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Review

A Review on the Use of Microalgae for Sustainable Aquaculture

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Abstract: Traditional aquaculture provides food for humans, but produces a large amount of wastewater, threatening global sustainability. The antibiotics abuse and the water replacement or treatment causes safety problems and increases the aquaculture cost. To overcome environmental and economic problems in the aquaculture industry, a lot of efforts have been devoted into the application of microalgae for wastewater remediation, biomass production, and water quality control. In this review, the systematic description of the technologies required for microalgae-assisted aquaculture and the recent progress were discussed. It deeply reviews the problems caused by the discharge of aquaculture wastewater and introduces the principles of microalgae-assisted aquaculture. Some interesting aspects, including nutrients assimilation mechanisms, algae cultivation systems (raceway pond and revolving algal biofilm), wastewater pretreatment, algal-bacterial cooperation, harvesting technologies (fungi-assisted harvesting and flotation), selection of algal species, and exploitation of value-added microalgae as aquaculture feed, were reviewed in this work. In view of the limitations of recent studies, to further reduce the negative effects of aquaculture wastewater on global sustainability, the future directions of microalgae-assisted aquaculture for industrial applications were suggested.

Keywords: aquaculture; microalgae; wastes treatment; resource utilization; sustainability

1. Introduction

In recent years, the depletion of wild fishery resource is driving the fast development of aquaculture worldwide [1]. In some countries, the farming of aquatic animals has surpassed the yield of wild fisheries [2]. It is expected that in the coming future, aquaculture will become the main industry providing aquatic products to human beings. However, with the continuous expansion of the scale of aquaculture and the increased production, water pollution has become a serious problem posing threats to the environmental protection and hindering the sustainable development of aquaculture [3,4]. Moreover, in aquaculture practice, with the water deterioration, high incidence of diseases would also increase the commercial risks of the whole industry [5].

To overcome the aforementioned problems, a lot of efforts were devoted into aquaculture to control wastewater pollution and improve survival efficiency of aquatic animals. The most common and straightforward method to control the pollution of aquaculture wastewater pollution is using traditional environmental remediation technologies, such as aeration, filtration, and anaerobic-anoxic-oxic (A^2O) system, to remove nutrients in wastewater [3,6,7]. In a real-world application, the treatment of wastewater by these technologies with high energy consumption or investment improves the total cost of aquaculture and increases the financial burden of industry [3,8]. By traditional technologies, nutrients, including nitrogen, phosphorus, and carbon, in wastewater could not be fully utilized and recycled as resources. Some technologies may produce a large amount of carbon dioxide and sludge, causing secondary environmental pollution [9]. In aquaculture, antibiotics and medicine are commonly used as a feasible way to prevent animals' diseases and reduce aquaculture risks. However, the overuse of antibiotics or medicine may negatively impact the meat quality of aquatic animals and cause food safety problems [10]. The accumulation of residual antibiotics or medicine in the water body may also become a trigger of antibiotics-resistance and even some ecological disasters [11]. Therefore, in recent years, interests in the development of economically feasible and environmentally friendly technologies to deal with the problems occurring in aquaculture are growing worldwide.

Microalgae, which could efficiently assimilate nutrients in a eutrophic water body, have been proven to be a good way for wastewater remediation [12,13]. The great performance of microalgae for nutrients assimilation has been widely observed in the remediation of food industry effluent, agricultural waste stream, municipal wastewater, and many other types of wastewater [14–16]. In recent years, more and more studies confirmed the beneficial role of microalgae in aquaculture wastewater treatment [17,18]. In addition to treating wastewater, microalgae could synthesize value-added components, including protein, lipid, and natural pigments. Previous studies have successfully applied various microalgal species, such as *Chlorella* sp., *Dunaliella* sp., and *Scenedesmus* sp., for the production of value-added biomass, which could be exploited to partly replace aquaculture feed and enhance the immunity of aquatic animals [15,18,19]. Last but not the least, microalgae with a high capacity of generating oxygen could act like a bio-pump for aeration in aquaculture and adjust the microbial community in a water body [20]. Thus, the water quality in aquaculture practice could be properly controlled to avoid algal bloom or oxygen depletion. Owing to aforementioned benefits, the use of microalgae for aquaculture wastewater remediation has recently emerged into the limelight.

In recent years, the concept of using microalgae in aquaculture has been proposed and a lot of efforts are devoted to promote the industrial implementation of microalgae-assisted aquaculture [19,21]. This work provides a state-of-the-art review on the use of microalgae, which plays important roles in water quality control, aquaculture feed production, and nutrients recovery, for the sustainable development of the aquaculture industry. This paper also focuses on the technologies and mechanisms related with microalgae-assisted aquaculture. Finally, challenges and prospects of the integration of microalgae with aquaculture are discussed. It is expected that the industrial implementation of microalgae technology in the near future could be a promising way to overcome problems in traditional aquaculture and upgrade the whole aquaculture industry for global sustainability.

2. Progress of Traditional Aquaculture

Although traditional aquaculture made a great contribution to the supply of aquatic products, problems associated with environmental protection and food safety seriously limited its sustainable development in the future. Problems commonly occurred in the traditional aquaculture industry include water deterioration and antibiotics abuse [9].

2.1. Problems in Aquaculture

Water deterioration, which refers to oxygen depletion, harmful algal bloom, and eutrophication of water body in aquaculture, may lead to the failure of aquatic animals rearing and even cause serious environmental disasters [9,22,23]. As shown in Figure 1, the water deterioration mainly occurs in three aspects: (1) The addition of an excessive amount of traditional aquaculture feed, which consists of biomass rich in protein and lipid, may not be fully eaten by aquatic animals and the residual feed would be converted to soluble nutrients, driven by some bacterial activities, partly contributing to the eutrophication in water body. (2) The water deterioration is attributed to the wastes secreted by aquatic animals. In the aquaculture with high stocking density, this could be the main reason for water deterioration. The mechanisms of water-deterioration induced animals' diseases or death have been widely documented by previous studies [24,25]. One of the key mechanisms is that elevated NH_4^+ comes into cells and displaces K^+, causing the depolarization of neurons and the activation of the N-methyl-D-aspartate (NMDA) receptor, which leads to an influx of excessive Ca^{2+} and subsequent cell death in the central nervous system [26]. (3) In the closed aquaculture system, eutrophication would accelerate the microbial reproduction and cause harmful algal bloom. A previous study discovered that some harmful algal bloom species, particularly cyanobacteria, could consume oxygen, at the same time, produce toxins [23]. As a result, owing to the oxygen depletion, bacterial reproduction, and toxin accumulation, survival and health of aquatic animals would be seriously threatened. Even if the water deterioration does not cause the failure of aquaculture, aquatic animals with diseases or toxins may cause serious food safety problems and negatively impact human health. Therefore, the negative effects of aquaculture problems on environmental protection and human health merit attention from both academia and the industry.

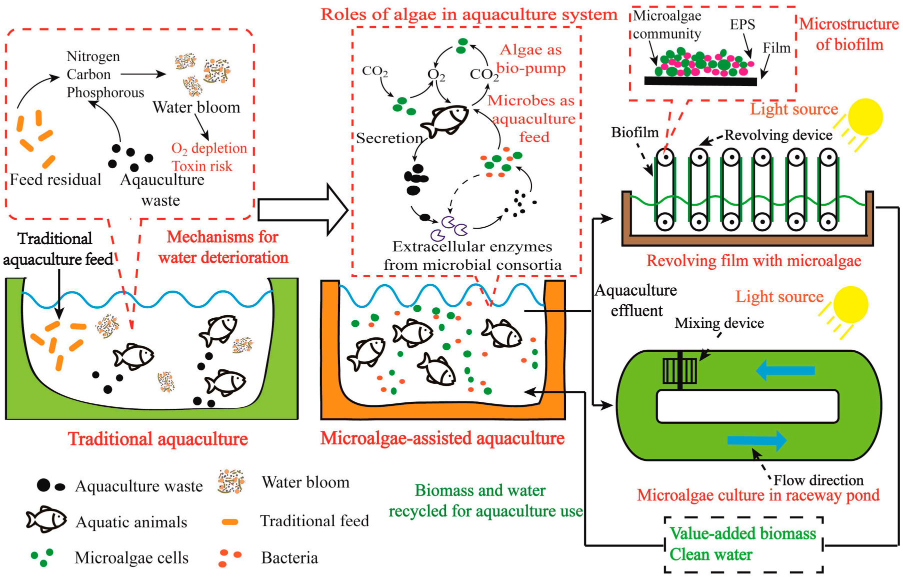

Figure 1. Comparison of conventional aquaculture and microalgae-assisted aquaculture.

2.2. Conventional Technologies and Solutions

2.2.1. Control of Water Quality

The most straightforward methods to control water quality in aquaculture include reducing the stocking density and frequent water replacement. However, in the aquaculture industry, owing to the low profitability and the high cost, the applications of these two methods are limited.

Wastewater treatment for water reuse is a possible way to reduce water replacement frequency and control the operation cost of the aquaculture system [7,27]. Technologies commonly applied for the aquaculture wastewater treatment include anaerobic treatment and aerobic treatment, which performed well in wastes removal and water purification. For example, Boopathy et al. (2007) reported that removal efficiency of chemical oxygen demand (COD) of sequencing batch reactor (SBR) under aerobic condition reach 97.34% in eight days [27]. In the study of Mirzoyan et al. (2008), aquaculture sludge was subjected to anaerobic treatment for methane production and up to 70% sludge-mass reduction was demonstrated [28]. However, from the perspective of nutrients recovery, these traditional treatment technologies are not highly recommended [9]. For example, organic carbon in aquaculture wastewater is converted to CO_2 and CH_4 by aerobic and anaerobic treatment. As a result, the aquaculture wastewater is treated at the expense of greenhouse gas emissions and the resources in wastewater could not be efficiently reused. An artificial wetland was considered as an environmentally friendly way to recover nutrients from aquaculture wastewater and improve water quality, however, its commercialization is hindered by the large ground occupation area and the high management cost. Longo et al. (2016) reported that the maintenance of a high content of dissolved oxygen (DO) by aeration is a technically feasible way to improve the stocking density, but the high electricity consumption of the aeration process increases the aquaculture cost [8]. Therefore, until now, the eco-friendly and economically promising technologies for water quality control have not been widely applied in aquaculture.

2.2.2. Use of Antibiotics or Medicines

Due to the unfavorable environment caused by water deterioration, in aquaculture practice, antibiotics or medicines are used to control the diseases of aquatic animals [29]. Commonly used antibiotics and medicines for aquaculture include ampicillin, oxacillin, penicillin, ceftazidime, cefazolin, and so forth [30]. However, the diseases in aquaculture are controlled by antibiotics at the expense of human health, environmental protection, and ecological stability. First, one of the problems created by the abuse of antibiotics is the presence of residual antibiotics in commercialized aquaculture products, of which the consumption is increasing continually in recent years. Second, antibiotics-resistance caused by the residual antibiotics has been considered as a trigger of ecological disasters. According to the survey of Hossain et al. (2012), in some cases antibiotics are no longer effective in treating bacterial diseases as the aquaculture pathogens have become antibiotics-resistant. Therefore, it is not a sustainable and eco-friendly way to control aquaculture diseases by the overuse of antibiotics or medicines. Previous studies mainly focused on the removal of antibiotics or medicines by conventional technologies, such as anaerobic and aerobic biological treatment processes [31,32], while did not devote much effort in the source reduction of antibiotics or medicines in aquaculture.

3. Microalgae-Assisted Aquaculture

3.1. Principles of Microalgae-Assisted Aquaculture

3.1.1. Principles

The concept of microalgae-assisted aquaculture is to convert organics in eutrophic effluents to biomass by microalgae growth and exploit value-added biomass to partly replace aquaculture feed and enhance aquatic animals' immunity. The construction of microalgae system also could accelerate the carbon dioxide fixation and promote oxygen release, acting like a bio-pump and creating a good environment for aquatic animals.

The specific scheme of microalgae-assisted aquaculture is shown in Figure 1. First, microalgae, which are inoculated into the fish rearing tank or pond, could enhance the self-purification capacity of aquaculture system by digesting some wastes secreted by aquatic animals and also act like a bio-pump to maintain the content of dissolved oxygen in water body. Second, microalgae cultivation system is constructed to assimilate nutrients in aquaculture effluent. Third, harvesting technology, which is suitable for the aquaculture system, is employed to obtain the microalgae biomass in an environmentally friendly and cost-saving way. Fourth, harvested fresh biomass is used as value-added aquaculture feed to reduce the fish rearing cost and treated effluent is recycled to the aquaculture system.

3.1.2. Advantages

According to the previous study, in a real-world application, the integration of microalgae with fish rearing not only brings technical advantages, but also creates economic benefits: (1) Oxygen production by microalgae alleviates the risks of oxygen depletion and reduces the energy consumption of traditional aeration devices. (2) Survival of microalgae in a fish rearing tank or pond may limit the growth of unfavorable or toxic microorganisms, creating a good environment for aquatic animals. Consequently, the water replacement frequency of a fish tank or pond would be minimized and the related cost would be reduced significantly. (3) As the immunity of aquatic animal is enhanced by the microalgae feed, overuse of antibiotics or medicines in aquaculture could be avoided, increasing the safety of aquaculture products and maximizing the market acceptance. Generally, pollution-free aquaculture products have much higher prices and larger market demands than traditional products. (4) The aquaculture effluent could be treated by advanced microalgae biotechnology at low cost.

Thus, the wastewater treatment cost that was paid by the aquaculture industry to sewage treatment plant in the past could be avoided, reducing the financial burden of industry to some extent. (5) As the harvested biomass is used to partly replace the traditional aquaculture feed, the cost of fish rearing could be controlled.

Therefore, considering the aforementioned advantages, it is expected that the aquaculture industry could be upgraded with the assistance of microalgae, bringing great benefits to factory, consumer and society. In a real-world application, to truly use microalgae for aquaculture wastewater remediation, some important issues should be considered. First, cost-saving cultivation systems for efficient biomass production are needed to grow microalgae for large-scale treatment of aquaculture wastewater. Second, microalgal strains with value-added components should be selected for both wastewater remediation and aquaculture feed production. Third, advanced harvesting technologies are needed to simplify the harvesting process and reduce the biomass cost. Fourth, effects of microalgae on the growth of aquatic animals and relevant mechanisms should be fully understood. Recently, researchers developed a lot of efforts in aforementioned issues and had a lot of important findings that merit attention from the fields of environmental protection and aquaculture.

3.2. Microalgae-Based Wastewater Remediation

3.2.1. Mechanisms of Wastes Assimilation

Nitrogen Assimilation

Nitrogen is one of the compositions in wastes secreted by aquatic animals and a high concentration of ammonium in a water body is unfavorable or even toxic to the aquatic animals. Common forms of nitrogen in wastewater include ammonium (NH_4^+–N), nitrate (NO_3^-–N), and nitrite (NO_2^-–N) [26,33]. Ammonium could be absorbed by microalgae cells through active transport and directly utilized for amino acids synthesis while nitrate and nitrite absorbed by microalgae through active transport have to be converted to ammonium by nitrate reductase and nitrite reductase before the further assimilation process [33]. In microalgal cells, amino acids are synthesized from ammonium through glutamine synthetase-glutamine oxoglutarate aminotransferase (GS-GOGAT) pathway and glutamate dehydrogenase (GDH) pathway. Since α-ketoglutarate, a metabolic intermediate of the Krebs cycle, is an essential substrate in both the GS-GOGAT pathway and GDH pathway for nitrogen assimilation, carbon metabolism and nitrogen metabolism are closely connected [34]. In microalgae cultivation, parameters of the C/N ratio, light intensity and quality, and carbon forms could be adjusted to enhance carbon assimilation, further promoting the nitrogen assimilation.

Carbon Assimilation

Carbon sources in aquaculture wastewater for microalgae growth include inorganic carbon (CO_2 and HCO_3^-) and organic carbon (saccharides and volatile fatty acids). As the assimilation of CO_2 or HCO_3^- is driven by photosynthesis, it is a feasible way to promote the fixation of inorganic carbon by creating favorable conditions, particularly light and temperature, for photosynthesis [35]. With the fixation of CO_2 and the release of O_2, content of dissolved oxygen (DO) in a water body will be increased, constructing an oxygen-rich environment for aquatic animals. Compared with inorganic carbon assimilation, organic carbon assimilation might be more complicated and time-consuming in some cases since some forms of organic carbon could be utilized efficiently by microalgal cells [36]. For example, owning to the large size, some insoluble solids rich in carbon could not be absorbed by microalgal cells directly [14].

To promote the carbon assimilation in aquaculture wastewater, the construction of algal-bacterial consortia has been widely studied [37,38]. In consortia, CO_2 released by bacteria could be utilized by microalgae for photosynthesis, which generates O_2 for heterotrophic metabolisms in bacterial cells. As described by Hernández et al. (2013), in wastewater remediation, bacteria convert indigestible carbon forms to digestible carbon forms, such as volatile fatty acids, amino acids, and glucose, by secreting extracellular enzymes. Meanwhile, digestible carbon forms could be assimilated by microalgae in an efficient way. Therefore, compared with pure microalgae system, algal-bacterial consortia have a much better performance in nutrients recovery.

3.2.2. Properties of Aquaculture Wastewater

Boyd (1985) reported that the production of one kilogram of live catfish releases about 51 g of nitrogen, 7.2 g of phosphorus and 1100 g of COD into the water body as organic wastes, which accumulates in the form of soluble nutrients or insoluble sludge [4]. According to the data in Table 1, aquaculture wastewater should be a good medium for microalgae cultivation and value-added biomass production. First, aquaculture wastewater is rich in essential macro-elements, such as nitrogen, phosphorus, and carbon, for microalgae growth. Since nutrients of aquaculture wastes are impacted by stocking density, water replacement frequency, feed addition, and some other factors, different nutrients profiles were observed (Table 1). In most cases, supernatant of aquaculture wastewater contains lower concentrations of nutrients than sludge obtained from the bottom of aquaculture system. Second, different from industrial effluent, municipal waste stream and mining wastewater, aquaculture wastewater contain much less toxic components, such as heavy metals [39]. On one hand, the stream without toxic components may create a good environment for microalgae growth. On the other hand, harvested microalgae without contamination by toxic components have the potential to be used for aquaculture feed production. Third, concentrations of NH_3–N and COD in aquaculture wastewater are not too high to threaten the survival of microalgae. As shown in Table 1, the highest concentration of NH_3–N was about 100 mg/L while ammonium toxicity for most Chlorophyceae occurs when the concentration of NH_3-N reaches 300 mg/L [40].

Table 1. Nutrients profiles of aquaculture wastewater.

Animal Type	TN (mg/L)	NH_3-N (mg/L)	TP (mg/L)	COD (mg/L)	Total Solids (g/L)	Reference
Shrimp	361	90	*NA*	1321	*NA*	[41]
NA [a]	1023.84	28.08	239.76	904.2	21.6	
NA	777.87	50.25	383.91	348.8	20.1	[28]
NA	533.42	23.84	458.92	2494	14.9	
Shrimp	>365	83.7	*NA*	1593	*NA*	[42]
NA	110.8	0.07	*NA*	19.7	*NA*	[43]
Shrimp	>395	101.7	*NA*	1201	13.1	[27]
Rainbow trout	1.18	0.27	0.19	17.6	0.01	[44]
Crucian carp	6	0.9	>0.7	*NA*	*NA*	[45]
Water eel	12.4	4.6	5.2	48	*NA*	Our lab [b]
Crucian carp	47.6	72.0	*NA*	368	1.02	Our lab [c]

[a] "NA" is short for "Not Available". [b] Aquaculture wastewater collected from water eel rearing factory. [c] Aquaculture wastewater collected from crucian carp rearing factory.

3.2.3. Microalgae Cultivation Systems

Although various microalgae cultivation systems have been developed for different purposes by previous studies, not all of them are suitable for microalgae-based aquaculture wastewater remediation. Generally, cultivation systems with high land utilization efficiency, a low investment cost, and high photosynthesis rate, have the potential to be used in aquaculture. Two systems, including a raceway pond and revolving algal biofilm (RAB), which meet the criteria mentioned above, have more advantages in the aquaculture industry.

Raceway Pond System

As shown in Figure 1, a raceway pond system consists of a closed circulation channel with a depth of 0.2–1.0 m and one or two paddle wheels drive the circulation of water body [41,46]. As the raceway pond system has been intensively studied in both lab research and an industrial application, a couple of problems have been identified, including: (1) Contamination risks of microalgae biomass by bacteria and other microbes are high; and (2) microalgae grown in a raceway pond system might be limited by unfavorable conditions, such as temperature fluctuation and light deficiency. However, compared with closed glass photo-bioreactors, a raceway pond system has a much lower investment cost but higher volume, making it more suitable for the treatment of aquaculture wastewater [47]. In addition, a raceway pond system has lower annual operating expense ($42.65 M) and yields lipids at a lower cost ($13/gal) than a photo-bioractor ($62.80 M and $33/gal) [48]. A life cycle analysis (LCA) conducted by Sfez et al. (2015) indicated that it is a sustainable way to use microalgae-bacterial flocs in a raceway pond system for aquaculture wastewater remediation and recycle biomass for aquaculture feed. As a lot of efforts have been devoted into the fundamental research and parameters optimization, recent studies are heading towards the demonstration scale of microalgae cultivation for either wastewater remediation or value-added biomass production.

Considering the turbidity and bacteria co-growth in aquaculture wastewater, in the demonstration scale of microalgae cultivation in the raceway pond system for aquaculture wastewater remediation, two critical factors should be considered. First, location and pond depth should be selected or designed reasonably to improve the light transmittance and photosynthesis rate. Second, the relationship between microalgae and bacteria in the raceway pond system should be fully understood. Van Den Hende et al. (2014) reported that microalgal bacterial flocs contributed to the removal of 28% COD, 53% BOD_5, 31% TN, and 64% TP in aquaculture wastewater (12 m^3 raceway pond), suggesting that with the establishment of beneficial cooperation in microbial community, threat of bacteria to microalgae growth in raceway pond system could be reduced to a low level. A similar phenomenon was reported by the studies on the interaction between microalgae and bacteria or fungi in other wastewater sources [49,50]. Therefore, in the application of a raceway pond for a microalgae-based aquaculture wastewater treatment, research interests are gradually moving from the bacterial contamination control to the establishment of cooperation between microalgae and bacteria for nutrients recovery.

The use of a raceway pond system for an aquaculture wastewater treatment has a low investment cost and low operation cost, but this system is more likely to be influenced by the external environment. In addition, after algae cultivation in a raceway pond system, the harvesting process of biomass is time-consuming and energy-intensive.

Revolving Algal Biofilm (RAB) System

The RAB system, which was developed to grow microalgae on a film, was considered as a potential technology to improve land utilization efficiency and simplify the harvesting process [51,52]. As shown in Figure 1, revolving algal biofilm is a system consisting of a microalgal biofilm, drive unit, and open pond with wastewater. Since the biofilm is established vertically on the open pond, theoretically, the RAB system has higher land utilization efficiency and biomass productivity than the standard raceway pond system [51]. Besides, compared with conventional harvesting methods, such as centrifugation and chemical-floculation, it is more cost-saving and eco-friendly to harvest biomass attached on film by using a scraper [53]. In a real-world application, to construct biofilm with a high biomass density, previous studies compared a couple of film materials and found that cotton is a good material for biofilm construction, yielding a biomass at 16.20 $g \cdot m^{-2}$ [52]. In addition to the research in the lab, a pilot-scale RAB system performed well in a wastewater treatment and biomass yield. According to the studies of Gross and Wen (2014) and Christenson and Sims (2012), in the pilot-scale RAB system, nutrients removal rates were 2.1 and 14.1 $g \cdot m^{-2} \cdot day^{-1}$ for total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN) and biomass productivity could reach 31 $g \cdot m^{-2} \cdot day^{-1}$ [51,53]. Another advantage of the RAB system is that the harvesting process does not reply on any chemicals, thus, the harvested biomass has a high safety level for aquaculture use. Therefore, from the perspectives of nutrients recovery and biomass reuse, the RAB system is a promising technology for the wastewater remediation and resource recycle in aquaculture.

As the RAB system is exposed to the atmosphere during operation, bacteria or fungi would grow together with microalgae on the biofilm. In spite of the competition on nutrients, the cooperation between microalgae and other microbes in the biofilm formation and nutrients uptake merits more attention. It was reported that extracellular polymeric substances (EPS) released by the bacteria function as "glue" to promote the formation of biofilm and extracellular enzymes produced by bacteria convert high-molecular-weight organics to low-molecular-weight organics, which could be assimilated by microalgae more efficiently [54–56] (Figure 1). However, to our knowledge, until now, mechanisms related with biofilm formation, nutrients conversion and assimilation, and algal-bacterial interaction in the RAB system have not been fully understood [57]. In the coming future, with the wide use of RAB system for aquaculture wastewater remediation, those scientific questions and technical problems currently bothering researchers will be addressed.

The most important advantages of the RAB system is that this system integrates algae cultivation with biomass harvesting. Besides, compared with the raceway pond system, the RAB system has better performance in land utilization and harvesting cost control. However, the RAB system has a much higher investment cost than the raceway pond system. In a real-world application, the appropriate microalgae cultivation system should be selected according to the actual conditions.

3.3. Technologies for Biomass Production

To improve the biomass productivity and increase the economic performance, previous studies devoted a lot of effects into the technologies for biomass production in wastewater, particularly wastewater pretreatment and construction of algal-bacterial consortia, which have been proven to play an important role in aquaculture wastewater remediation. The existence of solid organics and the unbalanced nutrients profile are two main problems that should be addressed by wastewater pretreatment.

3.3.1. Pretreatment of Wastewater

Solid Organics

Aquaculture wastewater contains some solid organics, which are regarded as sludge in some cases [27,28,55]. Without appropriate treatment, solid organics with a large size could not be assimilated by microalgae directly, and even hinder the photosynthesis of microalgae by increasing the turbidity of wastewater. The core principle of pretreating solid organics is to convert indigestible nutrients to digestible nutrients, such as volatile fatty acids, sugar, and carbon dioxide. Common pretreatment methods include anaerobic digestion and aerobic digestion. Mirzoyan et al. (2010) that summarized the results of employing anaerobic digestion to treat aquaculture sludge revealed that the removal efficiency of a total solid (TS) could reach 80%–100%. By controlling relevant parameters of anaerobic digestion, volatile fatty acids, which are good carbon sources for microalgae metabolisms, could be produced [58]. In the treatment of aquaculture wastewater, which is not suitable to be anaerobically digested due to lower concentrations of nutrients, aerobic digestion is commonly applied to convert solid organics to carbon dioxide. Dissolved carbon dioxide could be further fixed by microalgae through photosynthesis. Thus, by appropriate pretreatment, conversion efficiency of solid organics to microalgae biomass is highly improved.

Unbalanced Nutrients Profile

According to the redfield ratio for phytoplankton, the ratio of C/N in culture medium or wastewater should be controlled around 6.1:1, which is much higher than the ratio of C/N in aquaculture wastewater [59]. A comparison between aquaculture wastewater and a commonly used mixotrophic medium also showed that carbon deficiency might be a barrier to the microalgae growth in aquaculture wastewater. For some ammonia-sensitive microalgal strains, a high concentration of ammonia could cause toxicity or even lead to the failure of microalgae growth [40]. Similar problems have been reported in the application of microalgae to treat other sources of wastewater, such as food processing wastewater and livestock industry effluent [36,60].

To address the aforementioned problems in aquaculture wastewater, some methods used in other wastewater may be useful. First, the most straightforward method to balance the nutrients profile is adding certain nutrients in wastewater. It was verified that with the addition of glucose and acetic acids, nitrogen assimilation ability of microalgae cells was improved since carbon metabolism and nitrogen metabolism are closely connected through the TCA cycle and GS-GOGAT pathway [31]. In a real-world application, Lu et al. (2016) found that mixed food processing wastewater from different sources have a more balanced nutrients profile and become suitable to microalgae growth. Thus, not only the biomass yield, but also nutrients recovery efficiency is dramatically improved by appropriate mixing. For example, the biomass yield of algae grown in mixed wastewater ranged between 1.32 g/L and 2.68 g/L while in non-mixed wastewater the biomass yields were less than 1.16 g/L. Second, Wang et al. (2015) found that microalgae cells (*Chlorella* sp.) pretreated by nitrogen starvation performed amazingly in ammonia removal, assimilating NH_3–N at 19.1 mg/L/d in wastewater with 160 mg/L NH_3–N. Therefore, it is a possible way to quickly remove ammonia in aquaculture wastewater by appropriate nitrogen starvation of microalgae.

3.3.2. Algal-Bacterial Cooperation

As mentioned above, to control the total cost, open systems are commonly used for microalgae-based wastewater remediation. In some cases, bacteria and microalgae in wastewater may form synergistic consortia, which perform much better in nutrients recovery than individual microbial system [54,56,61]. First, some substances secreted from microalgae and bacteria contribute to the formation of a synergistic relationship. Croft et al. (2005) reported that vitamins released by bacteria have beneficial effects on microalgae growth. Besides, some intermediate metabolites of microalgae could be partly released to the extracellular environment, thus providing organic carbon for bacteria growth. Second, bacterial metabolisms may accelerate the breakdown of solid organics in aquaculture wastewater and provide more digestible nutrients for microalgae growth [54,55]. It was discovered that extracellular enzymes, such as lipase and protease, released by microbes are critical to the yield of digestible nutrients [62]. Third, gas exchange between microalgae (O_2 producer) and bacteria (CO_2 producer) is beneficial to both biomass production and wastewater treatment. Such a cooperation mechanism has been fully documented by previous studies that used microalgae to treat wastewater rich in suspended solids [50,54]. With the accumulation of dissolved oxygen in wastewater by microalgal photosynthesis, an aerobic environment is created in the aquaculture wastewater, promoting the bloom of beneficial bacteria, such as nitrifying bacteria, *Bacillus subtilis*, and yeast. Thus, after microalgae-based treatment, waste, containing beneficial microorganisms, reused for aquaculture may have positive effects on the health of aquatic animals [63,64].

To establish the interspecies cooperation, competition between microalgae and bacteria in nutrients utilization should be mitigated. The study of Ma et al. (2014) studied the effects of inoculation concentration and inoculation ratio on microalgae growth in wastewater, revealing that the maximum biomass yield was obtained when inoculation concentration was set as 0.1 g/L. In addition, physical or chemical sterilization methods can be used to control bacteria in wastewater and adjust inoculation ratio. In the coming future, cost-saving technologies for bacteria control in aquaculture practice will be developed and widely used for microalgae-based aquaculture wastewater remediation.

3.4. Technologies for Biomass Harvesting

3.4.1. Criteria for Harvesting Technology Selection

To use microalgae biomass in various industries, previous studies have developed many harvesting technologies, such as centrifugation, filtration, gravity-driven sedimentation, flocculation by positively charged ions, harvesting by edible fungi, and flotation [65,66]. In a real-world application, however, not all of these technologies are suitable to the biomass harvesting for aquaculture practice. To our knowledge, in the aquaculture industry, the biomass harvesting technologies should meet three specific requirements. First, the harvesting process should be performed at a low cost to improve the competitiveness of microalgae feed over traditional feed. Second, no toxic or unhealthy chemicals could be used in the harvesting process. Otherwise, contaminated biomass may negatively impact the safety of aquaculture products or even cause the failure of the aquaculture. For example, the accumulation of aluminum ions and polyacrylamide, which have been widely used for microalgae biomass harvesting at full-scale, in food chain may cause serious safety problems. Third, as the harvesting step could directly impact the hydraulic retention time (HRT) of the wastewater treatment system and water circulation frequency of aquaculture system, the harvesting process should be efficient and time-saving. According to the criteria mentioned above, two advanced technologies, fungi-assisted harvesting and flotation, may be applicable in microalgae-assisted aquaculture (Table 2).

Table 2. Comparison of harvesting technologies for aquaculture.

	Harvesting Cost	Safety Level	Time Consumption
Centrifugation	High (Energy-intensive centrifugation equipment)	High	Short
Filtration	High (Frequent replacement of filter blocked by algal cells)	High	Short
Gravity-driven sedimentation	Low	High	Long (Repulsive force among negatively charged algal cells)
Flocculation by chemicals	Low	Low (Addition of toxic or unhealthy chemicals)	Short
Harvesting by edible fungi	Low	High	Short
Flotation	Low	High	Short

3.4.2. Fungi-Assisted Harvesting

Fungi-assisted microalgae harvesting refers to the addition of filamentous fungi, including either fungal pellets or fungal spores, into medium with microalgae. The study of Zhou et al. (2013) found that that microalgal cells could attach to or be entrapped in the fungal pellets, which could be harvested by simple filtration. It was discovered that the use of fungal pellets could harvest over 95% of microalgae biomass in 1.5 h, revealing the great performance of fungi in microalgae harvesting [67]. In practice, however, the production of fungal pellets has a strict requirement on equipment and fermentation conditions, which increase the total cost of harvesting.

Recently, to further simplify the harvesting procedure, the co-cultivation of fungal spores with microalgae in wastewater or medium has been intensively studied. In some cases, the co-cultivation will not only harvest microalgae biomass, but also promote the nutrients recovery from aqueous phase, which has been fully confirmed by the study of Gultom et al. (2014) that co-cultivated *Aspergillus* sp. with *Chlorella* sp. in molasses wastewater. According to previous studies, the parameters that impact the fungal-algal pellet formation and determine the pellet size include inoculation ratio of fungi/algae, pH value, carbon content, cultivation temperature, and so on [67–69]. Table 3, which lists some examples of fungi-assisted microalgae harvesting, confirms that it is an efficient way to collect biomass from culture medium or wastewater by employing fungal spores or fungal pellets. For aquaculture practice, fungi used for microalgae harvesting should have a high safety level, meaning that they do not contain or secrete toxic components. Otherwise, harvested biomass used as aquaculture feed may threaten the survival of aquatic animals or even lead to the failure of aquaculture. Some fungal strains, such as *Aspergillus oryzae* and *Monascus purpureus*, isolated from food production have a high safety level and contain value-added compounds [69,70]. However, beneficial effects of these fungi on aquatic animals have not attracted attention from researchers yet.

Table 3. Fungi-assisted microalgae harvesting.

Microalgae	Fungi	Harvesting Efficiency	Conditions	Reference
Chlorella sp.	*Penicillium* sp.	98.2%	Fungal pellets; 30–34 °C; pH: 4.0–5.0; Agitation speed: 120–160 rpm	[67]
Chlorella sp.	*Penicillium* sp.	99.3%	Fungal spores; 40 °C; pH: 7.0; Agitation speed: 160 rpm	
Chlorella vulgaris	*Aspergillus oryzae*	93%	Fungal spores; Heterotrophic culture; 25 °C; Agitation speed: 150 rpm; 3-day	[68]
Chlorella vulgaris	*Aspergillus* sp.	Almost 100%	25 °C; pH: 5.0–6.0; Agitation speed: 100 rpm; 2-day	[71]
Chlorella vulgaris	*Aspergillus niger*	>60%	Fungal spores; 27 °C; pH: 5.0; Agitation speed: 150 rpm; 3-day	[72]
Chlorella vulgaris	*Aspergillus fumigatus*	>90%		
Scenedesmus quadricauda	*Aspergillus fumigatus*	>90%	Fungal pellets; 28 °C; Agitation speed: 150 rpm; 2-day	[73]
Pyrocystis lunula	*Aspergillus fumigatus*	Around 30%		

In addition to the simplification of the harvesting process, co-cultivation of fungi with microalgae could promote nutrients recovery from aquaculture wastewater with high contents of solid organics. Previous work, which presented the metabolic mechanisms of fungi and microalgae, showed that fungal cells could convert high-molecular-weight organics to low-molecular-weight organics easily utilized by microalgal cells in the co-cultivation system [69]. At the same time, carbon dioxide released by fungi through heterotrophic metabolism could be assimilated by microalgae through photosynthesis, preventing the greenhouse gas emission and improving the carbon utilization efficiency. Such a synergistic relationship between microalgae and fungi has been proven by the study of Gultom et al. (2014). Therefore, the co-cultivation of fungi and microalgae in aquaculture wastewater for simple biomass harvesting and efficient nutrients recovery merits more attention from academia and industry.

3.4.3. Flotation and Modified Flotation

Flotation has been assessed as one of the most economic technologies for microalgae harvesting [74]. The main process of flotation-based harvesting is generating fine air bubbles continuously in wastewater or a culture medium with microalgae. As the air bubbles attach on suspended microalgae cells, microalgae cells will rise to the surface of aqueous phase. Flotation can be considered as an inverted sedimentation, having a small footprint, low detention period, and high overflow rate [74].

A negative repulsive charge on the surface of microalgae cells is the main reason for suspension of microalgae cells. In a real-world application, to improve the harvesting rate, flotation can be combined with flocculation, which partly neutralizes the negative charge on microalgae cells [75]. Previous studies have explored various types of flocculating agents, such as metal ion and polymer, for microalgae flocculation [66,76,77]. Sirin et al. (2012) optimized the addition content of aluminum ion in microalgae flocculation process and found that the harvesting efficiency could reach 82%. For aquaculture practice, to recycle the harvested microalgae as feed, biomass safety should be strictly controlled. Owing to the serious threats caused by metal accumulation in food chain, metal-ions based flocculation can not be considered as a safe and eco-friendly technology. Recently, some studies reported the coagulation effects of natural polymers, such as protein and polysaccharide, secreted by microorganisms [78,79]. Generally, by natural-polymer based flocculation, the harvesting efficiency of microalgae could reach 90% [78,80]. Therefore, it is suggested that in aquaculture, to harvest microalgae by flotation-flocculation, natural polymers can be used for safety purpose.

3.5. Microalgae-Based Aquaculture Feed

3.5.1. Algal Species with Commercial Potential

In aquaculture practice, microalgae are important nutrition sources of fish or shrimp either by direct consumption or as indirectly prepared feed. Advantages of microalgae feed over traditional feed in aquaculture include the abundance of nutrients and the maintenance of water quality. Microalgae are rich in proteins, lipids, and carbohydrates, which are essential nutrients to aquatic animals. In addition, determined by the regulatory gene and growth condition, microalgae could intensively synthesize a variety of value-added components, such as antioxidants and pigments [81,82]. For the purpose of aquaculture use, microalgae biomass with value-added components is highly needed. Therefore, in addition to screening robust algal strains for wastewater remediation, we need to obtain value-added algal strains with commercial potential in aquaculture. Table 4 listed some algal strains with great potential to be used for wastewater remediation and value-added biomass production. Generally, for aquaculture practice, the value-added components in microalgae could be classified into three categories. First, microalgae rich in protein and carbohydrate can be used to partly replace traditional feed, reducing the aquaculture cost. Second, antioxidants in microalgae could be exploited to enhance the immunity of aquatic animals, overcoming the problems of antibiotics abuse. Third, some components play important roles in the growth of special fish. For example, astaxanthin, which determines the skin and flesh color of some fish, is an essential pigment in salmon production industry.

Table 4. Nutrition profile of microalgae biomass.

Strain	Culture Medium	Protein (%)	Lipid (%)	Carbo Hydrate (%)	Value-Added Compound	Reference
Thraustochytrium sp.	Medium with glycerol	NA	38.95	NA	EPA and DHA (37.88% of total lipid)	[83]
Chlorella zofingiensis	Cane molasses	NA	30–50	NA	Polyunsaturated fatty acids (36.89–49.16% of fatty acid profile)	[84]
Scenedesmus sp.	Soybean oil extraction effluent	53.3	33.4	NA [a]	EPA (15.89% of fatty acid profile)	[15]
Galdieria sulphuraria	Modified Allen Medium	26.5	1.14	69.1	Dietary fiber (54.1% of carbohydrate)	[85]
Galdieria sulphuraria	Modified Allen Medium	32.5	1.77	62.9	Astaxanthin (575 mg/kg)	
Chlorella zofingiensis	Cane molasses	NA	NA	NA	Astaxnathin (56.1 mg/L)	[86]
Chlorella zofingiensis	Cane molasses	NA	30-50	NA	Astaxnathin (13.6 mg/L)	[84]
Haematococcus pluvialis	OHM medium	NA	NA	NA	Astaxnathin (>15 mg/L)	[87]
Haematococcus pluvialis	Primary-treated wastewater	NA	NA	NA	Astaxnathin (80 mg/L)	[88]
Botryococcus braunii	NA	39.9	34.4	18.5	Essential amino acids (54.4 g/100 g protein)	[89]
Tetraselmis chuii	NA	46.5	12.3	25.0	Essential amino acids (45.5 g/100 g protein)	
Phaeodactylum tricornutum	NA	39.6	18.2	25.2	Essential amino acids (45.2 g/100 g protein)	
Porphyridium aerugineum	NA	31.6	13.7	45.8	Essential amino acids (63.9 g/100 g protein)	

[a] "NA" is short for "Not Available".

3.5.2. Microalgae Feed for Aquaculture

Protein

Protein synthesis in microalgae is impacted by both growth conditions and nutrients supply in culture medium. Table 4 indicated that protein content in dry microalgae biomass ranged between 26.5% and 53.3%. Particularly, compared with soybean protein, microalgae protein has much higher productivity, making it a feasible protein source for aquaculture. According to previous studies that used microalgae for aquaculture practice, feed conversion ratio (FCR) of fish fed by microalgae is higher than that of fish fed by traditional feed [81]. However, in some cases, due to palatability problems, microalgae feed may not perform well in aquaculture. For example, in the culture of Atlantic cod, with the increase of microalgae content (0%–30%) in fish-meal, most growth parameters, including final body weight, absolute feed intake, and specific growth rate, decreased but mortality increased [90]. Hence, in a real-world application, to ensure the sustainable operation of microalgae-assisted aquaculture, palatability of microalgae feed should be comprehensively evaluated.

Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA), arachidonic acid (AA), docosahexaenoic acid (DHA), and α-linoleic acid (ALA), have been proven to be essential for the growth of larvae. For example, EPA, functioning as a precursor for eicosanoids synthesis that partly regulates the developmental and regulatory physiology, plays a pivotal role in the growth of aquatic animals. Compared with traditional aquaculture feed, microalgae biomass contains much higher concentrations of PUFAs. As shown in Table 4, percentages of PUFAs in microalgae could reach almost 50% under some special conditions, such as low temperature. Compared with soybean and peanut, which are usually exploited as feedstock for traditional fishmeal production, microalgae with higher contents of PUFAs have much greater potential to be used for aquaculture. Therefore, microalgae can be considered as an affordable and productive source of PUFAs for aquaculture.

Special Pigments

The natural pigments, such as astaxanthin, chlorophyll, and carotene, in microalgae are important to the growth of some fish species. First, biochemical characteristics of fish are partly determined by the intake of microalgae pigments. Choubert et al. (2006) found that rainbow trout *Oncorhynchus mykiss* fed by *Haematococcus pluvialis* contained higher concentration of astaxanthin (around 20 mcg/g dry weight). In most cases, rainbow trout with more astaxanthin and a better flesh color is preferred by consumers in the market. Second, some pigments play a pivotal role in the immunity of aquatic animals. It was discovered that lysozyme activity, an indicating factor of fish immunity, of large yellow croaker *Pseudosciaena crocea*, increased with the increase of astaxanthin and *Haematococcus pluvialis* levels, suggesting that the fish immunity has a positive relationship with the intake of microalgae pigment (astaxanthin) [81]. Traditionally, for specific purposes, feed with pigments, of which the production, extraction, purification, and preservation cost are high, are added into aquaculture [91]. In microalgae-assisted aquaculture, biomass rich in natural pigments is directly added to feed aquatic animals, thus reducing the total cost and prevent the pigments degradation.

Other Applications

Besides directly using microalgae as feed, researchers also studied the microalgae-based artificial ecological system, in which herbivorous fish relying on microalgae feed is preyed on by carnivorous fish. Thus, microalgae biomass can be indirectly used to feed carnivorous fish. Considering the poor performance of microalgae in the remediation of a water body with low concentrations of nutrients, recently, some studies proposed a novel concept of integrating microalgae culture with a hydroponics system, through which microalgae and leafy plants cooperate simultaneously for nutrients recovery from aquaculture.

4. Problems and Prospects

4.1. Potential Problems

Although the concept of microalgae-assisted aquaculture has emerged into the limelight owing to aforementioned advantages, further developments are required before the industrial implementation is feasible. This novel concept itself has a couple of potential problems, which may hinder its wide application.

Firstly, the safety level of biomass recycled from wastewater as aquaculture feed has not been assessed comprehensively. As the biomass production in wastewater is coupled with bacteria growth, harvested biomass consists of both microalgal biomass and bacterial biomass. To our knowledge, some bacterial species may contain toxic components or release bio-toxin, thus, the survival of aquatic animals will be seriously threatened if the biomass contaminated by bacterial toxins is used as aquaculture feed. Hence, strict control of toxic bacteria in wastewater treatment is pivotal to the successful application of microalgae-based aquaculture.

Secondly, flotation and fungi-assisted harvesting have a couple of problems related with economic feasibility and biomass safety. For example, flotation may bring solid wastes in aquaculture wastewater to the surface of the aqueous phase with microalgae biomass together, thus reducing the safety level of harvested staff in aquaculture practice. In addition, the cost of fungi-pellets production in an artificial medium is not low enough to support the commercial application of fungi-assisted harvesting. Hence, without addressing the problems associated with economic feasibility and biomass safety, harvesting technologies are not mature enough to support the industrialization of microalgae-assisted aquaculture.

Thirdly, the lack of knowledge on economic assessment and life cycle analysis is a barrier to the industrialization of microalgae-based aquaculture. Based on previous studies on technologies, the use of microalgae in aquaculture industry, including feed production, water quality control, and nutrients recovery could promote nutrients recycle, mitigate greenhouse gas emission, and reduce aquaculture cost [92]. However, the economic performance of microalgae-based aquaculture has never been fully studied. Besides, the life cycle analysis has not been conducted yet to evaluate the effects of microalgae-based aquaculture on natural environment. Therefore, aiming at controlling potential threats of microalgae-based aquaculture, it is necessary to comprehensively evaluate its economic performance and environmental impacts.

4.2. Prospects

As the eco-friendly aquaculture and the nutrients recovery from wastewater are becoming more important to the global sustainability, microalgae-assisted aquaculture bringing great beneficial benefits to natural environment merits more attention from both academia and industry. With the solution of aforementioned problems, microalgae-assisted aquaculture will move forward from lab research to industrial application. Generally, the great advancement brought to aquaculture by the wide use of microalgae biotechnology can be classified into environmental and economic aspects.

Firstly, the threats of aquaculture to the environmental sustainability can be controlled to some extent. Owing to the great performance of microalgae in carbon dioxide fixation and wastewater remediation, pollution caused by aquaculture can be alleviated. In addition, since microalgae feed has beneficial effects on the health of aquatic animals, the abuse of antibiotics or medicines could be prohibited in microalgae-assisted aquaculture. Thus, the antibiotics-resistance, which is becoming more serious in aquaculture, may be controlled. Therefore, with the wide application of microalgae-assisted aquaculture, its positive effects on the environmental safety and sustainability will contribute to the global sustainability.

Secondly, the cost of aquaculture can be reduced by the nutrients recovery and biomass production. The cost of feed accounts for a large portion of the total aquaculture cost in a real-world application, so the financial burden is a barrier to the development of aquaculture. By microalgae-assisted aquaculture, nutrients in wastewater are converted to value-added biomass, which can be further exploited to produce aquaculture feed. Thus, the nutrients recovery in aquaculture by microalgae biotechnology can reduce material input and the total cost of the whole system.

Considering the advancement in environmental and economic aspects, with the wide application of microalgae-assisted aquaculture, it will make great contribution to the aquaculture sustainability and global sustainability.

5. Conclusions

To overcome the problems in traditional aquaculture, herein, the use of microalgae for sustainable aquaculture is introduced by this work. Based on the principles of microalgae-assisted aquaculture, this novel system could convert wastes to value-added biomass as aquaculture feed, alleviate water deterioration, and reduce energy consumption for aeration. In recent years, technologies have been widely developed to promote the application of microalgae-assisted aquaculture, key technologies or theories include microalgae-based nutrients assimilation, design of the raceway pond system and RAB system, wastewater pretreatment methods, establishment of algal-bacterial cooperation, fungi-assisted harvesting and flotation, composition analysis of microalgae biomass, and beneficial effects of microalgae on aquatic animals.

In spite of the great progress in the aforementioned fields, microalgae-assisted aquaculture still has some problems related with the biomass safety level, economic feasibility, and the lack of knowledge on economic assessment and life cycle analysis, which hinder its industrialization or commercialization in a real-world application. In the near future, with the solution of these problems, microalgae will play a more pivotal role in aquaculture for sustainable development.

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