Örverur til auðgunar fiskeldisseyru /

Microorganisms for aquaculture sludge enrichment

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Report Summary
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Summary in English:	The primary objective of the project "Microorganisms for aquaculture sludge enrichment" was to develop a method for treating side streams from aquaculture (sludge) using microorganisms, thereby rendering the sludge suitable for use as agricultural fertilizer. Given the rapid expansion of aquaculture in Iceland, finding solutions for side streams is imperative to sustain the industry and enhance circular economy practices. Implementing solutions that encourage side stream utilization aligns with the United Nations' sustainable development goals. The legal landscape for utilizing fish farm sludge as fertilizer is extensive and, in certain aspects, complex, delineating what is permissible and who grants permission. For instance, applying sludge to pasture for grazing requires adherence to specific timelines, such as application before December 1st, with grazing permitted no earlier than 5 months later or on April 1st. The project focused on enriching the sludge's nitrogen content with microorganisms. An enrichment culture was established to promote ammonia-oxidizing bacteria in the sludge, increasing its potential as a fertilizer. Chemical analysis of the sludge was conducted to evaluate its nutrient content. The results indicate that the sludge can serve as an ideal supplement or additive, for instance, with biodegradable livestock manure. Continuing projects that enhance the value of like sludge is crucial for maintaining nutrient cycles within the circular economy. The use of sludge as fertilizer is mutually beneficial for both aquaculture companies and Icelandic agriculture.
English keywords:	Aquaculture fish sludge, microorganisms, fertiliser, sustainability, circular economy.
Ágrip á íslensku:	Megin markmið verkefnisins „Örverur til auðgunar fiskeldisseyru" var að þróa aðferð til að meðhöndla hliðarstrauma frá fiskeldi (seyru) með örverum svo seyran geti nýst sem áburður fyrir landbúnaðinn. Miðað við hraðan vöxt fiskeldis á Íslandi skiptir sköpum fyrir sjálfbærni iðnaðarins að finna lausnir fyrir hliðarstrauma og efla þannig hringrásarhagkerfið. Innleiðing lausna er stuðla að nýtingu hliðarstrauma, og

	efla hringrás, eru í samræmi við markmið Sameinuðu þjóðanna um sjálfbæra þróun. Lagaumhverfi um nýtingu fiskeldisseyru sem áburð er bæði umfangsmikið og á köflum nokkuð flókið, þ.e. hvað má og hver veitir leyfi. Sem dæmi um kröfur til nýtingar seyru sem áburð þá verður að bera seyru á beitartún fyrir 1. desember ef nýta á svæðið til beitar, skepnum má þá beita á svæðið 5 mánuðum síðar eða í fyrstalagi 1. apríl. Í verkefninu var unnið að því að auðga nítrat í seyrunni með örverum til að auka möguleika á nýtingu seyrunnar sem áburðarefnis. Stofnað var til auðgunarræktunar með það að markmiði að auðga fyrir ammoníak-oxandi bakteríum í seyrunni. Einnig var gerð efnagreining á seyrunni til að meta næringarefnahlutfall hennar. Niðurstöður efnamælinga benda til þess að seyra geti verið tilvalin sem viðbót eða íblöndunarefni við til dæmis lífbrjótanlegan búfjáráburð Mikilvægt er að halda áfram með verkefni er stuðla að því að auka verðmæti hliðarafurða á borð við seyru til að halda næringarefnum innan hringrásarhagkerfisins. Nýting seyru sem áburðar er til hags fyrir bæði fiskeldisfyrirtæki sem og íslenskan landbúnað.
Lykilorð á íslensku:	Fiskeldisseyra, örverur, áburður, sjálfbærni, hringrásarhagkerfið.

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1. Background

The solid biodegradable waste produced from aquaculture is rapidly increasing in volume in Iceland, from increased production at sea and on land of farmed salmonids. This solid biodegradable waste includes uneaten food, faeces, and fish mortalities (of differing category) which this report will refer to collectively as fish "sludge".

Land-based fish farming in Iceland represents a unique opportunity to capture and re-utilize the sludge from uneaten food and faeces - to create value. Currently this material is pumped to the ocean which is environmental and economically impactful. Furthermore, there are increasing volumes of fish mortalities as the industry grows in scale from both land-based and sea-cage aquaculture which are currently capture but do not create a value for the Icelandic economy.

This project was developed with the ethos that there is high potential for value creation from fish sludge for the Icelandic circular economy and seeks to answer key knowledge gaps to support the sustainable exploitation of this value. The following section will describe the background relevant to this project.

1.1 Fish Sludge from Commercial Aquaculture

Fish sludge as a side stream in aquaculture is a mostly unknown and unexploited raw material for further use. This project, "Microorganisms to Enrich Fish Sludge", aims at observing and analysing the microbial content by a metagenomic approach of these resources, in fish sludge, to better understand its composition and potential in delivering bioavailable nutrients. In parallel the N, P, K content will also be determined. A microbial trial by using specific bacteria adapted to the sample nature, could be then attempted to balance the bioavailability of nutrients in the potential fertiliser made from fish sludge.

This approach of assessing the potential of using fish sludge that is an integral side stream in land-based aquaculture farming, is important since these biodegradable materials are flushed into the nearby ecosystem. Such act is not only a danger for the existing ecosystem in the surrounding of land-based aquafarming, but also illegal to do so since January 2023 (IS 103/2021). Furthermore, there is a loss of important nutrients from depleting resources (like phosphorus) which are essential in fertiliser for agriculture.

The objective of this project "utilizing fish sludge as fertilizer" aligns with several "UN Sustainable Development Goals," which represent an urgent call for action towards sustainability by all countries. This project intersects with various UN goals, including Goal 12 on responsible consumption and production, Goal 14 on life below water, and Goal 15 on life on land.

Icelandic agriculture is in a vulnerable situation concerning nutrients for meadows and fields whereas farmers need to fully rely on importing of industrial fertiliser (Sturludóttir, E. et al., 2021). The volume of domestically produced manure is not adequate to meet the needs for Icelandic agriculture and leaves the sector vulnerable and dependent on import of industrial fertilisers and both environmental and economic cost. Therefore, it is relevant to seek all solutions that aim to benefit multiple criteria such as the aquaculture and agriculture sectors and is beneficial for the ecosystem.

Using fish sludge that is an integral side stream from land-based aquaculture as fertiliser has a two-fold advantaged; it contributes to the circular economy and is beneficial for the sustainability of the Icelandic agriculture. Furthermore, by capturing this resource, this will reduce negative environmental impact to the Icelandic marine ecosystem.

Project carried out by Matís in (2020) "Greining á magni lífrænna áburðarefna og tækifæri til aukinnar nýtingar" laid the groundwork of importance of analysing fish sludge as a potential fertilizer. In this project, the amount of biodegradable waste in Iceland was identified and calculated. The results show that land-based farming produced 8.660 tonnes of live fish in 2019, and based on those numbers, the estimated amount of fish sludge is 2.305 tonnes (Baldursson, J., et. al. 2020). Since 2019, land-based farming has increased significantly and this sector aims to produce approximately 100.000 tonnes (live fish) in the coming years (Þórðarson, G. and Arason, S. 2022) and that gives us estimated amount of fish sludge around 27.000 tonnes per year.

For the reasons stated above, it is important in the context of rapid growth in the land-based aquaculture industry to reduce the environmental impact of such production. Furthermore, it is needed to increase sustainability and circular economy for this sector and that can be done by producing fertiliser from fish sludge that will benefit the agriculture sector which needs such materials.

1.2 The Environmental Challenge of Sludge

Food scarcity amongst increasing global populations and unprecedented climate change has become a critical issue. Wild fisheries around the world are no longer able to meet the increased demand for seafood, which has resulted in expansion into the aquaculture industry in order to meet these growing demands. Aquaculture, also known as fish farming or the growth of aquatic plants, animals, and other organisms, has thus become one of the fastest-growing food sectors globally (Choudhury A., Lepine C., Witarsa F., Good C., 2022). According to the United Nations Food and Agriculture Organization, since 1990, there has been a significant increase in global aquaculture production - approximately 527% - and the production of nearly 115 million tons of fish and aquatic plants through aquaculture alone in 2018. In several aquaculture locations, such as Norway and Iceland, biological challenges regarding the sea phase of salmon farming have forced the salmon industry to move more of its production onto land, resulting in increased volumes of capturable sludge production (Sandvold HN., Gregg JS., Olsen DS., 2019). Aquaculture sludge is a form of waste that is produced when the uneaten feed and fecal matter from farmed species collects in aquaculture tanks. Unfortunately, this sludge cannot be simply released into the environment in a sustainable manner, as it contains high levels of nutrients which cause the environmental deterioration of the receiving water bodies and sediments (Choudhury A., Lepine C., Witarsa F., Good C., 2022).

Aquaculture production has moved towards intensive land-based farming globally to meet higher demands for seafood, providing higher yields as well as greater economic value to farmers. However, intensive land-based fish farming also leads to an increase in contaminants, and wastes such as uneaten feeds, inedible harvest products, and faecal solids which accumulate at the bottom of aquaculture tanks. These waste products, which comprise sludge, can cause significant environmental impacts if not mitigated (Choudhury, A., et. al. 2022). The major adverse impact of aquaculture sludge is negative effects on water quality, causing serious damage to aquatic resources. The high levels of nutrients such as nitrogen and phosphorus, as well as minerals, are essential elements for life that are often present in sludge yet can cause adverse ecological impacts such as eutrophication[1], algal blooms[2], and anoxic conditions[3] - therefore, the extraction of these nutrients can cleanse wastewater while capturing the nutrients, allowing the wastewater to be reused (Christiano, S., et. al. 2022). Luckily, there is potential to use sludge from aquaculture farms for value, a concept that has attracted interest in recent years due to the potential to create new products and uses for aquaculture sludge.

1.3 Key Existing Knowledge Gaps Addressed by this Project

As the aquaculture industry continues to grow in Iceland, there is an increasing need for further research in this sector to ensure sustainable development and expansion without compromising value or negatively impacting nature and the environment. The demand for environmental considerations has risen significantly in tandem with the substantial and rapid growth of fish farming.

In recent years, Matís has actively participated in studies aimed at enhancing sustainability in aquaculture production. This research is designed to facilitate value creation while minimizing environmental impact. One prominent project contributing to the pursuit of sustainability in the aquaculture sector is Accelwater. Funded by the EU Horizon 2020, Accelwater's primary objective is to optimize freshwater consumption in the food and beverage industry. The synergy between this project and Accelwater underscores the importance of reducing environmental impact.

Additionally, Matís has been involved in other significant projects that have contributed valuable data on the quantity of by-products and waste integral to aquaculture production. These initiatives collectively support the overarching goal of promoting sustainability in the aquaculture industry.

1.4 Project Outline

In this report, several key knowledge gaps were addressed that will support sustainable value creation from this new Icelandic waste streams from fish sludge. The project was divided in five work packaged as followed:

- WP1. Divided in four tasks:
    - 1.1. Collecting existing reports and information's from research and industry.
    - 1.2. Supply chain and source mapping.
    - 1.3. Reporting of the results and the mapping.
    - 1.4. Preliminary industry SWOT analysis of the supply chain. Results will fuel in WP in a shape of a workshop.
        - D1.1. Deliverable: Report (2-5p) on the situation in Iceland. Make it open source.
            - Published on Matís webpage and IOC webpage, June 2023.
            - Press release in June 2023.
- WP2. Divided in three tasks:
    - 2.1. Analyses of the N,P,K, content of the sludge. Atmonia ratio N/NH4.
    - 2.2. Microbial diversity analysis of the sludge using a 16SRNA sequencing approach (metagenomic analysis).
    - 2.3. Virtual diversity analysis: what viruses can be found?
        - D2.1. The results were published in workshop in June 2023.
- WP3. Divided in three tasks:
    - 3.1. Report of applicable processing technologies.
    - 3.2. Regulatory approach: mapping of regulation in Iceland and in Europe.
    - 3.3. Opportunities and challenges of fish sludge as side stream.
        - D3.1. Overview report on sludge in Iceland.
- WP4. Project management

1.5 Project Lead and Participation

This project was led by Matís which conducted all the microbiology work. The Icelandic Ocean Cluster (IOC) collaborated with Matís in analyzing the supply chain and source mapping. Matís and IOC worked together on the workshop “Co-Products and Coffee. Chat about sludge from Fish Farms.” and processing the results. The fish farms Núpar and Silfurstjarnan contributed fish sludge for analysis and participated in project meetings, providing relevant information.

1.6 Report Outline

The report will provide in-depth information on the methods, results and key outcomes of the project work packages. Section 2 explored the supply chain and source mapping for sludge in Iceland which reports on the SWOT analysis carried out during a sludge and aquaculture industry stakeholder workshop hosted at the Iceland Ocean Cluster as well as key laws and regulations relevant to the proper utilization of sludge at both the national and the European level. Section 3 presents the microbial investigation of this project carried out by the team at Matís. Key conclusions from the entire project are then drawn in the final section of this report.

2. Supply Chain and Source Mapping

Aquaculture practices in Iceland commenced in the 1950s, initially in small ponds, land-based tanks, and ocean-based enclosures. Over the years, the aquaculture sector has undergone periods of experience and development. In recent years, there has been notable expansion in the industry, particularly in the domain of ocean-based enclosures. Concurrently, land-based aquaculture farming has seen a marked increase, especially the last few years. As a consequence of these developments, there has been a heightened focus on finding effective solutions for fish sludge from land-based farming.

Figure 1 illustrates the distribution of land-based Salmonid farms in Iceland, with each farm's size represented proportionally to the maximum production permitted, as specified by permits from the Icelandic Food and Veterinary Authority (MAST). Notably, most of the production is concentrated along the south coast of Iceland, particularly in areas of Grindavík and Þorlákshöfn. In 2021, land-based aquaculture accounted for 16% of total domestic aquaculture production. Arctic char (*Salvenius alpinus*) is the main bred species onshore, followed by Atlantic salmon (*Salmo salar*). According to the Ministry of Food, Agriculture, and Fisheries, 400,000 metric tons/year of salmon could be produced domestically by 2032. The Marine and Freshwater Research Institute (MFRI) is tasked with estimating the carrying capacity of the affected areas of each operation. This visual representation provides insights into the geographic concentration and scale of salmonid aquaculture, offering a valuable perspective on the industry's regulatory framework, spatial dynamics, and future growth potential.

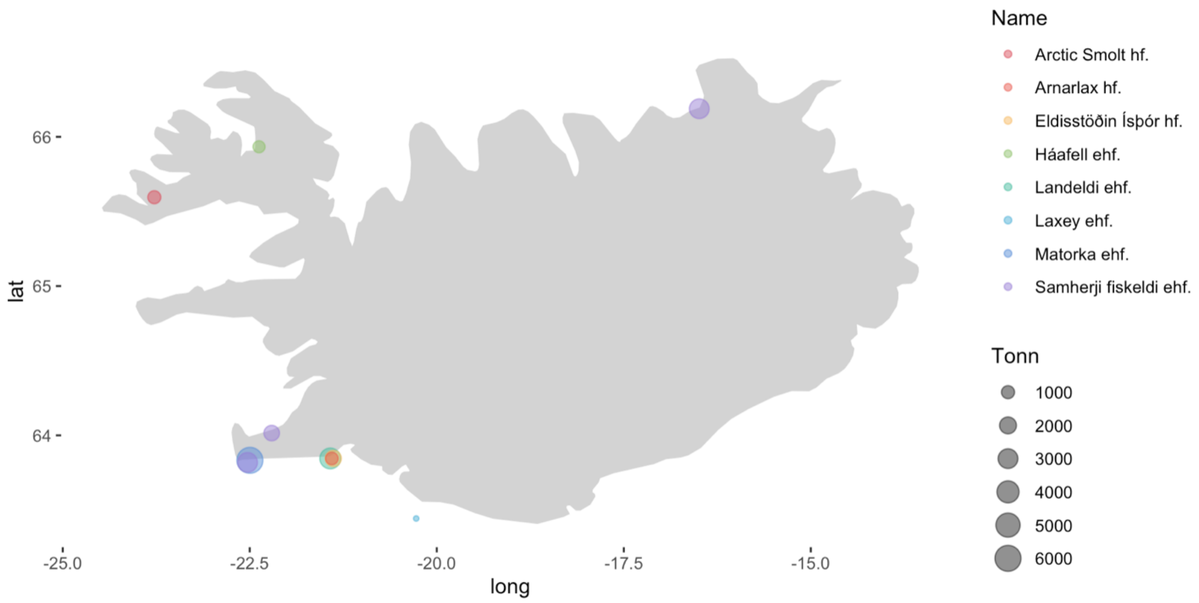

Figure 1 - Mapping Iceland's land-based Salmonid Farm with sizes proportional to permitted maximum production (based on permits from MAST).

2.1 Analyses

As we delve into the valorisation of aquaculture sludge, a comprehensive SWOT analysis reveals critical insights into the project's dynamics. In June the project did hold a workshop and invited stakeholders from the land-based and sea aquaculture sector, as well as research, innovation, energy, and technology actor from across Iceland. The aim of the workshop was to share knowledge about the state of research of fish sludge and to explore the challenges and opportunities around sludge from fish farms. This event was hosted at the Icelandic Ocean Cluster in collaboration with Matís, for this project exploring the issue, valorisation of aquaculture sludge.

The event was an interactive workshop, sharing information from experts about ongoing research and existing applications of sludge value creation globally, and splitting the stakeholder participants into break-put groups to map the strengths, weaknesses, opportunities and threats, SWOT, for sludge valorisation. Following is a brief overview of the key elements in each section:

2.1.1 Strengths

The project reveals several inherent strengths, including the potential for local utilisation, extensive know-how in fish farms, and a commitment to transparent sludge management. The abundance of resources, such as large volumes of heated wastewater and Iceland's appeal to foreign experts, further fortifies our foundation. Additionally, economic incentives, inexpensive energy, and the presence of valuable nutrients like phosphorus in sludge contribute to the project's robustness.

2.1.2 Weaknesses

While acknowledging our strengths, it is imperative to address the weaknesses. Challenges related to sea cages, the youthfulness of the industry, and the geographical spread of operations demand careful consideration. We must grapple with critical mass/volume issues, a lack of demonstrated technology, and the associated costs. Infrastructure, supervision, and adherence to laws and regulations pose additional hurdles.

2.1.3 Opportunities

Turning our attention to opportunities, the project aligns with Iceland's potential to lead in technology and sustainability. Embracing dynamic ideas in circular economies opens avenues for innovation. Opportunities beckon investors, both domestic and international, as we explore the potential of geothermal energy, localised food production, and the replacement of imported fertiliser. The diversification into biogas production and other value-added products signifies exciting prospects.

2.1.4 Threats

Navigating through potential threats is crucial for project resilience. The speed of changes, market pressures, and considerations linked to global issues like climate change and biosecurity are looming challenges. Accommodating community needs, policy shifts, and fluctuations in the global economy warrant our attention. Addressing funding gaps and ensuring financial sustainability are paramount to mitigate threats effectively.

S	W	O	T
Strengths	Weaknesses	Opportunities	Threats
• Potential for local utilisation • Know-how in fish farms • Transparent sludge management • Abundant resources • Iceland's attractiveness to foreign experts	• Sea cages • Young industry • Geographical spread • Lack of demonstrated technologies • Cost	• Iceland leading in technology • Circular economy initiatives • Geothermal energy usage • Localised food production	• Speed of changes • Market pressure • Climate change • Biosecurity issues • Lack of funding

Table 1 - SWOT analysis

This SWOT analysis encapsulates the multifaceted nature of the valorisation of aquaculture sludge, offering a strategic roadmap for leveraging strengths, mitigating weaknesses, capitalising on opportunities, and fortifying against potential threats.

2.2 Law and Regulations

Those intending to utilize by-products from animal production must, in the early stages of the process, precisely define the final purpose of the product—whether it is intended for human consumption or external applications such as fertilizer. This initial categorization is crucial due to the divergent laws and regulations governing products meant for human consumption compared to others, like fertilizers. Failure to appropriately classify by-products can lead them to be legally considered as bio waste. Even a minor oversight or hesitation in the work processes can result in the by-product being labeled as unsuitable for human consumption and relegated to bio-waste, potentially ending up in landfill. Therefore, establishing the purpose for the by-product is of utmost importance from the early stages of the process.

Following are reviewed key topics from both environmental (EB) and food safety (IS) law and regulations concerning by-products. It is essential to note that this review primarily focuses on by-products, particularly sludge from fish. Additionally, it is crucial to recognize that distinct laws and regulations apply to specific by-products, such as sludge from aquaculture. The applicability often depends on the producer's considerations during primary production and processing procedures.

The law and regulations that where analysed for this project where following:

- EB 1009/2019 Fertilzing products
- EB 1069/2009 laying down 1774/2002 animal by-products ...
- IS 22/1994 lög um eftirlit með fóðri, áburði og sáðvöru (e. Act on Control of Feed, Fertilizer and Seed Production)
- IS 7/1998 lög um hollustuhætti og mengunarvarnir (e. Act on Hygiene and Pollution Prevention)
- IS 55/2003 lög um meðhöndlun úrgangs ásamt breytingum samþ. af alþingi 13. Júní 2021 (e. Act on Waste management)

- IS 71/2008 um fiskeldi (e. Act on Fish Farming)
- IS 103/2010 reglugerð um hollustuhætti er varða matvæli (e. Regulation on Hygiene related to Food)
- IS 738/2003 reglugerð um urðun úrgans (e. Regulation on Waste Landfill)
- IS 540/2020 reglugerð um fiskeldi (e. Regulation on Aquaculture)

Regarding regulatory oversight, two governmental authorities manage laws and regulations for by-products in Iceland: The Environment Agency of Iceland (UST) and The Icelandic Food and Veterinary Authority (MAST). The Environment Agency of Iceland oversees another governmental authority, Heilbrigðiseftirlit (e. Health Surveillance), which also operates under these laws and regulations. Heilbrigðiseftirlit is subdivided into nine regions and is governed by the political committees of the municipalities in Iceland.

Following a comprehensive examination of laws and regulations pertaining to the further development of fish sludge as fertilizer, it is noteworthy to consider that fish sludge is categorized as a by-product from animals and is consequently governed by IS law for waste no. 55/2003. The regulation of waste falls under the purview of the Environmental Agency. However, this complicates the process of converting fish sludge into fertilizer, as the regulations for fertilizer fall under the jurisdiction of the Food and Veterinary Authority. Additionally, the Health Surveillance authority issues work permits and oversees production.

Given that fish sludge is categorized as a by-product, specific requirements must be met when using it as a fertilizer. For instance, in the case of grazing land used for animals or as a hay field, the sludge must be applied before the 1st of December the year preceding the field's use for animals or mowing. Grazing is permissible starting from the 1st of April the following year. Notably, the use of sludge as fertilizer for growing vegetables intended for human consumption is prohibited. Additionally, in fields designated for grain or other forage crops, the application of sludge is allowed in the springtime, provided it is incorporated into the soil through ploughing.

3. Microbiology

3.1 Cultivation of Nitrifying Bacteria

The original screening of the Nupar and Silfurstjarna samples indicated the presence of nitrifying bacteria. Here, it was attempted to isolate these bacteria on agar-plates and enrich them in liquid culture to obtain a native nitrifying species that could work under these conditions. Samples were collected from both sites and each diluted 10-fold, 100-fold and 1000-fold. All dilutions from both sites were plated on DSMZ media 1583 (with NH3 and 0,5% KNO3) and 756a (with NO2 and 0,5% KNO3) and incubated at 22°C and 30°C for approx. 4 months. The samples were plated on Nytran membranes on top of the agar media and the membranes were moved to fresh agar once a week during the cultivation. This did not result in many colonies and none that had the appearance of nitrifying bacteria and the plate colony isolation approach was therefore discontinued. To enrich for nitrifying bacteria in liquid media, all the dilutions were also inoculated into liquid media DSMZ 1583 with 0,5% KNO3 and incubated at 22°C and 30°C for approx. 4 months. Growth could be observed in these as a thin biofilm on the inside of the tubes and some turbulence in the medium. One dilution from each site and each temperature was selected for re-inoculation into fresh media for continued enrichment. The reinoculation culture was done in DSMZ 1583 media with and without 0,5% KNO3 and cultivated for one month before sequencing.

3.2 Microbiome Analysis

DNA was extracted using the MasterPure GramPos DNA Purification kit with 2 hours in lysozyme. From sludge samples, 150 uL were used directly as input into DNA extract. For enrichment cultures, cells were collected after scraping a thin layer of biofilm from the inside of the tube with a sterile spatula and inverting to mix. Approximately 30mL of the culture were poured to a fresh 50mL conical tube and spun down at 3000g for 20 minutes. Supernatant was poured over to new tubes and store at 4°C for ammonia/nitrite tests. The pellets were resuspended in 150uL TE and used as input into DNA extraction.

From each extracted DNA sample, the V4 hypervariable region of the 16S rRNA gene was amplified using primers A519F and 802R with Illumina overhang (TCGTCGGCAGCGTCAGATGTGTATAAGAGA-CAGCAGCMGCCGCGGTAA and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-TACNVGGGTATCTAATCC) in duplicate reactions, using the Q5® High-Fidelity DNA Polymerase (NEB). Pooled duplicate reactions were purified using the PCRClean™ DX beads (Aline), indexed using the Nextera XT V2 indexes (Illumina) and normalized using the SequelPrep™ Normalization Plate Kit (Applied Biosystems). Pooled amplicons were sequenced on the iSeq 100 instrument with v2 reagents (Illumina).

Analysis was done using using packages DADA2 and Phyloseq in RStudio, with the Silva v138.1 database.

3.3 Microbial Enrichment

Analysis of the most abundant bacteria in each sample show that samples of sludge from both Núpar and Silfurstjarnan (NupOrgA and SilOrgA, respectively) hold a very similar microbial composition with the same 14 genera representing a median relative abundance of more than 0.1% (Figure 2 and Figure 3). These can be broadly divided into three groups with one group containing bacteria typically found in marine environments, fresh water and sediments, i.e. *Aquamicrobium*, *Marinicella*, *Pseudoalteromonas*, *Psychrobacter*, *Sulfitobacter* and *Truepera*. Within this group, there are some differences in bacterial metabolism and their optimum temperatures and pH ranges, but a diversity of such bacteria is to be expected within samples such as aquaculture sludge where a range of nutrients is available and access to oxygen may vary depending on exact location.

The second group contains genera that all have some associations with human or animal microbiota, i.e. *Corynebacterium*, *Cutibacterium*, *Escheria-Shigella*, *Faecalibacterium*, *Lawsonella*, *Staphylococcus* and *Streptococcus*. Few of these have been specifically described in the microbiota of fish but most likely they may still thrive there, particularly the ones related to gut contents. Among these are a few potential pathogens, including *Staphylococcus* and *Lewsonella*, there is no clear cause for concern here, but this underlines the fact that if sludge were to be introduced into other ecosystems, some form of monitoring for pathogens and harmful microbes would be advised.

Thirdly, a single genus of archaea was among those most abundant, i.e. *Thermococcus*. This is a group of thermophilic archaea, strictly anaerobic and often found near hydrothermal vents terrestrial hot springs. They are heterotrophic, organotrophic sulfanogens the use elemental sulfur. The presence of this genus here among the most abundant taxonomic groups in the sludge may suggest that there is sufficient elemental sulfur present in the material to sustain this group of microbes and that this may be an important consideration when using the sludge as fertilizer.

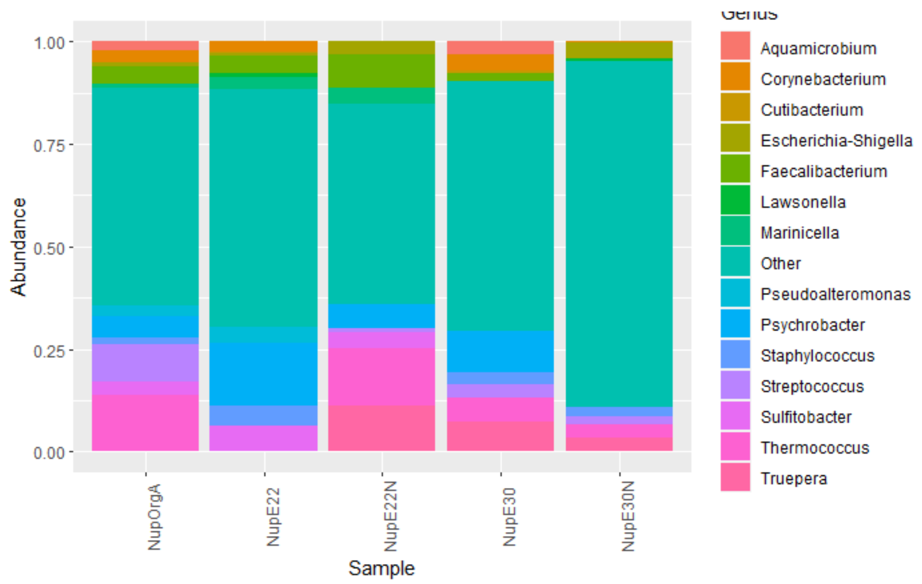

Figure 2: Relative abundance of bacteria grous at the genus level in sludge from Núpar (NupOrgA) and in enrichment cultures at 22°C and 30°C (as indicated) with and without added 0.5% KNO3 (indicated by "N"). Genera representing more than 0.1% of the sample are listed but those making up less than 0.1% of the sample and are grouped and shown as "Other".

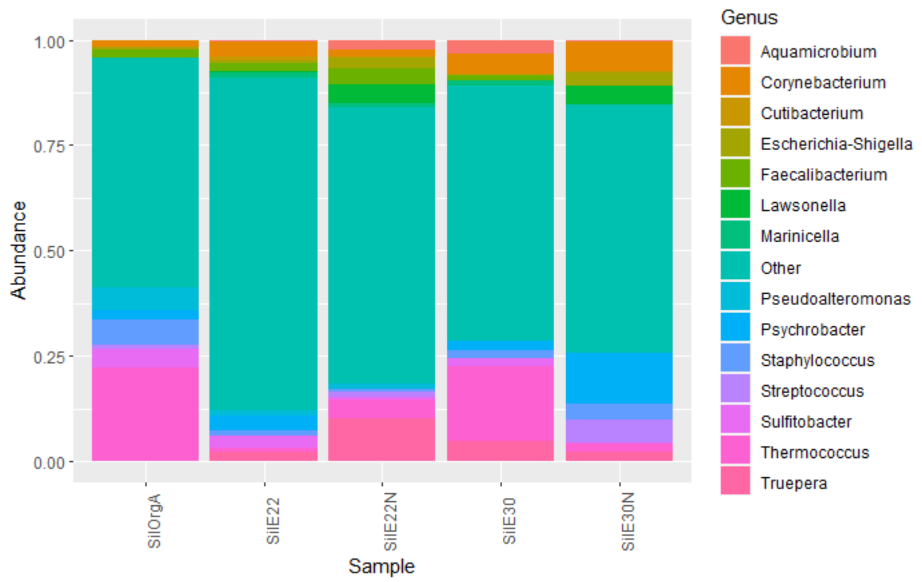

Figure 3: Relative abundance of bacteria grous at the genus level in sludge from Silfurstjarnan (SilOrgA) and in enrichment cultures at 22°C and 30°C (as indicated) with and without added 0.5% KNO3 (indicated by "N"). Genera representing more than 0.1% of the sample are listed but those making up less than 0.1% of the sample and are grouped and shown as "Other".

Here, the emphasis was put on the nitrogen metabolism in the sludge and enrichment cultures set up with the aim to enrich for known Ammonia Oxidising Bacteria by using enrichment medium DSMZ 1583. Overall, this enrichment was not successful in the sense that the overall composition of the microbiota is not greatly affected, the same genera are still found above the 0.1% median relative abundance threshold and no Ammonia or Nitrite Oxidising bacteria (AOB and NOB, respectively) are among the most abundant genera.

A closer look for AOB and NOB in the samples and enrichments revealed that none were detected in the original sludge samples (”Sludge”) used here as input in the enrichment cultures. A very small enrichment of *Nitrosomonas* and *Nitrobacter* was shown in enrichments supplemented with 0.5% KNO3, particularly for *Nitrobacter* in enrichments done at 30°C where nitrobacter reaches 1-2% relative abundance (Figure 4). This shows us that they were present in the original samples but in a very low abundance and therefore not detected. However, previously analysed sludge samples (”Sludge (pre)”) from Núpar had showed a higher abundance of both *Nitrobacter* and *Nitrosomonas* on which the selection of enricment medium here was based. This finding clearly shows us temporal variation in the sludge microbial consortium and if this biomass is to be used for any agricultural, biological or industrial processes, it is very important to monitor and maintain the microbial flora.

A look at the alpha diversity in the original sludge samples an enricment cultures also shows that the diversity is not decreased by the enrichment culture as would be expected. This further confirms that the enrciment culture has not been successfull (Figure 5).

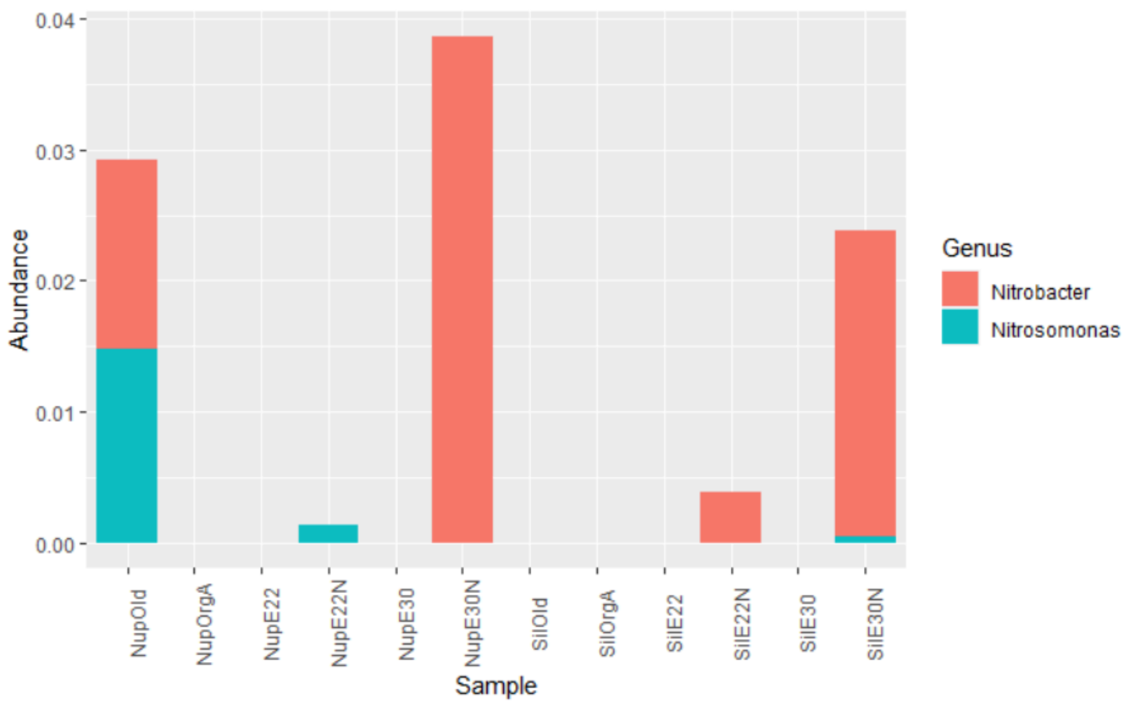

Figure 4: Relative abundance of Nitrobacter *and* Nitrosomonas *in sludge samples and enrichment cultures.*

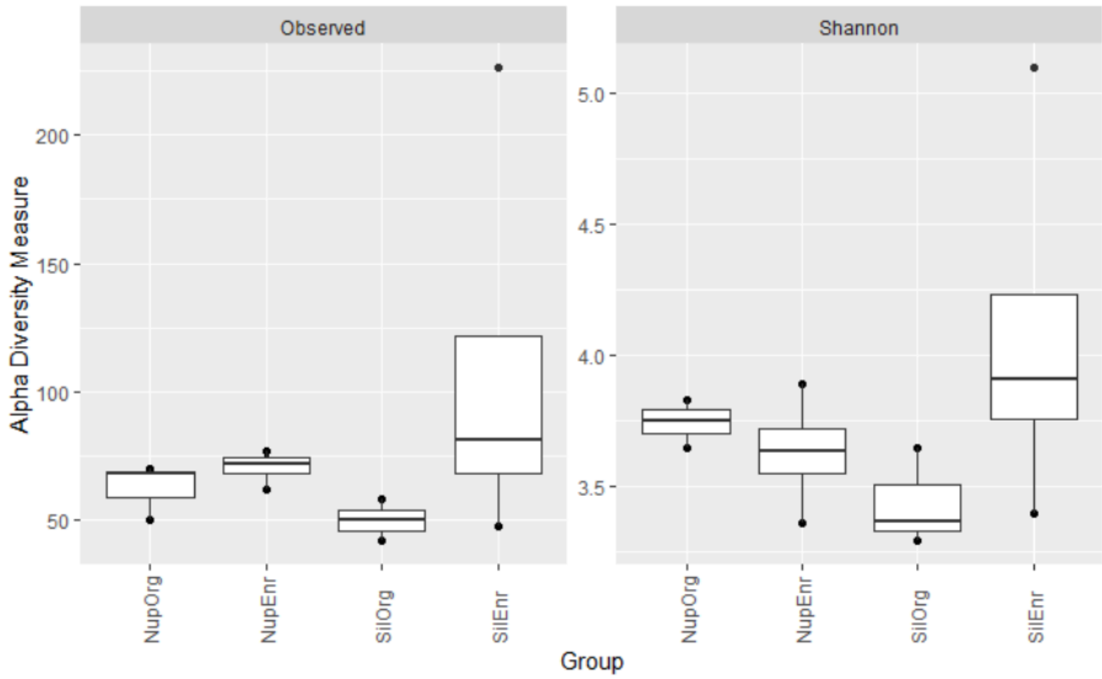

Figure 5: Alpha diversity in original sludge samples (NupOrg and SiOrg) and enrichment cultures (NupEnr and SilEnr) shown as the number of Observed taxonomic groups (left) and the Shannon index of sample richness and evenness (right).

Based on the results of the microbiome analysis showing a very low abundance of nitrifying bacteria in the samples, an analysis of the ammonia/nitrite oxidation in the liquid was not performed. Furthermore, the enrichment is most efficient in cultivation that contains KNO3, which interferes with the detection of ammonia/nitrite oxidation and testing of the liquid media is therefore not likely to produce reliable results.

4. Chemical Analysis

Chemical analysis of the sludge from Núpar and Silfurstjarnan was done at the Matís chemical laboratory applying standardized protocols. The results are shown in Table 2.

Chemical composition – Wet weight	Núpar	Silfurstjarnan
Water (g/100g sample)	90.3±0.1	90.6±0.3
Dry matter (g/100g sample)	9.7±0.1	9.4±0.3
Nitrogen (g/100g sample)	0.5±0.0	0.2±0.0
Fat (g/100g sample)	1.9±0.0	0.5±0.1
Ash (g/100g sample)	2.2±0.0	2.2±0.1
Salt (g/100g sample)	<0.1	0.7±0.0
Natrium (Na) (g/kg sample)	0.09±0.0	3.1±0.1
Potassium (K) (g/kg sample)	0.05±0.01	0.3±0.0
Phosphorus (P) (g/kg sample)	2.91±0.06	2.55±0.19
Calcium (Ca) (g/kg sample)	6.13±0.12	4.52±0.41
Magnesium (Mg) (g/kg sample)	0.21±0.01	0.90±0.04
Ammonia (g/100g sample)	0.08±0.00	0.07±0.00

Table 2. Chemical composition of fish sludge from Núpar and Silfurstjarnan.

The main results in the context of applying the sludge as fertilizers are the following: The dry matter of the sludge was 10%. The nitrogen content was ~5% of the dry matter of the Núpar sludge, thereof 0,7-0,8% ammonia which is 16% of the total nitrogen content. Phosphorus was measured ~3% of the sludge dry weight. Potassium was ~0,05% of the dry weight from the Núpar sludge (fresh water) and 0,3% of the dry weight of the Silfurstjarnan sludge (salt water). Sulphur, molybdenum, and boron which are important components in fertilizers where not measured. Summarized, the nitrogen content is similar to what is found in cow manure. The sludge is rich in phosphorus. The content is more than in cow manure and enough for utilization as fertilizers. On the other hand, the potassium content is too low as well as the content of calcium and magnesium. Accordingly, the sludge should be ideal as a co-ingredient for example with cow manure in fertilizer products, especially as a source of phosphorus.

5. Conclusions

Aquaculture in Iceland has evolved considerable since its inception in the 1950s, transition from small ponds to diverse setups. The geographical concentration of land-based Salmonid farms and their proportional sizes provide insights into the industry's spatial distribution, regulatory framework, and potential for future growth. Understanding these dynamics is crucial for sustainable aquaculture development in Iceland.

A SWOT analysis was conducted to assess the valorisation of the fish sludge. Stakeholders' participation in the workshop, sharing expertise and mapping with the SWOT method provided a strategic roadmap for leveraging strengths, mitigating weaknesses, capitalizing on opportunities, and fortifying against potential threats in the valorisation of aquaculture sludge.

The legal framework surrounding the utilization of by-products, such as fish sludge involves considerations from multiple laws and regulations. The categorization of the by-product is pivotal, given the divergent regulations for products intended for human consumption and those for external applications like fertilizers. Compliance with laws, especially waste management regulations, is critical for the sustainable development of the aquaculture sector.

Utilizing fish sludge as fertilizer is subject to specific requirements, varying based on the intended use, such as for grazing land or vegetable cultivation. The specific requirements for utilizing fish sludge highlight the need for future planning and researches to consider present regulations if the sludge is to be used as fertilizer in the future.

The results and discussions presented underscore the multifaceted nature of aquaculture practices in Iceland, the strategic considerations for sludge valorization, and the legal framework governing the utilization of side streams for fish farming. The integration of these elements is crucial for the sustainable development of the aquaculture sector in Iceland, that also is beneficial for Icelandic agriculture.

The microbiome analysis reported here shows that the abundance of ammonia and nitrite oxidising bacteria varies in time and is generally quite low. Further study would be required to map what factors influence this difference and subsequently how the microbiome in the sludge can be driven towards a more favourable composition. The focus here was on ammonia and nitrite oxidising bacteria but other groups of bacteria and archaea may also be favourable in the context of re-cycling nutrients. One example detected here are *Thermococcus* and the same applies as for the AOB/NOB, more extensive mapping of the microflora over time and in relation to environmental factors and chemical composition is required. Such mapping would provide a basis for enrichment efforts and should consider factors such as temperature, pH, salinity and availability of nutrients and oxygen.

In addition to enrichment of the native microbial flora, addition of cultivated microbes needs to be considered. This approach would not only boost the abundance of microbes favourable for fertilizer applications but may also aid in keeping less favourable microbes at a low abundance. Here we show that the sludge microflora contains several groups of microbes of animal origin and some that may be pathogenic. For that reason, routine monitoring of the microflora of all sludge used in industrial applications is advised.

Furthermore, the findings of this study confirm the chemical suitability of fish sludge to be used as fertilizer, opening opportunities to develop new domestic value chains for this material.

For the future, what is needed now is resilient policy for the implementation of strategies and business models to utilize fish sludge environmentally and economically.

6. References

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Lög um breytingu á lögum um hollustuhætti og mengunarvarnir, lögum um meðhöndlun úrgangs og lögum um úrvinnslugjald (EES-reglur, hringrásarhagkerfið) nr. 103/2021.

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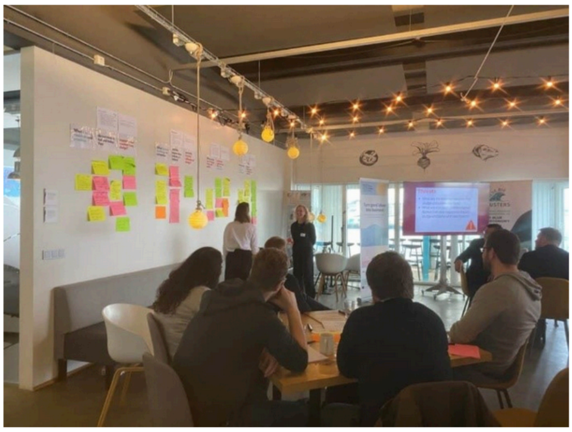
7. Appendix



The sludge stakeholder workshop images:

Following is the link to the project page:
https://matis.is/matis_projects/orverur-til-audgunar-fiskeldisseyru/

Following is the link to press release:
https://matis.is/frettir/hlidarafurdir-og-hugmyndir-spjallad-um-seyru-fra-fiskeldi/

8. Appendix

Skráning/starfsleyfi	Nafn starfsstöðvar	Heimilisfang	Póstnúmer	Lýsing	Fisktegund	Tegund eldis	Leyfilegur hámarkslífmassi (tonn)	Sludge estimate
FE-1057	Víkurlax ehf.	Ystuvík, Grenivík	610	Ystuvík	Regnbogasilungur	Matfiskeldi	10	2
FE-1074	Benchmark Genetics Iceland hf. - Kalmanstjörn			Kalmanstjörn, Höhnum	Lax	Klakfiskeldi	190	40
FE-1216	N-Lax ehf. - Laxamýri	Laxamýri	641	Laxamýri	Bleikja, Lax	Matfiskeldi, seiðaeldi	50	10
FE-1175	Benchmark Genetics Iceland hf. Kirkjuvogi	Kirkjuvogi við Hafnir	233	Kirkjuvogi	Hrognkelsi, Lax	Seiðaeldi	90	19
FE-1186	Fjallalax - Hallkelshólar	Hallkelshólum	801	Hallkellshólum, 801 Selfoss	Bleikja, Lax	Matfiskeldi, seiðaeldi	100	21
FE-1208	Bleikja ehf.	Laugar	851		Bleikja, Lax	Matfiskeldi, seiðaeldi	100	21
FE-1170	Matorka ehf. Húsatóftir, svæði i6	Hlíðasmára 6	201	Húsatóftir i6	Bleikja, Lax, Regnbogasilungur	Seiðaeldi	190	40
FE-1211	Laxey ehf - Seiðaeldi í Friðarhöfn	Garðavegur 14	900	Vestmannaeyjar	Lax, Regnbogasilungur	Seiðaeldi	199	42
FE-1081	Arnarlax hf. - Tálknafjörður			Gileyri, Tálknafjörður	Bleikja, Lax	Seiðaeldi	200	43
FE-1094	Tungusilungur ehf (IS-36125) Þórshamri	Tálknafjarðarþorp við Þórsberg og á Hvítalæk, á Mjóparti og á Keldeyri	460	Tálknafirði	Bleikja, Lax, Regnboasilungur	Matfiskeldi	200	43
FE-1156	Hólaskóli á Hólum í Hjaltadal - Verið	Háeyri 1	550	Verið vísindagarðar	Ýmsar tegundir	Rannsóknaeldi	14	3
FE-1160	Hafrannsóknastofnunin tilraunaeldi	Stað Grindavík	240	Stað, Grindavík	Ýmsar tegundir	Klakfiskeldi	21	4
FE-1053	Fiskeldið Haukamýri ehf (IS-36080) Haukamýragil	Haukamýragil	640	Haukamýragil, Húsavík	Bleikja, Lax	Matfiskeldi, seiðaeldi	450	96
FE-1181	Benchmark Genetics Iceland hf. - Vogavík	Bæjarhrauni 14	220		Lax	Matfiskeldi, seiðaeldi	500	107
FE-1189	Fiskeldi Austfjarða hf. - Lónin Kelduhverfi	Lónin Kelduhverfi	671	Lónin Kelduhverfi	Lax	Matfiskeldi	600	129
FE-1146	Háafell ehf.			Nauteyri, Hólmavík	Lax, Regnbogasilungur	Seiðaeldi	800	172
FE-1179	Arnarlax hf. - Þorlákshöfn	Laxabraut 5	815		Bleikja, Lax	Matfiskeldi, seiðaeldi	900	193
FE-1137	Arctic Smolt hf.			Norður-Botni II	Lax, Regnbogasilungur	Seiðaeldi	1000	215
FE-1154	Samherji fiskeldi ehf. Stóru-Vatnsleysu	Glerárgata 30	600	Stóru-Vatnsleysu, Vogum	Bleikja, Lax, Regnbogasilungur	Matfiskeldi	1600	344
FE-1162	Eldisstöðin Ísþór hf.			Þorlákshöfn	Lax, Regnbogasilungur	Seiðaeldi	1800	387
FE-1201	Bleikja ehf. - Skráning	Laugar, Landsveit	105		Bleikja, Lax	Matfiskeldi, seiðaeldi	20	4
FE-1141	Samherji fiskeldi ehf. Stað Grindavík	Stað	240	Stað, Grindavík	Bleikja, Lax	Matfiskeldi, seiðaeldi	3000	645

FE-1142	Samherji fiskeldi ehf. Núpsmýri Öxarfirði	Glerárgötu 30	600	Núpsmýri Öxarfirði	Bleikja, Lax	Matfiskeldi, seiðaeldi	3000	645
FE-1185	Landeldi ehf. - Þorlákshöfn	Urðarhvarfi 8	203	Laxabraut 21-25, Þorlákshöfn	Bleikja, Lax, Sjóbirtingur	Matfiskeldi, seiðaeldi	3450	742
FE-1153	Matorka ehf. - Vestan Grindavíkur, svæði i5	Húsatóftum	240	Húsatóftir i5, Grindavík	Bleikja, Lax, Regnbogasilungur	Matfiskeldi	6000	1291

*Table 3 - List of the permits from MAST. *Estimated Volume of Sludge from Land-based Fish Farms*

The amount of sludge was estimated with P(sludge) = f x FCR x Annual Biomass Production x e ; with P(sludge) is the sum of Total Suspended Solids (TSS) in tons/year; f is the mass fraction of wasted solids produced per unit of feed. For salmonids, the literature recommends to use f=0.25; FCR is the Feed Conversion Ratio, meaning the amount of feed an animal needs to gain one kilogram of body weight. For salmonids, FCR is close to 1:1; e is the efficiency factor which represents the performance of all the removal devices. In the Nordics, it is safe to use e=87.5% to project sludge production.Based on Del Campo, L. M., Ibarra, P., Gutiérrez, X., & Takle, H. R. (2010). Utilization of sludge from recirculation aquaculture systems. Nofima rapportserie.