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Microalgal applications in aquaculture and animal husbandry

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Abstract

Although the use of microalgae for food has endured for a long time now, their full potentials have not yet been exploited. Several important species of microalgae are currently serving as sustainable substitutes to some agricultural processes, helping in boosting-up food production. Microalgae are gradually replacing fishmeal and fish oil in aquaculture feed production as they have been successfully applied up to a maximum inclusion level of 10% in Atlantic salmon's diet without recorded defect, and even up to 100% replacement in other fish diets such as carp. Breakthrough have also been recorded in the use of both live and concentrated microalgae in the farming of other essential aquatic animals such as clams, oyster, shrimps, mollusks and sea cucumbers, as well as in ruminant and non-ruminant animals. Here, we have presented a comparative evaluation of various applications of microalgae in aquaculture and animal husbandry, indicating the enormous advantages of adopting microalgal substituted meals and their applications in aquaculture water management and pH modulation. We have also holistically addressed the challenges restricting microalgae feed commercialization, using schematic illustration to present possible approaches to obtain cost-effective biomass production needed in microalgae feed.

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Introduction

If the world must attain the sustainable development goals in terms of food security, adequate sustainable sources of food and feed for both humans and animals, respectively, is indispensable. Aside from increasing production of already dwindling food sources, the current pressure on the use of co-food stuffs for human and animals such as fish-protein and –oil, as well as soybeans and corn amongst others, must be reduced or totally eliminated. This may be achieved by the adoption of efficient sustainable microbial alternatives such as microalgae. The application of algae in agriculture dates back to ancient times [1,2] with numerous trendy applications evolving in modern days.

Algae have become an alternative source of protein and food for a long time in human history and their use is quite popular in modern aquaculture industry. Aside the basic protein, lipid and carbohydrate composition of algae, some genera contain high amounts of carotenoids with antioxidant properties [3,4]. High concentrations of some polyunsaturated fatty acids such as eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA) are also common in such genera [5-8]. These nutritional constituents contribute significantly to the assessment of a microalgal species as adequate diet for marine organisms [9-11]. Furthermore, added advantages that microalgae can be easily grown almost anywhere with cheap media source such as wastewater on relatively smaller land present them as important "modern agriculture tools."

In the last few decades, there have been a good number of recent research publications on the applications of some microalgal species in several aspects of aquaculture and animal husbandry (especially in feed production). Review articles have however been more focused on important bio-active and nutritional compounds from microalgae [12-15]; microalgae wastewater bio-remediation and treatment [16-19]; as well as microalgae biomass production [20-23] and generation of bioenergy [24-27]. Some researchers have reviewed the enormous prospects and applications of microalgae in some animal feed [28-30]. However, almost none of these studies have been very holistic in discussing microalgal feed options nor were the differences between these microalgae meals and the conventional ones properly evaluated like we presented here. More so, thorough integrated solutions to the challenge of microalgal biomass production for feed have not been considered in details in previous studies as we have done. It is therefore important to note that as more studies continue in the field of phycology and agriculture, more and more agricultural applications of microalgae will evolve while some others may be modified.

Microalgae in agriculture

Microalgae have been in use for centuries as food and feed [1,2]. Microalgal biomass provide not only the protein, carbohydrates and n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA), but also essential vitamins (such as vitamins C, E, B1, B2, B6, B12, folic acid and pro-vitamin A) and important minerals like calcium, potassium, iron and magnesium [31]. They are also sources of a good number of other health benefiting compounds and pigments (Table 1). There are diverse applications of microalgae in agriculture which include: usage as aquaculture feed and feed supplements [32-34]; feed supplements for other livestock such as poultry, ruminants and non-ruminants [35-38]; source of *in vivo* colorants/pigments in animals [10]; biological agents for aquaculture water purification (bio-purifier) and as pH bio-stabilizers [39,40], among others.

Table 1: Some essential components of microalgae and their physiological effects.

Components	Source	Physiological effects	Reference
Alpha-tocopherol	*Euglena gracilis, Stichococcus bacillaris, Dunaliella tertiolecta, Tetraselmis suecica*	Antioxidant, anti-carcinogenic	[41-44]
Astaxanthin	*Haemotococcus pluvialis, Chlorella sorokiniana, Tetraselmis* sp.	Antioxidant, pigmentation	[45-48]
Beta-carotene	*Tetradesmus obliquus, Dunaliella salin, Porphyridium cruentum, Isochrysis galbana, Phaeodactylum tricornutum, T. suecica, Nannochloropsis gaditana*	Antioxidant, pigmentation	[3,49,50]
Zeaxanthin	*P. cruentum, N. gaditana, Nannochloropsis oculata, Scenedesmus almeriensis*	Pigmentation, antioxidant, anti-age-related macular degeneration (AMD), ophthalmo-protection	[3,51,52]
Lutein	*T. suecica, Coccomyxa onubensis, S. almeriensis, N. oculata*	Pigmentation, antioxidant	[3,51,53,54]
Fucoxanthin	*Odontella aurita, Nitzschia laevis, P. tricornutum, Chaetoceros muelleri, Amphora* sp., *Chrysotila carterae, Tisochrysis lutea, Navicula* sp.	Pigmentation, antioxidant, anti-inflammation, anti-tumoral, anti-hypertension	[7,55-59]
Violaxanthin	*Eustigmatos* cf. *polyphem, N. oceanica, T. suecica*	Pigmentation, anti-oxidant, anti-arterio-sclerosis, anticancer	[60,61]
Flavonoids	*N. gaditana, P. tricornutum, Nannochloropsis* sp., *T. suecica, Chlorella pyrenoidosa*	Pigmentation, antioxidant	[62,63]
Chrysolaminarin	*O. aurita, P. tricornutum*	Antioxidant, anticancer	[55,64]
Eicosapentaenoic acid (EPA)	*O. aurita, N. laevis, N. gaditana, N. oceanic*	Antioxidant, anti-obesity, anti-diabetes, anti-inflammatory, cardiovascular neural and mental development	[7,8,55,65]
Docosahexaenoic acid (DHA)	*Schizochytrium* sp., *Thraustochytrium* sp., *Isochrysis* sp.	Antioxidant, anti-inflammatory, cardiovascular neural and mental development	[5,6,66]

Microalgae in aquaculture

Aquaculture involves the farming of aquatic organisms including fish of all kinds, mollusks, crustaceans and diverse kinds of aquatic plants [67]. Aquaculture sector has been declared as world's fastest producer of food for some decades with an annual net worth of about $166 billion US dollars [68,69]. Microalgae have several applications in aquaculture [70,71] which include: primary and/or secondary feed for fish and other aquaculture animals such as mollusks, and crustaceans [11,69,72-74]. This is basically because of their high contents of protein, vitamins, carbohydrate, lipid and well balanced chemical constituents [9,75]. Microalgae are also applied as bio-purifiers in aquaculture ponds to remove poisonous nitrogenous waste substances such as ammonia, nitrite and nitrate [39,40], and as

pH bio-stabilizing agents in aquaculture systems [40].

Microalgae as aquaculture feed

FAO [76] predicted that aquaculture sector and fisheries will reach 172 million tonnes production capacity by 2021. There have also been continued global increase in the demand for aquaculture products. Therefore, the quest to increase aquaculture production and consequently source alternative sustainable and adequate feeding options keep increasing [77-79]. Some of such aquafeed components needing urgent and sustainable replacement options are fish protein and fish oil that are majorly sourced from fish-meals/oil and terrestrial plants, as well as a few from invertebrate and nut meals [77,79,80]. To further corroborate the need to source fish protein and oil from other sources, it was reported that in 2008 alone, about 73.8 and 60.8% of fish oil and fishmeal were consumed just in aquaculture production [76,81].

Some products of terrestrial plants (such as soymeal, gluten meal, rapeseed meal and wheat meal) have been adopted at low inclusion levels as substitutes to these aqua-nutrients [82-85]. Some studies have however shown that these terrestrial plants are not the best sources for these nutrients, especially for carnivorous fish such as salmon [86-88]. A suitable source of such aqua-nutrients is microalgae [71,77]. Both live and microalgae concentrates have been demonstrated in several works to be appropriate feed substitutes and/or nutrient supplements in aquaculture [31,34,89-91]. However, for microalgae to be used as a supplement or aquaculture feed, it must first meet a number of important criteria [11,92]. Primarily, such algae must have adequate nutritional composition and not be toxigenic among other features (Table 2).

Table 2: Important features considered in microalgae adoption for aquafeed.

Feature	Description/Importance	Reference
Appropriate ingestion size	(i) 1-15 µm for filter feeders, (ii) 10-100 µm for grazers	[9,92]
Easy digestibility	Preferably without rigid cell wall, e.g. *Boekelovia hooglandii* and *Euglena* sp.	[9,11,92,93]
High growth rate	Even in relatively poor growth medium and under fluctuating conditions	[9,11]
High level of stability to fluctuations in light, temperature and nutrients	These fluctuating conditions are quite common in aquaculture systems, especially in hatchery set-ups	[92]
Appropriate pigmentation	The right pigmentation is very important because it can influence the colour, and thus the price of some types of fish	[9-11]
Good nutrient composition	Adequate amount of protein, carbohydrates, essential PUFAs, vitamins, minerals, etc	[9,11,70,92]
Absence of toxins	To prevent both intoxication of the aquaculture animals and ultimately, the transfer up the food chain to humans	[9,92]
Immune stimulation[1]	Presence of molecules such as β- 1,3-glucan that could play some immune-regulatory functions in shellfish and fish	[70]

[1]Could be a secondary benefit and not necessarily a primary consideration.

A number of *in vitro* studies have demonstrated and proved that many species of microalgae meet up many of the requirements listed in Table 2 and have therefore been used in aquaculture. Tibbetts et al. [69] examined the prospects of using *Nannochloropsis granulate* as a source of digestible protein for rainbow trout. They reported that *N. granulate* degree of protein hydrolysis and apparent digestible coefficients (ADC) were quite similar to some important aquafeed ingredients like that of fishmeals as reported by Lemos et al. [94], giving this microalga a good prospect as aquafeed source. The hydrolysate of *Laminaria digitata* was also used as carbon source to grow three potential aquafeed *Chlorella sp*. heterotrophycally [79]. *Chloella protothecoides* gave the best performance with the shortest lag phase, growing to a biomass concentration of 11 g/L, accumulating 42% dry weight protein, and six-folds greater amino acid (in comparison with *L. digitata* used as carbon source). C. *protothecoides* was therefore regarded as an adequate supplement in fish feed. Similarly, *Scenedesmus quadricauda* biomass and nutritional composition profiling results obtained from its application in carbon dioxide sequestration also suggested its potential use as dietary feed source for fish [95].

Several microalgae have been applied at different inclusion levels to practically substitute fishmeal in Atlantic salmon's diets in many studies. These include *Nanofrustulum* and *Tetraselmis* [89], *Desmodesmus* sp. [78], *Phaeodactylum tricornutum* [81], *Nannochloropsis oceania* [81], *Scenedesmus* sp. [91] among others (Table 3). Interestingly, most of these microalgal supplemented diets did not result in any significant difference from the standard control diet treatments as presented in Table 3. A maximum 10% optimal microalgae inclusion value was obtained in Atlantic salmon trial studies above which there were noticeable defects in some measured parameters (Table 3).

Beyond fishmeal substitution, microalgae have been recognized as a prominent source of sustainable n-3 LCPUFA [31,75]. Microalgae have been used as a replacement to fish oil and served as a veritable source of lipid in fish. Microalgae such as *Schizochytrium* sp. having a very rich lipid content (55-75 % DM) with about 49% DHA [96] have been a choice species used in several studies [31,97-99]. Several studies with *Schizochytrium* sp. substituting fish oil have reported no significant difference in important salmon's growth and health factors as well as feed quality parameters in comparison with the conventional control feed (Table 3) [31,100,101]. However, reduction in pellet durability was reported by Kousoulaki et al. [31] as inclusion level reached 5%. This is considered smaller than the maximum *Schizochytrium* sp. inclusion level of 13.2% reported to obtain adequate feed hardness and durability by Samuelsen et al. [99]. The most significant defect of n-3 LCPUFA reduction in salmon is one of great nutritional concern to consumers [101]. This reduction concurs with that of Sparus aurata in which fish oil was also replaced by microalgae [97,102]. This nutritional reduction could pose a big challenge in microalgae substituted fish diets.

Microalgae have also been successfully used as substitute to fishmeal and/fish oil in other fish diets (other than that of Atlantic salmon's) even in higher inclusion levels reaching up to 100% [89,104-106]. Impressive outcomes have been obtained with microalgal supplemented meals and even better outcomes than control treatments were reported in some studies (Table 4).

Aside from fishery aquaculture, microalgae have been extensively applied in the diets of other aquaculture animals [33,107,108]. Studies on applications of both live and microalgae concentrates as feed at different growth stages of sea cucumber (*Holothuria scabra*) have been demonstrated with interesting results (Table 5). Other important aquaculture animals such as winged pearl oyster and giant clams have been reported to thrive well with microalgae feed as well (Table 6). Positive

Table 3: Effects of microalgae inclusion in Atlantic salmon's diets

Microalgae species	Inclusion level (%)	Substituted aqua-nutrient	Effects	References
Nanofrustulum & *Tetraselmis*	5 & 10	Fishmeal	No significant difference in growth performance, body composition and feed performance among the treatments groups and the control.	[89]
Desmodesmus sp.	10 & 20	Fishmeal	No significant difference in the survival rate, specific growth rate and condition factor among the treatment groups and control; Inferior feed conversion rate in the treatment groups as against the control.	[78]
Phaeodactylum tricornutum	3, 6[1]& 12	Fishmeal	No significant adverse effect on growth rate, feed conversion ability (in terms of ADC) of dry mater, protein, lipid, ash and energy between optimum inclusion level treatment group and control.	[81]
Nannochloropsis oceania	10[1]& 20	Fishmeal	Negative effect of 20% inclusion level on salmon's health, feed intake, feed conversion ratio (FCR), lipid and energy conversion as well as reduced weight gain and specific growth in comparison with control.	[103]
Scenedesmus sp.	10[1]& 20	Fishmeal	No significant difference in growth and feed utilization between control and 10% inclusion treatment group.	[91]
Schizochytrium sp.	1, 6 & 15	Fish oil & Fishmeal	No significance difference in survival, feed intake, feed conversion, protein efficiency rates, technical quality of fillet and total fillet lipid among treatment groups and control; Increased number of slim cells and oxidative stress in intestine with increasing inclusion level.	[98]
Schizochytrium sp.	2.5 & 5	Fish oil	No significant difference in the growth rate and feed conversion ratios among all the treatment categories; No difference in protein composition, energy digestibility and pellet technical quality among all the diets; Reduction in pellet durability at 5% inclusion level.	[31]
Schizochytrium sp.	5.5 & 11	Fish oil	Significant reduction in persistent organic pollutants compared to control; No difference in fish health status and overall weight gain among all the treatment categories; Significant reduction in n-3 LCPUFA (especially EPA) in treatment groups compared to control.	[101]

[1]Optimal inclusion level. Control: control group fed with conventional diet devoid of microalgae feed, i.e. 0% microalgal inclusion level.

Table 4: Effects of microalgae inclusion in other fish's diets.

Type of fish	Microalgae species	Inclusion level (%)	Substituted aqua-nutrient	Effects	References
Carp	*Nanofrustulum* & *Tetraselmis*	25 & 40	Fishmeal	No significant difference in growth performance, body composition and feed performance among treatment groups and control.	[89]
European sea bass	*Tisochrysis lutea* & *Tetraselmis suecica*	[1]15, 30 & 45; [2]12, 24 & 36	[1]Fishmeal and [2]lipid (oil)	No significant difference in growth performance, body composition and feed performance among treatment groups and control.	[106]
Indian carps (*Catla catla* and *Labeo rohita*)	*Spirulina platensis*	25, 50, 75 & 100	Fishmeal	No significant difference in specific growth rate (SGR), weight gained and protein efficiency ratio among *Catla catla* at all microalgal inclusion levels and control; Significant improvement with increasing microalgal inclusion levels diets compared to control in *Labeo rohita.*	[105]
Common carp (*Cyprinus carpio* L.)	*S. platensis*	25, 50, 75 & 100; [3]Sole protein	Fishmeal	No negative effect on FCR, SGR, weight gain and organoleptic qualities; No significant difference in carcass moisture and protein contents among the treatment groups and control; [3]Better net protein retention compared to control	[104]
Nile tilapia (*Oreochromis niloticus*)	*Chlorella* spp. & Sc*enedesmus* spp.	10, 25, [4]50 & 75	Fishmeal	Increased growth performance in a direct proportional relationship with the inclusion level of microalgae, peaking at 50% above the control, and then dropped below the control at 75% inclusion level	[110]

[1]Fishmeal substituted inclusion levels, [2]lipid (oil) substituted inclusion levels, [3]*S. platensis* used as sole protein source, [4]Optimal microalgal inclusion level.

Table 5: Microalgal application in *Holothuria scabra* (sea cucumber/sandfish) feed

Microalgae used	Effects	Reference
Two live microalgae: *Isochrysis* aff. *galbana* (TISO) & *Chaetoceros muelleri*; Six concentrates: *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weisflogii, Thalassiosira pseudonana* &[1]Shellfish Diet 1800®	Seven of the tested microalgae were ingested by the larvae with varying rate of digestion depending on the age of the larvae with TISO giving the best outcome	[107]
Commercial concentrates: *Isochrysis* sp., *Pavlova* sp. & *T. weisflogii*	All microalgae gave steady increase in larvae auricullariae stomach width and total length as against the control setup which resulted in reduction of these parameters. *T. weissflogii* gave the best outcome with auricullariae mean lengths of 918.20±3.36 and 1011.64±5.93 µm on day 7 and 9, respectively	[111]

Commercial concentrates: *Isochrysis* sp., *Pavlova* sp. & *T. weisflogii*	All microalgae supported the growth and development of larvae into proficient doliolariae as against the unfed larvae. There was also formation of hyaline spheres in all the larvae fed with microalgae with varying sizes depending on microalgae nutritional composition but the unfed larvae failed to develop hyaline sphere.	[112]
Two live microalgae: TISO &*C. muelleri*; Six commercial concentrates: *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *T. Weisflogii, T. pseudonana* &[1]Shellfish Diet 1800®	Seven of the microalgae were ingested except for TISO. There was cell wall digestion in five ingested microalgae with *C. muelleri,* giving the best cell wall digestion and growth rate of sandfish juveniles	[90]

[1]A mixture of several microalgae: *Isochrysis* sp., *Pavlova* sp., *T. pseudonana* and *Tetraselmis* sp.

Table 6: Applications of microalgae in feed of other aquaculture animals

Animal	Microalgae used	Effects	Reference
Tridacna noae (Giant clams)	*Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii*	Selective ingestion and faster digestion of the smaller sized *Isochrysis* sp. (5-7 µm) and *Pavlova* sp. (4-7 µm) over their larger sized counterparts by larvae	[34]
Pteria sterna (winged pearl oyster)	*Phaeodactylum tricornutum, Chaetoceros calcitrans, Chaetoceros muelleri, T. weisflogii, Dunaliella salina, Nannochloris* sp., *Tetraselmis tetrathele, Tetraselmis suecica, Isochrysis* aff. *galbana, Pavlova lutheri*	Ingestion of only *Pavlova lutheri, Isochrysis* aff. *Galbana* and *Nannochloris* sp., and digestion of just the first two	[113]
Pteria penguin (winged pearl oyster)	Concentrate microalgae: Isochrysis 1800® and Pavlova 1800®	Superior growth and development of larvae, greater antero-posterior measurement (APM) of larvae (10.3 µm) compared to previous study with live microalgae	[114,115]
P. penguin	Concentrate microalgae: Isochrysis 1800® and Pavlova 1800® and [1]Shellfish Diet 1800®	Optimal larvae stocking density of 6 and 1 larvae mL^{-1}, and feeding ration of 10 x 10^3 and 20 x 10^3 cells mL^{-1} for post-fertilized larvae at 1 to 8 and 8 to 17 days, respectively	[116]

[1]A mixture of several microalgae: *Isochrysis* sp., *Pavlova* sp., *T. pseudonana* and *Tetraselmis* sp.

growth impact has also been recorded in shrimps' aquaculture with microalgae by some other studies [89,109].

Microalgae as bio-purifiers and pH bio-stabilizers

Microalgae can efficiently absorb nutrients and other pollutants (such as nitrogenous wastes) from waste effluent [19]. Poisonous nitrogenous wastes - ammonia, nitrite and nitrate - can cause harm to aquatic organisms, especially their seedlings. As a result of the toxicity of most of these wastes, they are required to be quite low in aquaculture water for high productivity. Unionized and ionized ammonia, nitrite and nitrate are expected to be below the recommended limits of 0.0125, 1.0, 1.0 and 400 mgL^{-1}, respectively, in re-circulating aquaculture systems (RAS) [117]. However, in practice, there may be several variations in these threshold concentrations depending on species of aquaculture organism and their age as well as other water parameters such as pH, temperature, and dissolved oxygen.

In fishery for example, nitrogen primarily enters the pond from the fish feed [118]. Nitrogen from such feed and some other sources undergo some reactions (largely facilitated by bacteria) to generate toxic nitrogenous wastes such as ammonia and nitrite [119,120] (Figure 1). Considering the great danger of these nitrogenous wastes, especially ammonia and nitrite which are over a hundred times more poisonous than nitrate [121-123], it is necessary that their concentrations are kept close to zero [118] in aquaculture ponds. But this could be a daunting challenge. This is conventionally addressed by constant water change in aquaculture. Regular change of aquaculture water is however expensive and leads to a significant increase in the cost of production. The need for this constant change of water limits aquaculture to areas with adequate and guaranteed source of water. However, microalgae have been reported to be efficient bio-purifiers of such wastes in aquaculture ponds without continuous water change [40,109].

Microalgae are responsible for about 70% of total global nitrogen assimilation with about 65% consumed in form of reduced nitrogen (such as ammonia and organic nitrogen), about 10% through nitrogen fixation and the balance as nitrate [39]. Microalgae have been broadly applied for nutrient removal in wastewater both as free cells [124] and in immobilized forms [125]. Application of microalgae in sustainable aquaculture, i.e. as bio-purifiers in the cleansing of aquaculture water for longitivity and reuse [40,109], is therefore based on the fact that microalgae can consume and/or assimilate nitrogenous substances, using them as sources of nitrogen, as operational in sewage wastewater treatment scheme [2,124]. These nitrogenous substances are the poisonous nitrogenous waste in aquaculture water that hamper sustainable aquaculture significantly [40,118]. Therefore, microalgae assimilate these nitrogen wastes for their normal growth while producing oxygen from photosynthesis to increase dissolved oxygen content in the aquaculture water [39,109,126,127].

Dissolved oxygen (DO) is a very important water quality parameter for fish cultivation and survival in aquaculture [127,128]. The minimum daily DO concentration in aquaculture ponds is therefore of great importance. DO affects the survival, growth, behavior, distribution as well as the general physiology of aquatic organisms [129]. The major physical sources of oxygen in water bodies are through atmospheric air, wind and wave actions. The principal biological source of oxygen is through

photosynthetic planktons such as microalgae [129,130]. Oxygen demands by fish varies among species, age, and culture conditions [130]. Generally speaking, a DO level >5 mgL^{-1} is required to adequately support a good fish production. DO between 1-3 mgL^{-1} could have sub lethal effects on the growth of most fish species and their feed utilization efficiency, while a DO of 0.3-0.8 mgL^{-1} is quite lethal to fish and could lead to total stoppage of fish feeding, increased stress and eventual disastrous fatalities [129,130]. To tackle this challenge of DO deficiency, some aquaculture systems adopt the use of either electrically or mechanically powered aerators or regular change of aquaculture water (using flow-through technology) which could be expensive [131-134].

In situ cultivated microalgae can simultaneously supply oxygen into the ponds through photosynthesis while they bio-remediate nitrogenous and phosphorous wastes or/and also serve as aqua-feed [40,127]. However, since consumption of oxygen by microalgae at night affects total DO in the pond, adoption of electrically powered lamps only at night for continuous photosynthesis and oxygen generation may be necessary to keep the DO within/above the acceptable limits. A schematic description illustrating the connections among the multiple applications of microalgae in aquaculture is presented in Figure 2.

Microalgae have been reported to be better efficient systems in nitrogen bioremediation than higher plants, partly because of higher rates of biomass production but also because microalgae do not have the large stores of structural carbon (i.e. cellulose) present in land plants [39]. The most important process that results in the loss or transformation of ammonia is its uptake or absorption by algae. Therefore, algae co-cultured with aquaculture animals can aid in the removal of ammonia [40,109] and other nitrogenous waste while producing useful biomass simultaneously [39,40,109]. We demonstrated the efficiency of in situ microalgal application in reducing or totally eliminating some toxic nitrogenous waste in *Clarias gariepinus* (African catfish) seedling's aquaculture with interesting results [40] (Table 7). While Ge et al. [109] reported similar *in situ* concept in shrimp's aquaculture (Table 7).

Table 7: Microalgae in bio-purification and bioremediation of aquaculture water

Kind of aquaculture	Microalgae species used	Type of purification	Effects	References
African catfish(*Clarias gariepinus*)	*Chlorella lewinii* & *Scenedesmus dimorphus*	*In situ* bio-purification	Reduction and/or total elimination of toxic ammonia and nitrite in the nursery ponds.	[40]
Shrimps (*Litopenaeus vannamei*)	*Platymonas helgolandica, C. vulgaris, Chaetoceros mulleri*	*In situ* bio-filtration/ bio-purification	Regulation of total ammonia nitrogen (TAN) and nitrite nitrogen within recommended levels.	[109]
Silver Sea Bass (*Lates calcarifer*) wastewater treatment	Co-culture *Chlorella* sp. & effective microorganisms	Bioremediation of organic matter	Total removal of ammonia and phosphorus by day 7.	[136]
Rainbow trout (*Onkhorynchus mykiss*) wastewater treatment	*Oocystis*sp.	Bioremediation of organic waste matter	Total removal of ammonia; 70% of phosphate removal.	[137]
Tilapia fish	*Chlorella vulgaris* & *Oscillatoria okeni*	Bioremediation of organic matter waste	Reduction of TAN and nitrite concentrations to 0.01 mgL^{-1} in effluent	[138]
Nile Tilapia (*Oreochromis niloticus*) aquaponics system with [1]RAS	*C. vulgaris* & *Tetradesmus obliquus*	Bioremediation of organic waste	99.7 and78.7%; 99.7 and 97.0% removal of nitrate and phosphate from sterile and non-sterile samples by *C. vulgaris,* respectively. 69.3 and 80.6%; 99.7% removal of nitrate and phosphate from sterile and non-sterile samples by *T. obliquus,* respectively	[135]

[1]Recirculating Aquaculture System (RAS).

Another potential application of microalgae in this industry is their use in the recycling of used aquaculture wastewater to facilitate future reuse as obtainable in some recirculating aquaculture systems, RASs (Figure 2) [135]. This, of course, will go a long way in boosting up food production (especially aquaculture products) in areas of the world where there are total and/or seasonal water shortage and water scarcity emanating from several reasons. A similar concept of bioremediation (Figure 2) was explored by Lananan et al. [136] and Riano et al. [137] in the treatment and removal of organic matter from aquaculture wastewater using a consortium of microalgae and other effective microorganisms (Table 7).

A secondary or "by-product" benefit of microalgal bio-purification application in aquaculture is a simultaneous pH bio-stabilizing effect [40]. High acidic and alkaline pH values affect the growth and survival of fish (especially fry because of their large surface area to volume ratio) in aquaculture systems. Uzoka et al. [139] demonstrated that there was 100% mortality of *C. gariepinus* fry at acidic pH 2–3 and alkaline pH 10–11 by day 2 of their experiment. They further reported increasing survival rate of fry as the pH approached 7 and 8, with pH 7 giving the best growing condition. We also observed similar effect of alkaline pH on the fry of *C. gariepinus*. We discovered that the mortality rate of *C. gariepinus* increased as the pH of the control aquaculture ponds without microalgae increased, resulting largely from ammonia accumulation [40]. Although both high acidic and alkaline pH are detrimental to fish survival in aquaculture, alkaline pH is the most encountered case since it largely results

from ammonia accumulation which is a very common aquaculture waste.

Ammonia is very soluble in water, producing hydroxyl ions on dissolution [140]. It is both the primary waste in aquaculture [120,126] and the primary nitrogenous source of eukaryotic microalgae [141]. Therefore, a system using microalgae as bio-purifiers is sure to give pH stability as the ammonia responsible for pH increase and fluctuations is assimilated by the microalgae (Figure 2). We applied this concept in our *C. gariepinus* aquaculture study. *Scenedesmus dimorphus, Chlorella lewinni*, and the co-culture of *S. dimorphus* and *C. lewinni*, that were used in our experiment, gave very minimal pH fluctuations from the

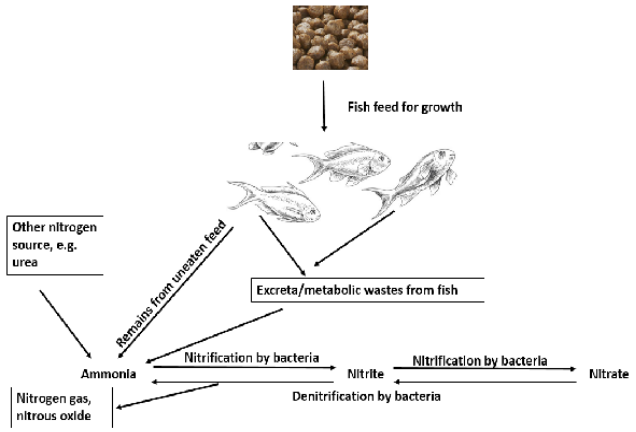

Figure 1: Nitrogen cycling in aquaculture ponds

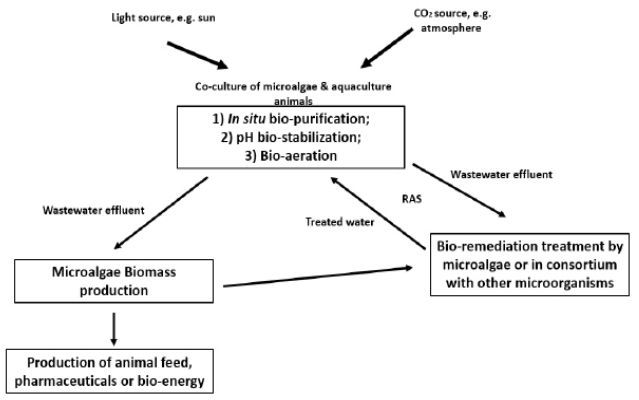

Figure 2: Schematic illustration of microalgae applications in aquaculture.

initial 6.3 ± 0.03 to 6.7 ± 0.6, 6.5 ± 0.2 and 6.4 ± 0.1, respectively, throughout the period of the study. However, the control (without microalgae) peaked at 9.0 ± 0.06 which was detrimental to the health and survival of the fish seedlings [40], and as previously reported by Uzoka et al. [139].

Microalgae as feed and/or feed supplements of livestock

FAO [142] reported that human demand for animal-derived products will be doubled by 2050 because of global population rise coupled with increase in income. This definitely will place a big pressure on food such as corn and soybeans supply, which are popular conventional animal feedstuffs [28,143]. This therefore necessitates an adequate and sustainable replacement of these livestock ingredients. Microalgae present an interesting alternative that is rapidly gaining reputation in livestock feed substitution [144,145], and have been applied in the supplementation and replacement of some ingredients in feeds of poultry, ruminants, and non-ruminants like pig and rabbits.

There is currently high interest in the application of microalgae in poultry feed. Poultry has been reported to be the most consumed source of meat in some parts of the world such as the United States of America and Europe with an annual average per capital consumption of 38 and 22 kg, respectively [146-148]. Therefore, there are currently research projects on how to improve the quality of poultry meat and enhance egg production. Microalgae feed supplementation has produced favorable results in both meat and egg production and quality in a good number of practical meal replacement studies (Table 8).

In broiler farming for example, algae-derived n-3 LCPUFAs were reported to be very efficient in supplementing broiler's diet. It led to better bird productive performance and improved fatty acid composition [147] as well as promoted carcass yield [148] when compared to other meals tested. However, meat acceptability was negatively affected (especially at 7.4% DHA inclusion level) due to reduction in meat oxidative stability [147,148]. This was addressed by selenium-*Chlorella* supplementation as demonstrated by Dlouhá et al. [149]. A good number of other studies have demonstrated that microalgal supplemented diets do not result in significant differences in broilers' features and meat quality when compared to diets without microalgae (Table 8). Some other studies have however reported that microalgae improved broilers quality and performances (Table 8). *Spirulina* is a regular choice microalga in broiler's meal supplementation and its application has resulted in a good number of positive attributes (Table 8).

Microalgae have also been applied in the supplementation of layer's diet. Several microalgae such as *Porphyridium* sp. [150], *Chlorella* sp. [151], *S. platensis* [152], *Schizochytrium* sp. [153], and *Nannochloropsis oceanic* [36] have been used to improve egg quality of layers. There were no significant differences observed in layers' body weight, feed intake and feed conversion rate (FCR), number and weight of eggs produced and some other features when compared with the control treatments without microalgae (Table 8). Some other studies have also demonstrated that microalgae are efficient in improving layers' health status as well as improving their egg quality compared to conventional meals (Table 8). Similar improvements were also reported in Pekin duck whose diets were supplemented with commercially fermented *Chlorella vulgaris* at inclusion levels ranging from 0 to 0.2% [154]. These studies and a good number of others have successfully demonstrated microalgae as good feed supplements in poultry.

Supplementation of diets of some ruminants such as sheep and cows (both dairy and meat producing ones) with microalgae has also been reported to improve their productivity. This is due to the importance of n-3 LCPUFA (especially EPA and DHA) mostly in humans (being the final consumers of dairy products) and the preference of natural nutritional supplements [160]. The use of microalgae therefore comes handy as an interesting substitute over cod-liver oil [161], fish and linseed oil [162] that have been adopted overtime. Microalgae were reported to both increase milk production and quality (by increasing its n-3 LCPUFA concentrations) (Table 9). Lamb's meat quality has also been improved beyond the conventional feed treatments using different species of microalgae (Table 9). EL-Sabagh et al. [163] reported quite a good number of such improvements in final body weight and daily live weight gain, feed intake and FCR, total white blood cell and hemoglobin count, serum globu-

Table 8: Application of microalgae in poultry's feed.

Kind of bird	Microalgae species used	Inclusion level (%)	Effects	References
Broiler	*Spirulina platensis*	0.5, 1[1]&1.5	Increased in FCR, body weight and intestinal villi length were more than the control.	[155]
Broiler	*Spirulina* sp.	4 & 8	No significant difference in total body weight gain, internal organs weight gain, FCR and mortality rate among all treatment categories; Increasing yellowness of fillet with increasing microalga inclusion.	[156]
Broiler	*S. platensis*	0.5 & 1.0	No significant difference in gained body weight, FCR, mortality rate and meat lipid oxidation among the treatment groups and control.	[157]
Broiler	*Spirulina* sp.	6, 11, 16[1]& 21	No significant difference in feed intake, final bird weight and live weight gain among all treatments (except 21%) and the control; Higher digestible methionine in algae diets compared to control; 16% gave the highest digestible cysteine and lysine compared to other treatments; 21% gave a nominally low production rate and increased temperature of hot pellet compared to other treatments.	[158]
Broiler	[2]*Chlorella vulgaris*	1	No significant difference in feed intake and conversion among the treatment groups and control; Significant increase in the concentration of plasma IgA in all *Chlorella*-supplemented treatments (CST) compared to antibiotics growth promoters (AGP) and control; Significant higher bird weight (BW) in CST and AGP compared with the control; FLC best improved BW, immunity and *Lactobacillus* production in intestine.	[159]
Layer	*Porphyridium* sp.	5 & 10	No difference in BW, egg number and egg weight among treatment groups and control; Significant lower serum and egg yolk cholesterol levels but increased linoleic and arachidonic acids levels in egg yolk compared to control; 10% reduction in feed consumption among treatments groups compared to control.	[150]
Layer	*Chlorella*	2 & 10	Improved hen's laying capacity and egg's morphological features; Increased intensity of yolk pigmentation by 2.5 units by Roche's scale compared to control.	[151]
Layer	*S. platensis*	1.5, 2 & 2.5	No significant changes in feed intake and FCR; egg production and weight; yolk index and Haugh unit; shell thickness and weight; specific gravity and yolk cholesterol among treatment groups and control; Significant increase in egg yolk colour in treatment groups compared to control.	[152]
Layer	*Schizochytrium*	0.5 & 1	Higher egg production with 1% at 44-46 weeks; Increased egg yolk colour, shell thickness and DHA compared to control; Reduced serum triglyceride and cholesterol compared to control.	[153]
Layer	*Nannochloropsis oceanica*	3 & 5	No change in BW, egg production rate and weight compared to control; Increase in n-3 fatty acids in yolk and plasma with increasing inclusion levels.	[36]

[1]Optimal microalgal inclusion level.
[2]The *Chlorella vulgaris* used is in three forms: Dried *Chlorella* powder (DCP); *Chlorella* growth factor (CGF); and fresh liquid *Chlorella* (FLC).

Table 9: Application of microalgae in ruminant's feed

Kind of ruminant	Microalgae species used	Inclusion level (%)	Effects	References
Cow	*Arthrospira* (*Spirulina*) *platensis*	~ 3	Fatter cows (8.5-11%) obtained in treatment group than in control; Average more milk (34 kg) produced per day from treatment group than control.	[164]
Cow	*Schizochytrium* sp.	3.97	Presence of more conjugated linoleic acids, n-3 LCPUFA (particularly DHA) and trans-vaccenic acid; and lower concentrations of total saturated fatty acids in treatment groups with microalgae compared to control.	[160]
Cow	*S. platensis, Chlorella vulgaris* & *Nannochlopsis gaditana*	-	No effect on the quantity of dry matter (DM) but on DM intake, DMI, (due to poor palatability of microalgae diets) in treatment groups compared to control; No significant differences on arterial concentrations (of histidine and methionine), nutrients' digestibility, and milk or energy corrected milk yield among treatment groups and control; Significant increase in milk fat, arterial acetic acid and non-esterified fatty acids concentrations in microalgal treatment groups compared to control.	[145]
Lamb	[1]DHA-Gold™	1.92	No difference on performance, carcass weight and [a]GR fat content among the treatment group and control; EPA and DHA were significantly greater in microalgae treatment group than in control.	[165]
Lamb	*Schizochytrium* sp.	1, 2 & 3	Similar daily DMI, average daily gain (ADG), gain to feed ration (G:F), wool yield and quality among treatment groups and control; Similar carcass features except thickness of body wall that increased which increased quadratically with increasing inclusion levels; Significant increase in EPA and DHA in adipose tissues with increasing inclusion levels; Decreased SFA:PUFA ration with increasing inclusion levels.	[166]

Lamb	*Schizochytrium* sp.	3.89	No significance effect on carcass traits except a trend tilting to greater adipocyte diameter in microalga treatment group compared to control; Increase in EPA, DHA and α-linolenic acid in treatment group than in control; Negative effect on meat quality with higher lipid oxidation and lower ratings for odor and flavor in microalga treatment group than in control; Lower AVG and greater slaughter age in treatment group compared to control.	[167]
Lamb	[2]DHA-Gold™	2	Modification of fatty acid composition in all studied anatomical locations in treatment groups compared to control; Increased DHA and total n3 fatty acids in intramuscular fats of treatment group than in control.	[37]

[1]GR site: This is the depth of muscle and fat tissue located from the surface of the carcass to the lateral surface of the twelfth rib 110-mm from the midline usually measured with a GR knife.
[2]A commercial microalga produced by Martek Biosciences Corporation, Maryland, USA.

lin as well as vitamin A using *S. platensis* to supplement lambs' feed. Furthermore, traits such as cholesterol, aspartate amino transferase, alanine amino transferase and blood glucose were significantly reduced in the study.

Microalgal supplemented meal research trials seem to be on the increase in popular non-ruminants, such as pigs and rabbits. There are several studies demonstrating how best to improve weaned piglets' health [168,169] and increase the quality of pork meat produced [170-173] with different species of both fresh and defatted microalgae (Table 10). On the other hand, rabbits are known zootechnical herbivores rich in the production of meats with LCPUFA [174], and this has led to the increased interest in the use of antioxidants in their feed formulation [175]. Microalgae being a natural source of exogenous antioxidants have been tried in several studies. These serve not only as a source of antioxidants but also supplement other nutrients and improve several important rabbit's features as well as the final meat quality [174-177] (Table 10). Peiretti and Meineri [176] reported a high maximal *S. platensis* inclusion level of 10% in rabbit meal that gave the highest feed intake while also noting no significance differences in weight gain and feed efficiency. However, dry matter, organic matter, crude protein, gross energy, neutral detergent fibre (NDF), acid detergent fibre (ADF) and digestibility of their control feed were higher than those supplemented with *S. platensis.*

Challenges

The major challenge with achieving a total microalgae adoption for feed in aquaculture and animal husbandry is the high cost of microalgae biomass production [28-30]. Although the large scale markets (e.g. the commodities and energy markets) have the potential of absorbing a very huge amount of microalgae biomass (reaching up to 104 $ktyr^{-1}$), the current price of feeds in the markets (i.e. €0.01–0.50 kg^{-1}) is still far below the current production cost of microalgae biomass [178]. The current production cost of microalgae which is about $7.7 kg^{-1} (i.e € 6.20 kg^{-1}) is still quite above the acceptable economic feasibility threshold (i.e. < 1 € kg^{-1}), thereby making microalgae biomass noncompetitive for animal feed industry [29,179]. To produce enough microalgae biomass for the aquafeed market at competitive prices (with a demand price tag < $5 kg^{-1}; i.e. ~€4 kg^{-1}) [179], several techniques and processes are being explored for reduced production cost. Some of these approaches include: adoption of efficient cultivation systems, use of wastewater as culture medium, as well as low cost but efficient harvesting methods.

Cultivation systems include the type of bioreactors and cultivation methods used for microalgae biomass production. This is very important as it largely determines the biomass productivity for any given medium used for the cultivation. Several bioreactors ranging from indoors to outdoor photobioreactors (PBRs) have been optimized for large scale production of microalgae biomass and various productivities have been reported under photoautotrophic and mixotrophic growth conditions (Table 11). Some of these bioreactors have also been adequately modified to increase the efficiency of biomass harvesting. Examples

Table 10: Application of microalgae in non-ruminant's feed

Kind of non-ruminant	Microalgae species used	Inclusion level (%)	Effects	References
Pig	*S. platensis*	[1]0.2, 0.5 & 2; [2]0.1 & 0.2	[1]No differences observed in performance from 0-14 days among treatment groups and control; Cubic response for ADG and average daily feed intake (ADFI) observed from 14-28 days with 2% inclusion level giving the greatest ADG among the microalga treatment groups compared to control; [2]No differences in ADG and ADFI observed among the treatment groups and control; Significantly better feed efficiency obtained in treatment groups than in control.	[168]
Piglet	*S.platensis*(SP) & *C. vulgaris* (CV)	1	No significant effect on ADG, ADFI &G:F of microalgae treatment groups compared to controls; CV significantly reduced diarrhoea incidence compared to SP, positive (antibiotics) and negative control groups; Significantly greater tract digestibility for gross energy, organic matter, dry matter and NDF in microalgae treatment groups than in the controls; Significantly greater villus height at jejunum in microalgae treatment groups compared to controls.	[169]
Pig	*Schizochytrium* sp.	0.25 & 0.5	No effect of microalgal supplementation on growth and slaughtering parameters; No significant differences in pH values, loin composition, meat colour, iodine number of subcutaneous fat and fatty acid composition among treatment groups and control.	[170]
Pig	Iodine (I)-enriched *Chlorella* spp.	2 mg Ikg^{-1}	Significant higher iodine concentration in muscle tissues, thyroid and serum compared to KI supplemented diet (at the same inclusion value); No significant difference in meat quality traits between microalga supplemented group and that of KI.	[171]

Pig	*S.platensis*	0.2	9.26 and 2.02% higher average daily weight gain and carcass output, respectively, in treatment group compared to control; 0.33% lower intramuscular fat in the control compared to treatment group.	[172]
Pig	*Schizochytrium* sp.	0.3, 0.6 & 1.2	More DHA in algae treatment groups compared to linseed oil and soybean oil treatment groups; No significant differences in consumer sensory analysis among all the groups; More lipid oxidation in algae treatment group than in control.	[173]
Rabbit	*Schizochytrium* sp.	0.4	Similarity in reproductive efficiency, slaughtering and zootechnical performances of rabbits; Influence of both loin and thigh's lipid content by administered algae diet.	[174]
Rabbit	*Arthrospira platensis* (*Spirulina*)	5	No effect on apparent feed intake, daily weight gain, mortality, morbidity, digestibility of dry matter, acid digestibility fibre, organic matter, digestible and gross energy in treatment groups compared to control. Lower crude protein (CP) total tract apparent digestibility in algal treatment group compared to control.	[177]
Rabbit	*S.platensis*	1	No effect on digestibility of dry matter, organic matter and gross energy among treatment groups and control; Increase in CP digestibility in algae treatment groups than in control.	[175]

[1]Experiment 1: A total number of 203 pigs used in a 28-day growth trial.
[2]Experiment 2: A total number of 180 weaning pigs used in a 42-day growth trial.

Table 11: Bioreactors and their impact on microalgae biomass production

Name of Bioreactor	Type of Cultivation	Description/Capacity	Microalgae cultivated	Impact on biomass production	Reference
Photobioreactors/ Raceway circulatory system combined with alkaline-CO_2 capturing medium	Indoor batch cultivation	Consists of: (i)12 cylindrical glass photobioreactors (PBRs) of 4-L capacity, each with length and diameter of 100 and 8 cm, respectively, arranged in series; (ii) 1,000-L raceway; and (iii) a circulation pump	*Chlorella* sp. ATI	Doubled biomass production at pH 11; 50% and 1.2 kgd^{-1} CO_2 utilization and fixation rate, respectively	[197]
	Outdoor semi-continuous cultivation	Consists of : (i)12 cylindrical glass PBRs of 50-L capacity, each with length and diameter of 250 and 16 cm, respectively, arranged in series; (ii) 10 tons raceway; and (iii) a circulation pump			
Horizontal photobioreactor (HPR)	Semi-continuous cultivation	Made of inexpensive transparent polyethylene sheet and measures 133.5 by 68 cm with 5cm deep raceway	*Nannochloris atomus* Butcher CCAP 251/4A	High biomass concentration and productivity of 4.0 gL^{-1} and 12.9 $gm^{-2}d^{-1}$ for indoors; and 4.3 gL^{-1} and 18.2 gm^{-2} for outdoor cultivation, respectively	[198]
A spraying adsorption tower merged with an outdoor open raceway pond	Outdoor batch cultivation	Spray measuring 1.8 m high and 0.8 m in diameter with two top spraying nozzles. Towel was made from poly-methyl acrylate.A culture volume of 8000-L.	*Chlorella pyrenoidosa*(FACHB 9)	Maximum biomass productivity and yield of 0.114 $gL^{-1}d^{-1}$ and 0.927 gL^{-1}, respectively; 50% peak CO_2 fixation efficiency	[199]
Flat plate air-lift PBR with broth circulation guides	Mixotrophic batch cultivation	Uses reflective broth circulation guides to increase mass transfer and light distribution inside a 4-L PBR.	*Desmodesmus subspicatus* LC172266	Increased biomass and lipid productivities to >1.5 and 0.217 $gL^{-1}d^{-1}$, respectively	[200]
Attached cultivation PBR	Indoor and outdoor phototrophic cultivation	[1]PBR consists of algae chamber (0.3x0.4x0.1 m) with inserted glass plates (0.3x0.1 m); there is adjacent gap of 0.02-0.06 m among glass plates. Aluminum foil is used to cover five faces of the glass chamber in order to isolate unwanted illumination leaving only one to receive light	*Scenedesmus obliquus*; *Botryococcus braunii*SAG 30.81; *Nanochloropsis* OZ-1; *Cylindrotheca fusiformis*	Good growth of both fresh water and marine microalgae; *S.obliquus* gave an outdoor biomass productivity of 50-80 $g/m^2/d$ which corresponds to 5.2-8.3% photosynthetic efficiency. *B. braunii* gave a biomass productivity of 5.7 $g/m^2/d$ which is 150% increase compared to the traditional glass PBR.	[183]
Aquarium PBR	Autotrophic and mixotrophic cultivation	Aquarium's dimension: 50.8 x 25.4 cm with fluid depth of 4 cm and total fluid volume of 10-L	*Chlorella vulgaris; Scenedesmus dimorphus*	Mixotrophic condition gave 2-3 times higher biomass concentrations than autotrophic condition for both algae; i.e. 75.2 gm^{-2} compared to 44.8 gm^{-2} for *C. vulgaris* in 9 days	[202]
Revolving algae biofilm (RAB) cultivation system	Continuous autotrophic cultivation	Consists of cotton duct fabric-made flexible cell material stretch around drive shafts to form either triangular or vertical configuration. System made up a 8.5 m^2 raceway pond retrofitted with 2 triangle of 6 vertical RAB systems	*Chlorella vulgaris* (UTEX #265)	302% average increase in microalga biomass productivity compared to that of standard raceway pond; 18.9 $gm^{-2}d^{-1}$ maximum biomass productivity (ash free)	[181,182]
Closed PBRs	Indoor and outdoor batch cultivation	10-L tubular methacrylate containers (0.65 and 0.125 m height and radius, respectively) was used in indoor cultivation. Two outdoor PBR: (i) 30-L polyethylene hanging bags (PHB) (0.20 x 1.0 m); (ii) 50-L polymethylmethacrylate bubble column PBR (BCP) (1.0 and 0.125 m height and radius, respectively)	*Phaeodactylum tricornutum*	Indoor PBR gave maximum growth performance of 16.66 x 10^6 at day 15 while that of the outdoor PHB and BCP were 3.90 x 10^6 and 5.13 x 10^6 at day 7 and 10, respectively	[203]

Continuous sequential heterotrophic/ autotrophic cultivation system	Heterotrophic and photoautotrophic cultivation	Heterotrophic phase consists of a 2.5-L mini-jar fermentor with a working volume of 2.0-L while the autotrophic phase consists of tubular PBR with 450 mL working volume	*Chlorella pyrenoidosa* C-212	High biomass concentration and protein content of 14 gL^{-1} and 60.1%, respectively	[204]
Rotating floating PBR (RFP)	Outdoor mixotrophic cultivation	RFP rotates on a water body by means of wave power, It has a PVC plastic axis (9 cm in diameter); 6 Plexiglas paddles (6 mm thick); and six 5-L barrels made of polyethylene terephthalate plastic filling the space between paddles	*Dunaliella tertiolecta* strain LB-999	Increased biomass productivity (3.10 $gm^{-2}d^{-1}$) and 4.61 % photosynthetic efficiency	[201]

[1]Type 2 prototype PBR adopted for mass cultivation, however, it is very similar to type 1in basic structure.

of such bioreactors include the attachment cultivation of microalgae systems [180], the revolving algae biofilm cultivation system [181,182], and the attached cultivation PBR [183]. These modifications have been demonstrated to help in cutting down the cost of microalgae biomass production.

Aquaponics have also been demonstrated to help in reducing the cost of producing microalgae biomass. This involves the use of nutrient-rich aquaculture water for algae cultivation [184,185]. The adoption of the narrow wavelength bandlight-emitting-diodes [186] and luminescent solar concentrators [187-189] over the conventional fluorescent lambs present interesting modifications of microalgae cultivation system that is helping to enhance microalgal growth and biomass productivity. Although these sources of light led to higher productivities, their large-scale application is yet to be demonstrated.

Different kinds of wastewaters have been demonstrated to be adequate nutrient sources for microalgae biomass production while the algae simultaneously bio-remediate the wastewater by consuming both the organic and inorganic pollutants (C,N, P) for growth (Table 12). However, since the microalgae of interest here is for animal feed production, not all forms of wastewater may be applicable for biomass production in order

Table 12: Use of wastewater as media for cultivation of microalgae.

Name/Source of wastewater	Microalgae cultivated	Effect/impact on microalgae	Reference
Concentrate wastewater and crude glycerol	*Chlorella vulgaris* UTEX2714	Average biomass productivity of 16.7 $gm^{-2}d^{-1}$ and 23.3% lipid content obtained in 34 days of semi-cultivation mode	[205]
Anaerobic digester effluent	*Scenedesmus* sp., *Chlorella sorokiniana* HS (KCTC12171BP), *C. vulgaris* &*Micractinium inermum* NLP-F014 (KCTC 12491BP)	Wastewater supported growth of microalgae giving similar biomass productivity with BG11 medium, however, *M. inermum* had the best effect from the effluent nutrients with such biomass and fatty acid methyl esters (FAME) productivity: 0.16 $gL^{-1}d^{-1}$ with 3.23 gL^{-1} of dry cell weight, and 0.04 $gL^{-1}d^{-1}$ with 27.54% (w/w) of FAME contents, respectively	[206]
Biodiesel wash water	[1]*Monoraphidium contortum, Ankistrodesmus* sp., *Chlorococcum* sp., Chlorophyceae species[2]	Four microalgae grew well with *M. contortum* giving the best growth capacity and the second highest fatty acid content (267.9 mgg^{-1} of dry weight)	[207]
Tilapia pond effluent	*C. vulgaris* & *Oscillatoria okeni*	*C. vulgaris* gave the highest growth capacity and rate of ~4.0$gL^{-1}d^{-1}$ and 0.58 d^{-1}, respectively	[138]
Municipal wastewater & pig biogas slurry	*Chlorella zofingiensis*	8% slurry in wastewater gave significant effect in algae growth – 2.5 gL^{-1} biomass and 8% increase in lipid content - compared to BG11	[208]
Dairy wastewater	*Coelastrum* sp.	Maximum cell growth and lipid content of 2.71 gL^{-1} and 50.77 %, respectively, compared to the maximum biomass productivity of 0.281 $gL^{-1}d^{-1}$ obtained from semi-batch culture	[191]
Food & green waste compost	*C. vulgaris* FSP-E	25% compost mixture gave the best biomass, lipid and protein concentrations of 11.1, 10.1 and 2.0%, respectively, compared to modified BG11 medium	[193]
Dairy wastewater	[3]*C. zofingiensis, Chlorella* sp. & *Scenedesmus* spp.	*Chlorella* sp. gave biomass and lipid productivities of 674.3 and 142.2$mgL^{-1}d^{-1}$ while *Chlorella* sp./ *C. zofingiensis*/ *Scenedesmus* spp./ (1:1:1) consortium gave the highest biomass productivity of 758.9 $mgL^{-1}d^{-1}$ by day 7	[190]
Primary effluent (PE) & secondary effluent (SE) from meat-processing industry	*Scenedesmus* sp.	Biomass productivity in PE and SE was 1160 mgL^{-1} and 371 mgL^{-1} of volatile suspended solids, respectively while the highest lipid productivity (3.7 $gL^{-1}d^{-1}$) was recorded in PE	[194]

[1]Four of the microalgae (out of 11 species) that grew effectively in the biodiesel effluent.
[2]Unidentified species.
[3]Most were used in consortium.

Name/Source of wastewater	Microalgae cultivated	Effect/impact on microalgae	Reference
Anaerobic digestate of Municipal wastewater, sewage sludge & agro-waste	*C. sorokiniana, C. vulgaris, N. gaditana, Scenedesmus I* and *II* strains	Highest dry weight biomass density obtained for *C. vulgaris* and *Scenedesmus I* (2.0 – 2.5 gL^{-1}) with municipal wastewater effluent medium. Agro-waste gave over 300% lipid increase per volume in *C. vulgaris.*	[209]
Blend of 4 wastewaters: liquid digestate from compost, landfill leachate from rainwater, liquid from septic sludge & wastewater treatment plant effluent	[4]*Clorella* sp.	Maximum alga biomass of 22.76 $mgL^{-1}d^{-1}$ in a blend of 60% water, 19% treated effluent and 21% digestate.	[210]
Industrial wastewater and flue gas	*Chlorella* sp. & *Chlorococcum* sp.	Overall 1.7 times improvement in microalgae biomass productivity. *Chlorella* sp. recorded the highest biomass of 1.52 gL^{-1} on the fifth day of batch cultivation	[211]
Human and animal waste	*Chlorella singularis, Micractinium pusillum,* &*C. sorokiniana*	*C. sorokiniana* grew in all concentrations of animal & human wastes unlike *C. singularis* &*M. pusillum* which did not grow in some concentrations of human waste. *C. sorokiniana* gave a maximum growth rate and lipid production of 140 mg/L/d and 45.5 $mgL^{-1}d^{-1}$, respectively in poultry waste	[212]
Waste nutrient solution (WNS) from plant factory	*C. vulgaris* &*Acutodesmus* sp.	Both microalgae grew well in the wastewater, however, *Acutodesmus* sp. gave almost the same specific growth in WNS (0.685) as in standard OHM medium (0.673)	[195]
Digestate from agro-waste mixtures	[5]*Parachlorella kessleri, Acutodesmus obliquus, C. vulgaris* & *Tetraselmis tetrathele*	The biomass yield of *P. kessleri, A. obliquus, C. vulgaris* &*T. tetrathele* were 1.075, 1.117, 0.570 and 0.845 gL^{-1}, respectively, and fatty acids (FAs) content ranging between 3.9-24.5% by 25 days of cultivation	[213]
Raw and recycled dairy wastewater	*Scenedesmus quadricauda* & *Tetraselmis suecica*	Dry weight biomass of *S. quadricauda* and *T. suecica* after cultivation in raw and the recycled dairy wastewater were 0.43 and 0.58 gL^{-1}; 0.36 and 0.65 gL^{-1}, respectively, after 12 days for each setup	[192]
Piggery effluent	*Rhizoclonium* sp. & *Ulothrix* sp.	Dry weight mean biomass productivity of 31.1 $gm^{-2}d^{-1}$ (ash free). Total protein and carbohydrate contents of 43.4-45.0 and 42.8-54.8%, respectively.	[214]

[4]Used in a consortium with bacteria.
[5]Four out of the 7 microalgae strains that acclimatized to the waste medium.

to ensure the safety of the products. Wastewater from dairy products [190-192], aquaculture [136-138], agro-waste and food industries (18,193-196) have been reported to enhance microalgal biomass productivity and thus have great potentials as cheap nutrient sources for cultivating microalgae biomass for animal feed production.

Furthermore, the method adopted in harvesting of microalgae plays significant role in determining the final cost of production [7]. Several harvesting technologies ranging from mechanical to biological approaches use the following methods: centrifugation, filtration, flotation, magnetic separation and flocculation or a combination of them in microalgae harvesting [215-217]. Harvesting technique like centrifugation is not cost-effective, especially for large scale biomass production, because of cost of power and depreciation [178,179]. Filtration and a good number of other methods may also not be adequate for large scale biomass production due to several reasons as outlined by Lu et al. [217]. Flocculation have been evaluated to be a cheap and effective harvesting method for microalgae biomass [217-219] compared to most methods of harvesting. However, chemical flocculants may not be safe for harvesting microalgae biomass for animal feed because of possible effects of such chemical residues on animal's health [217]. Thus bioflocculants (flocculants of biological origins) have been extensively explored [220,221].

Microorganisms-assisted flocculation has been proposed by several researchers as a very effective and cheap alternative to both chemical agents and biologically derived polymers used as flocculants. This involves co-cultivation of the microorganisms with the microalgae or addition of the microbial culture to the microalgae at the point of harvesting. Several kinds of bioflocculation approaches have been demonstrated in some studies, these include: microalgae-bacteria [222,223], microalgae-fungi [224-226], and microalgae-microalgae [227]. For bioflocculation, the safety of the microorganism employed must also be considered. It is important to note that no one method is suitable for all species of microalgae and for all scale of production. We therefore summarized some possible process pathways for achieving cost-effective microalgae biomass production in animal feed production in Figure 3. We believe that the collaboration of several industries in need of one or more components of microalgae would drastically reduce the cost of production to the economic feasible price.

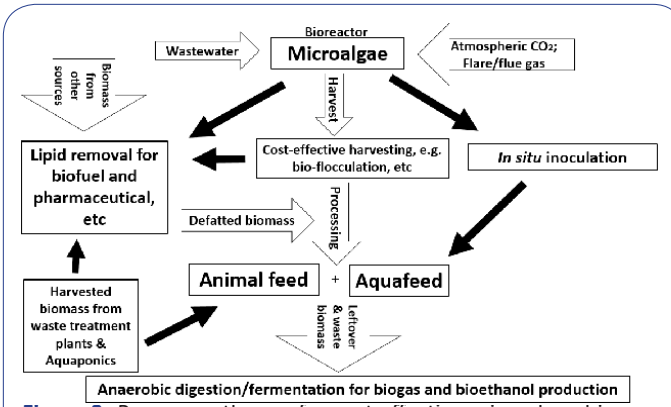

Figure 3: Process pathways for cost-effective microalgae biomass production for animal feed

Another challenge aside the basic limitation caused by microalgae relative scarcity and high market price [106], is the problem of meal palatability [228]. This may result to a cascade of several other secondary negative effects depending on the microalgae inclusion levels. Walker and Berlinsky [228] used *Nannochloropsis* sp. and *Isochrysis* sp. to feed juvenile Atlantic

cod at inclusion levels ranging from 0 to 30%. They reported that the feed intake (and consequently growth) of the tested fish decreased with increasing microalgal inclusion level, possibly due to palatability problem of the algae supplemented feeds. This problem eventually manifested to an almost starvation when the inclusion level was increased to 30%. Davies et al. [229] also reported the negative effect of the alga, *Porphyra purpurea*, on the growth of *Chelon labrosus* (grey mullet), though such reduction was not directly linked to feed palatability. Tackling this problem of palatability of microalgal diets, Vizcaíno et al. [77] had to include 5% squid meal to their microalgal treatment diets for gilthead sea bream.

Some studies have reported the problem of antinutritive components in some microalgae diets which may possibly lead to several forms of interferences in digestive processes [230]. However, a whole lot of other researchers did not acknowledge the presence of antinutritional components in their microalgal substituted fishmeal trial experiments. Varying results obtained with microalgae incorporated diets in several studies were summarized by Vizcaíno et al. [77] to be due to a number of factors which include: the type of fish and microalgae tested, the inclusion level of algae adopted as well as the original nutritional composition of the supplementing algae.

The challenge of nutrient digestibility and availability to aquaculture animals resulting from algal rigid cell wall was addressed by Teuling et al. [231]. They examined several physical and mechanical techniques of microalgal cell wall disruption to enhance their *in vivo* nutrient digestibility using *Nannochloropsis gaditana* and the juvenile of Nile tilapia (*Oreochromis niloticus*). They reported that the mechanical treatment (bead milling) gave the highest nutrient (protein, fat, dry matter, ash, calcium and energy) digestibility. There was increase in ADC of both fat and protein from 50 to 82% and from 62 to 78%, respectively, in the study. This agrees with an initial study by Tibbetts et al. [232] in which homogenization was used to increase the ADC of *Chlorella* sp. protein from 79.5 to 85.4% in Atlantic salmon. Furthermore, Gong et al. [108] reported that extrusion of microalgae substituted feed through a twin-screw cooking extruder significantly increased their meal digestibility. Ultimately, companies producing animal feed from such microalgae with recalcitrant cell wall apply enzymes such as Carbohydrate-Active enzymes (CAZymes) and proteases to process the algal biomass making the feed better adsorbable by animals [28,30].

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