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A desktop study on biofloc technology

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Report 9-2023 – NORCE Climate and Environment

Report title	A desktop study on biofloc technology
Institution	NORCE Climate and Environment
Classification:	Open
Report No.	9-2023 – NORCE Climate and Environment
ISBN	978-82-8408-300-1
No. of pages	40
Date of publ.:	June 2023
Geographical area	Norway, Europe
Keywords	sustainable aquaculture, recirculating aquaculture systems, integrated multitrophic aquaculture, biofloc technology, fish farming, shrimp farming

Summary

Aquaculture industry is a growing sector worldwide and is expected to contribute significantly to satisfying the increasing protein needs of a growing population. Biofloc technology (BFT) emerged over the past decades as a sustainable and cost-efficient cultivation system, mainly for warm-water species in tropical climates. This report begins by describing this technology, presenting its generally perceived benefits, continues with examples of BFT applications and finally, it presents a context analysis, aiming to identify where and how BFT could contribute to the future of Norwegian aquaculture industry.

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Abbreviations

AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
BFT	Biofloc Technology
COMAMMOX	Complete Ammonia Oxidation
C:N	Carbon to Nitrogen ratio
DO	Dissolved Oxygen
DOM	Dissolved Organic Matter
EPS	Extracellular Polymeric Substances
IMTA	Integrated Multi-Trophic Aquaculture
NOB	Nitrite Oxidizing Bacteria
RAS	Recirculating Aquaculture System
SVI	Sludge Volume Index
TAN	Total Ammonia Nitrogen
TSS	Total Suspended Solids

1 Preface

The aquaculture industry is a growing sector worldwide and is expected to contribute significantly to satisfying the increasing protein needs of a growing population in the future[1]. Aquafarming can be defined as a controlled cultivation of aquatic organisms, *e.g.*, fish, crustaceans, molluscs, algae and other low trophic organisms of commercial value. Globally, freshwater fishes are the leading production category, followed by seaweeds according to 2017 data[2]. Crustaceans, diadromous fishes, marine fishes, and miscellaneous other species constitute the third largest group with production scales approximately equal to the fourth group, molluscs. The diversity of aquaculture systems, adapted to small- and large-scale cultivation, is nearly as broad as the species range, constituting of net pens in deep lakes and marine habitats (fjords and near shore waters), earthen ponds or raceways, tanks, and various recirculating systems. Each one of these systems is characterized by a unique set of challenges, with the major and common environmental issues being: (1) excess organic matter (from faeces and uneaten feed) introduced to the surrounding environment, (2) medications used to manage diseases and parasites being released to environment and potentially harming non-target organisms, (3) excess inorganic nutrients in the water near the cages or released to the surrounding environment causing eutrophication, (4) dispersal of pathogens into the environment, (5) escapees as well as (6) animal welfare.

In case of marine aquaculture, which is the major form of aquaculture in Norway, farming in offshore installations (further away from the shore), in (semi-)enclosed cages or in recirculating aquaculture systems (RAS) on land are viewed as potential solutions to many of the above challenges and are considered ways towards sustainable aquaculture. However, the benefits associated with these approaches come at a high investment price, and these systems are in the early phases of large-scale testing. Nevertheless, there is a great research effort invested in technological improvements of high-technology clear water RAS.

In countries where aquaculture is traditionally practiced on land and where clear-water RAS is too costly to establish, alternative land-based recirculating systems have been established. Among these, BioFloc Technology (BFT) emerged over the past decades as a sustainable and cost-efficient solution for warm-water species in tropical climates[3]. Originally, BFT was developed to better control the environmental impact of land-based aquaculture production, *i.e.*, as a waste treatment system, and to prevent disease outbreaks. In brief, BFT is an aquaculture approach that relies on massive microbial growth in the rearing water, which results in the formation of microbial aggregates, so-called bioflocs. The role of these flocs is much like that of the bacteria living in the biofilms of RAS biofilters, namely, to remove harmful nitrogen forms. Bioflocs in BFT and biofilters in RAS are both taking advantage of biological processes for this, however in very different formats. Due to a simpler design, BFT systems are cheaper to establish than RAS, and may provide various additional advantages over conventional clear-water RAS[4,3,5]. Biofloc technology became widespread mainly in Asia, and it is used in Australia, USA and recently Brazil as well, while little information is available regarding Europe. This report aims to provide an introductory overview of BFT, its potential benefits and limitations as well as some consideration for its application in the Norwegian context.

2 An introduction to biofloc technology

Briefly summarized, biofloc technology (BFT) is an aquaculture approach where massive growth of microorganisms is promoted through careful manipulation of the organic carbon content in the rearing water with the aim of promoting microbial growth and floc formation[6]. The role of the bioflocs is to **control the nutrients content** (ammonia, nitrate, and nitrite) in the rearing water by converting waste nitrogen into microbial biomass (protein), as well as to **serve as additional feed** for the cultured species[7,8]. The bacteria constituting the bioflocs provides the additional **health benefit** of outcompeting potentially pathogenic microbes and serving as an *in situ* produced natural probiotic[9]. BFT can also been defined as *'the use of aggregates of bacteria, algae, or protozoa, held together in a matrix along with particulate organic matter for the purpose of improving water quality, waste treatment and disease prevention in intensive aquaculture systems'*[10].

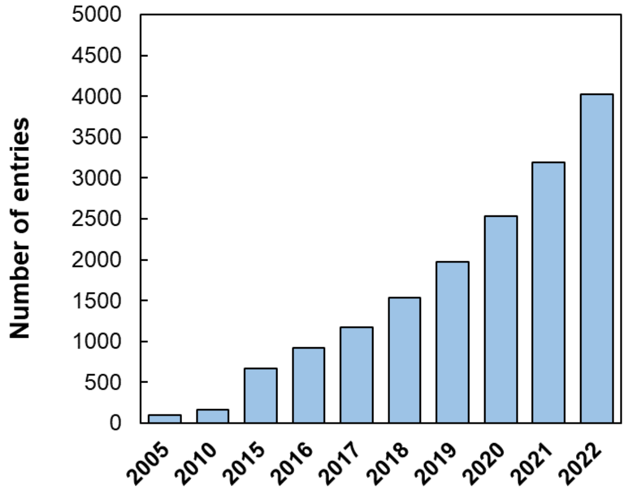

Figure 1 Number of Google Scholar hits for ["biofloc technology" aquaculture].

The major underlying principle of BFT, biofloc formation (or flocculation), has been adopted from conventional wastewater treatment systems known as activated sludge reactors. BFT has been researched and applied for many decades, beginning in the 1970's at Ifremer-COP, maturing into commercial application by the end of the 1980's according to Emerenciano et al.[11]. A more comprehensive review on the history of BFT is provided by Dauda[12]. As of 30 June 2023, a Google Scholar search for ["biofloc technology" aquaculture] returned 5,340 entries, corresponding to 0.18% of all aquaculture entries (compare with [salmon aquaculture] accounting for 17.8% of all aquaculture entries). The number of publications on BFT in aquaculture (indexed in Google Scholar) has been increasing exponentially since 2015, when the technology began to be seriously considered as a major contributor to the 'blue revolution' (Figure 2). There is certainly a great interest and enthusiasm towards BFT as a **sustainable alternative** to other forms of aquaculture, especially in tropical shrimp production[5,13-21]. The potential benefits highlighted above in purple represent the pillars on which BFT should be evaluated on, in comparison to other systems. Importantly, the framework for sustainability assessment (*e.g.*, economic sustainability, ecological sustainability considering planetary boundaries or merely a smaller environmental footprint) should be clearly defined a priori to such analysis. In addition, **animal welfare**, a factor rarely mentioned in BFT research, should be added as fifth pillar.

2.1 What bioflocs are?

By definition, a biofloc is an aggregate of suspended particles and microscopic organisms held together in a matrix of extracellular polymeric substances (EPS)[3]. The key functions within BFT, *i.e.*, nitrogen removal (maintenance of good water quality) and serving as feed supplement, are performed by these so called bioflocs. Bioflocs in engineered aquatic systems such as those in aquaculture and wastewater treatment are comparable to aggregates in natural ecosystems, like marine snow. Since the activated sludge process in biological wastewater treatment also relies on (bio)flocs for the removal of organic matter and nutrients through the same processes as BFT, much can be learned from the experience gained in that research area.

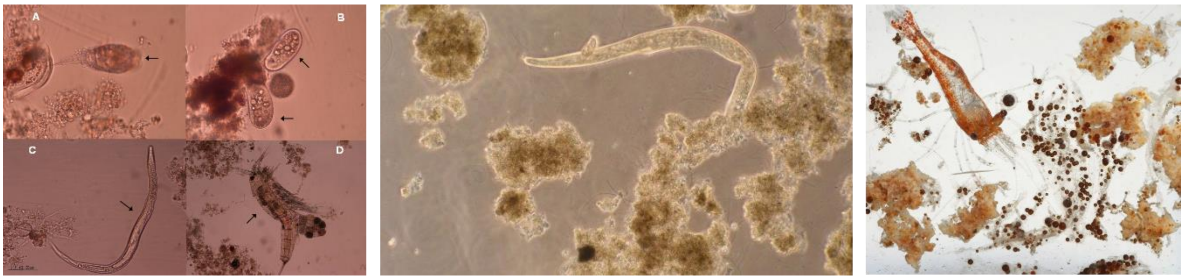

Figure 2 Illustrations of different biofloc types from aquaculture (left), activated sludge (middle) and marine snow (right). Photo credits in footnote[a].

Generally, bioflocs are composed of a consortium of bacteria, diatoms, filamentous microalgae, protozoa, micro- and macroinvertebrates, as well as particulate organic matter trapped in the EPS matrix. The EPS matrix consists mainly of polysaccharides and proteins but contains other macromolecules as well (*e.g.*, polyhydroxyalkanoates, polyamides and polyphosphates) and its role is to keep biomass and particles attached to each other through a different forces[22]. EPS is produced by the microorganisms within the biofloc through pathways that can be engineered for biotechnological applications[23]. EPS composition depends on the microbial species involved in its production, the available carbon and nitrogen sources and environmental parameters. The EPS matrix can accumulate metals and adsorb unwanted organic compounds, enhance flocculation, and improve settling characteristics. These properties may pose some challenges for BFT (*e.g.*, accumulation of metals could prevent its suitability as feed), however, they also make EPS an interesting substance and a potentially environmentally friendly polymer for wastewater treatment purposes. In natural ecosystems, for example in the marine environment, bioflocs (*i.e.*, marine snow) are key players in the global carbon pump exporting large quantities of organic material to the benthic realm (Figure 3)[24]. Whether in a natural or in an engineered system, biofloc formation is advantageous for the microorganisms involved, because it provides a

[a] Photo credits:https://www.linkedin.com/pulse/filament-count-activated-sludge-ahmed-omar; https://www.intechopen.com/chapters/44409; https://blog.education.nationalgeographic.org/tag/marine-snow/

protective habitat where commensal interactions between the members are facilitated. In addition, the matrix holding the flocs together (EPS) can serve as an emergency reserve of organic matter. The formation of bioflocs takes place spontaneously through a series of physical, chemical and biological processes when sufficient carbon and nutrient sources are available[8]. In BFT, this process is facilitated by introducing external carbon source to the rearing water, which is naturally rich in nitrogenous compounds (nutrients). A mature biofloc community[b] can contain organisms from several trophic levels, including primary and secondary producers, grazers, and predators[25]. Although most of the focus in BFT literature is dedicated to the prokaryotic microorganisms (bacteria and archaea) in the biofloc, it is important to note that uni- and multicellular eukaryotic organisms will, over time, populate the bioflocs as well and subsequently influence the nitrogen dynamics. To illustrate this, Figure 3 shows the major flows of organic matter in a BFT system. Dissolved organic matter originating from leftover feed, fish faeces and dead organisms is first and foremost utilized by heterotrophic bacteria. In outdoor or light exposed BFT systems, sunlight (or artificial light) promotes microalgal growth as well. Both bacteria and microalgae are consumed by unicellular eukaryotic grazers, such as ciliates and heterotrophic nanoflagellates, which play an important role in controlling their turnover and activity. Metazoans such as rotifers and nematodes perform similar functions, by feeding on the grazers, asserting an additional level of top-down control[26].

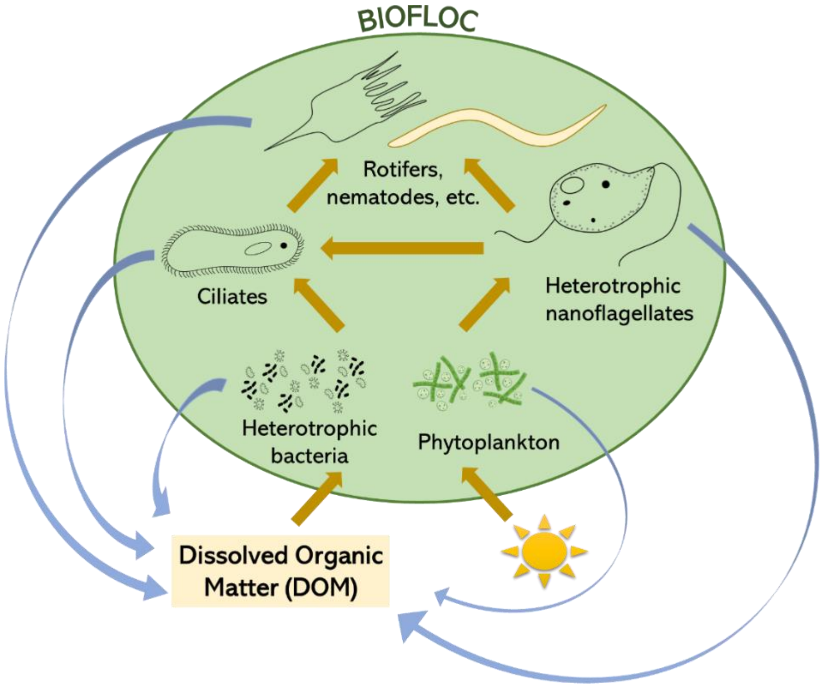

Figure 3 A schematic illustration of a simplified microbial loop and food web within a biofloc system in an outdoor setting, where sunlight is expected to promote microalgal growth (algae utilizing dissolved nitrogenic compounds, *i.e.*, ammonia released from the organic matter).

There is no general formula describing the composition of biofloc as each BFT system is unique and dynamic. The biomass within bioflocs represented by heterotrophic bacteria can vary greatly, constituting between 3 and 35% of the total biofloc biomass[16]. Many researchers classify bioflocs into 3 different systems (types) based on the proportion of photoautotrophic, chemoautotrophic, and heterotrophic microbes.

Bioflocs predominantly composed of photoautotrophs take advantage of sunlight and incorporate excess and potentially harmful nitrogen compounds into microalgal biomass. Chemoautotrophic systems are dominated by nitrifying organisms, which convert toxic nitrogen forms into inert ones. Lastly, the heterotrophic system relies on external organic carbon amendment for the uptake and storage of nitrogen in the form of heterotrophic biomass[6,27]. A recent study suggested that a simple image analysis system of filtered biofloc culture could provide useful information regarding the biomass and ratio of photoautotrophs vs heterotrophs, and thus inform about water quality parameters (*e.g.*, TSS, C:N, N:P ratio)[28]. In addition, bioflocs can take up different shapes, sizes and densities and vary considerably in their community composition depending on the environmental conditions, the carbon source provided, and the species cultivated[29]. Each of these biofloc features affect the performance of BFT systems and thus are essential to regulate (or at least monitor)[30].

2.2 How does BFT work?

BFT systems are engineered mini-ecosystems, where uneaten feed, excess nutrients and feces are converted into edible bioflocs through microbial processes[3]. A BFT production cycle typically begins with establishing the biofloc culture over a few weeks' time and only when a stable state is achieved is the cultivated species introduced either *in situ* in the single BFT-production tank or *ex situ* in a separate production unit from the BFT tank. Start-up time may be reduced by reusing mature bioflocs[b] from a previous production cycle or by optimizing the carbon and nitrogen supply at start[31]. In fact, it is often the case that shrimp and biofloc production begins at the same time, since the feces and mucus from the animals help in the formation of the microbial aggregates. At the beginning (first cycle), the shrimp density is kept lower (250 individuals/m^2). This is then increased to double (500 individuals/m^2) during the second cycle. In all systems, once the cultivated species is introduced, commercial feed is supplied on a regular basis providing the main source of food for the farmed organism, and depending on the system setup, external carbon is supplied to maintain the bioflocs.

Over the course of a production cycle, the composition of the biofloc consortium evolves as the conditions in the rearing tanks (particularly in terms of supplied feed) change. The floc formation process needs to be supported by effective water movement (both vertical and horizontal) which is generated through various types of mechanical mixing and the oxygenation (aeration) itself. Appropriate mixing and maintaining adequate oxygen concentration is extremely important to avoid settling of organic matter and formation of anoxic zones on the bottom of the tanks. Biofloc concentration is controlled to maintain its levels below empirically established thresholds, and if accumulated, the excess is removed. Figure 5 shows a simplified schematic of an *in situ* biofloc rearing tank, with the input (*i.e.*, energy for mixing and aeration, feed, and carbon source) and control requirements as well as the main outputs (*i.e.*, nitrate, fish and microbial protein). Ensuring optimal water quality required by the

[b] Biofloc is considered mature when nitrate is observed in the water and little or no dissolved ammonia and nitrite is present.

cultivated species is key for obtaining maximal growth rate and yield. This can be difficult, particularly due to the oxygen demand of the bioflocs. As mentioned earlier, in BFT, bioflocs are responsible to maintain good water quality, *i.e.*, for the removal of toxic nitrogen species. The next section describes the major microbial processes involved in that.

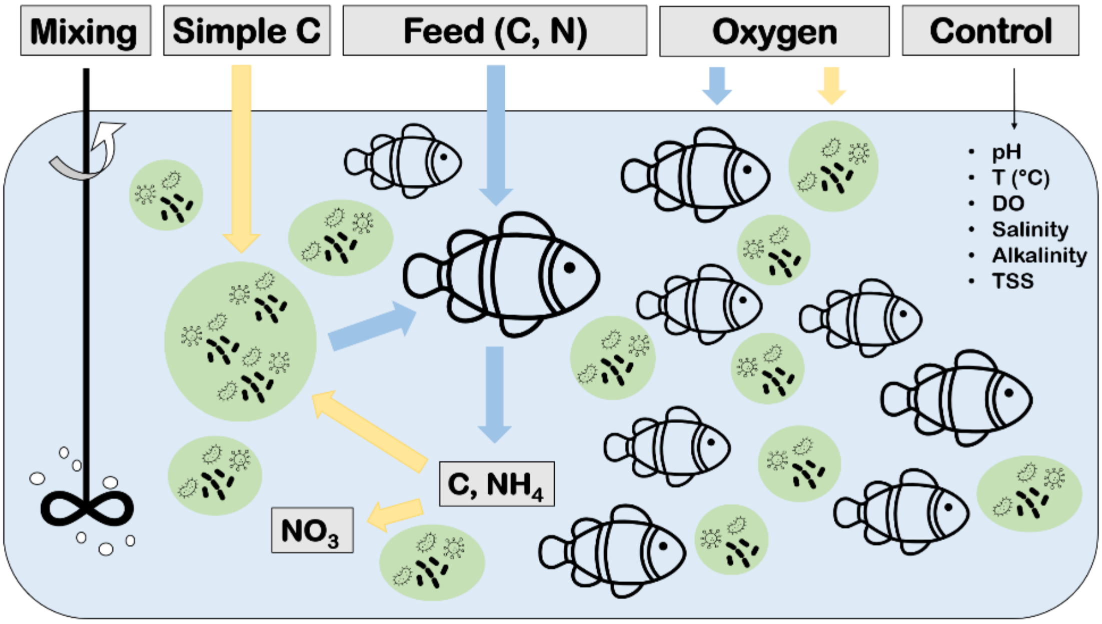

Figure 4 A schematic illustration of an *in situ* biofloc system. Blue arrows represent streams to and from the fish and yellow arrows represent streams to and from the bioflocs. Simple C = simple carbon source in the form of easily degradable carbohydrates, C = carbon and N = nitrogen.

2.2.1 Processes involved in maintaining good water quality

Like in any zero-water exchange (recirculating) system, in BFT as well, there is nitrogen accumulation over time, which can result in detrimental effects to the cultivated species. Nitrogen forms in the rearing water include (1) organic nitrogen originating from leftover feed, feces, animal corpses, urea etc. and (2) inorganic nitrogen such as ammonia and ammonium (NH_3/NH_4^+-N), nitrite (NO_2-N), nitrate (NO_3-N) and to some extent nitrogen gas (N_2). Ammonia can cause gill irritations, gill lesions and affect oxygen transport at concentrations >0.02 mg/L, and nitrite excess (>2 mg/L) may result in hypoxia, reduced activity of enzymes involved in ion transport, or water retention in the kidneys due to impaired production of the hormone T4[32]. Although the concentrations of nitrogenous products that cause problems for animals confined in conventional systems have been identified, in the BFT system the tolerance to these same nitrogenous compounds is much higher and the reason for this is not known. The success of BFT relies mostly on the successful removal of the harmful forms, *i.e.*, ammonia (total ammonia nitrogen or TAN) and nitrite nitrogen through microbial transformation into non-harmful compounds and biomass. In natural ecosystems, microorganisms form complex networks that link ubiquitous nitrogen-transforming reactions, collectively named the nitrogen cycle. The dominant processes are dependent on the resident microbes, the environmental conditions, including the level of available carbon sources. The diversity of the reactions involved in nitrogen cycling mediated by microorganisms is extensive and still far from fully understood, particularly in the marine environment[33]. The major, well-known processes are summarized below.

Microorganisms can metabolize nitrogenous compounds in several ways, depending on the availability of light, carbon sources and the level of dissolved oxygen concentration and therefore, either transform harmful nitrogen forms into less harmful ones remaining in the dissolved nitrogen pool (*i.e.*, nitrification) or eliminate nitrogen from the dissolved pool (*i.e.*, assimilation and denitrification) (Figure 5). In outdoor BFT systems, where light is available, **algal assimilation** (process #2, Figure 5) may become the dominant pathway for ammonia nitrogen removal through assimilation. Algae uses carbon dioxide (CO_2) as carbon source, thus there is no need for supplementing the biofloc culture with external organic carbon, unless mixotrophic growth is to be encouraged[34]. Maintaining adequate mixing and aeration, however, is still crucial for appropriate oxygen concentration in the rearing water. Although algae produce oxygen during the daytime, their oxygen consumption during the nighttime can be significant[35]. Since ammonia is directly packaged into algal biomass, the formation and accumulation of nitrite or nitrate is eliminated. Unfortunately, this process can be short-lived due to the possibility of a sudden algal population crash which can be caused by the same natural processes controlling algae blooms in non-engineered environments, *i.e.*, predation and viral lysis. Such crashes of algal communities result in the release of the nitrogen stored in algal cells back into the rearing water.

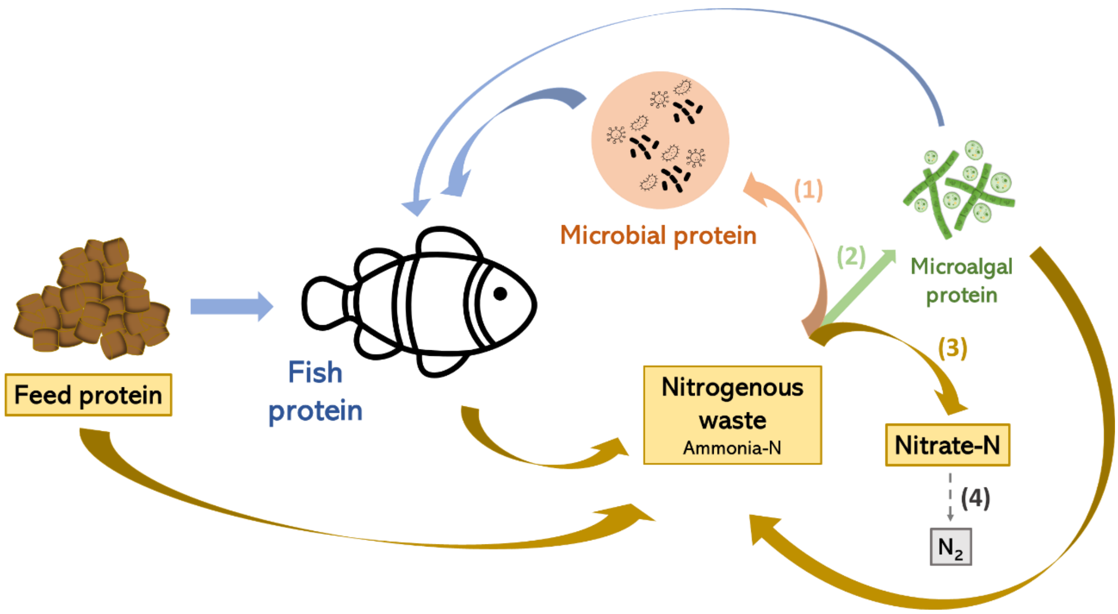

Figure 5 A schematic illustration of the fate of nitrogenous waste (ammonia) in a biofloc system. Three main routes are highlighted: (1) assimilation by heterotrophs, (2) assimilation by algae, (3) nitrification by autotroph nitrifiers, and hypothetically: (4) aerobic denitrification by heterotrophs.

Algal assimilation is typically the main course of ammonia removal in well-controlled semi-intensive outdoor pond BFT systems[36]. The second route of ammonia removal is **nitrification, a process carried out by autotrophic prokaryotes** (process #3, Figure 5) either in two consecutive steps (ammonia oxidization followed by nitrite oxidation) or in a single step (complete ammonia oxidation, *i.e.*, comammox). This process requires aerobic conditions and again only CO_2 as carbon source. In contrast to the algal removal, the final product here is mainly nitrate nitrogen. Only minimal biomass is produced, as nitrifiers are slow-growing microorganisms, thus not contributing greatly to the biofloc mass. Many of the key players performing nitrification are well-known, including the ammonia oxidizing bacteria/archaea (AOB/AOA) from the genera: *Nitrosovibrio*, *Nitrosolobus*, *Nitrosomonas*, *Nitrosococcus* and *Nitrospira*, and the nitrite oxidizing bacteria (NOB) from the genera: *Nitrospina*, *Nitrococcus*, *Nitrobacter*. Comparably less is known about microbes performing comammox, however, their role could be just as important as AOB and NOB. A third, however, much less likely fate of ammonia in BFT could be ammonia oxidation followed by **denitrification, the conversion of nitrate into gaseous nitrogen (N_2)** (process #4, Figure 5). Although this has been observed in aquaculture systems, the process typically requires anoxic conditions that can only exist in micro-niches within the biofloc structure due to the heavy aeration of BFT systems. Bacteria capable of aerobic denitrification have been found and it may be the case that such mechanism can also take place in biofloc tanks[37,38]. Finally, a key process in BFT, which not only removes ammonia nitrogen but also generates the largest bacterial biomass contributing to biofloc formation, is the **assimilation by heterotrophic bacteria** (process #1, Figure 5). This process requires both aeration and supplementation with an external organic carbon source (*e.g.*, carbohydrates). Since heterotrophic bacteria are fast-growing organisms, the addition of sufficient organic carbon in the form of simple sugars quickly induces massive growth and a subsequent rapid removal of ammonia. However, a continuous supply of carbon will result in a continuous increase in biofloc concentration (total suspended solids, TSS) which can lead to unsuitable condition for the cultivated species.

Assimilation by heterotrophic bacteria is considered ideal route of ammonia removal, as it allows for complete recycling of waste nitrogen into fish protein through biofloc formation and the fish feeding on biofloc (Figure 5). Such ideal scenario is rarely (if ever) obtainable, nitrogen waste will always remain in the form of excess biofloc, nitrite and nitrate nitrogen. Thus, realistically, a partial recovery of nitrogen combined with accumulation of nitrate is achieved at best. This is because heterotrophic bacteria rapidly packages ammonia nitrogen into biomass, however, this is only partially consumed by the farmed species. The remainder will re-enter the dissolved organic matter pool (thus expose the cultured species to toxic nitrogen forms) when the bacterial cells die and go through the same cycle all over again. It is then only the autotrophic nitrification process which removes ammonia nitrogen from this cycle at the expense of nitrate accumulation. All the above processes (assimilation and nitrification) in combination are at the disposal of BFT operators, whose task is to carefully balance between each one of them to control the levels of harmful nitrogenous compounds. A stoichiometric framework, based on microbial growth kinetics and metabolic reaction kinetics, has been developed by Ebeling et al in 2006[27]. This framework explains in detail how the three major pathways of nitrogen removal (*i.e.*, algal assimilation, autotrophic nitrification and heterotrophic assimilation) differ in their substrate requirements, resulting biomass and generated by-products. Since all these processes may be contributing at the same time in any given culture setup, a better understanding of the microbial community composition (*i.e.*, the metabolic potential of the system) could facilitate a more reliable management approach. Knowing which microbes, *i.e.*, which metabolic potentials are present could lead to better founded decisions regarding C:N ratio adjustments and solids removal strategies on a system and cultivation stage-dependent manner.

2.3 How can bioflocs be produced?

In principle, it is easy to generate bioflocs. As soon as organic or inorganic fertilizers (carbon and nitrogen sources) are added to natural freshwater or saline water, microbial growth, a prerequisite of biofloc production, will be stimulated. Thus, it can be as simple as adding easily available fertilizers (for example molasses) to the water which is going to be used for cultivation and ensuring mixing and aeration over a certain period, while closely monitoring water quality parameters[39,40]. The type of microbial community evolving in response to this depends largely on the type of fertilizer added and the environmental conditions, *e.g.*, salinity, temperature, and light availability. Inoculation with various unspecific natural sources of bacteria (manure or soil) can be employed to facilitate the process, as well as the use of designer probiotics composed of defined bacterial or algal cultures[41]. As mentioned earlier, the production of bioflocs may be initiated separately, prior to introducing the cultivated species, or simultaneously in the same tank as used for growing the species of interest. In the latter case, fertilization is done both by the externally supplied carbon and feed as well as the excreted feces and shed mucus of the cultivated animals. It is perhaps more common for the autotrophic biofloc system to undergo a separate start-up phase, where the rearing water is only fertilized with ammonium and nitrite, until a stable nitrifying community is established[42]. Regardless of the fertilization approach, during the start-up phase, fluctuations and peaks of the various nitrogen forms are expected, and their pattern will be depending on the biofloc type in maturation. There is no single universal recipe for biofloc production, as the goal is to achieve microbial biomass levels and thus biofloc performance that are adequate for removal of the nitrogenous waste in the particular rearing setup they are used. Nevertheless, there are generic guidelines one can follow to initiate the process. There is a small margin between the production and control of bioflocs, especially in the case of simultaneous introduction of fertilization and the cultivated animals. Guidelines for controlling the bioflocs are discussed in the next section.

2.4 How to control a BFT system?

As briefly mentioned above, operating a BFT system requires balancing several interdependent parameters that control microbial growth and nitrogen removal as well as fish health, welfare, and growth rate. It is an admittedly challenging task and 100% reproducibility of the result with identical operating procedures is not necessarily guaranteed[3,43]. Moreover, due to the diversity of existing and possible BFT configurations, general guidelines are difficult to provide[3]. Figure 6 shows a simplified overview of the major biological processes and their dependence on operational interventions as well as the result of these processes and interventions on the variables (conditions) crucial for animal growth and welfare. To ensure a good grip on the rearing water quality and protect animal welfare, temperature, pH, dissolved oxygen, carbon dioxide, ammonia-N, nitrite-N, TSS and alkalinity must be monitored regularly and adjusted to appropriate levels depending on the species being cultivated. The frequency of measurements is dictated by the expected dynamics (time scale) of the given parameter's fluctuations and the potential consequence for the cultivated species. For example, dissolved oxygen concentrations are taken more frequently, while TSS may only be determined once a week. Behavioral changes of the cultivated species, especially fish, are commonly used as indicators of water quality deterioration and a sign of the need for intervention. Restlessness and grasping for air can indicate elevated ammonia concentrations as well as a decrease of dissolved oxygen, urging the operator to increase aeration levels and adjust carbon supplementation. Since aeration and mixing are absolute requirements for safe BFT operation, emergency power supplies must be in place in case of power outages. Without proper aeration, the dissolved oxygen levels can very quickly (in less than an hour at worst) reach critically low levels and cause mortality[44].

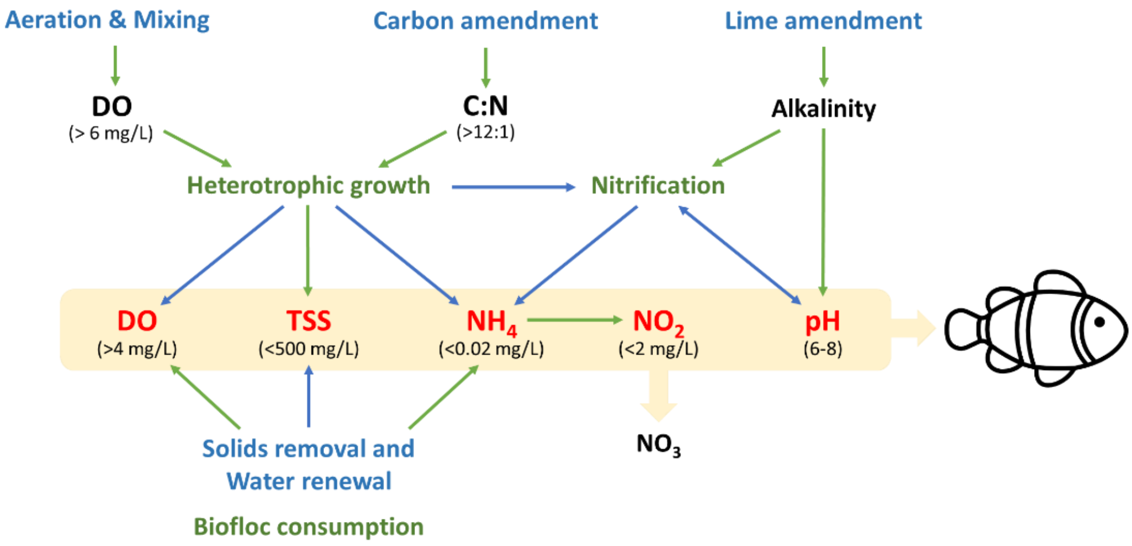

Figure 6 Key actions available for operators to modify (blue text), major biological processes (green text) and physicochemical parameters crucial for the farmed animal's survival and growth (red text). Arrows point to parameters that are influenced by the increase in biological or operator-performed processes. Blue arrows represent processes which lead to the decrease in the affected parameter while green arrows represent activities which lead to the increase in the affected parameter.

The key challenge: Controlling the biofloc

The most challenging aspect of BFT is maintaining an appropriate biofloc concentration (as in suspended solids, TSS) and a well-functioning microbial ecosystem within it, which reliably removes toxic nitrogen compounds without detrimental effects to the farmed organism and without the need for excessively high aeration. As highlighted on Figure 6, biofloc systems require aeration and mixing at all stages of the production cycle to maintain the bioflocs, ensure that they are suspended in the water and distributed homogeneously in the tank. Without mixing, the flocs would settle to the bottom of the tank, thus become unavailable for nutrient conversion in the water column, as well as not serving as feed any longer. Moreover, settled flocs will over time turn anaerobic and produce unwanted and toxic metabolic products.

2.4.1 Controlling floc morphology and concentration

Most bioflocs are not visible to the naked eye, although they can grow into large aggregates reaching up to 1 mm in diameter. Green water bioflocs (with microalgae as dominant organisms) are typically larger and have a diameter around 50-200 µm on average while brown water bioflocs (dominated by heterotrophs) are smaller (20-100 µm in diameter)[3]. Floc size, shape and density (collectively referred to as floc morphology) controls an important biofloc characteristic, settling velocity, which in turn affects the ability of bioflocs to remain suspended in water and thus perform their nutrient removal function and remain accessible for the cultivated species as feed. Oxygen concentration, temperature and ionic strength of the rearing water do influence floc morphology, by for example encouraging the growth of filamentous bacteria. Low DO and high temperatures (>30 °C) favour these types of organisms[8]. Filamentous bacteria, creating loose bioflocs with larger surface area and high sludge volume index (SVI; ~500 mL/g), tend to cause operational challenges due to bulking[5,45]. Temperatures between 20-25 °C and higher DO concentrations generally result in denser, more stable, and low SVI flocs (~200 mL/g) that settle faster[8].

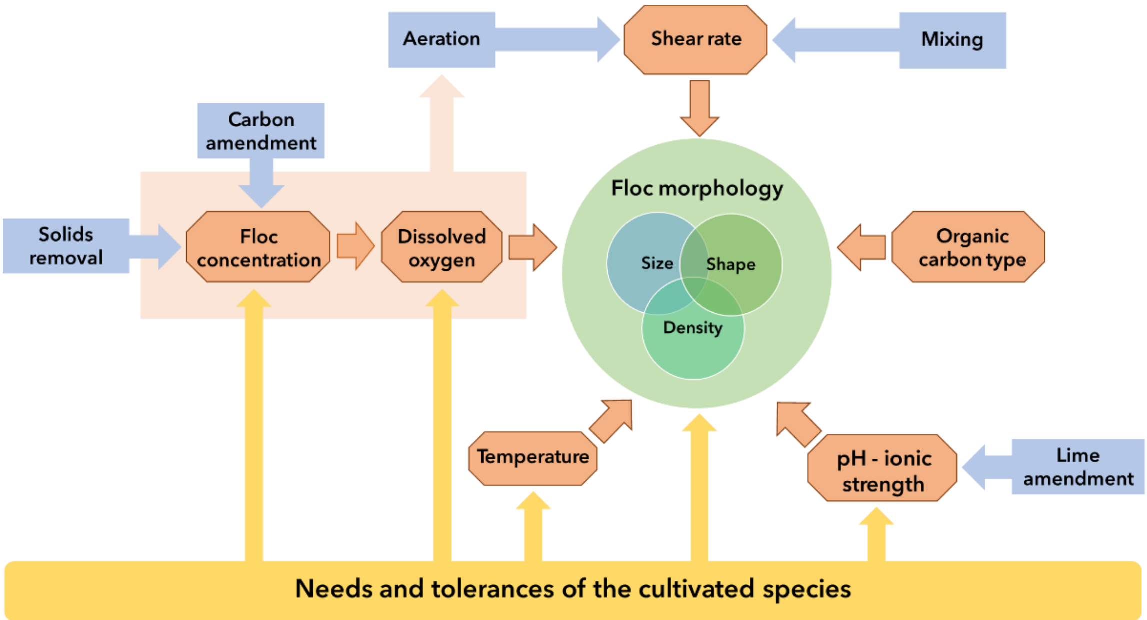

Figure 7 An illustration of the interdependence among operational parameters influencing morphological characteristics of the bioflocs.

Floc morphology is also influenced by the type and intensity of aeration and mixing, the type and amount of organic carbon added(Figure 5)[30]. Lower (mild) shear forces facilitate the attachment and immobilization of microbes and result in the formation of larger relatively loose flocs. Increasing share rate leads to the fragmentation of flocs and increased EPS production. Very high shear forces caused by intensive aeration and mixing result in the formation of smaller, round and dense flocs (granules) which may develop anaerobic zone inside the granule[46]. A shear rate between 0.1 – 10 Wm^{-3} is considered optimal. Since maintaining appropriate dissolved oxygen concentrations (> 4 mg/L) in the rearing tank is essential, there is a limited window for using aeration intensity to control floc morphology in BFT systems during the growing phase. Nevertheless, the choice of aeration type and positioning of the aeration unit can be used to influence biofloc formation dynamics[7,47,48].

Floc concentration controls the ammonia removal potential as well as directly affecting dissolved oxygen concentration and turbidity. Once the production cycle has begun, the amount of total bioflocs, as in suspended solids are regularly measured in a simple manner (using Imhoff cones) and TSS is kept within a desired range (preferably not exceeding 500 mg/L, or even less in an IMTA setup)[49-51]. As a practical example, 250–350 mg/L TSS (equivalent to 10–14 mL/L settleable solids (SS) and a turbidity between 75 and 200 NTU) is recommended for pacific white shrimp *Litopenaeus vannamei* production in BFT[52].Biofloc concentration can be regulated by either adjusting the amount of external carbon supply to limit bacterial growth or by removal of excess biofloc through water renewals or clarification[36]. The first approach may directly impair the ammonia removal process by halting bacterial assimilation. Therefore, it should be applied with care to avoid ammonia peaks during production. Water renewal is a safe and efficient measure; however, it increases water demand as well as the amount of effluent released to the environment. To circumvent these drawbacks, small clarifier tanks (~1% of the volume of the production area) can be operated on an on-demand basis instead. Although it has been proven to be efficient, this approach compromises the simplicity of BFT systems by introducing an additional operational unit as well as generating sludge which then needs to be treated. The removed biofloc biomass can this way serve as raw material for feed production for example or needs to be disposed of as waste.

2.4.2 Controlling the microbial community composition

Besides morphology and concentration, the composition of biofloc microbial community is extremely important. In a typical carbon amended BFT system, the main goal is to promote the growth of heterotrophic bacteria, while ensuring that nitrifiers also remain active. Nitrifying bacteria can easily be outcompeted by heterotrophs, who may scavenge most of the available ammonia from the slow-growing nitrifiers, leading to the gradual diminishing of nitrification potential. In order not to impair the nitrifying community, sudden changes in operational parameters also need to be avoided, pH and alkalinity must be controlled, and the heterotrophic assimilation needs to be maintained at a level where not all the available ammonia is consumed by them. Heterotrophic bacteria require organic carbon for their growth, and the easier it is to degrade the carbon the faster their growth takes place. The dissolved organic matter within the rearing water is usually enriched in nitrogenous compounds to the extent that the lack of organic carbon becomes the limiting factor for their growth. Thus, supplying simple sugars or other simple and preferably cheap (and easy to access) carbohydrate-like molecules (*e.g.*, glycerol) is used to boost bacterial growth by adjusting the carbon to nitrogen ratio[53]. The success of the heterotrophic bacteria in removing toxic nitrogen compounds is highly dependent on this carbon to nitrogen ratio (C:N) in the rearing water. There are different recommendations regarding the ideal ratio, although most agree that an absolute minimum C:N of 10:1 (optimally 12:1-20:1) should be maintained. A recent study found that supplementation with microalgae (*Platymonas*) allowed for reducing the C:N ratio to 6:1 while maintaining identical nitrogen removal efficiencies as only carbon amended systems with C:N of 10:1. Besides the quantity of the carbon source, its quality has an impact on biofloc characteristics as well. Examples of organic carbon used in BFT include acetate, dextrose, glucose, molasses, starch, wheat flour, cellulose, wheat bran, rice flour, palm sap and combinations of these[16,54].

Due to the recognition that better understanding of the microbial dynamics outlined above can lead to the development of more efficient and reliable intensive production systems, the microbial ecology (prokaryotic and eukaryotic composition and function) within the biofloc has become the focus of research attention in recent years[26,55-57]. For example, it was shown that the inclusion of settling tanks the BFT systems do impact the microbial community in the rearing tank, by selectively removing the best floc-forming members[55]. Another study demonstrated how floc size and microbial community composition are correlated[58]. Regardless, there is still relatively little research done on the microbial ecology of bioflocs[59]. Community composition is most commonly assessed by cultivation-based approaches, specific qPCRs (quantitative polymerase chain reactions) or FISH (fluorescence *in situ* hybridization) assays, employed to monitor primarily the abundance of nitrifying prokaryotes[37]. With regards to high-throughput sequencing, an Entrez search (03.06.2022) of the Short Read Archive (SRA) database for "biofloc" returned a total of 53 publicly available projects (BioProjects) since 2015, from 28 institutes covering 9 countries: Brazil, China, India, Iran, Netherlands, Malaysia, Mexico, and South Korea. Most of these sequencing experiments employed a metabarcoding approach, besides RNA (metatranscriptomics) and whole-genome sequencing, on Illumina as well as Oxford Nanopore and IonTorrent platforms. Since this date until 30.06.2023, an additional 22 projects have been made available through SRA. There is substantial work left to be done in uncovering the temporal dynamics of various types of bioflocs through meta-omics. Such knowledge is needed for successfully engineering the biofloc composition within the boundaries of a given production unit (the cultivated species, environmental conditions, e.g., temperature and salinity).

3 Benefits of biofloc technology

Numerous benefits have been suggested (and to some extent documented) in BFT systems, including (1) reduced operational costs due to reduction in feed requirement, and (2) overall better survival, increased wellness and growth rate of the cultivated species in comparison to non-BFT systems[19,20]. Besides the many studies advocating BFT, added benefits are not always observed in comparison to clear water or hybrid recirculating system[60,61]. With appropriate species being cultivated, BFT has either a neutral or a beneficial effect in comparison to alternatives.

3.1 Complementary feed

Biofloc can serve as replacement or supplemental feed for several aquaculture species which are able to directly consume particulate organic matter (filter, deposit or detritus feeders, certain herbivores and omnivores) due to its favorable nutrient profile[7]. Certain morphological structures are required for the cultivated species to be able to graze on bioflocs and also a capacity to digest and absorb nutrients from microbial aggregates[20]. Tilapia species possess micro-gill-rakers (microbranchiospines) lined with viscous mucus, which enables them to filter out small particles from the water (µm to mm size ranges)[62]. They can even ingest unattached bacterial cell provided as suspensions in their fry stage[63]. Similar structures exist in other fish groups, such as lates perches (a genus of the *Latidae* family with carnivorous, euryhaline species), scaled-fin grunts (*Haemulon* spp., plankton-feeding in open ocean, one of the most important fish groups of the coral reefs of Brazil) and *Gerres* spp. Juveniles of *L. vannamei* are assumed to use net-like setae located on their third maxillipeds to capture particles as small as 10 µm in diameter, including diatoms like *Thalassiosira* and *Amphiprora*. The following species were also shown to possess morphological features suitable for particle filtering (10-1200 µm): freshwater prawn *Macrobrachium rosenbergii*, silver carp (*Hypophthalmichthys molitrix*), flathead grey mullet (*Mugil cephalus*).

Table 1 Average ranges of percentages reported for the major components in bioflocs and some examples of commercial tilapia feeds. Values for bioflocs are calculated based on data in Khanjani et al.[16], ranges for tilapia feed are retrieved from FAO[64], prawn feed reference diet from and ranges for fishmeal from Smith et al[65].

Component	Biofloc	Tilapia feed	Prawn feed	Fishmeal
Crude protein	22.35-49%	22-45%	34%	70.2-73.2%
Carbohydrate	20.4-36.4%	>25%	32%	na
Lipid	0.1-2.85%	4-8%	9%	9.9-11.4%
Crude fibre	0.8-16.2%	4-10%	na	na
Ash	8.4-47.75	10-16%	17%	13-17%

In order for biofloc to serve as feed substitute, it is essential that the composition of the biofloc matches the nutritional requirements of the species produced besides the ability of the species to consume biofloc (Table 1). Crude protein, lipid, carbohydrate and ash content are the major guiding characteristics; however, the fatty acid and amino acid profile are equally important to assess to avoid deficiency in essential compounds.

The nutritional value and composition of bioflocs is a dynamic parameter which depends on the entire culture setup from temperature, salinity, species produced to organic carbon added[54]. Moreover, there is a large difference between green water and brown water bioflocs. Certain microalgae that dominate in green water have better macronutrient distribution (e.g. essential fatty acids) as feed than bacteria, contain pigments (*e.g.*, astaxanthin already used in feed formulations) with antioxidant properties, and some can produce abundant vitamins and immunostimulants[66]. With regards to brown water BFT systems, the type of carbon source (glycerol, acetate or glucose) was shown to significantly influence dry weight, crude protein, lipid and carbohydrate content[67].

Several studies demonstrated that white shrimp and tilapia does indeed consume biofloc and utilizes its macronutrients for growth[67]. Ekasari et al found that white shrimp (*L. vannamei*), red tilapia (*Oreochromis niloticus*) and mussels (*Perna viridis*) all consumed bioflocs and benefited from its nutritional value, with the best results obtained for shrimp[45]. Isotope-labelled nitrogen tracing showed that the total amount of nitrogen that could be derived from biofloc (nitrogen recycling efficiency) was also highest in case of shrimp. In the same experiment, bioflocs larger than 100 µm caused mortality in the mussel culture. The reasons for this could be associated with gills becoming clogged and filtration rates reduced as a result of exposure to too many large particles. Further demonstrating their nutritional value, bioflocs have been assessed as a raw material for feed formulation, for example as fishmeal replacement[68]. These experiments point towards an interesting direction for microbe-based feed production, through a combined use of bacterial, fungal and microalgal raw materials[69-71]. One key issue with this approach appears to be the bioflocs' consistent deficiency in methionine and certain types of fatty acids, that are generally supplied through fish oil amendment in feed[72]. This was demonstrated by a study where biofloc could completely replace fishmeal in shrimp feed, so long as fish oil and methionine were still supplied[73]. Nevertheless, use of biofloc in feed production should be evaluated on a case-by-case basis, due to the varying nutritional requirements of candidate aquaculture species the feed is to be formulated for[74].

3.2 Enhancing growth performance and health of cultivated species

Fish and shrimp reared in biofloc systems are mostly free of parasites that would otherwise plague open cage-reared fish and a general higher wellness and growth is commonly reported. Several studies have shown that biofloc consumption can enhance growth performance, strengthen the immune system of the cultured species and improve the activity of digestive enzymes, which then results in better utilization of the feed (increased feed conversion ratio)[75]. These effects are attributed to prebiotic components (*e.g.*, polyhydroxybutyrate), bioactive compounds (*e.g.*, various antioxidants, carotenoids and vitamins) and beneficial microorganisms (probiotics *e.g.*, *Bacillus* and *Lactobacillus*). Stress experiment (through exposure to sub-lethal hydrogen peroxide) revealed a differential gene expression response of blue shrimp (*Litopenaeus stylirostris*) when BFT and clear water reared animals were compared. Based on quantitative PCR observations, the authors concluded that BFT shrimps were better able to protect their cells against oxidative stress (through increased expression of catalase and constant levels of superoxide dismutase and glutathione transferase encoding genes), while the clear water reared shrimp immune competence seemed to have decreased after stress[76]. Vibriosis caused by pathogenic strains of *Vibrio* sp. represent a major threat to shrimp and prawn cultivation. Thus, a diverse set of strategies have been developed to counteract or prevent the disease. According to a recent review, BFT has proven to be a very effective tool to manage the occurrence and severity of vibriosis in shrimp farming[77]. Displacement of pathogens by biofloc bacteria, suppression of virulence genes and disruption of communication between pathogens by excretion of quorum quenchers has been proposed as possible mechanisms behind benefits. Prebiotics (watermelon rind and other food waste products) and probiotics (*Lactobacillus sporogenes*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Bacillus amyloliquefaciens* etc.) additions to BFT systems are also being tested with promising results, showing improved nitrogen removal and better growth performance in comparison to non-amended systems[78-80]. The mechanisms behind increased fitness of farmed animals are not yet fully understood, although researchers are now exploring these questions with state-of-the-art molecular tools (high throughput sequencing and proteomics)[81,82]. Metabarcoding studies showed associations between enhanced immunity and gut microbiota composition, and identified influences of stocking density and type of carbon source on gut microbiota composition[82]. Results from more than a decade of research into the link between immune system and gut microbiota ascertain such claims and should be further investigated in the context of BFT[83,84]. Techniques for modulating biofloc composition and gut microbiota represent interesting new avenues in growth enhancement and disease control. For example, simple adjustments in stocking density and carbon amendment can already result in restructuring of the gut microbiota in shrimp and an improved immune response in Nile tilapia[85,86].

4 Applications of biofloc technology

In general, BFT can be implemented **indoors** and **outdoors**, and in many different configurations for both **juvenile** and **adult** animal cultivation. The main goal is to produce the maximum amount of biomass (marketable product) with the lowest amount of water use, *i.e.*, to run BFT as an intensive or super intensive production unit[87]. Typically, BFT production systems are composed of rearing tanks (or lined ponds and raceways) with either an ***in situ*** or an ***ex situ*** biofloc formation process. *In situ* meaning that the biofloc formation takes place in the rearing unit, while the *ex situ* approach requires an additional tank where bioflocs are maintained. The second approach, where the biofloc unit's sole function is to remove harmful nitrogen, makes BFT similar to clear water RAS, where the biofloc unit represents a replacement for the biofilter. Indoor BFT systems are used where better control of the environmental parameters such as temperature is necessary and possible, while outdoor systems can be established in stable climates, with reduced investment and maintenance cost. As a result of different light availability, indoor and outdoor biofloc development will differ significantly. Outdoor BFT systems often promote the initial growth and bloom of microalgae, resulting in green color of the water. Such systems are therefore referred to as "**green water**" BFTs. However, algae-dominated BFTs tend to switch to bacteria-dominated ones towards later stages of the production cycle as feeding rate increases. A BFT dominated by heterotrophic bacteria has a characteristic brownish color, thus called "**brown water**" BFT. Indoor systems do not necessarily have a green water phase due to light limitation while outdoor systems may be operated as solely green water throughout the production cycle. In general, biofloc systems work best with species that:

- Are omnivorous or can utilize biofloc as food
- Can tolerate changes in dissolved oxygen and nitrogenous compounds
- Tolerate (extremely) high stocking density
- Tolerate high suspended solids

Any species chosen as candidate for BFT cultivation needs to meet these basic criteria.

4.1 Monoculture BFT

BFT today is mostly employed as a monoculture system, primarily in warm water shrimp farming. Approximately 16 shrimp species represent the most cultivated BFT organisms worldwide, largely *L. vannamei* (King prawn, White leg shrimp, Pacific white shrimp), *Penaeus monodon* (giant tiger prawn, Asian tiger shrimp, black tiger shrimp), and *Macrobrachium rosenbergii* (giant freshwater prawn, Malaysian prawn, giant river shrimp). The second most cultivated are tilapia species, as well as a handful of tropical freshwater fish species grown outdoors in countries where the climate is suitable for this.

Shrimp and **tilapia** are well-adapted to BFT conditions, as well as being able to consume biofloc, thanks to their anatomy. By far, these two species are the most researched organisms cultivated in BFT, followed by **catfish** and **carp** species (Table 2).

An extensive list of species grown in BFT is available in the review of Ulloa Walker et al, together with references to a number of studies investigating other species as candidates for BFT[20]. Based on this review, it can be concluded that relatively little research has been done on marine fish species, with **mullet** (*Mugil liza*) and **flatfish** (*Paralichthys olivaceus*) being successfully cultivated examples[88,89]. Until 2020, low trophic species have not been the focus of monoculture BFT, with the exception of sea cucumber (*Apostichopus japonicus*) and Asian green mussel (*Perna viridis*).

Table 2 Number of entries returned in Google Scholar for the search term "biofloc technology" AND [organism of interest, e.g., tilapia]. Percentage of total is shown in brackets.

	Total	In 2022	Since 2015
Shrimp	4380	679 (15%)	3810 (87%)
Tilapia	3740	644 (17%)	3280 (88%)
Catfish	2000	434 (22%)	1810 (91%)
Carp	1780	349 (20%)	1640 (92%)
Milkfish	459	110 (24%)	410 (89%)
Mullet	371	73 (20%)	325 (88%)

BFT has been successful as a nursery system for shrimp and tilapia and was shown to have beneficial effects on juveniles, even for the carnivorous African catfish, when specific carbon sources (fermented rice barn) and strictly controlled C:N ratios were maintained[90]. Moreover, it was demonstrated that carnivorous red drum can successfully be grown in a BFT system integrating, red drum, tilapia, and shrimp, with an FCR of 1.0[91]. On the contrary, juveniles of carnivorous largemouth bass (*Micropterus salmoides*) were shown to be unsuitable for BFT cultivation as the high solid concentration caused increased stress levels and gill remodeling[92]. Similar observations were made on the juveniles of another bass species, Japanese seabass (*Lateolabrax japonicus*)[93]. Thus, it remains an open question whether and how some of the carnivorous species could benefit from BFT. Besides the approach taken in the above studies, additional possibilities are (1) *ex situ* BFT and (2) incorporating biofloc as feed ingredient in their diet. Since BFT can be implemented in an *ex situ* manner with controlled low solids in the rearing water, cultivating species that cannot tolerate high solid content could be investigated in the future to understand whether any beneficial effects apply. Incorporating bioflocs as feed ingredient would be of interest for the aquaculture industry if biofloc could significantly reduce fishmeal requirement in formulated feed (and, thus, fishing pressure on harvested stocks of small pelagic fishes). It is estimated that by 2050, shortage ranging from 0.4 to 1.32 million metric tons of fishmeal could occur[94]. Establishing replacement options is thus becoming ever so urgent.

Temperature range of species cultivated in biofloc - The shrimp species cultivated worldwide have a temperature optimum range near 30 °C (28-30 °C for white leg shrimp, 27-33 °C for giant freshwater prawn and 28-32 °C for the giant tiger prawn) although can tolerate lower temperatures at the expense of reduced growth rate[c]. They reach large harvestable sizes (>20 g, >20 cm) within months thanks to their high growth rate (up to 3 g/week)[95]. In contrast, the Northern shrimp (*Pandalus borealis*) which has been an important fishery product in Norway takes several years to reach a commercially viable size (up to 16 cm and 20 g), which is still smaller than its warm-water counterparts. When it comes to fish, tilapia species (*e.g.*, Nile tilapia – genetically improved strain, *i.e.*, GIFT strain, red tilapia and blue tilapia) are grown at similar temperatures as shrimp (27-29 °C). Tilapia does survive under cold conditions (<5 °C) as demonstrated by overwintering practices, however it will not feed as long as the temperature remains under ~15 °C[96]. Channel catfish (*Ictalurus punctatus*) has a similar temperature profile, maintaining feeding activity down to 10 °C[d]. African catfish (*Clarias gariepinus*) and milkfish (*Chanos chanos*) also require relatively high temperatures while mullet species (*e.g.*, grey mullet *Mugil cephalus*), and to some extent common carp (*Cyprinus carpio*) can be cultivated below 30 °C (still requires temperature above 20 °C)[e]. In warm countries, temperature is little to no concern as an operational parameter, however, maintaining optimal temperatures above 20 °C can quickly become very costly in cold climates, where the ambient temperature is significantly lower than it would be required by typical biofloc species, *i.e.*, tropical fish and shrimp. Thus, it could be of interest to establish "cold-water" biofloc systems with cold-water species that do not require substantial heating. This requires identifying cold-water species of commercial interest which are suitable for BFT as well as understanding whether bioflocs can perform well at moderate or low temperatures.

Temperature effect on biofloc - Low temperature is known to limit all biological processes, including microbial activity[97]. Cold temperatures could therefore interfere with the growth rate of biofloc microbes and slow down processes such as nitrification. Most critically, such limitations occur when microbial communities are suddenly exposed to temperatures below *in situ* conditions they are adapted to and this phenomenon can be described mathematically[98]. Microorganisms adapted to cold temperatures (psychrophiles and mesophiles), however, can exhibit the same or similar activity levels as their warm-adapted (mesophile or thermophile) counterparts[99]. Considering this, bioflocs established under cold conditions (<15 °C) could perform equally well as those in warmer systems (>15 °C), however this has not been demonstrated. Drawing on experience from biological wastewater treatment's activated sludge systems, it is possible to achieve biodegradation of organic matter and nitrification at temperatures below 20 °C. However, very low temperatures are likely to interfere with these processes and cause nitrification to fail[100]. The exact reasons for these failures are yet to be fully understood and may involve intricate microbial interactions. When it comes to BFT, established bioflocs were able to maintain nitrogen removal activities in an overwintering experiment carried out with tilapia, despite temperatures dropping below 4 °C[96].

[c] https://www.fao.org/3/ad505e/ad505e06.htm
[d] http://extension.msstate.edu/content/biology-catfish
[e] https://thefishsite.com/

Considering the local climate conditions, implementation of BFT-based aquaculture production in Norway would need to begin by resolving two key aspects associated with the low-temperature constraint:

(1) Biofloc development and functioning under cold conditions

(2) Identification of commercially viable cold-adapted species that are able to take advantage of biofloc as feed and grow fast enough to marketable sizes

Alternatively, the farming of tropical species may be performed in BFT systems that are designed for cost-efficient temperature control. This may involve: (1) utilizing waste heat from other industries, instead of consuming costly electricity, (2) generating heat or electricity from the byproducts of BFT, i.e., excess sludge, or (3) installing small-scale BFT systems in greenhouses with coexisting plant farming.

4.2 Polyculture BFT

Polyculture or integrated multispecies aquaculture refers to the combined cultivation of several different species in a single production system. **Integrated Multi-Trophic Aquaculture** (IMTA) is a type of polyculture that combines the cultivation of several species from different trophic levels of the aquatic food web thereby mimicking natural ecosystems. In this context, it is worth mentioning the term aquamimicry, which refers to a farming approach where carbon source is added with some probiotics to generate phytoplankton and zooplankton blooms, simulating natural pond conditions[101]. Under the aquamimicry concept, an alternative to BFT, namely copefloc technology (*i.e.*, natural production of copepods in the system) has been developed as a solution for large outdoor shrimp production facilities[102]. Besides fed species, an IMTA setup contains extractive species of commercial value (*e.g.*, macroalgae, mussels, sea urchins), which retain excess nutrients (nitrogen and phosphorous), thus contributing to maintaining good water quality and increased nutrient utilization efficiency (Figure 8). In particular, **land-based closed-loop IMTA** has recently been suggested to have the highest nutrient retention potential among different IMTA systems[103]. In their 2021 review, Nederlof et al. concluded that maximum retention efficiency can be achieved for a conceptual four-species marine IMTA system (fish–seaweed–bivalve–deposit feeder) with 79%–94% of nitrogen, phosphorus and carbon supplied with fish feed being retained, theoretically. Chang et al demonstrated the viability of this approach on farm-scale (ponds for milkfish co-cultured with tiger shrimp, hard clam pond and seaweed pond with 2500, 800 and 1700 m^2 area, respectively) in Taiwan[104]. Like BFT, IMTA is recognized as a sustainable alternative that contributes to the development of circular food production since it enables better utilization of feed nutrients, minimizes the environmental impacts, and reduces fertilizer and water use. Thus, the major pillars of IMTA and BFT are very similar. With careful selection and integration of species, BFT has the potential to further improve the sustainability of IMTA systems by increasing overall productivity (profitability), reducing feed conversion ratio and further enhancing nutrient recycling[13]. Likewise, IMTA has the potential to improve BFT systems by integrating additional layers of nutrient control, *e.g.*, photosynthetic organisms removing nitrate that would otherwise build up or additional filter- and deposit feeders controlling the biofloc concentration. For example, in a polyculture experiment with red drum and pacific white shrimp, tilapia integration resulted in better control of the biofloc concentration (suspended solids), omitting the need for a settling tank, as well as providing an additional marketable product[91]. A successful integrated polyculture BFT of *Mugil liza* and *L. vannamei* was also demonstrated recently, with the shrimp being responsible for biofloc removal in this case[105].

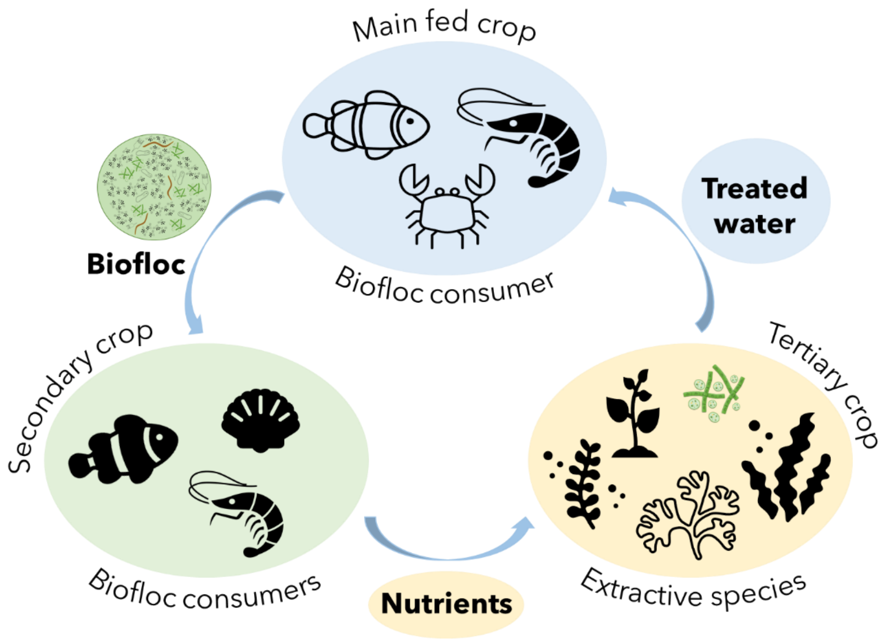

Figure 8 Conceptual schema of a biofloc IMTA system

Studies have also investigated the possibility and potential benefit of integrating oysters as biofloc consumers in a shrimp BFT. One experiment concluded that *Crassostrea gasar* does not necessarily reduce TSS but does feed on some fractions of the biofloc (flagellates)[106], while another showed the opposite result, with *C. gasar* being able to control solids (TSS) and nitrogen compounds, as well as reducing *Vibrio* abundances[107]. It is also possible to extend the range of species to include both biofloc-feeding secondary crop and extractive species such as seaweed as a means to remove nitrogen (ammonia, nitrate and urea)[108]. BFT effluent from *L. vannamei* has been shown to support the growth of some Ulva *ssp.* in a species-specific manner[109]. Identifying seaweed species which benefit most from BFT effluents and have the highest commercial interest could contribute to the growing industry of phyconomy[110,111]. While lower trophic organisms are generally considered as "waste removers" in IMTA, they can also provide live (*in situ* produced) feed for the main crop. Egesta of sea urchin (*Lytechinus variegatus*) was recently shown to be superior to commercial feed in terms of shrimp (*L. vannamei*) weight gain[112]. IMTA could also be beneficial because other macroorganisms may excrete secondary metabolites (bioactive compounds) with immunostimulatory effects for the cultivated species. It is known that macroalga contain a range of compounds with potential antioxidant and other immunostimulatory effects and trials with feed formulations containing bioactive compounds showed promising results. Resources could be saved if instead of feed formulations, an IMTA solution could supply these bioactive compounds. Immersing juvenile white shrimp in extract of brown algae (Sargassum sp.) increased their rate of survival when challenged with *Vibrio algynolyticus*[113]. Further trials could investigate whether merely the presence of macroalgae could have the same effect. The beneficial effects of combining biofloc and

IMTA were demonstrated with a combined cultivation of white shrimp, mullet and sea lettuce, where sea lettuce grown in the biofloc system contained more bioactive compounds than the control[114]. Such results are encouraging for future developments of biofloc-IMTA systems. Land-based recirculating IMTA does not need to be limited to a fully aquatic setup as illustrated in Figure 9. An IMTA, where production of aquatic animals is combined with farming of terrestrial plants (*e.g.*, vegetables and fruits), **aquaponics**, is also considered as a sustainable alternative for conventional farming practices (aquatic and terrestrial)[115,116]. In view of the multifaceted crises experienced by traditional terrestrial farming, and the emergence of hydroponics solutions (where plants are grown in water rather than in soil) addressing some aspects of this, advances in IMTA to include land-plant production appears to be another interesting avenue. Public interest in such systems has certainly increased and recent developments in saline aquaponics (maraponics or haloponics) are further widening the range of species to be grown in such systems[f,116-118]. In aquaponics, crop yield is highly dependent on nutrient management (mainly ammonia to nitrogen ratio) and physiochemical conditions (pH and salinity) in the outlet water of the fish (or other aquatic animal) grown. BFT could be integrated to facilitate improved regulation of nutrient composition, as it was demonstrated in a **flocponics** setup as well as in an earlier study experimenting with algal-bacterial amendment of aquaponics[119,120]. In the flocponics approach, combining BFT grown Pacific white shrimp, *Sarcocornia ambigua* and tilapia yielded increased productivity, reduced nitrate concentrations and reduced sludge production in comparison to a system without BFT[121]. *Sarcocornia* and *Salicornia* both have a long history of human consumption and in particular *Salicornia* is an emerging sustainable crop with properties that make it potentially interesting for other biotechnological applications[122,123]. Reduced nitrate concentration (better control of nitrate) was also reported in a flocponics system where Nile tilapia, freshwater shrimp, lettuce and watercress where grown in a single loop[124]. Besides nutrient regulation, the bioflocs may provide additional benefits to the hydroponically grown plants by allowing beneficial bacteria to colonize their roots (hydroponics setup). However, this colonization and deposition of solids on the roots may exert negative effects as well[125]. Thus, the pre-requisites and appropriate conditions for successful flocponics requires further research[126-128]. Alternatively, decoupled systems could provide a solution, but further research is needed to prove the stability and financially feasibility of long-term flocponics projects[129,130]. Lastly, BFT can also be combined with a so called **periphyton** approach, with added benefits regarding animal health and further reduced feed requirements as achieved with shrimp (*L. vannamei*)[131-133]. In essence, periphyton is very similar to biofloc, with the main difference being that the developing microbial community attaches to hard surfaces in the former scenario. It is also referred to as biofilm, grown on surfaces artificially introduced in the production system. Recent studies show that the combination of these two approaches: enhancing suspended microbial growth via fertilization and encouraging periphyton/biofilm growth through the introduction of artificial surfaces lead to better nitrogen removal efficiency as well as reduced need for external feed for the cultivated species[134-137].

[f] https://aquaponicsassociation.org/

5 Context assessment – Possibilities for BFT in Norway

It has been about 30 years ago that aquaculture industry in Norway "turned from ensuring livelihoods in rural areas to sustaining a national economic project" and it continues to be so[138]. On the other side of the Atlantic, aquaculture in Brazil still managed to become "a billion dollar industry", despite small farms producing the majority of revenue[139]. Besides large enterprises, there may be room for small scale land-based aquaculture farms that produce food for local market in Norway as well. With regards to agriculture, such transition already exists. A considerable share of vegetables and fruits are produced on small-scale farms and sold through local marketing channels, *e.g.*, specialty stores, food box schemes, farmers' markets, and community-supported agriculture in Norway[140]. This chapter aims to pinpoint how biofloc technology could be part of such transition within the aquaculture industry.

5.1 Existing land-based aquaculture in Norway

Land-based aquaculture in Norway is mainly practiced in the form of high-tech clear water RAS for smolt production. There is a long history of research and development on clear water RAS and Norway stands as a world-leading technology provider. Still, this technology is in its infancy when it comes to grow out production. Nevertheless, there is a great expectation towards RAS in fuelling the future growth of the Norwegian aquaculture industry, evidenced by the aquaculture strategy document[141]. Land-based grow out activities are established in inland Norway and include (1) cultivation of **brown trout** ('ørret', *Salmo trutta*) in ponds, small tanks in connection with other farming activities, and in cages in dammed lakes, as well as (2) production of **Arctic char** (*Salvelinus alpinus*) in RAS[141]. Brown trout is used and sold in similar manner to salmon (a common product that can be found in supermarkets) while Arctic char is a more culinary species that is sought after by chefs. Biofloc technology could play a role in increasing the diversity of species cultivated on land.

5.2 Novel species for aquaculture

Development of novel aquaculture species is a challenging and long process, nevertheless success stories do exist, and diversification in the future is necessary[g,h]. With regards to general future investments into new species, Akvaplan NIVA evaluated 31 potentially cultivable species for human consumption in Norway using input from informants including industry, academia and regulatory agencies[142]. One conclusion from the interviews with informants was to focus the developments on typical "Norwegian", *i.e.*, cold water species together with a general recommendation regarding future investments to focus on measures that can simultaneously benefit the aquaculture development of several species (rather than investing in a single species). This should however not exclude to possibility of experimenting with other alternatives, such as warm-water species that have been proven to suit biofloc technology best. The

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[g] https://www.urchinomics.com/

[h] https://www.nrk.no/nordland/aminor-as-pa-halsa-i-meloy-er-verdens-eneste-oppdretter-av-flekksteinbit-1.14916005

report also highlighted the importance of food safety and consumer trust, besides choosing species with good market potential, low environmental footprint and little conflict with wild fishing. When it comes to technological aspects, live feed production and preventative fish health improvements emerged as key points to consider when introducing new species to aquaculture. In this perspective, biofloc technology could be an avenue of technological development in Norway bringing additional benefits to already cultivated species and facilitate establishing new aquaculture species. The lack of information regarding the status of BFT systems in Norway suggests that the industry and the research community has so far shown little interest in this type of land-based aquaculture and the species cultivated in such systems.

5.3 Future directions for the aquaculture industry in feed development

From the aquaculture strategy document, it is evident that the Norwegian government demands[i] that future feeds rely on reusing raw materials and side streams from fishing, forestry and agriculture as much as possible[141,143]. Relevant projects in this area include OIL4FEED where oil-rich microorganisms are produced on byproducts from food industry and trees (Norlia AS, Borregaard AS, NMBU) and «ENTOFÔR: fra avfall til ressurs» (Havforskningsinstituttet, NIBIO) where various waste stream are evaluated as feed substrates for insects[144]. Through earlier projects, SINTEF identified 4 potential feed ingredient sources to be suitable for development or upscaling, among them hetero- and chemoautotroph microorganisms and microalgae. International research on new feed ingredients has also proliferated in the last decade, evaluating single-cell proteins (SCP), insect meal, and microalgae for their potential for replacing fishmeal and fish oil in aquaculture feed[2,145]. Bacterial and yeast protein has been shown to be a good replacement of fishmeal in salmon and trout as well as tilapia diets[146-148]. Considering these trends in feed development, biofloc technology appears to fit well with the national aquaculture strategy for its use of microbial protein, directly or in feed formulations. For species able to consume the bioflocs directly, the added benefit is the elimination of the feed production step.

5.4 Geographical distribution of existing BFT aquaculture

The vast majority of scientific publications about BFT originate from China, India, Egypt, and Brazil, followed by Iran, Israel, Indonesia and other Asian countries. Biofloc technology is expected to spread from Egypt to other parts of the African continent and some publications referred to biofloc aquaculture in Australia as well. Regarding North America and Europe, a few authors with affiliation from USA, Mexico, Spain, Germany, Belgium, Netherlands, and Ireland were identified. Nevertheless, Google searches focusing on Europe did not recover many companies that are currently in production (or appear to be in production) using BFT, with the exception of CreveTec (Belgium), Noray Seafood (Spain) and Happy Prawns (Norway). All three of these companies produce (or

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[i] «Fremtidens fôr må basere seg på ny og tilpasset bruk av eksisterende fôringredienser, og gjenbruk av restråstoff og sidestrømmer fra blant annet hav-, skog og jordbruk og gjennom en mer sirkulær økonomi.»

aim to produce) warm-water shrimp in BFT. Incentives and barriers to the application of biofloc systems in Canada has been evaluated through a Master project[149].

5.5 Norwegian research and industry actors engaged in BFT

A few indications of a building interest in BFT and BFT-reared species could be identified. Tilapia AS for example, located in Sunndalsøra, performed a market analysis and feasibility study in 2020 for tilapia production considering Eastern Norway and South Sweden, a population of people who are now consuming frozen tilapia from China, as target consumers[j]. It was not possible to trace the results of this analysis online. Nevertheless, it signals some industry interest in farming warm-water species in Norway (Håsøran Industripark[k]). A recent study from Sweden, one of the first LCAs evaluating tilapia and clarias farming in RAS under nordic conditions, demonstrated that land-based farming of warm-water fish in RAS can contribute to a sustainable future food sector in northern latitudes[150]. Small scale trials on combining indoor fish farming with plant growth also took place in Sweden[151]. In Norway, the Seafood Innovation Cluster AS, Mowi Genetics AS and Nofima are involved in a project, NewTechAqua, where biofloc technology will be tested as a rearing alternative (albeit not in Norway)[l]. A general shift in research efforts in the direction of integrated aquaculture systems could favor biofloc research and development in Europe, as illustrated by two large EU Horizon projects: ASTRAL (coordinated by NORCE) and AquaVitae (coordinated by Nofima), where research on the biofloc-IMTA combination is being carried out[m].

5.6 Integrated Multi-Trophic Aquaculture

The number of species combinations in an IMTA system is vast and such aquaculture setups are relatively unexplored, especially in the realm of land-based aquaculture in Norway (see SAFER-IMTA, AquaVitae, ASTRAL projects). Seaweed, blue mussel, sea urchin and sea cucumber can be named as the major low-trophic species considered in these marine-focused projects. Efforts have been based on the already existing salmon industry and utilizing salmon aquaculture waste as well as developing innovative ways to produce feed ingredients. Conceptually, the ideas behind these efforts are (1) establishing large-scale commercially viable waste management systems and (2) producing aquafeed sustainably. Considering that Rogaland is central to land-based food production in Norway, and reversing the above concepts (*i.e.*, taking the aquaponics approach, where aquatic animal production is the means to produce fertilizers for land plants directly), an avenue to establish small-scale commercial IMTA could be opened by taking existing edible land-plant production as starting point. Warm-water species could be cultivated in combination with greenhouse-grown vegetables, for example in a FLOC-ponics setting. Producer and consumer interest, compatibility of the species in terms of temperature and salinity, as well as

[j] https://tilapia.no/onewebmedia/2020-11-03_Aura_Avis_03-11_2020_print%20%283%29%20s%2010%2011.pdf
[k] https://suns.no/wp-content/uploads/2021/03/Presentasjon-akvakulturparken-19.03.2021-v2.pdf
[l] "NewTechAqua will demonstrate that investment in sustainable aquaculture research and innovation leads to the creation of new value chains, markets, growth and jobs in coastal, offshore and landlocked areas."
[m] https://aquavitaeproject.eu/biofloc-and-imta-two-fold-solutions-for-more-sustainable-aquaculture/

stoichiometric match (*i.e.*, the effluents produced by one member matching the uptake requirements of the other) would need to be mapped carefully. Reducing climate footprint through locally grown food is of great interest to Norwegian authorities[n].

5.7 Biofloc and biochar

Besides BFT being an alternative aquaculture system with reduced water use and improved nutrient retention, it can also be looked at as a production unit for a clean and valuable raw material, *i.e.*, the biofloc. Besides being a potential feed ingredient, biofloc waste could be raw material for solutions in the context of fertilizer shortage (particularly phosphorous), green energy production, sustainable feed development and eutrophication prevention[152]. Waste biofloc (sludge) can be used to produce fertilizer through the same processes employed in activated sludge treatment, *e.g.*, anaerobic digestion and pyrolysis. In addition, the liquid phase of biofloc waste can be used to load biochar, an emerging ingredient for soil improvement, with nutrients and thus producing biochar-fertilizer[153,154]. A 4-year project, CARBO-FERTIL has been carried out by NIBIO on this topic (biochar fertilizer) between 2018-2022[o]. Possible environmental benefits of using biochar are typically not considered beyond climate change mitigating effects (carbon storage). Nevertheless, it has been suggested that since biochar-based fertilizer is considered slow-release, it is not expected to cause massive leaching of nutrients into waters adjacent to farms. Consequently, its use can contribute to the prevention of lake water eutrophication, which is for example a local problem in Rogaland, Norway[155,156,157,158]. Moreover, biochar can be an important player in land-based saline FLOCponics due to its contribution to increased salt tolerance of some herbaceous plants[159]. Biochar production is still in its infancy in Norway, the first biochar production facility, Oplandske Bioenergi, opened just last year. Nevertheless, there is interest in using pyrolysis as a way of converting waste into valuable products. This technology will be evaluated in SLAM-DUNK project[p], led by NORCE.

Besides pyrolysis, the SLAM-DUNK project will also test using sludge from traditional RAS as raw material for biogas production and as nutrient source for microalgae production. Producing biogas from sludge through anaerobic digestion, in essence, a currency for energy and heat, holds the promise of making land-based aquaculture even more sustainable[150]. Anaerobic digestion of aquaculture sludge waste has been first reported in the 1990s, yet it has received little attention, until relatively recently[160]. Although methane production through anaerobic digestion of fish sludge is attainable, many challenges remain, including designing processes appropriate for higher salinity, lower alkalinity, lower C:N ratio and lower lipids than characteristic to more conventionally treated waste streams (wastewater sludge, food waste etc.)[160]. As reported recently, anaerobic digestion can also facilitate phosphorous recovery from treated RAS sludge (anoxic denitrification treatment) through microbial conversion of stored ploy-phosphate into soluble phosphate forms readily accessible for plants[161].

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n https://www.statsforvalteren.no/nn/Rogaland/Landbruk-og-mat/Jordbruk/norske-tomater-har-lagere-klimaavtrykk-enn-importerte-tomater/

o https://app.cristin.no/projects/show.jsf?id=591703

p https://www.norceresearch.no/en/projects/slam-dunk-the-sludge-appraisal-team---developing-a-sustainable-value-chain-from-tank-to-product

Other approaches for nutrient recovery from sludge include phototrophic bioconversion by anoxygenic phototrophic bacteria (APB). This has been suggested as an alternative treatment option which minimizes carbon and nutrient dissipation, resulting in (1) nutrients available for plant growth and (2) protein-rich microbial biomass that can be utilized by aquaculture animals as food[162]. Sludge from biofloc systems could be equally well-suited for similar purposes, extending the potential value-chain that can be built around a biofloc production unit (Figure 10). It remains to be explored how these possibilities can be practically realized and to demonstrate their actual sustainability performance.

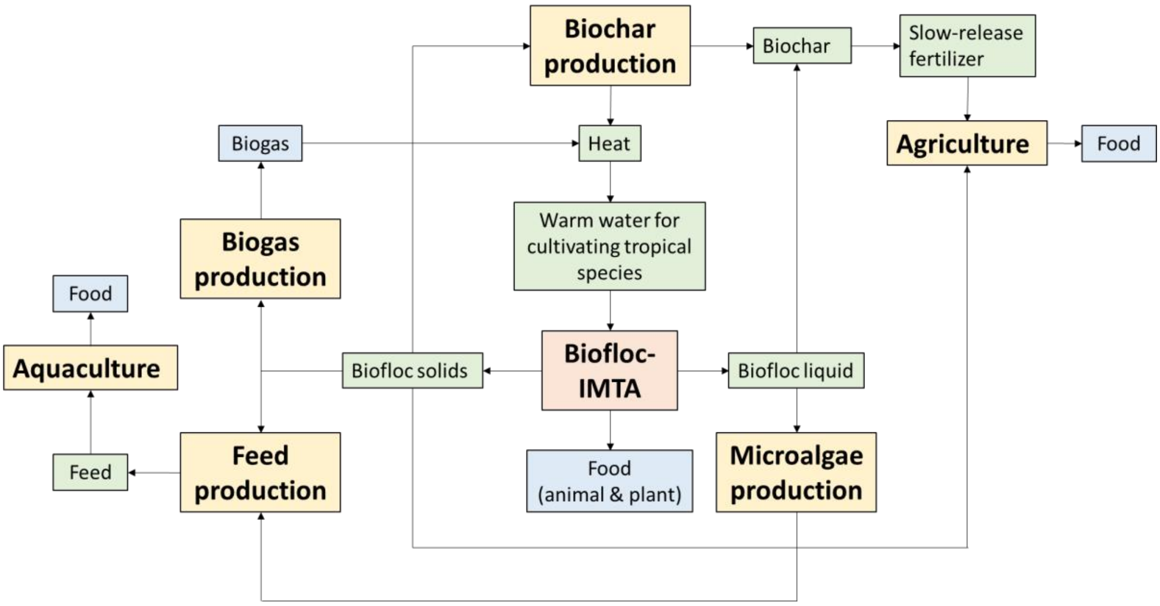

Figure 9 An example value-chain with freshwater biofloc-IMTA as central activity.

6 Closing remarks

BFT methodology is well established with warm-water species of shrimp and fish. It is widely used in Asian countries, to some extent in USA, Australia and Europe (*e.g.*, Germany and Belgium), mostly for land-based shrimp farming due to the high market price of the product[q]. BFT is potentially sustainable, but it is highly dependent on resources from larger economy and electricity, thus needs to undergo rigorous sustainability analysis and future improvements in terms of its scalability and resilience[19,163].

Challenges to address in BFT systems, potential limiting factors and knowledge gaps include:

- Besides the feed for the cultivated species, there is a need for additional **carbon source to maintain the bioflocs** -> identifying and testing sustainable alternatives for carbon source is essential as well as identifying strategies to reduce the carbon requirement
- **Energy requirement** that covers aeration and heating in temperate climate -> need to ensure renewable sources and optimal usage without high-tech and expensive solutions. This is a shared challenge across all RAS operators. Aquaculture actors and experts agree that Norway should emphasize development of technology that utilizes waste and excess heat generated by other producers, in line with the current aquaculture strategy[r].
- **Profitability, production capacity and market value of products** is a potential limiting factor -> market analysis in the Norwegian context is necessary
- It is still unclear whether the positive characteristics of BFT make it a real **sustainable** approach for aquaculture -> sustainability assessment under various scenarios
- Biofloc systems are **difficult to control** and the microbial ecology is still not well understood –> investigate and characterize the biofloc microbiome as they are responsible for maintaining water quality and conferring immunostimulatory effect on the cultured animals[17]. In addition, investigating the factors that influence nitrogen dynamics in BFT systems and the means of controlling contaminants other than nitrogen needs to be further studied[32]
- Novel strategies (*e.g.*, inclusion of microalgae) to reduce carbon supplementation and decrease **start-up time** are need –> knowledge regarding the development of nitrifying communities, phycosphere bacteria's contribution to nitrification, microbiome of macroalage in IMTA is needed
- Further knowledge on the **nutritional composition of the bioflocs** especially in terms of vitamins, essential amino acids and fatty acids and how is this connected to the community composition[45]

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[q] Belgium: http://www.crevetec.be/
[r] «Norge bør vektlegge utvikling av teknologi for utnyttelse av avfall og spillvarme.»

Despite its challenges, BFT is an interesting and growing research area within the topic of aquaculture worldwide. A recent analysis performed in Mexico concluded that the time for promoting and expanding BFT among small and medium sized producers there is now[164]. In the Norwegian context, there is a lack of publicly available information regarding the potential and feasibility of any form of BFT. There is some know-how about BFT within industry (*e.g.*, Happy Prawns and Aura Biofloc) but a general lack of research activities. It would therefore be beneficial to create a better understanding of stakeholder knowledge and opinion with regards to BFT as well as identifying research interest in this topic to map: (1) knowledge needs of current BFT operators, (2) potential of BFT to add value to current production systems (juvenile production or other land-based aquaculture in Norway) and (3) the BFT configuration which could have commercial potential in Norway.

7 Acknowledgements

The authors would like to thank the support and constructive comments of Renee Katrin Bechmann, PhD (NORCE), Shaw Bamber, PhD (NORCE) and Helena Hauss, PhD (NORCE).

This work was carried out as part of the SUREAQUA and ASTRAL projects. SUREAQUA is A Nordic Centre of Excellence on Bioeconomy, Funded by NordForsk, grant agreement No 82342. The ASTRAL project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 863034.

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