectrochemistry 110*, 69–78.

Chae, K.-J., Choi, M.-J., Lee, J.-W., Kim, K.-Y., & Kim, I. S. (2009). Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. *Bioresource Technology 100*, 3518–3525.

Chen, X., Liang, P., Zhang, X., & Huang, X. (2016). Bioelectrochemical systems-driven directional ion transport enables low-energy water desalination, pollutant removal, and resource recovery. *Bioresource Technology 215*, 274–284.

Cook, D., Newcombe, G., & Sztajnbok, P. (2001). The application of powdered activated carbon for MIB and geosmin removal: predicting PAC doses in four raw waters. *Water Research 35*, 1325–1333.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., & Verstraete, W. (2007, September 28). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture 270*, 1–14.

Dalsgaard, J., Lund, I., Thorarinsdottir, R., Drengstig, A., Arvonen, K., & Pedersen, P. B. (2013). Farming different species in RAS in Nordic countries: Current status and future perspectives. *Aquacultural Engineering 53*, 2–13.

Davidson, J., Good, C., Welsh, C., & Summerfelt, S. T. (2011). Abnormal swimming behavior and increased deformities in rainbow trout Oncorhynchus mykiss cultured in low exchange water recirculating aquaculture systems. *Aquacultural Engineering 45*, 109–117.

Davidson, J., Good, C., Welsh, C., & Summerfelt, S. T. (2014). Comparing the effects of high vs. Low nitrate on the health, performance, and welfare of juvenile rainbow trout Oncorhynchus mykiss within water recirculating aquaculture systems. *Aquacultural Engineering 59*, 30–40.

Davidson, J., Grimm, C., Summerfelt, S., Fischer, G., & Good, C. (2020). Depuration system flushing rate affects geosmin removal from market-size Atlantic salmon Salmo salar. *Aquacultural Engineering 90*.

Davidson, J., Schrader, K., Ruan, E., Swift, B., Aalhus, J., Juarez, M., … Summerfelt, S. T. (2014). Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon Salmo salar raised to market-size in recirculating aquaculture systems. *Aquacultural Engineering 61*, 27–34.

Davidson, J., Summerfelt, S., Schrader, K. K., & Good, C. (2019). Integrating activated sludge membrane biological reactors with freshwater RAS: Preliminary evaluation of water use, water quality, and rainbow trout Oncorhynchus mykiss performance. *Aquacultural Engineering 87*, 102022.

Davis, M. L. (2013). Water and wastewater engineering. In *Water and wastewater engineering*. New York: McGraw-Hill.

European Commissions, & Directorate-General for Maritime Affairs and Fisheries. (2020). *Recirculation aquaculture systems*. Publications Office.

FAO. (2008). Glossary of aquaculture. Available (accessed on 17.2.2022): http://www.fao.org/fi/glossary/aquaculture

FAO. (2020). *The State of World Fisheries and Aquaculture 2020. Sustainability in action*. Rome.

Freguia, S., Virdis, B., Harnisch, F., & Keller, J. (2012). Electrochimica Acta Bioelectrochemical systems: Microbial versus enzymatic catalysis. *Electrochimica Acta 82*, 165–174.

Ghamkhar, R., Boxman, S. E., Main, K. L., Zhang, Q., Trotz, M. A., & Hicks, A. (2020). Life cycle assessment of aquaculture systems: Does burden shifting occur with an increase in production intensity? *Aquacultural Engineering 92*, 1–14.

Gómez, S., Hurtado, C. F., & Orellana, J. (2019). Bioremediation of organic sludge from a marine recirculating aquaculture system using the polychaete Abarenicola pusilla (Quatrefages, 1866). *Aquaculture 507*, 377–384.

Guttman, L., & van Rijn, J. (2008). Identification of conditions underlying production of

geosmin and 2-methylisoborneol in a recirculating system. *Aquaculture 279*, 85–91.

Hall, P. O. J., Holby, O., Kollberg, S., & Samuelsson, M.-O. (1992). Chemical fluxes and mass balances in a marine fish cage farm. IV. Nitrogen. *Marine Ecology Progress Series 89*, 81–91.

Hamelers, H. V. M., Ter Heijne, A., Sleutels, T. H. J. A., Jeremiasse, A. W., Strik, D. P. B. T. B., & Buisman, C. J. N. (2010). New applications and performance of bioelectrochemical systems. *Applied Microbiology and Biotechnology 85*, 1673–1685.

Hau, H. H., & Gralnick, J. A. (2007). Ecology and biotechnology of the genus Shewanella. *Annual Review of Microbiology 61*, 237–258.

Heijnen, J. J., & Kleerebezem, R. (2010). Bioenergetics of Microbial Growth. In *Encyclopedia of Industrial Biotechnology* (pp. 1–66). John Wiley & Sons, Ltd.

Ho, N. A. D., Babel, S., & Kurisu, F. (2017). Bio-electrochemical reactors using AMI-7001S and CMI-7000S membranes as separators for silver recovery and power generation. *Bioresource Technology 244*, 1006–1014.

Hopkins, J. S., Sandifer, P. A., & Browdy, C. L. (1994). Sludge management in intensive pond culture of shrimp: Effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. *Aquacultural Engineering 13*, 11–30.

Jiaqi, S., Lifen, L., & Fenglin, Y. (2020). Successful bio-electrochemical treatment of nitrogenous mariculture wastewater by enhancing nitrogen removal via synergy of algae and cathodic photo-electro-catalysis. *The Science of the Total Environment 743*, 140738.

Kato, N., & Kawamata, M. (2017). Aquarium Recirculation System. In Takeuchi Toshio (Ed.), *Application of Recirculating Aquaculture Systems in Japan* (1st ed., pp. 237–255). Springer, Tokyo.

Kerzenmacher, S. (2019). Engineering of Microbial Electrodes. In F. Harnisch & D. Holtmann (Eds.), *Bioelectrosynthesis* (pp. 135–180). Cham: Springer International Publishing.

Kim, G., Duffy, L. N., Jodice, L. W., & Norman, W. C. (2017). Coastal Tourist Interest in Value-Added, Aquaculture-Based, Culinary Tourism Opportunities. *Coastal Management 45*, 310–329.

Kim, N., Choi, Y., Jung, S., & Kim, S. (2000). Effect of initial carbon sources on the performance of microbial fuel cells containing Proteus vulgaris. *Biotechnology and*

Bioengineering 70, 109–114.

Koch, C., & Harnisch, F. (2016). Is there a Specific Ecological Niche for Electroactive Microorganisms? *ChemElectroChem 3*, 1282–1295.

Kokko, M. E., Mäkinen, A. E., & Puhakka, J. A. (2016). Anaerobes in bioelectrochemical systems. In R. Hatti-Kaul, G. Mamo, & B. Mattiasson (Eds.), *Anaerobes in Biotechnology. Advances in Biochemical Engineering/Biotechnology* (1st ed., Vol. 156, pp. 263–292). Springer, Cham.

Kokko, M., Epple, S., Gescher, J., & Kerzenmacher, S. (2018). Effects of wastewater constituents and operational conditions on the composition and dynamics of anodic microbial communities in bioelectrochemical systems. *Bioresource Technology 258*, 376–389.

Koskue, V., Rinta-Kanto, J. M., Freguia, S., Ledezma, P., & Kokko, M. (2021). Optimising nitrogen recovery from reject water in a 3-chamber bioelectroconcentration cell. *Separation and Purification Technology 264*, 118428.

Kumar, P., & Kuppam, C. (2020). *Bioelectrochemical Systems: Vol.2 Current and Emerging Applications* (P. Kumar & C. Kuppam, Eds.). Springer Singapore.

Kuntke, P., Sleutels, T. H. J. A., Rodríguez Arredondo, M., Georg, S., Barbosa, S. G., ter Heijne, A., … Buisman, C. J. N. (2018). (Bio)electrochemical ammonia recovery: progress and perspectives. *Applied Microbiology and Biotechnology 102*, 3865–3878.

Lazado, C. C., & Good, C. (2021). Survey findings of disinfection strategies at selected Norwegian and North American land-based RAS facilities: A comparative insight. *Aquaculture 532, 736038*.

Ledezma, P., Jermakka, J., Keller, J., & Freguia, S. (2017). Recovering Nitrogen as a Solid without Chemical Dosing: Bio-Electroconcentration for Recovery of Nutrients from Urine. *Environmental Science & Technology Letters 4*, 119–124.

Lekang, O.-I., & Kleppe, H. (2000). Efficiency of nitrification in trickling filters using different filter media. *Aquacultural Engineering 21*, 181–199.

Lindholm-Lehto, P., Koskela, J., Kaseva, J., & Vielma, J. (2020). Accumulation of geosmin and 2-methylisoborneol in european whitefish coregonus lavaretus and rainbow trout oncorhynchus mykiss in RAS. *Fishes 5*, 13.

Lindholm-Lehto, P.C., Vielma, J., Pakkanen, H., & Alén, R. (2019). Depuration of geosmin- and 2-methylisoborneol-induced off-flavors in recirculating aquaculture system (RAS) farmed European whitefish Coregonus lavaretus. *Journal of Food Science and*

Technology 56, 4585–4594.

Lindholm-Lehto, Petra C., & Vielma, J. (2019). Controlling of geosmin and 2-methylisoborneol induced off-flavours in recirculating aquaculture system farmed fish—A review. *Aquaculture Research 50*, 9–28.

Logan, B. E. (2008). *Microbial fuel cells* (1st ed.). Hoboken, N.J: John Wiley & Sons, Incorporated.

Logan, B. E. (2009). Exoelectrogenic bacteria that power microbial fuel cells. *Nature Reviews. Microbiology 7*, 375–381.

Logan, B. E., & Rabaey, K. (2012). Conversion of Wastes into Bioelectricity and Chemicals by Using Microbial Electrochemical Technologies. *Science (American Association for the Advancement of Science) 337*, 686–690.

Lukassen, M. B., Menanteau-Ledouble, S., Jonge, D., Schram, E., & Nielsen, J. L. (2021). Impact of water quality parameters on geosmin levels and geosmin producers in European recirculating aquaculture systems. *Journal of Applied Microbiology 132*, 2475–2487.

Merck. (n.d.). Microbiology Introduction. Available (accessed on 22.3.2022): https://www.sigmaaldrich.com/FI/en/technical-documents/technical-article/microbiological-testing/microbial-culture-media-preparation/microbiology-introduction

Mirzoyan, N., Tal, Y., & Gross, A. (2010). Anaerobic digestion of sludge from intensive recirculating aquaculture systems: Review. *Aquaculture 306*, 1–6.

Monetti, J., Ledezma, P., Virdis, B., & Freguia, S. (2019). Nutrient Recovery by Bio-Electroconcentration is Limited by Wastewater Conductivity. *ACS Omega 4*, 2152–2159.

Mota, V. C., Nilsen, T. O., Gerwins, J., Gallo, M., Ytteborg, E., Baeverfjord, G., … Terjesen, B. F. (2019). The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (Salmo salar) in recirculating aquaculture systems. *Aquaculture 498*, 578–586.

Nancharaiah, Y. V, Mohan, S. V., & Lens, P. N. L. (2016). Recent advances in nutrient removal and recovery in biological and bioelectrochemical systems. *Bioresource Technology 215*, 173–185.

Naradasu, D., Long, X., Okamoto, A., & Miran, W. (2020). Bioelectrochemical Systems: Principles and Applications. In P. Kumar & C. Kuppam (Eds.), *Bioelectrochemical Systems* (1st ed., Vol. 1, pp. 1–33). Singapore: Springer Nature.

O'Shea, T., Jones, R., Markham, A., Norell, E., Scott, J., Theuerkauf, S., & Waters, T. (2019). Towards a blue revolution: catalyzing private investment in sustainable aquaculture production systems. In *The Nature Conservancy and Encourage Capital*. Virginia, USA.

Pedersen, L.-F., Oosterveld, R., & Pedersen, P. B. (2015). Nitrification performance and robustness of fixed and moving bed biofilters having identical carrier elements. *Aquacultural Engineering 65*, 37–45.

Perazzoli, S., De Santana Neto, J. P., & Soares, H. M. (2018). Prospects in bioelectrochemical technologies for wastewater treatment. *Water Science and Technology 78*, 1237–1248.

Potter, M. C. (1911). Electrical effects accompanying the decomposition of organic compounds. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character 84*, 260–276.

Pous, N., Korth, B., Osset-Álvarez, M., Balaguer, M. D., Harnisch, F., & Puig, S. (2021). Electrifying biotrickling filters for the treatment of aquaponics wastewater. *Bioresource Technology 319, 124221*.

Pulkkinen, J. T., Eriksson-Kallio, A. M., Aalto, S. L., Tiirola, M., Koskela, J., Kiuru, T., & Vielma, J. (2019). The effects of different combinations of fixed and moving bed bioreactors on rainbow trout (Oncorhynchus mykiss) growth and health, water quality and nitrification in recirculating aquaculture systems. *Aquacultural Engineering 85*, 98–105.

Pulkkinen, J. T., Kiuru, T., Aalto, S. L., Koskela, J., & Vielma, J. (2018). Startup and effects of relative water renewal rate on water quality and growth of rainbow trout (Oncorhynchus mykiss) in a unique RAS research platform. *Aquacultural Engineering 82*, 38–45.

Qin, M., Molitor, H., Brazil, B., Novak, J. T., & He, Z. (2016). Recovery of nitrogen and water from landfill leachate by a microbial electrolysis cell-forward osmosis system. *Bioresource Technology Journal 200*, 485–492.

Rabaey, K., Ossieur, W., Verhaege, M., & Verstraete, W. (2005). Continuous microbial fuel cells convert carbohydrates to electricity. *Water Science and Technology 52*, 515–523.

Robertson, R. F., Jauncey, K., Beveridge, M. C. M., & Lawton, L. A. (2005). Depuration rates and the sensory threshold concentration of geosmin responsible for earthy-musty taint in rainbow trout, Onchorhynchus mykiss. *Aquaculture 245*, 89–99.

Rodríguez Arredondo, M., Kuntke, P., ter Heijne, A., Hamelers, H. V. M., & Buisman, C. J. N. (2017). Load ratio determines the ammonia recovery and energy input of an electrochemical system. *Water Research 111*, 330–337.

Rozendal, R. A., Hamelers, H. V. M., Rabaey, K., Keller, J., & Buisman, C. J. N. (2008). Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnology 26*, 450–459.

Rusten, B., Eikebrokk, B., Ulgenes, Y., & Lygren, E. (2006). Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacultural Engineering 34*, 322–331.

San-Martín, M. I., Mateos, R., Carracedo, B., Escapa, A., & Morán, A. (2018). Pilot-scale bioelectrochemical system for simultaneous nitrogen and carbon removal in urban wastewater treatment plants. *Journal of Bioscience and Bioengineering 126*, 758–763.

Sander, E. M., Virdis, B., & Freguia, S. (2018). Bioelectrochemical Denitrification for the Treatment of Saltwater Recirculating Aquaculture Streams. *ACS Omega 3*, 4252–4261.

Schrader, K. K., Davidson, J. W., Rimando, A. M., & Summerfelt, S. T. (2010). Evaluation of ozonation on levels of the off-flavor compounds geosmin and 2-methylisoborneol in water and rainbow trout Oncorhynchus mykiss from recirculating aquaculture systems. *Aquacultural Engineering 43*, 46–50.

Schrader, K. K., & Summerfelt, S. T. (2010). Distribution of Off-Flavor Compounds and Isolation of Geosmin-Producing Bacteria in a Series of Water Recirculating Systems for Rainbow Trout Culture. *North American Journal of Aquaculture 72*, 1–9.

Schram, E., Schrama, J., Kusters, K., Kwadijk, C., & Palstra, A. (2016). Effects of exercise and temperature on geosmin excretion by European eel (Anguilla anguilla). *Aquaculture 451*, 390–395.

Scopus. (2022). Scopus Advanced search. Available (accessed on 4.3.2022): https://www.scopus.com/search/form.uri?#basic

Suhr, K. I., & Pedersen, P. B. (2010). Nitrification in moving bed and fixed bed biofilters treating effluent water from a large commercial outdoor rainbow trout RAS. *Aquacultural Engineering 42*, 31–37.

Summerfelt, S. T., Vinci, B. J., & Piedrahita, R. H. (2000). Oxygenation and carbon dioxide control in water reuse systems. *Aquacultural Engineering*, *22*, 87–108. Amsterdam: Elsevier B.V.

Tampereen Vesi. (2020). Veden laatu. Available (accessed on 21.3.2022): https://www.tampere.fi/vesi/toiminta/vedenlaatu.html#talousvedenlaatutampereella

Tidwell, J. (2012). *Aquaculture production systems* (J. Tidwell, Ed.). Ames, Iowa: Wiley-Blackwell.

Tucker, C. S. (2000). Off-Flavor Problems in Aquaculture. *Reviews in Fisheries Science 8*, 45–88.

Xiao, R., Wei, Y., An, D., Li, D., Ta, X., Wu, Y., & Ren, Q. (2019). A review on the research status and development trend of equipment in water treatment processes of recirculating aquaculture systems. *Reviews in Aquaculture 11*, 863–895.

Yamamoto, Y. (2017). Characteristics of Closed Recirculating Systems. In T. Takeuchi (Ed.), *Application of Recirculating Aquaculture Systems in Japan* (1st ed., pp. 21–53). Tokyo: Springer, Tokyo.

Zhang, F., Ge, Z., Grimaud, J., Hurst, J., & He, Z. (2013). Long-Term Performance of Liter-Scale Microbial Fuel Cells Treating Primary Effluent Installed in a Municipal Wastewater Treatment Facility. *Environmental Science & Technology 47,* 4941–4948.

Zhang, X., Li, X., Zhao, X., & Li, Y. (2019). Factors affecting the efficiency of a bioelectrochemical system: a review. *RSC Advances 9, 19748*.

Zhao, F., Heidrich, E. S., Curtis, T. P., & Dolfing, J. (2020). The effect of anode potential on current production from complex substrates in bioelectrochemical systems: a case study with glucose. *Applied Microbiology and Biotechnology 104*, 5133–5143.

APPENDIX A: SYNTHETIC MEDIUM COMPOSITION OF E100 ENRICHMENT STAGE

Feed component	Molarity (mmol L^{-1})	Concentration (mg L^{-1})
Inorganic salts		
NH_4Cl	0.111	5.932
$CaCl_2$	0.124	13.780
$MgCl_2$	0.0334	3.221
$MgSO_4$	0.0484	5.832
KCl	0.0767	5.720
NaCl	0.196	11.461
$NaNO_3$	0.190	16.148
Na_2HPO_4	0.158	22.421
Organic elements		
Sodium acetate	0.217	500
Glycine	0.103	217.040
Glucose	0.072	366.018
Buffers		
Na_2HPO_4	28.9	4102.644
NaH_2PO_4	29.1	2531.578
Trace elements		
$FeSO_4.7H_2O$	0.00360	1
$ZnCl_2$	0.00051	0.070
$MnCl_2.4H_2O$	0.00051	0.100
H_3BO_3	0.00010	0.006
$CaCl_2.6H_2O$	0.00059	0.130
$CuCl_2.2H_2O$	0.00001	0.002
$NiCl_2.6H_2O$	0.00010	0.024
$Na_2MoO_4.2H_2O$	0.00015	0.036
$CoCl_2.6H_2O$	0.00100	0.238

APPENDIX B: SYNTHETIC MEDIUM COMPOSITION OF CONTINUOUS PHASE

Feed component	Molarity (mmol L^{-1})	Concentration (mg L^{-1})
Inorganic salts		
NH_4Cl	0.111	5.932
$CaCl_2$	0.124	13.780
$MgCl_2$	0.0334	3.221
$MgSO_4$	0.0484	5.832
KCl	0.0767	5.720
NaCl	0.196	11.461
$NaNO_3$	0.190	16.148
Na_2HPO_4	0.158	22.421
Organic elements		
Sodium acetate	3.047	125
Glycine	1.445	54.260
Glucose	1.016	91.504
Buffers		
$NaHCO_3$	4.4	369.630
Trace elements		
$FeSO_4.7H_2O$	0.00360	1
$ZnCl_2$	0.00051	0.070
$MnCl_2.4H_2O$	0.00051	0.100
H_3BO_3	0.00010	0.006
$CaCl_2.6H_2O$	0.00059	0.130
$CuCl_2.2H_2O$	0.00001	0.002
$NiCl_2.6H_2O$	0.00010	0.024
$Na_2MoO_4.2H_2O$	0.00015	0.036
$CoCl_2.6H_2O$	0.00100	0.238

APPENDIX C: REACTORS' NH_4-N REMOVAL PERFORMANCE DURING CONTINUOUS OPERATION STAGES

Stage	HRT (h)	Reactor	TAN load ratio	TAN removal efficiency (%)	TAN removal rate (g_N m^{-3} d^{-1})
C2	18.7	1	11.02	58.67 ± 12.6	10.01 ± 2.22
		3	12.86	10.2 ± 4.68	1.32 ± 0.61
C3	13.6	2	8.79	14.58 ± 3.86	3.18 ± 0.84
		3	9.58	11.96 ± 4.71	2.36 ± 0.93
C4	9.8	3	9.28	11.33 ± 5.88	3.24 ± 1.68