

Review

Valorization of Macroalgae through Fermentation for Aquafeed Production: A Review

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Abstract: The increased development of aquaculture has resulted in increased demand for high-protein aquafeed. An increased demand for high-protein aquafeed means an increase in exploitation of unsustainable protein sources such as fishmeal for aquafeed production. Thus, alternative protein sources such as fermented macroalgae is explored. Fermented macroalgae had been tested as aquaculture diets in some studies, but with limited coverage in relation to aquaculture. Therefore, this review provides a new perspective regarding their nutritional qualities as aquaculture diets, and their impacts on growth performances of aquaculture animals.

Keywords: fish feed nutrition; feed formulation; microbial fermentations



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1. Introduction

Aquaculture as an industry has been booming in the last few decades. With the increased development of aquaculture, the demand for nutritional fulfilling commercial and compound aquafeeds are also increasing simultaneously. According to Troell et al. [1], 70% of the total global aquaculture production in 2012 depended on external feed input. In 2009, only around 4% of the global industrially produced animal feeds were used in aquaculture [1]. However, this trend will likely change due to the increasing reliance on commercial aquafeed [1]. In 2017, the estimated aquafeed usage was 51,230,000 tonnes [2]; however, that number was expected to achieve 58,850,000 tonnes in 2020 and 73,150,000 tonnes in 2025 [2,3]. Regardless, the supply of nutritious aquafeeds will also experience a similar growth rate as the demand increases. According to Hua et al. [4], some of the most common sources of aquafeed ingredients are fishmeal and fish oil. Fishmeal is a flour-type material that is rich in protein, made from milled and dried whole fish, fish trimmings or other fish by-products [5]. Similarly, fish oil is made from centrifugation and separation of pressed and cooked fish materials [5]. Fishmeal is nutritionally beneficial in aquafeed due to good digestibility and a good profile of essential amino acids, omega-3 fatty acids, vitamins, and minerals [6]. Fish oil is good source of essential polyunsaturated fatty acids. However, the increasing use of fishmeal and fish oil from capture fisheries for aquafeed production are unsustainable. Meals of aquatic animal origin that was produced in 2018 was 5,761,094 tonnes, whereas fish and aquatic mammals oils and fats (not including fish liver oils) that were produced in the same year was 1,236,730 tonnes [7]. The estimated total demand for fishmeal and fish oil will reach 3,490,000 tonnes and 908,000 tonnes, respectively, in 2020 [3]. Troell et al. [1] pointed out that the unsustainable harvest of wild forage fish for a continuous supply of fishmeal and fish oil for aquafeed production will become economically not viable. The shared use of fishmeal and fish oil by pigs and poultry from terrestrial animal agriculture, and the increased demands of these resources in aquaculture, drive up the prices [8]. This propels the terrestrial animal agriculture industry to shift their use of fishmeal and fish oil to alternative sources [8]. The situation is amplified

with the increased use of wild captured fish for human consumption [6]. Additionally, factors such as increased awareness to conserve the wild forage fish due to an increased understanding of their roles in the food chain, as well as tighter control on capture fisheries and unregulated fishing exacerbate the need to find alternative sources of fishmeal and fish oil [1,6]. Froehlich et al. [8] agreed that finding alternative sources is one of the strategies to reduce the overexploitation of wild forage fish.

2. Alternative Sources to Fishmeal and Fish Oil

Multiple alternative sources to fishmeal and fish oil for aquafeed production have been proposed, experimented, commercialized, industrialized, and marketed. Examples of alternative nutrient sources are fishery and aquaculture by-products, terrestrial animal by-products, plant nutrients, plant by-products, food wastes, insects, microbial proteins and oils (microalgae, bacteria, yeast and fungi), and macroalgae. There is vast literature addressing all of these different nutrient sources and ingredients for aquafeed production. However, the texts here will mainly mention, in brief, the pros and cons of these alternative nutrient sources.

2.1. Fisheries By-Product

The use of fishery by-products such as viscera, heads, fins, skin, scales, bones, intestines, tails, and blood from fish, as well as carapace, exoskeleton, shell, and debris from shellfish crustaceans for reduction into fishmeal and fish oil were much more sustainable than whole fish that was sourced fishmeal and fish oil [4,9,10]. Other examples are fish protein hydrolysates and by-products from aquaculture [10]. Currently, only a few promoted fishery by-products are utilized for the production of fishmeal and fish oil [8]. Europe's by-product usage in relation to fishmeal and fish oil is at 54% [5]. Nevertheless, the use of by-products in the production of fishmeal and fish oil is growing, occupying 25–35% of the production [5]. In general, fishmeal that is reduced from these by-products is nutritionally valuable with bioactive compounds and feed additives that are available [9,10]. However, by-products generally have a lower protein and some amino acid content, and higher ash content [4–6,9]. The higher ash content may cause interference to the absorption of some trace elements such as zinc [6]. Many fishery by-products are different in physical properties, nutritional composition, and seasonal availability [11]. Therefore, the complete replacement of whole-fish sourced fishmeal by fishery by-products is less likely. In addition, by-products are raw materials dependent. This means that constant supply of the raw materials as well as suitable facilities and logistic networks must be available to enable fishmeal and fish oil production is economically viable and their qualities are ensured [4,10,11].

2.2. Animal By-Product

Animal by-products from terrestrial sources are used too. Animal by-products are processed before their inclusion in aquafeed and are easily available and economical [11]. Some of the processed terrestrial animal by-products of meals and fats are meat and bone meal, blood meal, horn meal, feather meal, poultry by-product meals, lard, tallows, greases, and poultry fat [3,6,12]. Processed animal by-products not only have better digestibility, but they are a nutritionally complete protein source with a balance of all nine essential amino acids [11–13]. However, limiting essential amino acids such as lysine, methionine, and isoleucine are found in poultry by-products, meat and bone meals, and blood meal, respectively [11]. The inability of the fish to digest feather meal in aquafeed also limits its use [11]. Additionally, there are many safety concerns stemming from the use of animal by-products in aquafeed. For instance, the fear of transmission of diseases to fish, and contamination of these by-products with dioxins [6]. Therefore, strict regulation is implemented when it involves the use of animal by-products in aquafeed production. According to the European Union regulation, which is one of the strictest regulations in the world, the usage of animal by-products from category 3 that are of low risk and not a health

threat to humans and animals are the only products that are allowed [3,12]. Moreover, in the scenario of aquafeed, processed non-ruminant by-products are allowed, but not processed ruminant by-products, except for hydrolyzed protein [12]. In the case for lard, tallows, and poultry fat, as long as they are not contaminated with animal protein, their use in aquafeed, as well as feed for other non-ruminant and ruminant livestock, is allowed [12]. Additionally, nutritional compositions of animal by-products vary according to sources and processing technology [12]. Consumer acceptance regarding the use of processed animal by-products in aquafeed is another challenge [11].

2.3. Plant Based Product

Plant nutrient sources such as cereals, oilseeds, and pulses are also used in aquafeed production. Examples of cereals that are primarily milled and processed are maize or corn, wheat, rice, barley, sorghum, oats, rye, millet, and triticale, as well as extracted oil from maize or corn and rice [3]. Examples of oilseeds that are used are primarily are solvent extracted soybean, rapeseed, cotton, groundnut or peanut, sunflower, palm kernel, and copra to make oilseed meals, as well as extracted oil from palm, soybean, rapeseed, sunflower, linseed, cottonseed, and olive [3]. Examples of pulses that are used are milled and processed peas and lupins [3]. Soybean meal is one of the main plant proteins in aquafeed production. It is easily available and low in cost [13,14]. However, its low protein content, lack of some essential amino acids, low digestibility carbohydrate, anti-nutritional elements, low palatability, and dependence on the species can affect a fishes microbiome, physiology such as gut morphology, immune and endocrine system, growth rate, and survival negatively [4,6,13–16]. The replacement of fish oil with plant oil lowers omega-3 fatty acids in farmed fish [13,16]. Plant products can be processed to increase protein, lipid, and mineral content to improve nutrients solubility, palatability, and digestibility, and to reduce anti-nutritional elements, fiber, and toxins [6,14]. The lacking essential amino acids and bioactive compounds can be supplemented to improve the nutritional qualities of the plant proteins as well [6]. The processing is time-consuming, causes nutrient deterioration, and increases expenses to aquafeed and aquaculture production [13]. Price volatilities of plant sources such as cereals and oilseeds are higher than the products from meat, aquaculture, and capture fisheries sectors [1]. This means that the prices of these plant sources fluctuate easily, probably resulting in a cascading effect of increase in cost for aquaculture fish that utilize these plant sources for aquafeed production. Additionally, most of the plant protein sources are primarily consumed by humans [3]. The substitution of fishmeal with plant nutrient sources comes with heavy environmental impacts. Fishmeal substitution with plant nutrient sources increases freshwater, land, and phosphorus demands, thus creating subsequent effects of biodiversity loss from land clearing, pollution from fertilizer and pesticide use, and altogether, causes a rise in carbon emission [15,16].

As solutions to some of the problems of utilizing plant nutrient sources, plant by-products are considered. Most of the plant by-products are not consumed by humans and have lower environmental impacts. Plant by-products are derived from the three categories of plant nutrient sources mentioned previously. Cereals by-product meals are maize or corn gluten, wheat gluten, dried distillers grains with solubles, rice protein concentrate, rice bran, and wheat bran [3]. Oilseed by-product meals are made with soybean protein concentrates and rapeseed or canola protein concentrate, whereas oil cakes are leftover from oil extraction like canola, sunflower, coconut, sesame, mustard, palm kernel, soybean, groundnut, cottonseed, olive, and rapeseed oil cakes [3,17]. Pulses that are made by by-product meals are pea protein concentrate and lupin protein concentrate [3]. Additional plant by-products are leaf, stalk, seed pod, stem, molasses, husk, bagasse, seed, straw, shell, pulp, stubble, peel, and root [17]. Many of these plant by-products are nutritionally different to one another. Bran is rich in starch, fiber, protein, lipids, iron, vitamin B, phenolic acid, phytosterol, and antioxidants [18]. Straw and stover (leaf and stalk) are high in lignocellulose, which has low protein and digestibility [18]. Soybean by-products, pulp and fruit processing waste are also high in cellulolytic content, making them unsuitable for

feeding non-ruminant animals [18]. Spent grain from the brewery industry has high protein content, but the high lignocellulose content is indigestible to non-ruminant animals [18]. Capsicum processing waste powder has high protein content, but also contains capsaicin, which is an irritant [18]. Others like okara from tofu and soymilk production has a good essential amino acid profile, digestibility, and B vitamins levels, as well as being cheaper [9]. However, okara is easy to putrefy and has anti-nutritional factors [9].

2.4. Food Waste

Food wastes are originally fit for human consumption, but are discarded [4]. Food wastes can be generated from market, domestic household, and commercial sectors such as restaurants [4]. Food wastes are sustainable, however, face challenges such as various nutritional composition depend on different food wastes; reduced growth performance in certain species; high in moisture; are perishable, so they have health and safety concerns due to pathogens; anti-nutritional elements from plant wastes; difficult wastes separation; lack of logistics network for transportation of food wastes; energy intensive and requirement for large area during treatment; and regulatory barriers that must be addressed [4,9,12,19,20]. In response to some of these problems, the sterilization of pathogens, feed additives to improve nutritional contents, food wastes separation and tracing, bioconversion of food wastes by insects, and biotransformation by microorganisms can be done [4,9,20]. Specifically, the use of insects and microorganisms in aquafeed production are discussed below.

2.5. Insect Based Product

The use of insects in aquafeed does not compete with human protein sources. The insects that are commonly studied for aquafeed production are black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), green bottle fly (*Lucilia sericata*), stable fly (*Stomoxys calcitrans*), common fruit fly or vinegar fly (*Drosophila melanogaster*), and yellow mealworm (*Tenebrio molitor*). The larvae are most frequently used in aquafeed for flying insects. Insects are nutritionally good with highly digestible amino acids from protein, lipid, vitamins, and bioactive components [19,21–23]. Insects normally have fast growth, short life cycle, high survival, high number of eggs laying, high feed conversion ratio, can survive in various substrates, and in high density numbers, while thermal and humidity conditions are easy to control, and in addition, insects are less susceptible to diseases [19,21,22,24]. Since insects can survive in various substrates, bioconversion of food wastes and by-products by insects not only reduces the initial cost but also their disposal cost [21]. However, cost, process performances, and nutritional compositions (amino acids, fatty acids, vitamins, and mineral content) of insects, animal feed safety, public, and environmental health depend on species; life stages; substrates (types, availability, nutritional compositions; the protein:fat:digestible carbohydrate ratio; depth in production system; moisture; pH; microbes; pesticides; insecticides; heavy metals and toxins such as mycotoxins, polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs); pretreatment methods); nutritional requirements; feeding rate; population density; environmental conditions (daytime, light, humidity, temperature, oxygen); production systems, and downstream processing [4,10,11,19–31]. Therefore, controlling these parameters for optimal and scaling up of insect production are technical challenges. Another thing to consider are the continuous experiments to figure out the optimal production of insects that are necessary. This is because the lack of standardized feeding experiments will eventually affect their process performances [31]. The scaling up the insects production is important in making the future price more competitive [32]. Currently, profitability would reduce even with partial introduction into the aquafeed [32]. Ethereal extract from insect meal causes oxidation of fat and so this prevents higher inclusion in aquafeed [22]. The indigestible anti-nutritional chitin could reduce protein digestibility of insects by fish, whereas anti-nutritional phytate can limit the bioavailability of minerals [4,19,22,25]. Even when insect production is maintained at an optimal level, the growth performance of

the aquaculture animals that are fed the insect-based diet still depends on the species of aquaculture animals and the inclusion level [19].

Bioconversion of food wastes and by-products by insects are sound strategies, but they come with disadvantages. For instance, lignocellulolytic fiber is usually difficult to bio-convert by most insects, and, therefore, reduces the process performances of insects [25,26,31,33]. Microbes can bio-transform some compositions such as fibers and carbohydrates within food wastes into simpler molecules or microbial mass that are later inactivated in the guts of fly larvae, thus enabling the release of nutrients for larval development [19,24,26,31,33]. However, this unique feature of microbial inactivation relies on the microbe strain, microbial dose, nutrient availability, and the feeding duration of the larvae [31]. Some pesticides and insecticides can be decomposed by the fly larvae in their guts and their gut microbes or be excreted, making these chemicals less of a concern in animal feed safety [31]. However, whether the products health risks that are posed from pesticide and insecticide decomposition still requires investigation [31]. Within the excretion, antimicrobial proteins that are present can decrease pathogens in food wastes [23,31]. However, mechanisms of these antimicrobial proteins are still poorly understood [31]. Fly species, except for black soldier fly, are pathogen vectors [20,23,24,29,31]. Escape of these insects into the environment, or pests and pathogens that are entering the breeding sites would be biosecurity hazards [21]. Therefore, microbial inactivation treatments after harvesting of the insects must be done to maintain feed safety [24,31]. Microbial involvement could also produce excessive carbon dioxide production and modify the conditions of the substrates, and thus may negatively affect the process performance of insects [33]. Biotransformation and bioconversion of food wastes and by-products by microorganisms and insects, respectively, can enable added benefits to the final insect aquafeed product. However, microorganisms may compete with the insects for nutrients within the food wastes and by-products [28,31].

Huge costs arise from the employment of manpower in its operation in maintaining hygienic insect breeding conditions, and the processing of the harvested insects [20,21,29]. Harmful by-products and gases that are produced during bioconversion of food waste and by-products by insects may be safety hazards to personnel [24]. The regulatory framework and consumer acceptance are another challenge to overcome in some countries before the inclusion in aquafeed [10,19,21,24,29,32]. Insect production requires low water consumption, low energy, emits low greenhouse gas emission, and has less land usage, with less competition with agricultural lands [19,21–23]. Less competition with agricultural lands may be due to the farming of insects in three dimensional settings that utilizes only a small area [19,21,22,29]. Thus, initial investment costs and operational costs are lower [21,29]. Although generally energy usage is low, electricity consumption from the bioconversion and insect drying process may increase the environmental burden of utilizing insects in aquafeed [34,35]. Energy usage for heating during insect rearing in colder climates is not only environmental burdening, but it also contributes to higher costs [24,36]. When insect meal production is compared with soybean meal production, insect meal has higher global warming potential and energy usage, but lower land usage [35]. Not only the environmental issues that are associated with food waste and by-products are tackled, but bioconversion leftover and frass can be used as natural fertilizers [21,23]. However, ammonia emission from these bioconversion residues used as fertilizer may cause higher global warming potential, particulate matter, acidification, as well as terrestrial and marine eutrophication [33,34].

2.6. Microbial Based Product

Microbial proteins and oils from microorganisms such as microalgae, bacteria, yeasts, fungi, and protists have vast potential as fishmeal and fish oil replacement. Most of the microbial protein sources are almost nutritionally similar to fishmeal [37]. Some microbial species can be processed into microbial oils. One of the potential microorganisms that is high in nutritional quality of crude protein and lipid is microalgae. Although the rest

of the microorganisms offer some lipid too [37–41]. The high content of polyunsaturated omega-3 fatty acids in microalgae would solve the low polyunsaturated omega-3 fatty acids problems of plant oil [16,37,42]. Bacteria have a high crude protein content [39,40]. Yeasts offer high protein (generally high glutamic acid content), mannan, and β -glucan contents, with mannan and β -glucan offering health-promoting effects [41,43]. Unlike microalgae, yeasts are low in lipid and high in ash content [43]. Additionally, bacterial, yeast, and fungal proteins provide B-complex vitamins [38,43]. It is difficult to be specific regarding the nutritional compositions of these different microbial proteins because they are dependent on multiple factors which will be mentioned later.

A specific look at the nutritional contents of these microorganisms uncovers some problems. These proteins have high nucleic acid content [38,42–44]; high nucleic acid concentration causes interference in macronutrients and uracil metabolism in monogastric animals [43]. Feeding yeasts to rainbow trout increases kidney uric acid to a harmful level and causes hemolytic anemia [41]. On the other hand, some fish like salmonids can metabolize high nucleic acid concentration, and Bharti et al. [40] mentioned that nucleic acid helps the hepatic function and lipid metabolism of fish [43]. However, processing to decrease nucleic acid is encouraged [38]. Although mannan and β -glucan can promote good health in aquaculture animals, the digestive enzymes that are produced by some of the aquaculture animals are unable to digest the polysaccharides [13,41–45]. Therefore, processing is required to break down the cell walls of microorganisms to enable increased accessibility to protein and nutrient digestibility [13,38,39,41–44]. Processing also allows convenience in storage and the assessment of these concentrated microbial products [42]. However, processing may release anti-nutritional factors, thus negatively affecting the protein and other components (primarily amino acids and fatty acids), as well as the production cost [38,42–45]. Another disadvantage are disintegrated and aggregated processed microbial proteins, which make filter feeders that prefer whole cell microbial protein much difficult to feed [42,45]. Next, microbial cells that settle in the culture system will contribute to the leaching of nutrients that lowers the water quality, resulting in the proliferation of pathogens [42,45]. Data concerning digestibility of microbial oil are lacking [41]. Even though digestibility data are reported for microalgal oil, the data are highly variable [41,42]. The palatability of microalgae and bacteria differ according to their species, fish species fed, and the inclusion level [13,41,42,44]. Nevertheless, yeast proteins decrease the pellet quality and palatability, whereas microalgal pigments improve the feed texture and can give color, as well as acting as an antioxidant to oil [41,42]. The safety of the microbial protein and feedstock is another concern, for instance the presence of toxins in fungal protein (mycotoxins) and bacterial protein (exotoxins and endotoxins), and heavy metals in feedstock [38,42]. According to Gold et al. [31], although microorganisms are capable of metabolizing mycotoxins, pharmaceuticals, and pesticides, these components must be carefully monitored and assessed before approval.

In terms of cultivation methods, microalgae are primarily cultured photoautotrophically, including some bacteria such as purple photosynthetic bacteria [39]. Photoautotrophic culture with open pond systems is cheaper, lower in energy input, and has no competition with agricultural lands, but have greater difficulties with maintenance of consistent growth conditions and nutrient supply, low light penetration with increased water depth, high freshwater consumption, contamination, lower biomass production, higher cost for harvesting and downstream processing, and the technical difficulties in their execution [37,38,42,45–50]. Matassa et al. [37] pointed out that although autotrophic culture of microalgae does not compete with agricultural lands, the land usage is high. Although the photoautotrophic culture of microalgae with a closed system reduces contamination and harvesting cost, enables complete control of microalgae growth conditions, as well as having higher biomass production, the cost for closed photo-bioreactors is higher, light penetration is also limited, and the production cost in general is higher [42,46–48,50].

The input of external carbon sources, instead of carbon dioxide fixation during photoautotrophic culture, or for heterotrophic and mixotrophic culture of microalgae, fur-

ther improves growth rate, biomass, lipid, and protein production [39,46,48,50,51]. Heterotrophic culture is another cost-effective and simpler alternative microbial protein cultivation method [48,51]. Heterotrophic cultivation of microalgae does not require light, has a lower fermenters cost, the growth conditions can be controlled, input of external carbon can be derived from diverse sources, and it is easier to scale up [47,51]. Therefore, biomass production from heterotrophic culture is also consistent [48]. However, heterotrophic cultivation of microalgae has flaws such as limited heterotrophic microalgae species, growth inhibition when substrate is in excess, difficult prediction of complementary microalgae species with specific substrate, contamination, no production of light-induced metabolites, and cost-ineffectiveness can happen if improper management of operation with huge energy consumption and unsuitable substrate selection and usage occurs [48,51,52]. Contrary to photoautotrophic culture that is primarily for microalgae, heterotrophic culture such as solid-state fermentation is mostly for bacteria, yeasts, and fungi. It requires low to no water, small size fermenters, low usage of electricity, less downstream processing due to the easy recovery of the product, and overall, lower production costs [17]. However, biomass production for bacteria and yeasts are lower due to them requiring more moisture [17]. Yeasts can be more costly due to shared uses in human food production [41]. Therefore, fungi like molds are commonly grown with solid state fermentation [17]. The input of external carbon and nitrogenous sources during heterotrophic culture are costly [13,41]. Carbon substrates such as glucose, acetate, and glycerol could be expensive and price volatilities of these sources will become more prevalent [50,51]. Therefore, alternative inputs, for instance carbon sources, can be explored to reduce cost.

Diverse selections of external carbon sources that are derived from pretreated food wastes; plant and animal by-products, for example food waste; by-products hydrolysate; as well as food processing wastewater such as nitrogen and phosphorus sources are cheap and drive the production costs down [13,18,37–39,44,46,53]. This concept is known as biotransformation, and other microorganisms such as bacteria, yeasts, fungi, and protists can be used. Not only does this concept support the idea of circular economy, those microorganisms in combination of transformed food wastes, plant by-products and animal by-products will surely improve the nutritional profile [9,18]. However, suitable food wastes, plant or animal by-products as substrates, and suitable microorganisms must be used together to ensure effective microbial proteins and oils production [18]. These carbon sources may drive the cost lower, but food wastes need to undergo pretreatment to remove pathogens and the harmful effects of solid particles [9,51]. These pretreatments that function to breakdown complex nutrient molecules for the utilization by microorganisms can increase production cost, degrade some nutrient molecules, and produce inhibitory by-products [18,39,46,51–53]. Although supplementation of external carbon nutrient sources can be done into pretreated food waste, this can cause the wastes to become more polluted than the original, even after microorganism cultivation [51]. These food wastes not only require pretreatments, but plant or animal by-products must be sourced properly and locally to avoid impurities such as fermentation inhibitors and toxic compounds, and increase in transportation cost, as well as to ensure a consistent supply of materials for microbial production [18,39,51]. Some by-products such as molasses is increasingly being used as substrate in the culture of microorganisms for bioethanol and other biotechnological purposes, thus increasing market competition [52]. Regulatory barriers need to be overcome to enable the use of these feedstocks for microbial proteins and oils production [39]. The public perception and acceptance of these microbial proteins and oils that are produced from these substrates are another hurdle [38].

Other cultivation methods include chemoautotrophic culture that uses carbon dioxide, hydrogen, and oxygen gases as substrates for bacteria [39]. Hydrogen-oxidizing bacteria are primarily selected for chemoautotrophic culture with the benefit of cheap substrates and carbon dioxide fixation [39]. The culture of microbial mass using natural gas such as methane and other C1 organic carbon substrates, such as methanol, is known as methylo-trophic culture [37,39]. Due to this process being exothermic, the heat that is generated

must be removed [39]. The mixotrophic cultivation method is the combination of heterotrophic and autotrophic cultivation methods in the growing of microorganisms. Similar to photoautotrophic culture, the mixotrophic cultivation method can be implemented into chemoautotrophic culture [39]. In mixotrophic cultivation of microalgae with open-pond photoautotrophic culture concept, carbon is added only during daytime and can prevent the excessive growth of bacterial contaminants [48]. The mixotrophic cultivation method also enables high growth rates and thus produces a large biomass of microalgae. In addition to that, biomass that is lost during dark respiration is reduced, and photo-inhibitory and photo-oxidative damage are eliminated or reduced [42,48,51]. However, the input of carbon substrates can increase production cost while limited mixotrophic microalgal species, and contamination are problems to deal with [51].

Reducing the production cost with the input of external carbon substrates from food wastes is not enough. Although processing can affect the quality and quantity of nutritional components, Glencross et al. [41] pointed out that processing might allow the production of protein-rich co-products that result in production cost reduction. Therefore, downstream processing can be diversified into multiple valuable products or services to have higher value products that support lower value products [51]. This can push the price of the microbial proteins lower without incurring economic loss. In the production of microbial proteins, other valuable produced components such as lipids as microbial oils or biodiesel, carbohydrates as chemical building blocks or for energy generation, and oxygen can be separated, as well as services such as wastewater treatment can be provided [51]. However, it is difficult to separate the different components without damaging the other [51].

In short, microbial proteins and oils production are subjected to many factors. Regardless of the types of microorganisms that are cultured, the metabolism, growth rate, and nutritional compositions (amino acids, polyunsaturated or saturated fatty acids and its quantity, vitamins, and minerals) of microbial proteins and oils depend on factors such as microbial species and strains; types and concentrations of carbon substrates; types and concentrations of nitrogen sources; cultivation methods (autotrophic, heterotrophic, or mixotrophic); environmental and culture conditions (oxygen, carbon dioxide, pH, light spectra and intensity, photoperiods, heavy metals, temperature, nutritional imbalance of carbon, nitrogen, phosphorus and silicate, salinity, mixing, viscosity, foam, turbidity, or culture duration); production systems, modes of cultivation (batch mode, fed-batch, continuous, semi-continuous, or two-stage sequential regime); and downstream processing [13,14,40–44,47–52]. Thus, many technical difficulties in controlling these factors must be overcome to enable the most efficient production of microorganisms. Moreover, in general, with power and labor costs in the equation, currently the production cost and price for microbial proteins and oils are high, they have upscaling difficulties, and further studies of digestibility and bioavailability of nutrients are required [4,6,37,39,51].

Microbial production is environmentally friendly (low water consumption, low land usage, low impact on biodiversity, low greenhouse gas emission, and less contribution to global warming) [18]. However, improper operation and production will still contribute to high environmental impacts. Other than high water and land usage, the maintenance of temperature of the cultivation system will consume a lot of energy [54]. Even if energy is not spent in the maintenance of temperature, energy could be used to pump seawater into the cultivation system [36]. Moreover, microorganisms that are under heterotrophic cultures do not capture carbon like those in the photoautotrophic culture, and carbon substrates such as glucose have higher environmental impacts (land use, energy use, and global warming potential) [13,36,44,50,54]. The environmental impacts vary according to different downstream processing methods [54] and these problems can be overcome. For instance, heterotrophic culture of microalgae in a closed system for oil (docosahexaenoic acid, DHA) production, with a carbon source from volatile fatty acids that are extracted from dark fermentation of pretreated food waste, and with a system that is powered by renewable energy, has a low environmental impact (global warming, terrestrial acidification, freshwater eutrophication, land usage, and biodiversity loss) [55]. Heterotrophic cultiva-

tion and production of whole-cell microalgae using sustainable sugarcane feedstock and renewable energy from the sugarcane waste has higher land use efficiency than beef, whey, rice, soy, and pea, as well as lower greenhouse gas emission and water consumption than beef and whey [47]. The net carbon dioxide emission is lower for mixotrophic cultivation of microalgae [51]. Bacterial protein that is produced from natural gas have low freshwater and land usage, but the carbon footprint is higher than soybean and fishmeal due to the use of natural gas as a bacterial substrate and to power the production process [37]. The culture of hydrogen gas oxidizing bacteria using direct carbon capture technologies, electrolysis to provide hydrogen and oxygen gas, and renewable energies such as wind and solar to power these processes, shows low land and water consumption, and eutrophication [56,57]. Yeast protein concentrate also has low environmental impacts (climate change impacts, acidification, freshwater eutrophication, marine eutrophication, land usage, water usage, and primary production requirement) [43].

2.7. Macroalgae as Alternative Source

Macroalgae, also commonly known as seaweeds, are macroscopic and multicellular algae that can be observed without magnification [58]. Macroalgae can be found in phyla Rhodophyta, Ochrophyta (class Phaeophyceae), and Chlorophyta [58]. Macroalgae have bioremediation properties from nutrient-rich water, bioactive compounds, can act as a feed additive, and, dependent on species, might have higher total amino acids than plant proteins and fishmeal. However, due to the high concentration of dietary fiber, the absolute concentration of amino acids per whole biomass and digestibility are low [4]. Although the direct extraction and protein isolation can concentrate protein from macroalgae, here we suggest the fermentation of macroalgae to increase the protein content.

Alternative nutrient sources have pros and cons. However, the combination of multiple sources to complement each other can be done. For instance, the bioconversion of food wastes as substrates for insects is used to address the health and safety concerns of using food wastes directly in aquafeed, and, at the same time, reduces the cost of insect meal production. The biotransformation of food wastes as substrates to microbial proteins and oils is another option. Unlike insect meal, microorganisms can break down lignocellulose while microbial proteins and oils do not contain indigestible chitin. Although food wastes come with lower cost, they are difficult to separate and the regulation for their use in aquafeed production is strict. Therefore, macroalgae are another potential substrate for the growth of microorganisms. Macroalgae are much reliable and consistent substrates than food wastes and are economical. The biotransformation of macroalgae using fermentation by microorganisms can reduce its high dietary fiber content and improve its nutritional profile. The biotransformation of macroalgae by microorganisms using fermentation for aquafeed is new in comparison to the bioconversion of food wastes by insects and biotransformation by microorganisms. The suggestion of biotransformation of macroalgae using fermentation by microorganisms does not serve to outcompete other alternative sources, but as an addition to the current alternative sources to reduce overdependence on fishmeal and fish oil.

3. Research Gaps

Previously, fermented macroalgae have been explored as alternatives in aquaculture diets by Uchida and Miyoshi [59], but with limited coverage. In-depth nutritional studies, proximate analysis, and in vivo testing of fermented macroalgae in aquaculture animals have never been reviewed before. Therefore, this review provides an overview of the nutritional qualities and the growth performances of fermented macroalgae in aquaculture animals.

4. Nutritional Studies of Fermented Macroalgae in Aquaculture

4.1. Protein Content

Many studies showed improvement in protein content after macroalgae fermentation. Solid state fermentation of *Caulerpa lentillifera* (Chlorophyta), *Kappaphycus alvarezii* (formerly *Eucheuma cottonii*) (Rhodophyta), and *Sargassum fulvellum* (Ochrophyta, Phaeophyceae) with palm kernel cake (PKC) by the fungus *Phanerochaete chrysosporium* and yeast *Cyberlindnera jadinii* (formerly *Candida utilis*) showed that *S. fulvellum* had the best nutrient improvement in protein content [60]. Other *Sargassum* sp. such as *Sargassum aquifolium* (formerly *Sargassum binderi*) (Ochrophyta, Phaeophyceae) have been experimented. In the study by Dewi et al. [61], increasing the fermentation period of *S. aquifolium* with the bacterium *Bacillus megaterium* was found to increase the crude protein content. Different microorganism fermenters were studied in fermentation of *Sargassum* sp. as well. In study by Ardiansyah et al. [62], the fungus *Aspergillus niger* that fermented *Sargassum* sp. recorded a significant increase ($p < 0.05$) in protein content compared to bacterium *Lactobacillus* spp. and yeast *Saccharomyces cerevisiae* fermented *Sargassum* sp. Although improvement of the protein content of fermented *K. alvarezii* with PKC was not the best in a study by Ilias et al. [60], other studies recorded improvement of protein. Protein contents of *K. alvarezii* increased after fermentation in a study by Felix and Alan Brindo [63], and in a study by Hardjani et al. [64]. The fermentation of *K. alvarezii* was done with *Lactobacillus* spp. and *Sa. cerevisiae* in a study by Felix and Brindo [63], and with *Sa. cerevisiae* in study by Hardjani et al. [64]. The fermentation of *K. alvarezii* was done with different macroalgae strains and species as well. Seaweed flour that comprised of a *K. alvarezii* green strain, *K. alvarezii* brown strain, *Gracilaria gigas* (Rhodophyta), *Sargassum* sp., and *Caulerpa* sp., was fermented with either bacterium *Bacillus* sp.; 1.5% of tape yeast *Rhizopus* sp.; 1.5% of baker's yeast *Saccharomyces* sp.; or a mix of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. [65]. Protein- and nitrogen-free extract (NFE) levels increased with the highest levels being recorded in seaweed flour that was fermented with mixed fermenters [65]. However, different results were shown in study by Sedanza et al. [66] that included a mixed-culture of bacterium and fungus. Cellulase enzyme pretreated *Rhizoclonium implexum* (formerly *Rhizoclonium riparium* var. *implexum*) (Chlorophyta) that was fermented through solid state fermentation by the fungus *Kluyveromyces* sp., was higher in crude protein by a significant difference ($p < 0.05$) than when fermented *R. implexum* by bacterium *Micrococcus flavus*, or a mixed culture of both the bacterium and fungus [66]. The use of enzymes was common among the macroalgae fermentation studies. In study by Fernandes et al. [67], *Ulva rigida* (Chlorophyta) fermented with the fungus *Aspergillus ibericus* with solid state fermentation produced enzymes xylanase and cellulase, which were used in sequential enzymatic hydrolysis with commercial cellulase. After the addition of antifungal thymol, the protein concentration increased [67]. Various macroalgae species for fermentation have also been done. *Padina tetrastomatica* (Ochrophyta, Phaeophyceae) that was fermented with *Lactobacillus* spp. and *Sa. cerevisiae* showed an increase in protein content [68]. Both *Undaria pinnatifida* (Ochrophyta, Phaeophyceae) that was fermented with bacterium *B. subtilis* and *Sargassum fusiforme* (formerly *Hizikia fusiformis*) (Ochrophyta, Phaeophyceae) that was fermented with fungus *Aspergillus flavus* var. *oryzae* (formerly *Aspergillus oryzae*), recorded increase in the crude protein content [69]. The protein contents of fermented *Und. pinnatifida* and *Saccharina japonica* (Ochrophyta, Phaeophyceae) with either the fungi *Monascus purpureus* or *Monascus kaoliang* increased significantly ($p < 0.05$) [70]. However, an unchanged protein content was also recorded in the bacterium *Lactobacillus plantarum* fermented *Saccharina latissima* (Ochrophyta, Phaeophyceae) [71].

4.2. Carbohydrate Content

The carbohydrate content also changed in fermented macroalgae. The carbohydrate content decreased after fermentation in *C. lentillifera*, *K. alvarezii*, and *S. fulvellum* with PKC by *P. chrysosporium* and *Cyb. jadinii*, thus suggesting the breaking down of cellulose by the microorganisms [60]. In comparison to non-fermented *Sargassum* sp., the carbohydrate

content of *A. niger* fermented *Sargassum* sp. decreased significantly ($p < 0.05$) [62]. The fermentation of *S. aquifolium* with *B. megaterium* for nine days significantly reduced ($p < 0.05$) the alginate content [61]. One of the common problems with the use of macroalgae as a protein replacement in fish-feed manufacturing, is the high content of dietary fiber. Fermentation had been found to reduce dietary fiber in macroalgae. *K. alvarezii* that was fermented with *Lactobacillus* spp. and *Sa. cerevisiae* had a drastic decrease in crude fiber content [63]. In a study by Hardjani et al. [64], *K. alvarezii* that was fermented with *Sa. cerevisiae* showed an increase in crude fiber but decrease in carrageenan content. *Sa. cerevisiae* may utilize galactose by breaking down carrageenan within *K. alvarezii* [64]. Therefore, the carrageenan content decreased, but with the release of galactose, the crude fiber content increased [64]. Fermented seaweed flour, as prepared by Aslamyeh et al. [65], showed a decrease in the crude fiber content, with the lowest level recorded in fermented seaweed flour that was with mixed fermenters of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. *Pad. tetrastomatica* that was fermented with *Lactobacillus* spp. and *Sa. cerevisiae* also showed a drastic decrease in crude fiber content [68]. After the addition of antifungal thymol into a fermented mix of *U. rigida* with *A. ibericus*, *A. ibericus* produced the enzymes xylanase and cellulase which caused more polysaccharides to be hydrolyzed [67]. An increase in carbohydrates was recorded in *Und. pinnatifida* that was fermented with bacteria *B. subtilis* and *S. fusiforme* that was fermented with fungus *A. flavus* var. *oryzae* as well [69]. The reducing sugar content of fermented *Und. pinnatifida* and *Sac. japonica* by either *Mon. purpureus* or *Mon. kaoliang* increased significantly ($p < 0.05$) [70]. After fermentation of *Sac. japonica* powder with *A. flavus* var. *oryzae*, the total sugar increased [72]. Generally, the carbohydrate content of macroalgae increased with fungal fermentation, but decreased with bacterial fermentation. Fungal species may produce enzymes that breakdown complex carbohydrates within the macroalgae. Bacterial species may use the carbohydrates from macroalgae for the production of functional products that may benefit fish-feed manufacture. However, dependent on the species of fungus, the carbohydrate content may decrease due to the utilization of carbohydrates for growth.

4.3. Lipid Content

Some studies of macroalgae fermentation recorded changes in the lipid content. In comparison to non-fermented *Sargassum* sp., the lipid content of *Sa. cerevisiae* fermented *Sargassum* sp. reduced significantly [62]. The fermentation of *K. alvarezii* by *Sa. cerevisiae* recorded a slight increase in fat content, with an increase in fatty acid content as well [64]. Fermented seaweed flour, as prepared by Aslamyeh et al. [65], showed a decrease in the fat content, with the lowest level recorded in fermented seaweed flour that was mixed fermenters of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. Fermented *Und. pinnatifida* by *B. subtilis*, and *S. fusiforme* by *A. flavus* var. *oryzae* showed increases in the crude fat content [69]. Fermented *Und. pinnatifida* and *Sac. japonica* by either *Mon. purpureus* or *Mon. kaoliang* also recorded increases in the fatty acid levels such as palmitic acid, stearic acid, oleic acid, linoleic acid, and arachidic acid [70]. Mixed positive and negative results were found in studies involving lipid content of fermented macroalgae. Generally, an external source of lipids instead of the fermented macroalgae is preferable.

4.4. Ash Content

The improvement of ash content was shown after macroalgae fermentation. The percentage improvement of ash was shown after the fermentation of *C. lentillifera*, *K. alvarezii*, and *S. fulvellum* with PKC by *P. chrysosporium* and *Cyb. jadinii* [60]. Fermented *Sargassum* sp. by *A. niger*, *Lactobacillus* spp., and *Sa. cerevisiae*, respectively, showed an increase in ash content as compared to non-fermented *Sargassum* sp. [62]. Significant increases in micromineral contents, such as magnesium and iron, were observed in fermented *Sargassum* sp., compared to non-fermented *Sargassum* sp. [62]. A significant increase of calcium content was also observed in *A. niger* and *Sa. cerevisiae* fermented *Sargassum* sp. [62]. However, negative results were recorded as well. Fermented seaweed flour, as prepared by

Aslamyeh et al. [65], showed a decrease in the ash content, with the lowest level recorded in fermented seaweed flour with mixed fermenters of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. Generally, the crude ash content in fermented *Und. pinnatifida* by *B. subtilis* and *S. fusiforme* by *A. flavus* var. *oryzae* decreased [69]. However, fermented *Und. pinnatifida* had an increase in minerals such as calcium, phosphorus, iron, and zinc, but had a decrease in magnesium, potassium, and sodium [69]. Fermented *S. fusiforme* had an increase in calcium, phosphorus, zinc, potassium, and sodium levels, but had a decrease in iron, and the magnesium level remained unchanged [69]. The level of sodium, magnesium, cadmium, and mercury were significantly reduced in *L. plantarum* fermented *Sac. latissima* [71]. Due to the bioremediative properties of macroalgae, beneficial macrominerals can be deposited in the macroalgae. However, dangerous minerals such as cadmium and mercury need to be monitored.

4.5. Other Compounds

The fermentation of macroalgae also produced functional products such as organic acids, antioxidants, phenolics, and flavonoid. Organic acids can significantly enhance the growth and health of fish and shrimp [73]. Lactic acid is one of the common organic acids that is produced during fermentation and is primarily produced by lactic acid bacteria. The lactic acid contents of cellulase enzyme hydrolyzed, and lactic acid bacteria (*Pediococcus acidilactici*, *Weissella paramesenteroides*, *Pediococcus pentosaceus*, and *Enterococcus faecium*) fermented seaweed broth, comprised of fine *Sargassum* sp. powder, showed a significant increase ($p < 0.05$); *E. faecium* fermented seaweed broth achieved the highest lactic acid content [74]. The cellulase enzyme that was pretreated and fermented *R. implexum* by *M. flavus* had a significantly higher lactic acid production ($p < 0.05$) than the mixed culture of *M. flavus* and *Kluyveromyces* sp. [66]. The fermentation of *Gracilaria fisheri* (Rhodophyta) with *L. plantarum* produced higher acidity due to predominantly lactic acid production [75]. Predigested *Ulva reticulata* (Chlorophyta) with enzyme cellulase was fermented with *L. plantarum* and *Sa. cerevisiae* to produce lactic acid and marine single-cell detritus (MSCD) product, which can be supplemental to the use of microalgae as feed for shrimp larvae [71,76]. Lactic acid was also detected from the fermentation of *Und. pinnatifida* by the bacterium *Lactobacillus brevis*, fungi *Debaryomyces hansenii* var. *hansenii* and *Candida* sp. [77]. However, in a study by Hardjani et al. [64], a decrease of the pH from 6.48 to 4.6 was recorded in fermented *K. alvarezii* with only the fungus *Sa. cerevisiae*. This suggested the production of organic acids such as pyruvic acid, citric acid, and succinic acid by *Sa. cerevisiae* through the metabolism of sugars in the fermentation medium. Antioxidants can prevent oxidative stress, immune suppression, pathological symptoms, and slow growth [78]. After the addition of antifungal thymol into a fermented mix of *U. rigida* with *A. ibericus*, an increase in antioxidant compounds was recorded [67]. Phenolic compounds are antioxidants and immunostimulants according to Ahmadifar et al. [79], whereas flavonoid compounds are antioxidants, antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and have vasodilatory actions, according to Chakraborty et al. [80]. Phenolic and flavonoid contents of fermented *Sac. japonica* and *Und. pinnatifida* by either *Mon. purpureus* or *Mon. kaoliang* increased significantly ($p < 0.05$) [70]. All of the total phenolic contents of yellow, purple, and green varieties of *K. alvarezii* were enhanced after solid state fermentation with *A. flavus* var. *oryzae* [81]. All *Eisenia bicyclis* extracts that were fermented with *Cyb. jadinii*, *Lactobacillus* sp., and *Bacillus* sp. had an increase in the total phenolic content, with the highest recorded in *Cyb. jadinii* fermented extract after one day of fermentation [82]. Fermented *Sac. japonica* powder by *A. flavus* var. *oryzae* had an increased total phenolic content, however, the total flavonoid content decreased [72].

By-products such as ethanol and anti-nutritional compounds were produced through macroalgae fermentation as well. Cellulase enzyme pretreated and fermented *R. implexum* by *Kluyveromyces* sp. was significantly higher in ethanol ($p < 0.05$) than in a mixed culture of *M. flavus* and *Kluyveromyces* sp. [66]. Anti-nutritional contents, such as phytic acid,

total polyphenols, tannin, and saponin, were significantly reduced ($p < 0.05$) in fermented *Sargassum* sp. compared to non-fermented *Sargassum* sp. [62]. However, oxalates in the *Sargassum* sp. were not significantly reduced after fermentation [62]. It was noted that in the study by Ardiansyah et al. [62], polyphenols such as tannin and saponin were considered as anti-nutritional compounds. However, polyphenols and saponin that are used in aquaculture, as highlighted by Ahmadifar et al. [79] and Chakraborty et al. [80], respectively, showed positive growth-promoting and health-promoting effects among aquaculture animals. Further studies regarding the effects of polyphenols and saponin from fermented macroalgae towards the survival and growth of aquaculture animals are required.

The fermentation of macroalgae with microalgae for the purpose of aquafeed production is a possible implementation; microalgae can grow using heterotrophic cultivation methods and the carbon source is derived from pretreated macroalgae. The pretreated macroalgae will release more nutrients for the growth of microalgae. However, heterotrophic cultivation of microalgae has only been done for the purpose of human food and the production of supplements [47].

5. In Vivo Testing of Fermented Macroalgae in Aquaculture

In vivo testing of fermented macroalgae in the aquaculture diet is still very limited. Currently, in vivo testing has only been done in *Macrobrachium rosenbergii* (giant freshwater prawn) and *Pinctada fucata martensii* (Japanese pearl oysters) [63,68,77]. In vivo testing of fermented macroalgae on the digestibility, weight gain, growth rate, feed intake, protein efficiency ratio (PER), feed conversion ratio (FCR), and body composition on aquaculture animals are the examples of parameters that can be examined.

In vivo testing of different species of macroalgae after fermentation have been done in *Mac. rosenbergii*. In the first study by Felix and Brindo [68], fermented *Pad. tetrastomatica*-incorporated diets showed a significant increase ($p < 0.01$) in apparent digestibility coefficient (ADC) for dry matter, apparent protein digestibility (APD), and apparent lipid digestibility (ALD) in juvenile *Mac. rosenbergii* compared to juvenile *Mac. rosenbergii* that were fed the non-fermented *Pad. tetrastomatica*-incorporated diets. A significant increase ($p < 0.01$) in mean weight gain, specific growth rate (SGR), mean feed intake, and PER was observed in fermented seaweed-incorporated diet fed juveniles of *Mac. rosenbergii*, than the juveniles that were fed the raw seaweed-incorporated diets [68]. The FCR of *Mac. rosenbergii* juveniles that were given the fermented seaweed incorporated diets were better than the juveniles that were given the raw seaweed-incorporated diets [68]. No variations in the whole-body composition (moisture, protein, lipid, and ash) of *Mac. rosenbergii* juveniles were observed between the raw and fermented seaweed-incorporated diets [68].

In the second study by Felix and Brindo [63], *Mac. rosenbergii* juveniles were fed the fermented *K. alvarezii*-incorporated diets. In this study, *Mac. rosenbergii* juveniles that were fed with the fermented *K. alvarezii*-incorporated diets showed a significant increase ($p < 0.01$) in ADC for dry matter, APD, and ALD in comparison to non-fermented *K. alvarezii*-incorporated diets [63]. The mean weight gain and mean feed intake of juvenile *Mac. rosenbergii* given the fermented seaweed-incorporated diets significantly increased in comparison to raw seaweed incorporated diets ($p < 0.01$) [63]. The FCR and PER of juvenile *Mac. rosenbergii* that were fed the fermented seaweed-incorporated diets, in general, were better than the juveniles that were fed the raw seaweed-incorporated diets [63]. There was no significant variation in the whole-body composition (moisture, protein, lipid, and ash) between *Mac. rosenbergii* juveniles that were fed the raw and fermented seaweed-incorporated diets [63].

Fermented macroalgae also have been tested in *Pin. fucata martensii*. Seven-month old hydrolyzed and fermented *Und. pinnatifida* that was fed to young *Pin. fucata martensii* at a concentration of 3×10^4 cells/mL per day, showed a significant ($p < 0.05$) growth rate of the hinge length when compared to the unfed control group [77]. However, when it was compared to the microalgae *Chaetoceros calcitrans* (Bacillariophyta)-fed control group,

the growth rate of the hinge length of the young *Pin. fucata martensii* from the latter group significantly increased ($p < 0.05$) [77]. Therefore, a combined feeding trial of hydrolyzed and fermented *Und. pinnatifida* with *Cha. calcitrans* was done. The growth rate of the hinge length of the young *Pin. fucata martensii* that were given the combined feeding of 2×10^4 cells/mL per day of eight days old fresh hydrolyzed and fermented *Und. pinnatifida* with 3×10^3 cells/mL per day of *Cha. calcitrans*, was significantly increased ($p < 0.05$) in comparison to the group that were given the hydrolyzed and fermented *Und. pinnatifida* only [77]. The benefit of fermentation was seen when this combined feeding showed a significant increase ($p < 0.05$) in the growth rate of the hinge length of the young *Pin. fucata martensii*, in comparison to combined feeding of the hydrolyzed and non-fermented *Und. pinnatifida* with *Cha. calcitrans* [77].

6. Limitations from Current Studies and Challenges

Based on the current studies of fermented macroalgae in aquaculture nutrition, the carbohydrate content is still high after fermentation. The carbohydrate content can be utilized more as an energy source for the growth of the microorganisms that are selected for fermentation. Different factors, such as the microbial species and strains, types of carbohydrates, and the culture and environmental conditions affecting the utilization of carbohydrates as energy sources for microbial growth, must be investigated. The increased growth of microorganisms within fermented macroalgae can potentially increase the protein content. Thus, making fermented macroalgae much competitive as an alternative to fishmeal.

The lack of nitrogenous sources to the fermentation medium is another problem. Nitrogenous nutrients are another requirement for the growth of microorganisms other than carbon. External nitrogenous sources can be added to boost the growth of microorganisms. Among the previous studies that were reviewed, urea is utilized as a nitrogenous source in Hardjani et al. [64], peptone in Ilias et al. [60] and Suraiya et al. [70], yeast extract in Nor et al. [81] and Shobharani et al. [74], and soya powder in Sedanza et al. [66]. External nitrogenous sources that are economical for commercial production may be industrially produced ammonia and urea, or the nitrates from wastewater.

Nutritional or proximate comparison of fermented macroalgae with fishmeal can be done in future studies. Even though most of the studies showed higher or a significant increase in the protein content, it is not clear how nutritionally similar their protein content to the one in fishmeal. Even if there is nutritional information regarding fishmeal from Institut National de la Recherche Agronomique—Centre de Coopération Internationale en Recherche Agronomique pour le Développement—Association Française de Zootechnie Feed Tables (INRAD—CIRAD—AFZ Feed Tables), primary nutrients such as protein as well as amino acids and fatty acid profiles are difficult to compare. This is because proximate analytical methods of current studies might not be the same as the ones used to determine fishmeal proximate composition in INRAD—CIRAD—AFZ Feed Tables. Therefore, comparison of fermented macroalgae to fishmeal can be done in future studies.

7. Conclusions

Fermented macroalgae has the potential to replace fishmeal in aquafeed production. The fermentation of macroalgae improves the protein content. Additionally, major nutritional contents such as carbohydrates, lipids, and ash show improvement. However, further studies are needed until fermented macroalgae can achieve an almost similar nutritional profile to fishmeal. Moreover, the palatability, physiological, and growth changes of fermented macroalgae in aquafeeds to aquaculture animals require extensive studies before industrial application.

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