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Authors

Arne Holst-Jensen, Anne R. Kijewski, Gro S. Johannessen, Gunnar S. Eriksen,
Hanne Nilsen, Hilde Sindre, Haakon Hansen, Magdalena Owczarek-Koscielniak,
Kristin S. Pettersen, Taran Skjerdal
Veterinærinstituttet/Norwegian Veterinary Institute

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Content

Norsk utvidet sammendrag

Mattilsynet ba i november 2024 Veterinærinstituttet om kunnskapsstøtte for å vurdere effekten av hygienisering av fiskeslam fra biogassproduksjon når bioresten skal benyttes til gjødsel eller jordforbedring. Bestillingen listet opp noen spesifikke rammer for vurderingen, og tre konkrete spørsmål skulle besvares, basert på Veterinærinstituttets egen forskning og tilgjengelig litteratur. I bestillingen framgikk det at rapporten skulle skrives på engelsk med utvidet norsk sammendrag.

Den fullstendige bestillingen er gjengitt i denne rapportens **Appendix 1**.

For å kunne gjennomføre oppdraget innenfor rimelige tids- og ressursmessige rammer ble det av den involverte faggruppen ved Veterinærinstituttet gjort noen ytterligere avgrensninger og presiseringer, basert på tolkning av mandatet/bestillingen og det tilgjengelige kunnskapsgrunnlagets egnethet m.m. Blant annet ble det tatt utgangspunkt i fiskeslam kun fra oppdrett av laksefisk i Norge. Dette fordi laksefisk utgjør mer enn 90% av all akvakultur i Norge, er basis for nesten all tilgjengelig og relevant kunnskap for norske forhold, og fordi det for industrielle formål er hensiktsmessig å risikovurdere et produkt som kan utnyttes i storskala med høy grad av standardisering både ifht. tilgang på råvare og antatt tilknyttet risiko.

Fiskeslam, selv hvis man avgrenser til slam fra norsk salmonide akvakulturproduksjon, er heterogent, både med tanke på struktur, kjemisk sammensetning og biologiske risikofaktorer. Det gjør det vanskelig å vurdere sikkert hvordan biogassproduksjon påvirker den mikrobielle metabolismen og transformasjonen av forbindelser under biogassproduksjon og hygienisering. I tillegg er det uklart i hvilket omfang de fysiko-kjemiske parameterne varierer, for eksempel pH eller aerobe/anaerobe forhold under disse prosessene, på mikroskala. Mandatet for denne rapporten listet opp tre spørsmål som skulle besvares.

De tre spørsmålene og tilhørende svar er kort oppsummert og lett redigert:

Spørsmål 1: Er behandling av fiskeslam med standardparameterne for omdanning i et biogassanlegg (minimum 70° C i en kontinuerlig periode i minimum 60 minutter, ved en partikkelstørrelse på <12 mm) tilstrekkelig for å hindre spredning av fiskesykdommer, når bioresten brukes som organisk gjødsel eller jordforbedringsmiddel?

> De **biologiske sykdomsagens** som her er vurdert inkluderer prioner, virus, bakterier, sopp og parasitter som kan være tilstede i fiskeslam benyttet til biogassproduksjon.
>
> For **prioner** konkluderes det med at risikoen for smitteoverføring og -spredning er ignorérbar. Prioner vil stamme fra (kadavere av) landpattedyr. Prionholdig materiale vil derfor med svært stor sannsynlighet bli oppdaget og fjernet i det svært usannsynlige tilfellet at slikt materiale skulle havne i akvakulturmiljøet. Det er derfor svært usannsynlig at prioner havner i fiskeslam. I tillegg vil prionene, hvis de i det hele tatt havner i fiskeslammet, være i svært lav konsentrasjon, bli fortynnet gjennom slaminnsamling og prosessering, og hygieniseringen vil verken modifisere eller oppformere prioner.
>
> For **bakterier** konkluderes det med at sporedannende Clostridiales er de mest resistente mot hygienisering. Sporer er generelt resistente mot mange ulike typer av stress, inkludert varmebehandling. Sporer av *Clostridium botulinum* er for eksempel rapportert å kunne motstå temperaturer betydelig høyere enn 100° C i > 15 minutter. Selv om motstandsevnen er sterkt påvirket av fysiko-kjemiske parametere, er det vanskelig å estimere hvor effektive standard hygieniseringsbetingelser vil være på sporer i biorester etter biogassproduksjon. Tilstedeværelse av bakterien er imidlertid sjelden, og oppvekst og toksinproduksjon vil være mer relevant under anaerobe enn aerobe betingelser. Videre er bakterien allerede tilstede i jord og andre nisjer i naturen, og tilleggsrisiko fra slam som varmebehandles raskt etter innsamling er derfor begrenset. Det er ikke sannsynlig at varmebehandling ved f.eks. 80° C vil ha noen vesentlig bedre hygieniseringseffekt på *C. botulinum* enn 70° C. Sporedannende bakterier har

ikke blitt rapportert i forbindelse med sykdomsutbrudd i norsk akvakultur, men *C. botulinum* kan være tilstede i signifikante konsentrasjoner i fiskeslam, for eksempel dersom død fisk ikke fjernes daglig. *Lactococcus garvieae* og *Mycobacterium* spp. er varmeresistente og vil kanskje tåle hygienisering, men er betydelig mindre varmetolerante enn *Clostridium* spp.

For **sopp** konkluderes det med at selv om hygieniseringsprosessen sannsynligvis vil eliminere de fleste sopparter så kan ikke overlevelse av mer resistente sopp utelukkes. Dessuten utgjør mykotoksiner en potensiell risiko da de kan være persistente mot varmebehandling. Gitt dagens kunnskapshull er ytterligere forskning nødvendig for å avdekke diversiteten av sopp i fiskeslam for å kunne evaluere hvor effektiv hygienisering vil være for å forebygge og redusere biologisk risiko. Bedre forståelse av disse faktorene er vesentlig for å sikre trygg gjenbruk av fiskeslam til ulike formål.

Parasitter er en svært mangfoldig og polyfyletisk (i betydelig grad ubeslektet) gruppe av organismer, med svært ulik biologi og livssykluser, og et mangfold av parasitter kan være tilstede i akvakultur og fiskeslam. Likevel vurderer vi det slik at vi har dekket et tilstrekkelig mangfold av parasitter som denne rapporten må vurdere med tanke på effekt av standard hygienisering. Ektoparasitter er tilpasset vertens miljøbetingelser og påvirkes direkte av endrete miljøbetingelser, slik som oppvarming, uttørking og oksygenmangel under hygieniseringen. Vi anser det derfor som usannsynlig at relevante ektoparasitter kan overleve hygienisering. Egg og hvilestadier eller cyster av endoparasitter er generelt ansett som de mest motstandsdyktige parasittstadiene. Trematoder (flatormer) framstår å ha særlig motstandsdyktige egg, men trematoder er generelt ikke tilstede i norsk oppdrettsfisk og vi har ikke funnet dokumentasjon på overlevelse av egg ved 70° C etter mer enn 10 minutter selv for de mest varmeresistente artene. Også for nematoder (rundormer) og cestoder (bendelmark) virker standard hygieniseringsbetingelser tilstrekkelige for fullstendig inaktivering. Hvis relevante nematoder eller cestoder likevel skulle overleve og bli ført ut i miljøet er det usannsynlig at det vil øke infeksjonspresset i de fleste områder, da disse parasittene allerede har utbredt forekomst. Oppsummert anser vi at det til tross for begrenset og mangelfull dokumentasjon på effekter av hygienisering på parasitter, og et behov for mer dokumentasjon, er usannsynlig at noen relevant parasitt vil overleve standard hygienisering.

Flere fiskesykdommer av betydning for norsk salmonideakvakultur skyldes **virus**, og utbrudd av slik sykdom er ikke uvanlig. Det er derfor svært sannsynlig at sykdomsgivende virus vil forekomme i fiskeslam. Mens kappevirus generelt er relativt følsomme for oppvarming og derfor kan forventes å bli inaktivert ved standard hygieniseringsbetingelser, er nakne virus gjennomgående mer resistente mot oppvarming og kan betinge høyere temperaturer og/eller lengre behandling for inaktivering. Infeksiøst pankreasnekrosevirus (IPNV) gir særlig grunn til bekymring. Inaktiveringsforsøk indikerer at oppvarming til minst 75° C i minst 60 minutter er nødvendig for å inaktivere IPNV. Vår anbefaling er derfor å øke kravet til hygieniseringstemperatur til minst 75° C for en kontinuerlig periode på minst 60 minutter for å sikre tilstrekkelig inaktivering av de mest robuste virus.

Spørsmål 2: Kan det oppstå uønskede forbindelser som en konsekvens av hygieniseringsprosessen, for eksempel gjennom kjemiske reaksjoner mellom komponenter i sjøvann og organisk materiale ved oppvarming?

Mangelen på data forhindrer klare konklusjoner, men risikoen for dannelse av potente forbindelser gjennom hygienisering under anaerobe betingelser vurderes å være begrenset. Varmebehandling gir begrenset sannsynlighet for endringer. Prosesser med oksidasjonsmidler eller syre/base har større sannsynlighet for å interagere med mineraler i sjøvann og organiske molekyler i slam.

Spørsmål 3: Hvis det mangler kunnskap for å besvare punkt 1, hvilken kunnskap er det behov for?

Det er behov for mer detaljert dokumentasjon på effekter av hygienisering og mulig overlevelse av sporedannende bakterier. Imidlertid krever studier av *Clostridium botulinum* fasiliteter med høyt biosikkerhetsnivå. En mulighet er å først utføre slike studier på stammer som er mindre farlige men har forskjellige varmetoleranseprofiler. *Bacillus* spp. er sporedannende bakterier som kan være egnet for et slikt formål.

Litteraturen som dokumenterer effekter av hygienisering på parasitter er svært begrenset og ytterligere studier og dokumentasjon er derfor sterkt ønskelige.

Litteraturen som dokumenterer diversiteten og forekomsten av sopp, diversiteten og persistensen av mykotoksiner, samt motstandsevne mot hygieniseringsbetingelser hos soppsporer er svært begrenset og ytterligere dokumentasjon er nødvendig for å kunne vurdere biologisk risiko forbundet med sopp og fiskeslam.

1 Hygienisation of fish sludge from biogas production

1.1 Introduction and mandate

The Norwegian Food Safety Authority (NFSA; Mattilsynet) on November 15th 2024 asked the Norwegian Veterinary Institute (NVI) for knowledge support to assess hygienisation of fish sludge in biogas facilities when the bio-remains ("biorest") are intended for use as fertilizer or soil improvement.

The knowledge support (this report) shall provide a basis to clarify which processing methods the NFSA can accept for hygienisation of fish sludge. The following three questions should be addressed based on NVIs own research and available scientific literature:

1. If processing of fish sludge using standard parameters for conversion in a biogas facility (minimum 70°C for a continuous period of minimum 60 minutes, with a particle size of <12 mm (Reg. EC 142/2011, Annex V, chapter III, section 1) is sufficient to prevent dissemination of fish diseases, when the bio-remains are used as fertilizer or soil improvement?
2. If any undesirable substances can emerge as a result of the hygienisation process, e.g. through chemical reactions between sea water and organic matter during heating?
3. If the available knowledge is insufficient to answer point 1, what additional knowledge is needed?

The following framing shall apply:

- Only Norwegian settings.
- The businesses operate in compliance with existing legislation, applicable to both the aquaculture entities where fish sludge is produced and entities processing the fish sludge.
- The biogas facilities receive unprocessed fish sludge. The fish sludge is not in any way processed during transport and storage on its way to the biogas facility.
- The biogas facilities mix the fish sludge with other materials prior to hygienisation.
- The biogas facilities receive fish sludge from aquaculture facilities all over the country, both from land- and sea-based sites.
- The bio-remains are used as fertilizer and soil improvement all over the country.

There is a great interest in increasing the use of fish sludge, and many biogas facilities wish to exploit fish sludge and use the bio-remains as fertilizer or soil improvement.

The legislation concerning animal byproducts (ABP) lists specific requirements for hygienisation processes, but fish sludge is exempt from the ABP legislation. The legislation concerning fertilizers does not include specific hygienisation requirements but states that the fertilizer shall not imply a risk of transmission of disease to humans, animals or plants. Furthermore, it shall not contain *Salmonella* bacteria or infective parasite eggs, and the content of thermotolerant coliform bacteria (TCB) shall be less than 2500/g dry matter (FOR-2003-07-04-951; §10, section 3). Experience indicates that businesses have different practices with respect to hygienisation of fish sludge in biogas facilities. The NFSA is often contacted by businesses asking which hygienisation method to use, i.e. which method(s) that will be sufficiently effective.

1.2 Relevant legislation

Fish sludge is not specifically mentioned or regulated in the European Union's (EU) legislation on animal by-products (ABP). The current ABP legislation is composed of two regulations, No. 1069/2009 and No. 142/2011 (European Commission 2009; 2011). The regulations group ABPs into three categories; Cat. 1 is the highest risk category (material considered to pose a risk of transmissible spongiform encephalopathy (TSE), but also including a.o. wild animals, when suspected of being infected with diseases communicable to humans or animals; Cat. 2. represent intermediate risk material (such as manure, slaughterhouse wastewater and products of animal origin containing residues of veterinary drugs); and Cat.3 represent the lowest risk category (parts of animals that are fit, but not intended for human consumption, including by-products from processing of fish). The categories were defined in Articles 3-5 of Regulation No. 1774/2002 (European Commission 2002b; no longer in force). Because fish sludge is not specifically mentioned, classified or regulated in the EU ABP legislation, it is not clear if and when fish sludge can be classified as Cat. 2 or Cat. 3 material. The definition of Cat. 1 suggests that if wild fish or derived material suspected of being infected with a disease communicable to humans or animals, including wild or farmed fish, is present in the sludge, the sludge will have to be classified as Cat. 1. Pettersen et al. (2025) concluded that if fish sludge is accepted in the EU as a feed source for invertebrates, it is likely to be classified as Cat. 2 material. The method referenced in the mandate’s question 1 is applicable to Cat. 3 material and to Cat. 2 material which is composted without prior processing in accordance with Article 13(e)(ii) of Reg. (EC) No. 1069/2009 (see Reg. EC 142/2011, annex V, chapter III, section 1). Norway is not a member of the EU but a member of the European Economic Association (EEA). The EU ABP legislation is EEA relevant and therefore also relevant to Norway. As with the EU ABP legislation the Norwegian regulation on ABP not intended for consumption (FOR-2016-09-14-1064; “animaliebiproduktforskriften”) is without reference to fish sludge. The hygienisation method referenced in the mandate’s question 1 is not applicable to Cat. 1 material. Any sludge that would be classified as Cat. 1 material would therefore require an alternative to this hygienisation method to be fit for further use as an ABP.

New Norwegian legislation on fertilizers was published in January 2025 becoming effective from February 2025 with further changes becoming effective in successive steps over the next 5 years (“gjødselvareforskriften”; FOR-2025-01-20-116 and “gjødselbrukforskriften”; FOR-2025-01-29-115). According to a press release from NFSA Feb. 3rd 2025, the revised legislation is intended to facilitate use of waste and byproducts in fertilizers, while maintaining soil for food production in a long term perspective. The “gjødselvareforskriften” includes a positive list of well known and much used raw materials, making it possible to apply to the NFSA for permits to use also other raw materials provided it can be documented that they are suitable and safe for use in fertilizers. The previous regulation (FOR-2003-07-04-951) only permitted use of animal manure as fertilizer in another (Norwegian) county after hygienisation or if a permit for use of non-hygienised animal manure was granted by the NFSA. With the new “gjødselbrukforskrift” the general rule will be that use of non-hygienised animal manure from another county will not be permitted. Non-hygienised animal manure can be transported unrestricted to a processing plant in another county for hygienisation.

1.3 Approach

1.3.1 Definitions and further delimitations of the mandate

The mandate clearly focused on hygienisation and potential transmission of fish diseases. In the recently terminated Norwegian regulation “Forskrift om gjødselvarer mv. av organisk opphav” (FOR-2003-07-04-951) hygienisation was defined as “*Treatment aimed primarily at reducing the risk (danger) of transmitting infectious agents to humans, animals, or plants through disposal or other handling of the organic material.*” The new regulation “Gjødslevareforskriften” (FOR-2025-01-29-116) that entered into force from 1. February 2025 has a slightly modified but related definition. Neither of these regulations (forskrift) include a definition of infectious agents or infectious (communicable) diseases. As far as we know, no directly applicable Norwegian or European legislation contains a specific definition of infectious/communicable disease or infectious agent. However, the Norwegian act on prevention of infectious diseases (smittevernloven; LOV-1994-08-05-55 defines infectious disease as “*a disease or carrier condition caused by microorganisms or other infectious agents that can be transmitted from, to or between humans*”. Although this definition specifically refers to agents in a human context, it may also be applied to fish diseases as “*caused by microorganisms or other infectious agents that can* *be transmitted from, to or between fishes*". The term infectious agent (smittestoff in Norwegian) is commonly, although not legally (e.g. Store Medisinske Leksikon [sml.snl.no] and Wikipedia [en.wikipedia.org/wiki/Pathogen]), defined as "*microbes or organisms causing diseases, i.e. bacteria, fungi, parasites, prions, viroids or viruses*." Although prions, viroids and viruses should not be classified as organisms, they fall within the scope as biological agents causing disease and being transmittable. All known viroids are inhabitants of angiosperms and therefore out of scope for the present report. For this report the above definitions relating to the biological hazards were adopted.

Spilling from transport of non-hygienised sludge can take place and therefore induce a risk of dissemination of fish diseases. The consequences depend among others on where the spilt material ends up. This part of the fish sludge risk assessment falls outside the scope of the present report.

The mandate largely excludes **chemical hazards**, except for undesirable substances emerging as a result of the hygienisation process, e.g. through chemical reactions between sea water and organic matter during heating (see mandate question 2). **Undesirable substance** is defined in Directive 2002/32/EC (European Commission 2002a; on undesirable substances in animal feed) as "*any substance or product, with the exception of pathogenic agents, which is present in and/or on the product intended for animal feed and which presents a potential danger to animal or human health or to the environment or could adversely affect livestock production*". With respect to elements, hygienisation will not reduce or increase their quantity. It may however affect their speciation, bioavailability and toxicity, depending on parameters like pH, whether it is aerobic or anaerobic conditions during the anaerobic digestion and hygienisation processes, and red-ox state in the digest. The production of biogas would also include anaerobic digestion prior to the hygienisation. The fate of chemicals in this step would depend on chemicals present, the microbiota either present in the sludge or added in the process, and conditions such as pH. Furthermore, the reductive environment during anaerobic digestion could potentially lead to a range of chemical reactions. The microbiota has a high metabolic activity and will metabolise a large number of organic compounds. The effects of anaerobic conditions are, however, outside the mandate for this report, as only questions related to the chemical reactions during the hygienisation was specified in the mandate.
The anaerobic digestion is likely to alter the oxidation state of minerals. As the toxicity of several minerals, for example selenium and chromium, are dependent on oxidation state, this environment will probably modify the toxicity. The changes in oxidation state may, however, be reversed in a later processing step or when stored and distributed as fertilizer. The potential for chemical reactions involving the organic compounds will also depend on the mix with other matrices prior to digestion and hygienisation, but the mix is not specified and cannot be assessed.

With respect to organic pollutants present in the digesta following digestion, many are persistent and therefore unlikely to be modified by hygienisation processes, but some, e.g. certain pesticides may be modified resulting in lowered or increased risks to animals, humans and/or the environment. Microplastics and plastic associated chemicals, biotoxins and veterinary pharmaceuticals may also be modified by hygienisation processes, with similar possible consequences. The chemical hazards present in fish sludge are not themselves within the scope of this report. However, the effects of hygienisation on chemical hazards are within the scope and will therefore be addressed in the following.

Most microbes are killed by heat treatment over time. By heating large volumes, the target temperature will be reached early near the contact points between heater and sludge, but later in other parts with increasing distance to the heater. Non-homogenous heating and sub-optimal distribution of temperature measuring will therefore increase the risk of survival of microbes. In case of high fat content, it is known that fat protect the microbe's membranes against heat and therefore increase survival rates. Grease around sealings are classical niches for surviving microbes. Homogenous temperatures and avoidance of lipid rich areas is therefore important to limit the options for survival of microbes during heat treatment. The efficacy of the hygienisation protocol is dependent on complete heating of the matrix to minimum 70° C for a continuous period of minimum 60 minutes, see mandate Q1. Any deviations resulting in incomplete and/or shorter heating may reduce the hygienisation efficacy and consequently increased risk of survival of pathogenic agents. The conclusions of this report build on the explicit condition that heating is complete as described.

Fish sludge is a highly heterogeneous matrix, originating from a wide variety of sources and consequently also exhibit a wide variety of contents including biological and chemical hazards. Pettersen et al. (2025) defined fish sludge as a "*waste product consisting of particulate organic matter collected from fish farming systems stemming from feed spill and feces*". That definition does not include the presence of dead fish derivatives. The "gjødselvareforskriften" (FOR-2025-01-29-116) that entered into force in February 2025 defines fish sludge as "*sludge from aquaculture facilities consisting of feed spill and feces, without dead fish remains*". In Norway, dead fish are removed daily following the routine operations on land-based farms. The "akvakulturdriftsforskriften" (FOR-2008-06-17-822) states that daily removal of dead fish is also mandatory in sea water farms. However, extreme weather or other conditions can make it impossible, and § 16 of the regulation (forskrift) states that "*removal of dead aquaculture animals can be omitted when it is obviously unnecessary*". The mandate for the present report includes sludge collected from sea-based locations and we therefore define fish sludge to include potential presence of dead fish derivatives. Consequently microorganisms, including pathogens, from these carcasses and derived materials may be present in sludge. The mandate specifically refers to fish sludge, and therefore sludge from other aquaculture systems, such as farming of algae and crustaceans fall outside the scope of this report.

Although the mandate refers to fish sludge from any type of aquaculture facility in Norway, the present report will further exclude fish sludge from aquaculture facilities other than those producing salmonid fish. This limitation may be justified by two facts: First, salmonids in 2023 represented more than 90 % of Norwegian fish aquaculture, both in terms of biomass, number of fish and number of aquaculture facilities (Fiskeridirektoratet, 2024). This is also reflected in the available and retrieved literature considered relevant for this report. Secondly, as the NFSA did not provide funding or other resources to NVI for the preparation of this report, it had to be prepared within the resource constraints of NVIs total funding and societal mission. It may also be argued that for industrial applications it is desirable with standardised raw materials, stable supplies and low degree of risk uncertainty. A focus on sludge from salmonid aquaculture is in line with such a desire. In spite of the limitation, this report largely covers the types of pathogens that would also be relevant for a broad range of fish sludge, and the conclusions presented at the end of this report will likely apply also to other types of fish sludge from Norwegian aquaculture.

1.3.2 Expertise and approach

A team of experts on transmissible fish diseases (bacterial, fungal, parasitic and viral agents), as well as food and feed safety, organic and inorganic chemistry, toxinology and risk assessments was established. The team did not include expertise on prions and prion derived diseases. Therefore, the report leans heavily on a recent review paper that also covered prions in fish sludge (Pettersen et al. 2025).

Initially, the experts reviewed the available literature to identify the infectious agents and chemical hazards of relevance. Then the effects of the standard method for hygienisation on the different hazards was assessed, and knowledge gaps identified. Finally, the findings were summarized with a conclusion and recommendations.

2 Fish sludge

Sele et al. (2024) characterized 47 samples of fish sludge from commercial land-based Atlantic salmon (Salmo salar) farms in Norway, with the aim to document the levels of a limited selection of desirable and undesirable components. They concluded that undesirable substances such as arsenic and cadmium, some pharmaceuticals, plastic-related products and the UV filter benzophenone may be present, but did not find detectable levels of veterinary medicines, salmonid virus or bacteria. The Norwegian Scientific Committee for Food and Environment (VKM) in 2011 gave a statement on probabilities of disease transmissions from fish sludge when used as fertilizer or soil improvement without further treatment (VKM, 2011). They concluded that for some bacterial and viral disease agents such transmission could take place. The risk of transmission from fresh-water facilities to fresh-water facilities was considered to be higher than from sea water facilities to fresh or sea-water facilities. And the probability was considered to depend on species and strain of agent, the ability of the agent to survive outside the fish, the quantity and concentration of agent in the sludge, processing of the sludge, and whether the sludge is spread on the surface or ploughed into soil.

Transport of untreated sludge involve a risk of spillover and spread of hazards to new areas. There are already regulations in place to handle such issues. However, spilling from transport of untreated sludge by boat or over land, will have different impact depending on which areas and species that are present in the areas the vehicles and sludge pass. Within county distribution will have a smaller impact on spread, but adaptation to local conditions may over time increase the likelihood of infective hazards. Long distance transport (between counties) contribute to spread of hazards and illnesses. The transport vehicles should therefore have a design that limit the spillover. As transport of non-hygienised animal manure to another county is allowed, while use in another county is not (cf. “gjødselbrukforskriften”; FOR-2025-01-29-115), we assume that the risks related to spillover by transport from this material is considered as neglible for transport on land. Fish sludge may also be transported by boats. Spread to other locations via spillover of sludge at sea or via the boat outer surface needs to be included in such assessments.

3 Biological agents and risks

The EFSA BIOHAZ Panel (2021) conducted an assessment of the biological risks to animal and public health deriving from the use of, among others, biogas digestion residues and compost as organic fertilisers and soil improvers. The types of materials evaluated included Cat. 2 and Cat. 3 ABP and fat fractions, but did not include fish sludge or similar matrices and the assessment is therefore only partially comparable to assessment of risks associated with fish sludge matrices. The probability of inactivation to the required levels (5 Log_{10} for indicator bacteria and 3 Log_{10} for indicator viruses and parasites) was judged to be 66-99% certain for the most resistant of the indicator microorganisms with methods defined in the legislation (European Commission 2011). These methods, linked to specific matrices, use higher temperatures (80°, 100° and 133° C) and mostly also longer transformation times 120, 100 and 20 minutes, respectively, than the hygienisation protocol assessed in the present report (cf. the mandate). The EFSA BIOHAZ Panel (2021) recommended to implement studies to fill knowledge gaps on intrinsic physicochemical properties of ABPs and biological hazards in them. They also pointed out that available data on inactivation of indicator microorganisms and biological hazards are only exceptionally derived from studies carried out in ABP matrices and full-scale systems, and recommended more of the latter type of studies. Finally, they recommended a full characterisation of the usage pathways of ABP as organic fertilisers and soil improvers in the EU, to facilitate future risk assessments.

3.1 Prions

A recent review of relevance for the risk assessment (Pettersen et al. 2025) concluded that fish sludge is unlikely to provide a significant source of prions. More specifically that if present those prions would derive from a terrestrial mammal source that it is highly unlikely would go undetected. This could for example be a ruminant carcass. The removal of the carcass and possibly also the immediately surrounding sludge, prior to collecting sludge for industrial use, should be feasible and required. The amount of released prions in the sludge collected for industrial use would then be negligible. Furthermore, prions would neither be modified or propagated by the hygienisation process. We therefore conclude that prions do not pose a significant risk in the relevant context. This report therefore does not include further assessment of risks associated with prions in fish sludge.

3.2 Bacteria

3.2.1 Pathogenic bacteria in and from fish sludge

VKM (2011) concluded their summary with the statement that there is a very low likelihood for transmission of disease (virus, bacteria and parasites) to humans from use of fish sludge as fertilizer or soil improvement. However, fish sludge is a complex matter, and the potential for spread of infectious agents therefore depends on the composition/content of the sludge. While removal of dead fish is striven for, the presence of dead fish derivatives in some of the sludge is inevitable, adding to the potential of human pathogens. VKM (2011) further highlighted *Clostridium* spp., *Vibrio* spp. and *Aeromonas* spp. as possible human pathogens that can be found in fish sludge/sediments. Of these, the bacteria with the strongest resistance to hygienisation (by heat treatment, or other treatment) are the spore forming Clostridiales. Spores are in general resistant to many types of stressors, such as heat treatment. *Clostridium botulinum* spores, for example, can withstand temperatures well over 100°C for 15+ minutes (Odlaug and Pflug 1977). The resistance to heat in these spores is, however, very affected by the composition and water activities of the matrix the spores are suspended in (Murrell and Scott 1966; Odlaug and Pflug 1977). This makes it difficult to estimate which time and temperature combination(s) that will efficiently kill/inactivate the spores, as the already heterogeneous fish sludge matrix becomes even more complex when mixed with other matter at the biogas-plant.

The remaining bacterial risk can be described as the potentially pathogenic bacterial microbiota surviving the hygienisation treatment. Given the explicit condition that the appropriate temperature/time combination has been reached and no re-contamination has taken place, the remaining bacterial risk will most likely be due to Gram positive bacteria such as lactic acid bacteria, rapid growing mycobacteria, and other bacteria known to be resilient towards heat treatment, e.g. sporeforming bacteria. However, the concentration of relevant bacteria present will be reduced. When spread on fields and followed by tillage, e.g. ploughing, there will also be a dilution effect with soil. Although the concentration of specific bacteria most likely will be reduced over time, sporeforming bacteria may survive for decades, if not centuries, while others will be considerably reduced over a shorter period, e.g. weeks to months. Worst-case scenarios could be spreading of fertilizer/soil amendments followed by severe precipitation causing direct run-off to waterways, ponds and lakes, or incidental, e.g. climate/weather driven changes of soil parameters creating optimal conditions for growth of persistent pathogens such as sporeformers.

Pathogenic bacteria in fish can be excreted from diseased fish through feces, urine, exudates, and from dead fish. In Norwegian aquaculture, recent years have seen great variation in production systems, including open facilities in the sea where the sludge is not currently collected, semi-closed sea-based facilities, and closed land-based systems that take in both seawater and freshwater, flow-through systems, and recirculating aquaculture systems (RAS), all of which collect sludge. Dead fish are collected and separated from the sludge in these systems, but pathogenic bacteria from dead fish can end up in the sludge produced. According to the Norwegian regulation FOR-1997-02-20-192 (Forskrift om desinfeksjon av inntaksvann til og avløpsvann fra akvakulturrelatert virksomhet), intake water must be disinfected, but if disinfection routines fail or pathogens enters the facility in other ways, disease-causing bacteria may become part of the sludge in the facility. The bacteria are therefore discussed together, regardless of the production types usually associated with outbreaks of various diseases. The general ability to survive in sludge/sediments and how the sludge is treated (drying, heat treatment, pH, etc.) is crucial for determining to what extent pathogenic bacteria can be present in the fish sludge. The bacteria discussed here are commonly occurring pathogens in Norwegian aquaculture.

3.2.2 Gram-negative Bacteria

Various species of the genera *Vibrio* and *Aliivibrio* can cause septicemias and are associated with skin lesions in several fish species in Norwegian aquaculture. Examples of bacteria in this group found in sick fish include *Vibrio* (*Listonella*) *anguillarum*, *Vibrio ordalii*-like species, *Vibrio salmonicida*, and *Vibrio* (*Aliivibrio*) *wodanis*. Other *Vibrio* spp. isolated from fish samples include *Vibrio alginolyticus*, *Vibrio vulnificus*, and *Vibrio* (*Aliivibrio*) *logei*. Species within these bacterial genera are widespread in the marine environment and adapted to lower temperatures (Sommerset et al. 2023). *Vibrio salmonicida* has been found in large quantities in bottom sediments near affected pens (Enger et al., 1989). *Moritella viscosa* causes lesions and bacterial spread in several fish species (Colquhoun et al. 2004; Sørgaard et al. 2023). It is found in seawater and can cause serious outbreaks of skin lesions in fish kept in closed aquaculture systems that intake seawater. Larger outbreaks in open pens in saltwater are also common (Sommerset et al. 2024).

Yersinia ruckeri causes outbreaks of yersiniosis in salmon in Norway. The bacterium is widespread, and several genotypes exist, with some believed to be more virulent and closely linked to environmental factors. In Norway, disease outbreaks in salmon are mostly associated with strains belonging to clonal complex 1 (CC1). It is believed that the bacterium is introduced in the freshwater phase and is widespread in hatcheries, flow-through systems, and RAS facilities. The bacterium forms biofilm both in the field and under laboratory conditions, and seawater triggers biofilm formation. Healthy carriers are common after yersiniosis outbreaks, and they may continue to shed bacteria into the environment/sludge (Gulla et al. 2018; Sommerset et al. 2023).

Flavobacterium psychrophilum causes disease in freshwater and brackish water fish. The bacterium is widespread, and several genotypes have been found in Norwegian aquaculture, with one variant (ST2) primarily associated with high mortality. Other genotypes are believed to be more linked to the environment. In Norway, the bacterium causes disease in rainbow trout in inland facilities, hatcheries, and farms located in brackish water zones. It has been shown that the bacterium can survive for more than 300 days in a laboratory environment (Madetoja et al. 2002; Nilsen et al. 2014).

Tenacibaculum species, such as *Tenacibaculum finnmarkense* (varieties gv. *finnmarkense* and gv. *ulcerans*), *Tenacibaculum dichentrarchi*, *Tenacibaculum piscium*, and *Tenacibaculum maritimum*, are widely distributed in the marine aquatic environment, where they are associated with the degradation of organic detritus. Some species have been isolated from sediments. It has been reported that the bacterium can survive for several months in laboratory conditions. *Tenacibaculum finnmarkense* is associated with ulceration in salmon in seawater. Outbreaks of *Tenacibaculum dichentrarchi* have been described in land-based fish farms (Olsen et al. 2011; Klakegg et al. 2019).

Various species of the *Aeromonas* genus can cause disease in fish. All salmonid fish are vaccinated against *Aeromonas salmonicida* ssp. *salmonicida*, but disease outbreaks occur occasionally. Motile *Aeromonas* spp. are sometimes detected in connection with disease outbreaks in hatcheries. *Pseudomonas fluorescens* occasionally appears in hatcheries. Bacteria in these groups have zoonotic potential, are considered ubiquitous in freshwater environments, and are abundant in sediments in such environments (Sommerset et al. 2023).

Pasteurella atlantica (varieties gv. *cyclopteri* and gv. *salmonicida* – new, unapproved names) and *Pasteurella skyensis* are “new” pathogenic bacteria in salmon and lumpfish in Norway, particularly in open pens in seawater. These bacteria have not been detected in closed systems but pose a potential threat to all production forms that use seawater (Gulla et al. 2023).

Francisella noatunensis ssp. *noatunensis* is a reportable facultative intracellular bacterium in cod (Olsen et al. 2006). Given the renewed interest in cod farming in RAS, it could be present in sludge after disease outbreaks. Fecal-oral transmission is a likely route. Laboratory studies have shown that the bacterium can survive for up to 60 days under both sterile and non-sterile conditions, although it loses its infectivity after a few days (Duodu and Colquhoun 2010).

Piscirickettsia salmonis is a facultative intracellular bacterium that causes disease outbreaks in salmon with liver damage and skin lesions intermittently in Norwegian aquaculture (Olsen et al. 1997; Sommerset et al. 2023).

3.2.3 Gram-positive Bacteria

Mycobacterium salmoniphilum is a fast-growing *Mycobacterium* species occasionally detected in connection with granuloma formation in sick salmon in Norway (Zerihun et al. 2011; Reed et al. 2023). The detections have been linked to RAS, with the disease following fish to seawater (Zerihun et al. 2019). Mycobacteria are resistant to environmental degradation. In aquariums, *Mycobacterium* spp. have been detected in sediments and biofilm.

Renibacterium salmoninarum is a facultative intracellular gram-positive bacterium. The bacterium is reportable, and its presence has increased in Norwegian aquaculture in recent years (Brynildsrud et al. 2014; Sommerset et al. 2023). The bacterium is resistant to environmental degradation. It has been shown that the bacterium is excreted with feces and can survive up to 21 days in feces/sediment (Austin and Austin 2016).

Carnobacterium maltaromaticum and other gram-positive lactic acid bacteria are frequently detected in several fish species (Ringø, 2008). This group of bacteria is associated with inflammation in serous membranes, and some have been found as part of the intestinal microbiome of fish.

Spore-forming bacteria like *Clostridium botulinum* have not been reported in connection with disease outbreaks in Norwegian marine aquaculture (Austin and Austin 2016), but cases of botulism with fermented fresh water fish (rakfisk) have been observed. The Norwegian Scientific Committee on Foods and Environment (VKM 2010) stated that where dead fish is not removed on a daily basis, the concentration of *C. botulinum* (in fish sludge) is reported to reach levels at which the possibility of survival of spores with toxin producing potential cannot be ruled out. High concentrations of *C. botulinum* is more associated with anaerobic conditions than with aerobic conditions. Minimising the periods with anaerobic conditions in the sludge, for instance by turning the sludge to give access to air, reducing storage time, etc. will therefore contribute to risk reduction. Temperature below 6° C and minimum 6 percent salt (Mattilsynet guideline for production of rakfisk) limit the growth of the bacterium. Storage of treated sludge above this temperature will not prevent germination of spores. Measures to prevent germination of spores and destruction of preformed toxins of type E in the sludge will therefore be important. The D-value for the bacterium is 0.20 min at 121° C, and inactivation at temperatures below 100° C cannot be guaranteed (Dahlsten et al. 2015). On the other hand, pH below 4.6 limit the growth. The toxins denaturate after 10 minutes at 100° C or 30 minutes at 80° C, but it should be noted that the toxins are more stable at stronger acidic conditions than at more neutral pH (Munir et al. 2023).

3.2.4 Survival after hygienization

There is limited documentation on the survival of fish pathogens at high temperatures. Dixon et al. (2012) studied the survival of various bacterial species at 60° C for 60 minutes, including *Aeromonas salmonicida* ssp. *salmonicida*, *Lactococcus garviae*, *Vibrio anguillarum*, *Mycobacterium chelonae*, *Photobacterium damselae* ssp. *piscicida*, *Renibacterium salmoninarum*, *Streptococcus iniae*, and *Yersinia ruckeri*. Oplinger and Wagner 2013 reported that temperatures ≥55°C killed *Flavobacterium psychrophilum*.

Renibacterium salmoninarum and *Streptococcus iniae* were inactivated at 60° C for 60 minutes, so they would also be inactivated at higher temperatures (70° C for 60 minutes). Concentrations of *Aeromonas salmonicida* ssp. *salmonicida*, *Vibrio anguillarum*, *Photobacterium damselae* ssp. *piscicida*, and *Yersinia ruckeri* were significantly reduced at 60° C for 60 minutes, suggesting that a 70° C treatment for 60 minutes would further reduce/inactivate these bacteria. Dixon et al. (2012) reported that *Lactococcus garviae* (a gram-positive lactic acid bacterium) was found to be more resistant to heat, but treatment at 70° C inactivated the bacterium, with some strain variation within 1-6 hours. *Lactococcus garviae* is not common in Norwegian aquaculture and more frequently present in warmer regions. For *Mycobacterium chelonae* (closely related to *Mycobacterium salmoniphilum*), 24-48 hours at 60° C was required for inactivation.

Hygienization of sludge through heat treatment at 70° C for 60 minutes is likely sufficient to inactivate the bacteria causing diseases in fish in Norwegian aquaculture, with few exceptions such as *Lactococcus garviae* and *Mycobacterium* spp. It is possible that gram-positive bacteria, such as lactic acid bacteria e.g. *Carnobacterium maltaromaticum* and fast-growing mycobacteria as *Mycobacterium salmoniphilum*, will not be fully inactivated by the outlined treatment, but we can expect a significant reduction in bacterial concentrations. Variations in temperature tolerance among genotypes within different bacterial species, as shown for *Lactococcus garviae*, should also be considered. The type and amount of organic material, as well as other factors such as pH, may influence the extent to which bacteria survive heat treatment.

Experimental assessment of the efficiency of hygienisation of particular presumed thermotolerant bacteria such as *Lactococcus garviae* may be justified to close current knowledge gaps of relevance to hygienisation of fish sludge and Norwegian aquaculture.

To verify the effectiveness of sludge hygienisation in fish farming, more reliable and new data based on experiments are required. It may be argued that the best way to do so is by establishing a "standard sludge" with characteristics representative of sludge from Norwegian fish farms, spiked with pathogens, treated at 70° C for 60 minutes, followed by an examination for viable pathogens. The alternative is to perform the verification experiments with samples of fish sludge representative of the structural and compositional diversity produced in Norwegian aquaculture. Data on the presence of potentially pathogenic microbes in representative fish sludge samples are currently very limited and may inform the choice of verification approach.

3.3 Fungi

Fish sludge is expected to contain fungi (including yeasts). These fungi may include pathogenic species, opportunistic pathogens, and saprotrophic species that utilize sludge as a nutrient source. To date, no studies have specifically investigated the presence of fungi and fungus-like organisms in fish sludge from land-based aquaculture systems (Pettersen et al. 2025). In this context, within this report we are assessing the potential risks associated with fungal presence in fish sludge, particularly in the context of hygienization.

The presence of fungi in fish sludge is a potential concern due to their adaptability and resilience (towards e.g. temperature variations, reduced water availability, high salinity, UV treatment). Fungi are known to inhabit diverse environments, where they exploit both natural and xenobiotic resources. Certain species exhibit high stress tolerance and adaptability, enabling them to persist in fresh and saltwater systems. These fungi may be permanent elements of their environments or opportunistic colonizers responding to nutrient availability. Freshwater fungus-like pathogens from the family Saprolegniaceae are commonly found in aquatic environments and frequently establish biofilms, including in aquaculture facilities. Consequently, they are expected to be present in fish sludge. However, it is unlikely that Saprolegniaceae oospores or zoospores would survive the hygienization process (Dyer et al., 2007).

Like bacteria, fungi can produce spores that exhibit high resilience to extreme conditions, including temperature variations, reduced water availability, high salinity, and UV treatment (Dijksterhuis, 2019; Liu et al., 2020). Given the lack of comprehensive studies on fungal diversity in fish sludge, it is currently impossible to rule out the presence of resilient fungi capable of withstanding hygienization processes.

Mycotoxins are toxic compounds produced by fungi, and although they fall outside the scope of this report, they deserve attention as they represent an important knowledge gap. While most mycotoxin-producing fungi originate from terrestrial environments, some have been identified in aquatic ecosystems, including both freshwater and marine environments. The production and occurrence of mycotoxins in aquatic settings remain understudied. Mycotoxins can also enter fish sludge through plant-based feed (Oliveira and Vasconcelos, 2020). A crucial concern is that mycotoxins are generally heat-stable (Bullerman and Bianchini, 2007; Kabak, 2009), meaning they are unlikely to degrade during heat treatment of fish sludge, if present within untreated matrix. This necessitates further investigation into the potential risks associated with mycotoxin presence in hygienized fish sludge.

3.4 Parasites

3.4.1 Parasites in salmonid fish farms in Norway- an evaluation of survival during heat treatment.

Fish parasites in general represent a number of taxonomic groups that have one thing in common: they need a host to survive and complete the life-cycle (Noble & Noble, 1976). Different parasitic species and groups have different biology, life-cycles and developmental stages, which are important aspects when evaluating the survival of the different species under different conditions (natural environmental conditions or treatment). Parasites range from single-celled organisms to multicellular organisms, and can have direct life cycles with no intermediate hosts or more complex life cycles involving intermediate or other final hosts than fish. Parasites can also live either on the outside of the fish (ectoparasite) or inside the fish (endoparasite). The species with direct life-cycles are the ones with the highest potential for causing diseases in aquaculture as they would multiply and spread fast in a net pen with a high density of fish. It can be assumed, that since fish parasites are adapted to their hosts, they are also adapted to the environment of their host and their temperature tolerance will therefore be similar to that of their hosts. However, some life stages have evolved to survive for shorter or longer periods in the environment (off the host). These are likely to be more resistant to environmental factors and treatment, such as heat treatment, than the adult parasite or other stages. These stages include eggs, for example from the nematode *Anisakis simplex* and the tapeworm *Eubothrium crassum*, and cysts, of e.g. the ciliate *Ichthyophthirius multifiliis* and probably also of the flagellate *Spironucleus salmonicida*.

It can also be assumed that parasites in general are highly dependent on environmental conditions, especially ectoparasites or life stages that exist outside the host. These conditions can limit parasite development or even cause mortality if physiological parameters are altered. Temperature, for example, can delay or stop development, as parasites tend to develop more slowly in colder and more rapidly in warmer conditions. In addition, salinity plays a crucial role; freshwater fish parasites usually cannot survive for long periods in seawater, and vice versa. Therefore, when assessing whether potentially surviving parasites in sludge can spread to new areas, it must be taken into account whether the sludge is collected from marine or freshwater farms.

According to the Norwegian Fish Health report (Sommerset et al., 2024 and see also reports from previous years), a number of parasite species are found in or on salmonid fish in Norwegian aquaculture, both in freshwater and marine facilities. The parasite with the highest impact on Norwegian aquaculture is a parasitic crustacean with direct life-cycle, the salmon lice, *Lepeophtheirus salmonis*. Another species with a direct life-cycle is the amoeba *Paramoeba perurans*, a protozoan causing amoebic gill disease (AGD) in both salmonids and several other fish hosts (Karlsbakk et al., 2013). Representatives and species with more complex and with indirect life cycles includes the myxozoan *Parvicapsula pseudobranchicola* (Karlsbakk et al., 2002), that need a polychaete final host to complete the life cycle, and the tapeworm *Eubothrium crassum* which alternates between a copepod intermediate host and the fish final host, in which it can develop into an adult (see Hansen et al., 2022 and references therein).

According to Pettersen et al. (2025) there is a knowledge gap on "*experimental data on the fate of bacteria and parasites through the processing chain, from the raw fish sludge to the use as a potential feed material for invertebrates, when following relevant treatment methods described in the regulation*" (i.e. Reg. 142/2011; European Commission, 2011), and the same is true for experimental data on heat treatment for inactivation of parasites, especially for fish parasites. However, some examples from human and other animal parasite species belonging to the same taxonomic groups as those species present in Norwegian aquaculture are available.
A list of some of the known parasites found in Norwegian salmonid aquaculture with their characteristics is found in **Table 1a**, freshwater parasites, and **Table 1b**, marine parasites. We have evaluated only a selection of species of important parasites found in Norwegian aquaculture. However, these parasites represent different taxonomic groups with different biology and life-cycles and can be considered representative of the diversity of parasites that the present report need to assess transmission risks for.

Table 1a. Some known freshwater parasites found in Norwegian salmonid aquaculture

Group	Species	Life-cycle	Endo- or ectoparasite	Fish host	Other host
Ciliophora	*Ichthyobodo necator*	direct	ectoparasite	Salmon	NA
Ciliophora	*Ichthyophthirius multifiliis*	direct	endoparasite	several, incl. salmon	NA
Diplomonadida	*Spirocucleus salmonicida* [1]	direct	endoparasite	Salmonids	NA
Myxozoa	*Myxobolus cerebralis*	indirect	endoparasite	Salmonids	Tubifex
Myxozoa	*Tetracapsuloides bryosalmonae*	indirect	endoparasite	Salmonids	Bryozoa
Monogenea	*Gyrodactylus salaris*	direct	ectoparasite	Salmonids	NA

[1] Has also caused disease in the marine environment.

Table 1b. Some known saltwater parasites found in Norwegian salmonid aquaculture

Group	Species	Life-cycle	Endo- or ectoparasite	Fish host	Other host
Amobozoa	*Paramoeba perurans*	direct	ectoparasite	several, incl. salmon	NA
Ciliophora	*Trichodina* spp.	direct	ectoparasite	several, incl. salmon	NA
Ciliophora	*Ichthyobodo salmonis* [1]	direct	ectoparasite	Salmon	NA
Microsporea	*Desmozoon lepeophtherii*	direct and indirect	endoparasite	Salmon	Salmon lice
Myxozoa	*Parvicapsula pseudobranchicola*	indirect	endoparasite	Salmonids	Polychaeta
Cestoda	*Eubothrium crassum* [2]	indirect	endoparasite	Salmonids	Copepods
Nematoda	*Anisakis simplex*	indirect	endoparasite	several, incl. salmon runts	Copepods, marine mammals
Nematoda	*Hysterothylacium aduncum*	indirect	endoparasite	several, incl. salmon runts	Copepods
Copepoda	*Caligus elongatus*	direct	ectoparasite	several, incl. salmon	NA
Copepoda	*Lepeophtheirus salmonis*	direct	ectoparasite	Salmonids	NA

[1] Both freshwater and marine.

[2] *Eubothrium* sp./*E. crassum* is found in both freshwater and marine fish (see text).

3.4.2 Ectoparasites

General comments: In general, ectoparasites are directly affected by the abiotic factors in the surrounding environment. This will influence the population dynamics, distribution and dispersal of the parasite species in question (Chubb, 1977). It is reasonable to assume that ectoparasites, being adapted to the environmental conditions of the host, will die when exposed to such high temperatures as required for the treatment of sludge (cf. the mandate for this report). Under such high temperatures there will likely also be a lack of oxygen, which can contribute to increased mortality of the parasites. Many or all of these ectoparasites will most likely die when they dry, and this is indeed one way of disinfecting fishing equipment. Based on the limited current knowledge we consider it unlikely that any of the ectoparasites will survive the outlined heat treatment. Comments on knowledge for some ectoparasites are found below.

Gyrodactylus salaris. This freshwater ectoparasite has a direct life-cycle. It has been reported from a number of hatcheries and rainbow trout farms earlier, but is now near extinct in Norway (Hansen et al., 2024). It has had devastating effects on wild salmon populations in Norway and an introduction to a new area would have large consequences. However, it is shown to be very sensitive to environmental factors like high temperature, high salinity, disinfectants, and chemicals including aluminium sulphate and chlorine (Hagen et al., 2014; Koski et al., 2016; Pettersen et al., 2007; Poléo et al., 2004; Soleng & Bakke, 1997) and thus will not survive the outlined heat treatment.

Lepeophtheirus salmonis and *Caligus elongatus*. These parasites are marine crustacean parasites with a direct lifecycle. Infections with salmon lice is the disease with the highest negative impact on Norwegian fish farming (Sommerset et al., 2024). To our knowledge, there are no studies that have exposed these lice to temperatures above 36° C. It is however, known that the salmon lice is negatively impacted, although probably not dying, when exposed to higher temperatures as free-moving stages will detach from the salmon at between 32 and 36° C (Nilsson et al., 2023). Short term exposure to these temperatures is today used as treatment procedure in salmon farming (Sommerset et al., 2024).

3.4.3 Endoparasites

General comments: Eggs and resting stages or cysts are the stages that are the most resilient to environmental factors, and trematodes is one group of parasites that seem to have particularly resilient eggs. Trematodes are generally not found in fish in Norwegian Aquaculture, probably due to the use of formulated feeds and the parasite's complex life-cycles, although they are common in wild fish where they are often introduced via the fishes diets. As an examples of resistance to heat treatment, the trematode *Opistorchis viverrini*, the Southeast Asian Liver fluke that infects humans, seems to have particularly heat resistant eggs (Boueroy et al., 2019). However, even for this species, experiments demonstrated that all eggs were killed after exposure to 70° C for 10 minutes. Based on this, it is reasonable to assume that trematode eggs in general will be killed under such conditions, especially those found in temperature regions. Although not known, we have reasons to assume that the hygienisation protocol described in the mandate Q1 will kill eggs of nematodes and cestodes.

Anisakis simplex and *Hysterothylacium aduncum*. These two nematodes have both been detected in salmon runts in a Norwegian fish farm, but not in fish intended for sale (Mo et al., 2013). Ingestion of larvae of *A. simplex* can result in acute or chronic gastrointestinal disease (see e.g. Audicana et al., 2003). These two species have a different life-cycle. *Anisakis simplex* has fish as an intermediate host where it can be found in fish muscles, and it matures into an adult in the intestine of marine mammals. *Hysterothylacium aduncum* on the other hand, has fish as its final host and becomes an adult in the fish intestine. They both shed a high number of eggs with infective stages to the environment from their site in the final host.

According to Wootten and Cann (Wootten & Cann, 2001) as cited in Franssen et al. (Franssen et al., 2019), Anisakis larvae are killed in 1 minute at a temperature of 60° C or above. Survival of eggs are not listed, but as another example of a heat treatment of nematodes, studies have been carried out on eggs of *Ascaris* sp., an important human pathogen (Naidoo et al., 2018). For this species, it was found that at 70, 75 and 80° C, a complete inactivation was observed when exposure time exceeded 5 seconds. This means that it is highly unlikely that any stages of roundworms found in salmon will survive the hygienisation protocol assessed in the mandate Q1. Further spreading, should it survive treatment, would also not contribute to increased infection pressure in an area as these parasites already have a large geographic distribution (Cipriani et al., 2022).

Eubothrium crassum. This parasite has salmon (salmonid fish) as the final host and is common in Norwegian salmon aquaculture in the south-western part of Norway (Hansen et al., 2022). In freshwater, the species is found in salmonids all along the Norwegian coast, both in freshwater and the marine environment. It is, however, discussed whether specific freshwater and marine strains or species of the parasite exists (see Hansen et al., 2022). *Eubothrium crassum* produces an enormous amount of eggs that are released into the environment and can be eaten by the intermediate copepod host. Nothing is known on the resistance of the eggs of *Eubothrium* sp. to heat, but we know from e.g. studies on eggs of the cestode *Taenia hydatigena* that treatment at 60° C inactivated the eggs (see Buttar et al., 2013 and references therein for more information). It is therefore not likely that eggs of *Eubothrium* sp. will survive heat treatment, and if they should, this will most likely not contribute to increased infection pressure in an area, at least south of Nordland, as the parasite already has a widespread distribution (Hansen et al., 2022).

Myxozoans. Myxozoans are a diverse group of parasites with a complicated life-cycle. Most relevant for Norwegian fresh- and saltwater farming are *Myxobolus cerebralis*, *Tetracapsuloides bryosalmonae* and *Parvicapsula pseudobranchicola*. *Myxobolus cerebralis* is the causative agent of whirling disease, a disease which is particularly severe in rainbow trout, *Oncorhynchus mykiss* (Hallett & Bartholomew, 2012). Infections with T. bryosalmonae causes proliferative kidney disease, PKD, in salmonids in freshwater and can contribute to mortality in wild fish populations (Sterud et al., 2007). *Parvicapsula pseudobranchicola* infects salmon in sea water and the disease, parvicapsulosis, can result in high mortality in farms in Northern Norway (Nylund et al., 2005).

All three have a complex life cycle with an oligochaete, a bryozoan and a polychaete as the final host respectively. In both the fish intermediate host and the final host, myxozoans produce infective spores. It is known that these spores are very resistant to environmental factors and can withstand both freezing and desiccation (El-Matbouli & Hoffmann, 1991). They have also been found to be able to pass unchanged through the alimentary tract of humans (Boreham et al., 1998). However, to the best of our knowledge, spores of Myxozoans have never been tested for survival at temperatures as high as 70° C and we consider it unlikely that they will survive such treatment.

Spironucleus salmonicida. This is a freshwater commensal species in the intestine of salmonids, but has caused outbreaks (spironucleosis) in Atlantic salmon in marine farms several times since 1989 (Sommerset et al., 2024). It is unknown how the species spread to new hosts, but most likely through cysts in the environment. Cysts are not confirmed for this particular species, but have been found in the closely related *S. salmonis* (Moore, 1923) and it is assumed that they can be present also in *S. salmonicida* (Xu et al., 2014). The cysts of the related *S. muris*, a parasite of mice, will not survive a temperature exceeding 45°C for 30 minutes (Kunstýr & Ammerpohl, 1978). We therefore consider it likely that the assessed hygienisation protocol will kill cysts also of *S. salmonicida*.

3.5 Viruses

Several viruses, like infectious pancreas necrosis virus (IPNV), piscine orthoreovirus 1(PRV-1), infectious salmon anemiavirus HPR0 (ISAV HPR0), and salmon gill pox virus (SGPV), may be found in fish sludge from hatcheries as they are frequently detected in salmonid fry and smolt. In addition to the viruses mentioned above, at sea water sites, the viruses piscine myocarditis virus (PMCV) and salmonid alphavirus (SAV) are also wide-spread. Virus is shed to the environment during infection/disease outbreaks and may become associated with organic waste products like sludge. However, the dilution effect will be much lower in land-based closed facilities than in open cages at sea. Multiple other factors may also influence the amount of infectious virus present in the sludge, like time outside the host, salinity, temperature, and pH. Enveloped viruses, like ISAV and SAV, are considered relatively sensitive to heat-treatment with a required temperature of 60° C or less for 30 minutes for 4 log10 inactivation. Naked viruses, especially PRV-1 and IPNV are expected to be more robust against various inactivation methods. IPNV was identified as a potential threat to fish health that could be transmitted if fish sludge was used as fertilizer (VKM 2011). For fish sludge to be used safely for this purpose, elimination of this virus must therefore be done. Nygaard et al. (2012) investigated at what temperatures and times the virus was inactivated. At 70° C, 1 log10 reduction was accomplished in 10 minutes, 2 in 42 minutes, 3 in 75 minutes, 4 in 107 minutes and 5 in 140 minutes. Only with an increase of five degrees up to 75° C, did the respective times decrease to 3,13, 23, 33 and 43 minutes. Inactivation tests for PRV-1 indicates similar results. Heat treatment at 70° C for 60 minutes as a hygienisation step may therefore be inadequate for inactivation of these types of viruses. Fish sludge is such a complex matter for the heat to penetrate and spread evenly in, especially if it is mixed with other matter in large batches where temperatures can vary. Inefficient treatment, resulting in infectious virus within the sludge may result in spread of virus in the environment and to aquaculture species. Nygaard (2010) stated that heating Cat. 3 material from farmed fish to 76° C for 20 minutes would result in a 3 Log_{10} reduction of IPNV. VKM (2012) concluded that heating by-products of farmed fish, fish meal and oil originating from such materials to 76° C for 20 minutes would result in low probability of presence of IPNV and PRV-1, and negligible probability of presence of other viruses and parasites assessed.

Our recommendation is therefore to increase the temperature to **at least 75° C** for a continuous period of minimum 60 minutes, to ensure adequate inactivation of the most robust viruses.

4 Chemical hazards

A range of chemical contaminants are present in fish sludge (Hagerman et al., 2025, Sele et al., 2024), of which many, like PCBs and PFOAs are very persistent compounds. Other compounds, like several plastic-related compounds etc. are less persistent. These compounds may have been transformed to unknown compounds during the anaerobic digestion step in the biogas production. The chemical fate of compounds during anaerobic digestion is currently unknown and a literature search did not reveal any relevant hit. The fate of chemicals during this step would depend on conditions including oxygen availability, pH, temperature and chemical composition of the digest. An assessment of the process would therefore require more information about the fish sludge, the other materials mixed with the digesta in the biogas production facilities and the exact fermentation process. It could, however, also be noted that soil-living microorganisms have a high biotransformation capacity that may degrade most non-persistent chemicals.

The chemical contaminants present following the anaerobic digestion are probably more stable compounds. Furthermore, the temperature (up to 70° C, residence time 60 min, with the presence of water) is not considered to favour formation of more bioactive compounds. A literature search did not reveal any conclusive data, however. The lack of data excludes any firm conclusion, but the risk of formation of potent toxic compounds during the hygienisation is considered to be limited.

5 Summary, conclusions and recommendations

Fish sludge, even when considering only fish sludge from Norwegian salmonid aquaculture facilities is heterogenous, both with respect to structure, chemical composition and biological risks. This makes it difficult to assess with sufficient certainty how the biogas production affects microbial metabolism and transformation of compounds during biogas production and hygienisation. Furthermore, it is not clear to what extent the physico-chemical parameters vary, e.g. with respect to pH or aerobic/anaerobic conditions in these processes, at microscale. The mandate of this report listed three questions that should be addressed (slightly modified texts):

Question 1: Is processing of fish sludge through hygienisation at minimum 70° C for a continuous period of minimum 60 minutes, with a particle size of < 12 mm, sufficient to prevent dissemination of fish diseases when the bio-remains are used as fertilizer or for soil improvement?

> The **biological disease agents** considered here include prions, viruses, bacteria, fungi and parasites that may be present in the fish sludge used for biogas production.
>
> For **prions** it is concluded that the risk of disease transmission and dissemination is ignorable. Prions will stem from a (carcass of a) terrestrial mammal. Prion containing material will therefore very likely be detected and removed in the very unlikely event that it is introduced to the aquaculture environment. It is therefore highly unlikely that prions end up in fish sludge. Furthermore, if at all present in the fish sludge, the prions will be at very low concentrations, will be diluted during sludge collection and processing, and the hygienisation will neither modify nor propagate the prions.
>
> For **bacteria** it is concluded that the spore forming Clostridiales are considered to be most resistant to hygienisation. Spores are generally resistant to many types of stressors, including heat treatment. Spores of *Clostridium botulinum* have for example been reported to withstand temperatures well over 100° C for > 15 minutes. Although resistance is strongly affected by physico-chemical parameters, it is difficult to estimate how effective the standard hygienisation will be on spores in the bio-remains after biogas production. Presence of the bacterium is, however, rare and outgrowth and toxin production will be more relevant under anaerobic conditions than under aerobic conditions. Furthermore, the bacterium is already present in soil and other niches in the nature, and the additional risk from sludge if heat treated shortly after collection is therefore limited. It is not likely that heat treatment at e.g. 80° C will have a better hygienisation effect on *C. botulinum* than 70° C. Spore forming bacteria have not been reported in connection with disease outbreaks in Norwegian aquaculture, but *C. botulinum* can be present at significant concentrations in sludge, e.g. if dead fish is not removed on a daily basis. *Lactococcus garviae* and *Mycobacterium* spp. may be resistant to heating and standard hygienisation but are considerably less heat-tolerant than *Clostridium* spp.
>
> For **fungi** it is concluded that while the hygienization process is likely to eliminate most fungal species, the survival of more resistant fungi cannot be entirely ruled out. Additionally, mycotoxins pose a potential risk as they may persist despite heat treatment. Given the current knowledge gaps, further research is needed to assess the fungal diversity in fish sludge and evaluate the effectiveness of hygienization processes in mitigating biological risks. Understanding these factors is essential for ensuring the safe reuse of fish sludge in various applications.
>
> **Parasites** are a very diverse polyphyletic group of organisms, with very different biology and life cycles, and multiple parasites can be present in aquaculture and fish sludge. Yet, we believe to have covered sufficiently the diversity of parasites that this report needed to assess with respect to the efficiency of the standard hygienisation protocol. Ectoparasites are adapted to the environmental conditions of their hosts and are directly affected by altered environmental conditions, such as heating, drying and lack of oxygen during hygienisation. We therefore consider it unlikely that any relevant ectoparasite will survive

hygienisation. Eggs and resting stages or cysts of endoparasites are generally the most resilient parasitic stages. Trematodes (flatworms) appear to have particularly resilient eggs, but trematodes are generally not found in Norwegian farmed fish and we have not retrieved documentation of survival of eggs at 70° C for more than 10 minutes even for the most resistant species. Also for nematodes (roundworms) and cestodes (tapeworms) the standard hygienisation appears to be sufficient for complete inactivation. Even if surviving and released into the environment the relevant nematodes and cestodes are unlikely to increase the infection pressure in most areas as these parasites already have a widespread distribution. In summary, although the documentation on effects of hygienisation on parasites is limited and further studies and documentation is warranted, we consider it unlikely that any parasite of relevance will survive the standard hygienisation.

Several fish diseases causing **viruses** are of concern in Norwegian salmonid aquaculture, and outbreaks are not uncommon. It is therefore highly likely that viruses that can cause disease will appear in fish sludge. While the enveloped viruses appear relatively sensitive to heating and therefore can be expected to be inactivated by the standard hygienisation protocol, naked viruses appear more resilient to heating and may require higher temperatures and/or longer treatment. Infectious pancreas necrosis virus (IPNV) is of particular concern. Inactivation experiments suggest that heating to at least 75° C for 60 minutes is necessary to inactive IPNV. Our recommendation is therefore to increase the required hygienisation temperature to at least 75° C for a continuous period of at least 60 minutes, to ensure adequate inactivation of the most robust viruses.

Question 2: Can undesirable substances emerge as a result of the hygienisation process?

The lack of data excludes any firm conclusion, but the risk of formation of potent toxic compounds during the hygienisation under anaerobic conditions is considered to be limited. Heating offers a low probability for transformations. Processes with oxidative agents or high/low pH yield higher probability for interactions with minerals in sea water and organic molecules in sludge.

Question 3: What additional knowledge is needed to answer question 1 (what are the knowledge gaps)?

More detailed evidence of the effect of hygienisation and possible survival of sporeforming bacteria is warranted. However, studies of *Clostridium botulinum* require high safety level facilities. An option is to perform such studies with less dangerous strains but divergent heat tolerance profiles first. *Bacillus* spp. are sporeforming bacteria that can be used for this purpose.

The literature documenting effects of hygienisation on parasites is very limited and further studies and documentation is warranted.

The literature documenting fungal diversity, presence, diversity and persistence of mycotoxins, and the resilience of fungal spores to hygienisation is very limited and further studies and documentation are needed to assess the biological risks associated with fungi and fish sludge.

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Appendix 1: Mattilsynets bestilling datert 15. november 2024.

BESTILLING TIL VETERINÆRINSTITUTTET OM HYGIENISERING AV FISKESLAM

Oppdrag / mandat

Mattilsynet ønsker kunnskapsstøtte fra Veterinærinstituttet vedrørende hygienisering av fiskeslam i biogassanlegg, når bioresten skal brukes som organisk gjødsel eller jordforbedringsmiddel. Kunnskapsstøtten skal benyttes som grunnlag for å avklare hvilke behandlingsmetoder Mattilsynet kan akseptere for hygienisering av fiskeslam. Ut fra resultatene av deres forskning og tilgjengelig vitenskapelig litteratur ber vi dere vurdere følgende tre spørsmål:

1. Om behandling av fiskeslam med standardparameterne for omdanning i et biogassanlegg (minimum 70°C i en kontinuerlig periode i minimum 60 minutter, ved en partikkelstørrelse på <12mm (forord. 142/2011, vedlegg V, kapittel III, avsnitt 1) er tilstrekkelig for å hindre spredning av fiskesykdommer, når bioresten brukes som organisk gjødsel eller jordforbedringsmiddel.
2. Om det kan oppstå uønskede forbindelser som en konsekvens av hygieniseringsprosessen, for eksempel gjennom kjemiske reaksjoner mellom komponenter i sjøvann og organisk materiale ved oppvarming.
3. Hvis det mangler kunnskap for å besvare punkt 1, hvilken kunnskap er det behov for?

Rammer for bestillingen er presisert under:

- Kun norske forhold.
- Virksomhetene følger gjeldende regelverk, både anleggene der fiskeslammet oppstår og anleggene som behandler slammet.
- Biogassanleggene tar imot ubehandlet fiskeslam. Fiskeslammet behandles ikke på noen annen måte under transport og lagring på vei til biogassanlegget.
- Biogassanleggene blander slammet med annet materiale før hygienisering.
- Biogassanleggene tar inn fiskeslam fra anlegg over hele landet, fra både land- og sjøanlegg.
- Bioresten brukes som gjødsel og jordforbedringsmiddel over hele landet.

Bakgrunnsinformasjon

Det er stor interesse for økt utnyttelse av fiskeslam, og mange biogassanlegg ønsker å ta inn fiskeslam og bruke bioresten som gjødsel eller jordforbedringsmiddel. Regelverket for animalske biprodukter stiller konkrete krav til hygieniseringsprosesser, men fiskeslam er unntatt regelverket for animalske biprodukter. Gjødselregelverket stiller ikke konkrete krav til hygieniseringsprosessen, men krever at gjødselproduktet ikke skal medføre fare for overføring av sykdomssmitte til mennesker, dyr og planter. Gjødselproduktet skal heller ikke inneholde salmonellabakterier eller infektive parasittegg, og innholdet av termotolerante koliforme bakterier (TKB) skal være mindre enn 2500 pr. gram tørrstoff (Forskrift om organisk gjødsel, §10, punkt 3). Erfaring tilsier at det er ulik praksis for hygiensiering av fiskeslam i biogassanlegg. Mattilsynet får mange henvendelser fra virksomheter som vil vite hvilke hygiensieringsmetoder som er gode nok. VKM uttalte i 2011 at ubehandlet fiskeslam kan føre til spredning av fiskesykdommer. Store biogassanlegg kan ta imot fiskeslam fra, og distribuere bioresten til, flere ulike aktører over hele landet. Det er derfor behov for å vurdere hvilken hygienisering av fiskeslam som vil være tilstrekkelig for å hindre spredning av fiskesykdommer.

Rapporten skal leveres på engelsk, med utvidet norsk sammendrag.

Healthy fish
Healthy animals
Safe food

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postmottak@vetinst.no
vetinst.no