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Review

Valorization of Food Processing Wastewater for Astaxanthin Production by the Mixotrophic Fermentation of Microalgae: A Review

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Abstract

Food processing wastewater (FPW) poses significant environmental risks due to its high nutrient load yet offers untapped potential as a low-cost feedstock for high-value compound production. This review critically evaluates the valorization of FPW for astaxanthin production through the mixotrophic fermentation of microalgae. Key microalgal species (e.g., *Haematococcus pluvialis* and *Chromochloris zofingiensis*) effectively remediate nutrients (nutrients removal of up to 100%) while synthesizing astaxanthin under stress-inducing conditions, such as nutrient starvation, salinity, and oxidative stress. Advanced strategies, such as two-stage cultivation, nutrient profile adjustment, and microbial co-cultivation, which could enhance astaxanthin yields and wastewater treatment efficiency were reviewed comprehensively. The resulting astaxanthin-rich biomass demonstrates multifunctional benefits in animal feed, improving meat quality, immunity, growth, and shelf life. However, this review identifies some challenges, including wastewater management risks, low digestibility of microalgae biomass, and astaxanthin instability during feed processing, which should be addressed properly in real-world applications. This integrated approach aligns with circular bio-economy principles, transforming FPW from an environmental liability into a resource for sustainable biotechnology.

Keywords: wastewater; nutrient removal; microalgae; astaxanthin; animal feed

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1. Introduction

The global food processing industry generates vast quantities of wastewater annually as a byproduct of various activities such as grain and fruit fermentation, dairy products processing, meat cutting, and beverage production [1–4]. Food processing wastewater (FPW) is characterized by high concentrations of organic matters (e.g., carbohydrates, proteins, and lipids, etc.), as well as variable levels of salts and chemical additives [5,6]. In the food processing industry, if FPW is discharged without appropriate treatment, it may cause severe environmental disasters. For instance, the discharge of nutrient-rich wastewater can trigger eutrophication, leading to algal blooms, oxygen depletion, and the collapse of aquatic biodiversity in natural waters [7]. As global food demand rises with the continuous increase in population, researchers are necessitating innovative and sustainable solutions to mitigate the environmental and ecological risks of FPW [8].

In fact, traditional approaches, such as anaerobic digestion, advanced oxidation, and filtration, have been widely adopted to treat FPW but face significant environmental

and operational challenges [9,10]. For instance, anaerobic digestion, which performs well in reducing organic loads, converts organic carbon in wastewater into CH_4 and CO_2, of which the intensive emission can accelerate greenhouse effects [3,11]. Similarly, advanced oxidation of FPW by the Fenton reaction is also accompanied with the emission of CO_2 [12]. In addition, filtration, though efficient in removing suspended solids, generates a large amount of sludge or solid waste that is difficult to dispose of sustainably [13]. These limitations highlight the unsustainability of conventional methods, which prioritize pollutant removal over resource recovery and often trade one environmental problem for another. As a consequence, there is a growing urgency to develop circular economy strategies that realize the valorization of FPW.

Microalgae (*Haematococcus pluvialis*, *Chromochloris zofingiensis*, *Chlorella sorokiniana*, etc.) are good sources of natural astaxanthin [14,15]. According to a previous report, the astaxanthin yield of *Chromochloris zofingiensis* could reach 12.5 mg/L and the astaxanthin content in the dry weight of *Haematococcus pluvialis* falls in a range of 0.5–2.5% [14]. In the past, significant progress was made in understanding the biosynthetic pathways of astaxanthin in microbial cells, optimizing cultivation conditions to enhance astaxanthin yields, and validating its bio-active efficacy through in vitro experiments [16,17]. These foundational studies have laid the groundwork for scaling up astaxanthin production using FPW as a fermentation feedstock.

Emerging research has demonstrated the potential of certain microalgal species to simultaneously remediate FPW and synthesize high-value compounds, such as astaxanthin. As a ketocarotenoid with potent antioxidant properties, astaxanthin is widely used in food, animal feed, nutraceuticals, and cosmetics, commanding a foreseeable market value of USD 167.50 million by 2030 [18–20]. Notably, some microbial species thrive in nutrient-rich environments and can utilize the organic carbon, nitrogen, and phosphorus in FPW as growth substrates. In this way, through the fermentation of certain yeast and microalgae in FPW, nutrients in wastewater are recovered to attenuate environmental pollution and astaxanthin-rich biomass is produced to create economic benefits [21]. For example, molasses wastewater has been shown to enhance astaxanthin yields in *Haematococcus pluvialis* while nitrogen-deficient conditions in starch processing effluents induce astaxanthin accumulation in *Haematococcus pluvialis* [22,23]. This dual-function approach, namely FPW bioremediation coupled with astaxanthin biosynthesis, aligns with the principles of a circular bio-economy.

This review critically evaluates the valorization of FPW for astaxanthin production through the mixotrophic fermentation of microalgae. First, this paper analyzes compositions and environmental risks of FPW, emphasizing the inadequacy of conventional treatment technologies. Second, mixotrophic fermentation of microalgae in FPW for astaxanthin production is summarized, with a focus on nutrient assimilation from wastewater and microbial biomass accumulation. Third, it reviews recent advances in wastewater pretreatment, two-stage cultivation, and nutrient profile adjustment of FPW for the improvement of the microalgae-based FPW remediation and astaxanthin production. Fourth, practical applications of microbial astaxanthin in feeds to increase growth rate, enhance immune response, and improve survival efficiency of animals are reviewed. Finally, this review discusses techno-economic challenges, including scalability, contamination risks, and market barriers, while proposing potential strategies to enhance the practical feasibility of fermenting FPW for astaxanthin production. By synthesizing interdisciplinary insights from microbiology, environmental engineering, and biotechnology, this work aims to catalyze the adoption of FPW fermentation for microalgal astaxanthin production as a cornerstone of sustainable industrial practices.

2. Environmental Footprint of Food Processing Wastewater

2.1. Nutrient Profile and Biochemical Characteristics

As shown in Table 1, FPW can be produced in various steps and sectors, such as oil extraction, bottle-washing, milk processing, and so on, of food industry. Due to the enrichment of food nutrients, some FPW contains high concentrations of nitrogen, phosphorus, and organic carbon (Table 1). For example, molasses wastewater collected from a sugar refinery contained 57,433.2 mg/L TOC (total organic carbon) and 128,723 mg/L chemical oxygen demand (COD) while defective soy sauce contained 13,500 mg/L TN (total nitrogen) [24,25]. In addition to the dissolved nutrients, FPW from some sources are enriched with undissolved nutrients (Table 1). Allison and Safferman (2024) reported that there was 11,311 mg/L SS (suspended solids) in winery wastewater [26]. The composition of SS is relatively complex and highly dependent on the source of FPW and specific processing techniques. These solids may include proteins, starch–protein complexes, colloids, and so on [27,28].

Table 1. Nutrient profile and biochemical characteristics of food processing wastewater.

Wastewater	Nutrient Profile (mg/L)			SS (mg/L)	Reference
	TOC/COD	TN	TP		
Molasses wastewater	TOC: 57,433.2; COD: 128,723	NH_3^+ and NO_2^-: 484.2	/	/	[24]
Molasses fermentation wastewater	COD: 63,500	834	/	/	[29]
Beverage industrial wastewater	COD: 4424	68	11	580	[30]
Equalization tank wastewater of soft drink industry	COD: 5533	NH_3^+ and NO_2^-: 1.5	5.9	39	[31]
Bottle-washing wastewater of soft drink industry	COD: 453	NH_3^+ and NO_2^-: 0.9	1.4	9.5	[31]
Food waste fermentation wastewater	TOC: 5500; COD: 15,000	350	85	1050	[32]
Cidery wastewater	COD: 8000	/	/	6000	[26]
Beverage wastewater	COD: 11,214	8.1	16–68	5600	[26]
Winery wastewater	COD: 3236	7.6	5.26	11,311	[26]
Milk processing wastewater	COD: 1237.0–1509.0	52.4–66.5	24.2–26.7	/	[33]
Soft drink wastewater	COD: 1229.0–1603.0	16.7–43.8	61.5–65.5	/	[33]
Soybean oil plant wastewater	COD: 1349.0–1436.0	44.1–87.9	116.4–185.0	/	[33]
Defective soy sauce	TOC: 158,500; COD: 790,000	13500	/	/	[25]
Starch processing effluent	TOC: 2680–2820	228–281	25.7–29.4	580–610	[34]
Vinegar production wastewater	COD: 740	20.5	7.4	/	[35]
Soybean fermentation effluent	TOC: 2042.2	394.44	46.78	/	[36]

Compared with artificial culture mediums, particularly an autotrophic culture medium, FPW contains more essential nutrients for microalgae growth. However, it should also be noted that not all FPW can be directly used for microalgae cultivation. For example, FPW from some fermentation factories may have low pH value, which is unfavorable to the survival of microalgal cells. It was discovered that the pH of whey wastewater was around 3.8, an environment that precludes the growth and survival of

most microalgae species [37]. In addition, undissolved organics with large particle size in FPW may not be efficiently absorbed by microalgae and an unbalanced nutrient profile could result in low biomass productivity of microalgae. Whey wastewater contained about 120 g/L total solids and starch processing wastewater contained 0.58–0.61 g/L suspended solids [34,37]. Therefore, practical application of FPW for microalgae cultivation should be evaluated on a case-by-case basis.

2.2. Environmental Risks

Traditional treatment technologies widely applied for wastewater remediation cannot fundamentally eliminate the negative environmental impacts of FPW. Firstly, conversion of organic carbon into CO_2 by chemical oxidation or aerobic digestion could reduce the TOC and COD concentrations in FPW, but greenhouse effects are exacerbated with CO_2 entering the atmosphere. For example, in the molasses wastewater collected from a sugar refinery, TOC concentration reached 57,433.2 mg/L [24]. If all the organic carbon in wastewater is converted to CO_2, the treatment of 1 m^3 molasses wastewater will generate around 210.59 kg CO_2, which is equal to 118.98 m^3 CO_2. Hence, FPW enriched with organic carbon is recognized as a major source of greenhouse gas emissions. Secondly, filtration or sedimentation could efficiently separate SS from FPW and reduce pollutant concentration in FPW. However, a large amount of sludge from the filtration or sedimentation process may cause soil contamination and air pollution. On one hand, when the sludge is landfilled in open areas, rainwater leaching may cause nitrogen, phosphorus, organic carbon, and other pollutants to seep into surface water, soil and even groundwater. On the other hand, anaerobic decomposition of organic matter in sludge results in the emission of CH_4 and H_2S whilst sludge drying may release dust, pathogenic microorganisms, and volatile organic compounds, degrading air quality and causing human respiratory diseases [38,39].

In real-world applications, governments encourage the planting of trees, grass, and vegetables to absorb CO_2 and mitigate the greenhouse effect. However, plants have long growth cycles (from several months to several years) and relatively low carbon sequestration rates. A previous study reported that the biomass yield of *Miscanthus* sp., one of the most productive perennial C4 grass species, reaches 40 tons/ha/year [40]. Taking the aforementioned molasses wastewater from a sugar refinery as an example, since the carbon content in plants is about 50%, to fix the CO_2 (210.59 kg) released by 1 m^3 of FPW, 14.36 m^2 of land and a one-year growth period are required. The reliance on plants for offsetting CO_2 emissions from FPW treatment by traditional technologies is constrained by the land-intensive requirements and low carbon uptake rates. As a result, with the application of traditional treatment technologies followed by plant-based carbon capture, the recovery of pollutants in FPW has evolved into a complex system which restricts the practical application effectiveness of this technological pathway. Therefore, innovative technologies are needed to effectively address the environmental risks of FPW.

2.3. Microalgae-Based Carbon Sequestration

Traditionally, the concept of growing plants, including trees, grasses, and vegetables, to increase the greening rate for CO_2 absorption was popular [41–43]. In terms of FPW remediation, traditional treatment technologies convert organic carbon to CO_2; a portion of the released CO_2 is then captured by plants. In this way, the carbon footprint of FPW treatment by traditional technologies could be reduced. However, this technical route is challenged by the low CO_2 absorption rate of plants, particularly C3 plants. In addition, low CO_2 concentration (~0.04%) in the air is unfavorable to plant-based CO_2 absorption [44]. For example, the average dry weight of biomass per lettuce fall in a range of 1–5 g while the growth period of lettuce is about 30 days [45]. If the carbon content in the dry biomass

of lettuce is 50% and ten plants are cultivated on 1 m^2 land, the CO_2 absorption rate will be only 0.17–0.83 g C/m^2/day. Therefore, if we rely on plant-based carbon sequestration to offset the CO_2 emission caused by FPW treatment, vast amounts of arable land will be occupied.

Microalgae are a group of microorganisms which can perform both photosynthetic and heterotrophic metabolisms [46,47]. Compared with plants, microalgae demonstrate more significant advantages in carbon sequestration. Firstly, microalgae growing in FPW can utilize both organic carbon and atmospheric CO_2 as carbon sources through mixotrophic metabolism, thereby enhancing carbon sequestration efficiency via diversified carbon absorption pathways. Particularly, the metabolism of organic carbon can directly provide energy for microalgal cells, eliminating the need for growth dependence on photosynthesis. Secondly, microalgae have much shorter growth periods than most plants. Normally, growth periods of microalgae in FPW are about 5–10 days while those of plants fall in a range of 1–3 months (Table 2). Thirdly, technological innovations enable high-density and vertical cultivation modes, significantly enhancing the land use efficiency, biomass productivity, and carbon absorption rate of microalgae cultivation. In a study by Kim et al. (2018), the biomass productivity of microalgae grown in an open pond system (depth: 20 cm) was 6.16 g/m^2/day [48]. If the carbon content in microalgae biomass is set as 50%, the CO_2 absorption rate of microalgae cultivation reaches 3.08 g C/m^2/day, which is much higher than that of the vegetables mentioned above. Last but not the least, microalgae can be cultivated on non-arable land, having no competition with traditional agriculture.

Table 2. Microalgal species and inducing conditions for astaxanthin production.

Microalgae	Source	Culture Medium	Inducing Conditions	Cultivation Period (Day)	Biomass Yield	Astaxanthin Yield	Reference
Haematococcus pluvialis	A microbial culture collection in Tsukuba, Japan	Kobayashi's basal medium	Light with short wavelength (380–470 nm, blue and purple light)	12–13	0.480–0.546 mg/cm^3	9.3–14 μg/cm^3	[49]
Haematococcus pluvialis	Germany	Artificial medium	Salinity stress (0.25, 0.50, and 1.0% salinity)	6–16	~9.5 g/L	~9 mg/L	[50]
Haematococcus pluvialis	A culture collection of algae in the United States	NSIII medium	magnesium acetate, sodium acetate, and potassium acetate (10, 50, and 100 mM)	24–30	0.22–0.35 g/L/day	3.79–10.21 mg/L/day	[51]
Haematococcus pluvialis	A microbial culture collection in Tsukuba, Japan	BG11 medium	Ethanol (1, 2, and 3%)	14	5.7 g/L	119.61 mg/L	[52]
Haematococcus pluvialis	A culture collection of algae in the United States	MES-Volvox culture medium	15% CO_2 and 300 μmol/m^2/s light	12	878 mg/L	36.23 mg/g	[53]
Haematococcus pluvialis	Lugu Lake in Yunnan Province, China	BG11 medium	Melatonin (10, 15, and 20 μM) and putrescine (50, 100, and 150 nM)	13	~1.2 g/L	36.4 mg/g	[54]
Haematococcus pluvialis	Industrial facility in Reykjanesbaer, Iceland	Bold's basal medium	Hydrogen peroxide (0.1 mM)	14	/	12.27 mg/L	[55]
Haematococcus pluvialis	Industrial facility in Reykjanesbaer, Iceland	Bold's basal medium	High light (175 μmol/m^2/s)	14	/	16.91 mg/L	[55]
Haematococcus pluvialis	Industrial facility in Reykjanesbaer, Iceland	Bold's basal medium	Nitrogen starvation	14	/	13.67 mg/L	[55]
Chromochloris zofingiensis	A culture collection, Rockville, USA	Modified Bristol's medium	1 mM NaClO	9	4.8 g/L	1.47 mg/g	[56]

Table 2. *Cont.*

Microalgae	Source	Culture Medium	Inducing Conditions	Cultivation Period (Day)	Biomass Yield	Astaxanthin Yield	Reference
Chromochloris zofingiensis	A culture collection, Rockville, USA	Modified Bristol's medium	0.1 mM H_2O_2	9	~7.5 g/L	1.7 mg/g	[56]
Chromochloris zofingiensis	A culture collection, Rockville, USA	Kuhl medium	Nitrogen starvation	5	1.2 g/L	4.45 mg/L	[57]
Chromochloris zofingiensis	A culture collection, Rockville, USA	Kuhl medium	Phosphorus starvation	5	2.7 g/L	2.16 mg/L	[57]
Chromochloris zofingiensis	A culture collection, Rockville, USA	Kuhl medium	Sulfur starvation	5	1.8 g/L	2.86 mg/L	[57]
Chromochloris zofingiensis	/	Kuhl medium	5 g/L glucose	8	/	~0.55% of dry weight	[58]
Chlorococcum sp.	A rocky wall of Victoria Peak, Hong Kong	Modified Kuhl medium	Illumination (22 μE/m^2/s) and hydrogen peroxide (0.1 mM)	7	/	7.086 mg/g	[59]

3. Astaxanthin Production and Food Processing Wastewater Treatment by Microalgae

3.1. Microalgal Species for Astaxanthin Production

In nature, astaxanthin are discovered in a variety of microalgal species, but not all of these species have been successfully selected for FPW treatment. In the view of the present authors, some important criteria for selecting microalgae for FPW treatment and astaxanthin production are listed as follows. (1) Microalgal species capable of withstanding harsh environments hold potential as astaxanthin producers using FPW as culture medium. For example, the high concentration of ammonia in some FPW specifically imposes cellular stress that inhibits microalgae growth. In addition, the water quality of FPW frequently fluctuates, posing challenges to the stable growth of algae. (2) Microalgal species should have high biomass productivity and astaxanthin content when they are grown in FPW. Otherwise, the economic viability of microalgae-based FPW treatment remains difficult to achieve at commercially feasible levels. (3) Microalgal species should not contain toxic or unhealthy components which may limit the use of biomass as an animal feed ingredient. To apply the microalgae biomass as feed ingredients, feed-grade microalgae should be cultivated for astaxanthin production.

Up to now, certain microalgae, such as *Haematococcus pluvialis* and *Chromochloris zofingiensis*, have demonstrated significant potential in the synthesis of astaxanthin and have been successfully applied at an industrial scale. The advantages of the aforementioned microalgal species for astaxanthin production are listed as follows. Firstly, these microalgal species have been documented as excellent producers of natural astaxanthin in previous studies. As reported by Wen et al. (2015), *Haematococcus pluvialis* can produce astaxanthin at a concentration of 119.61 mg/L [52]. Secondly, *Haematococcus pluvialis* and *Chromochloris zofingiensis*, which have been successfully commercialized and widely used in the industry, can be obtained easily. As shown in Table 2, *Haematococcus pluvialis* and *Chromochloris zofingiensis* can be obtained from the culture collections of algae in China, USA, and Japan. Thirdly, it has been proven that *Haematococcus pluvialis* and *Chromochloris zofingiensis*, which show significant resilience against wastewater-borne stressors, could grow well in different types of FPW [37,60,61].

Recently, with the application of genetic modification techniques, astaxanthin synthesis regulatory genes can be transferred into astaxanthin-nonproducing microalgal species, enabling heterologous astaxanthin biosynthesis. For example, in a study by Lin et al.

(2019), genes encoding β-carotenoid ketolase and β-carotene hydroxylase in *Haematococcus pluvialis* were integrated into the chloroplast genome of *Dunaliella viridis* via homologous recombination [62]. As a result, the maximum content of total astaxanthin in genetically modified *Dunaliella viridis* reached 77.5 μg/g while a wild-type strain lacks astaxanthin biosynthetic pathways, with high-performance liquid chromatography (HPLC) analysis confirming undetectable levels [62]. A similar result was reported by the experiment of applying genetic techniques in *Chlamydomonas reinhardtii* for astaxanthin production [63]. However, in real-world applications, genetically modified ingredients are subject to strict restrictions in animal feed by some countries [64]. Currently, *Haematococcus pluvialis* and *Chromochloris zofingiensis* remain the primary microorganisms employed for producing natural astaxanthin.

3.2. Inducing Conditions for Astaxanthin Synthesis

During astaxanthin synthesis, microalgae often undergo significant cellular morphological changes, which are closely linked to stress responses and metabolic reprogramming. In nature, *Haematococcus pluvialis* and *Chromochloris zofingiensis* growing in nutrient-sufficient environments are green microalgae. At this stage, microalgae allocate their primary metabolic resources toward cellular growth and reproduction rather than astaxanthin biosynthesis [21]. However, when subjected to adverse environmental conditions, microalgal cells enter a dormant state, undergoing morphological transformations and synthesizing astaxanthin to enhance their survival under such stressful conditions. Therefore, the transition of cells from normal physiological states to resting cysts constitutes a critical step in achieving efficient astaxanthin production via microalgal biotechnology.

As shown in Table 2, a couple of inducing conditions have been discovered by previous studies to enhance astaxanthin production in microalgae. In general, these inducing conditions can be classified into three categories, namely nutrient profile modification, oxidative substances/conditions, and salinity stress. Firstly, the starvation of some nutrients can limit microalgae growth and result in the formation of microalgal resting cysts [65]. Mao et al. (2018) evaluated three types of nutrient starvation, discovering that phosphorus starvation, sulfur starvation, and nitrogen starvation effectively enhanced astaxanthin in *Chromochloris zofingiensis* [57]. It should be noted that compared with phosphorus and sulfur starvation, nitrogen starvation improved astaxanthin yield to a much higher level (4.45 mg/L). In addition, the supplementation of organic carbon, such as glucose, acetate, and ethanol, favors the astaxanthin accumulation in microalgae [51,52,58]. Therefore, in previous studies, nitrogen starvation and organic carbon supplementation were widely adopted to adjust the C/N ratio for microalgae-based astaxanthin production (Table 2). Secondly, oxidative substances/conditions, such as hydrogen peroxide, sodium hypochlorite, and illumination, could be adopted to induce astaxanthin synthesis in microalgae [53,56]. Katsuda et al. (2004) found that blue and purple light with a wavelength of 380–470 nm, which can induce cellular oxidation, are more likely to cause microalgae to accumulate astaxanthin (9.3–14 μg/cm^3) [49]. The main mechanism for this phenomenon is that the aforementioned substances or conditions could cause oxidative stress in microalgal cells, which actively synthesize astaxanthin to eliminate oxidative stress. This can be regarded as a self-protection mechanism, which improves the survival of microalgal cells under oxidative environments. It should be noted that excessive oxidative stress may seriously impact the microalgal metabolisms, resulting in low astaxanthin yield. For example, when the concentration of H_2O_2 increased from 0.1 to 10 mM, the astaxanthin content in *Chromochloris zofingiensis* dropped from ~1.75 mg/g to 1.35 mg/g [56]. Therefore, in practical application, oxidative substances and conditions should be optimized to achieve the maximum astaxanthin yield in microalgae fermentation. Thirdly, salinity stress can be employed to enhance

astaxanthin accumulation in microalgae. Sarada et al. (2002) observed the significant improvement of astaxanthin content in *Haematococcus pluvialis* with the increase in NaCl from 0% to 0.5%, confirming the feasibility of employing salinity stress for astaxanthin synthesis induction [50].

In real-world applications, to select the most appropriate inducing conditions for astaxanthin synthesis in microalgae, actual situations should be taken into consideration. Firstly, physical and biochemical properties of FPW should be considered before the selection of inducing conditions. For instance, the strategy of using high-intensity light to enhance astaxanthin synthesis in microalgae proves less effective in FPW with a high content of SS, primarily because solid particles obstruct light penetration to the algal cells. Secondly, the total cost of applying inducing conditions should be evaluated comprehensively. In the aforementioned inducing conditions, illumination has high energy consumption while the supplementation of oxidizing agents raises the cost of culture medium preparation. Thirdly, the environmental footprint of the inducing conditions should not be neglected. To apply the strategy of supplementing organic carbon to improve the C/N ratio in wastewater for astaxanthin synthesis induction, potential risks must be addressed with corresponding countermeasures to prevent pollution caused by residual organic carbon after microalgae cultivation.

3.3. Nutrient Removal by Microalgae in Wastewater

Astaxanthin-producing microalgae have been widely adopted to treat a variety of nutrient-rich effluents, including FPW (Table 3). On one hand, the sugars in some FPW are favorable to enhance astaxanthin biosynthesis in microalgal cells. As mentioned above, the presence of glucose could promote astaxanthin accumulation in microalgae. On the other hand, microalgal cells could uptake organic carbon in FPW directly to produce biomass by performing heterotrophic metabolisms. Different from traditional treatment technologies, microalgae-based FPW fermentation has the potential to be carbon-neutral or even carbon-negative, attenuating the negative effect of CO_2 or CH_4 emissions on the environment.

Table 3. Nutrient removal and biomass production by astaxanthin-producing microalgae in wastewater.

Microalgae	Wastewater	Nutrient Removal (%) and Initial Concentration			Biomass Yield	Period (day)	Reference
		COD	TN	TP			
Haematococcus pluvialis (co-cultured with fungus)	Wastewater from wastewater treatment plant	100	83.3 (~550 mg/L)	88.2 (~90 mg/L)	1.39 g/L	12	[66]
Haematococcus pluvialis	Walnut shell extracts	39.18 (280.5 mg/L)	52.15 (8.71 mg/L)	45.46 (5.7 mg/L)	0.92 g/L	13	[67]
Haematococcus pluvialis (co-cultured with fungus)	Wastewater from wastewater treatment plant	/	100	100	1.95 g/L	12	[66]
Haematococcus pluvialis	Ethanol plant waste effluent	/	91.7	100	4.37 g/L	16	[61]
Haematococcus pluvialis	Domestic secondary effluent	/	93.8 (7.0 mg/L)	97.8 (0.46 mg/L)	207 mg/L	22	[68]
Chromochloris zofingiensis	Whey wastewater	85.47 (100.36 g/L)	92.69 (2.81 g/L)	77.08 (795.30 mg/L)	3.86 g/L	7	[37]
Chromochloris zofingiensis	Municipal wastewater and biogas slurry	/	93 (31.5 mg/L)	90 (10.15 mg/L)	2.5 g/L	4	[69]
Chromochloris zofingiensis	Piggery wastewater	79.84 (3500 mg/L)	82.70 (148.0 mg/L)	98.17 (156.0 mg/L)	2.96 g/L	10	[70]

Table 3. *Cont.*

Microalgae	Wastewater	Nutrient Removal (%) and Initial Concentration			Biomass Yield	Period (day)	Reference
		COD	TN	TP			
Chromochloris zofingiensis	Dairy wastewater	/	51.7 (118.0 mg/L)	97.5 (149.0 mg/L)	~1.1 × 10^7 cells/mL	6	[60]
Chromochloris zofingiensis	Swine wastewater and fishery wastewater	~95 (2596 mg/L)	~80 (586 mg/L)	~95 (62 mg/L)	~1.8 g/L	7	[71]

As shown in Table 3, astaxanthin-producing microalgae could effectively remove nutrients, including nitrogen, phosphorus, and organic carbon, in wastewater from different sources. In some studies, the removal efficiency of TN, total phosphorus (TP), and COD could even reach 100%, demonstrating that astaxanthin-producing microalgae can effectively utilize the nutrients in some wastewater for growth and cellular metabolism [66].

The cultivation of microalgae in wastewater also presents technical and environmental challenges that should not be overlooked. Firstly, in some cases, the high concentration of nutrients in FPW may negatively impact the growth of microalgae or even result in the failure of microalgae cultivation. For instance, high ammonia concentration in wastewater can cause ammonia toxicity since most microalgal species are sensitive to ammonia [72]. Therefore, a lot of wastewater could not be directly used to cultivate microalgae. Secondly, microalgae may not completely assimilate nutrients in wastewater and residual nutrients after microalgae production may cause water contamination if the treated wastewater is discharged. In some FPW, a high portion of nutrients are in the form of undissolved organics, such as protein residues, insoluble starch, and solid lipid, which cannot be assimilated directly by microalgal cells. Under this situation, nutrient removal efficiency will be low and the discharge of wastewater treated by microalgae may cause environmental pollution. For example, in the study of growing *Haematococcus pluvialis* in walnut shell extracts, only 39.18% COD, 52.15% TN, and 45.46% TP were removed by microalgae [67]. Concentration of COD in the treated wastewater was 170.63 mg/L, exceeding the regulatory thresholds for wastewater discharge [67]. In summary, wastewater could provide astaxanthin-producing microalgae with essential nutrients, but potential technical and environmental challenges should be addressed properly in the practical application.

4. Potential Technologies for Astaxanthin Production

In recent years, advanced technologies and methods with the potential to address current challenges have surfaced, thus promoting the microalgae-based astaxanthin production and microalgae production in wastewater. Herein, a two-stage cultivation model, nutrient profile adjustment of wastewater, and co-growth of microalgae with other microorganisms are introduced. It should be noted that due to the significant variations in the physical, chemical, and biological characteristics of FPW, it is challenging to establish standardized methods for wastewater pretreatment to achieve efficient microalgae cultivation. Therefore, the selection of an appropriate method to pretreat FPW and cultivate microalgae for astaxanthin production must be based on a case-by-case analysis.

4.1. Two-Stage Cultivation Model

As shown in Table 2, a couple of inducing conditions (e.g., salinity stress, oxidizing compounds, and intensive illumination) are unfavorable to microalgae growth although they are of importance to the accumulation of astaxanthin in microalgae. Under this situation, astaxanthin synthesis in microalgae would be induced at the expense of microalgae growth and biomass production. For instance, in the study by Difusa et al. (2015), it was

discovered that with the increase in light intensity from 27 to 94.5 μmol/m^2/s, cell density dropped significantly [73]. Similar results, which confirm the negative effects of inducing conditions on microalgae growth, were also observed in other study [74]. By contrast, microalgae cultivated under favorable environments without any inducing conditions exhibit rapid growth but demonstrate low efficiency in astaxanthin biosynthesis. Therefore, the conflict between biomass production and astaxanthin synthesis in microalgae should be addressed properly.

The two-stage cultivation model is developed to simultaneously enhance the astaxanthin synthesis in microalgae and achieve high biomass productivity [75]. In Stage 1, microalgae are cultivated in comfortable environments without any inducing conditions. During this period, microalgal cells grow rapidly, maintaining high biomass productivity. As microalgae undergo rapid proliferation and enter the stationary phase, specific inducing conditions are initiated in Stage 2 to enhance astaxanthin biosynthesis. In this stage, due to the inducing conditions, microalgae growth is limited while astaxanthin accumulation is enhanced. In this way, biomass production and astaxanthin biosynthesis are decoupled into distinct phases (Stage 1 and Stage 2), accommodating their divergent cultivation requirements.

Previous studies have widely adopted the two-stage cultivation model for microalgae-based astaxanthin production [53,76]. However, in the practice, to apply the two-stage cultivation model, some special factors should be taken into consideration. Firstly, properties of the FPW should be considered in the selection of inducing conditions. Due to the enrichment of suspended solids, the turbidity of some FPW is high, resulting in low light transmission. In this situation, high light intensity should not be adopted for astaxanthin induction in the microalgae since microalgae grown in wastewater with high turbidity may not be sensitive to the illumination. Secondly, the addition of specific chemicals, such as salt and oxidizing compounds, in the wastewater or culture medium can induce the astaxanthin accumulation of microalgae, but those chemicals may become new pollutants. For example, salt stress is a promising inducing condition, but the increase in salinity makes the subsequent treatment processes of FPW more complex.

With the application of the two-stage cultivation model, astaxanthin production and biomass yield can be increased simultaneously rather than achieving astaxanthin production at the expense of microalgae growth. This is one of the main advantages of the two-stage cultivation model for microalgae-based astaxanthin production. It should also be noted that this technical approach has some disadvantages. For example, the two-stage cultivation model may extend the total period of microalgae growth, thereby increasing the risks and uncertainties during the microalgae cultivation process.

4.2. Nutrient Profile Adjustment of Wastewater

FPW, which is different from artificial culture mediums, may contain excessive nutrients and some growth-limiting factors. For example, in whey wastewater, turbidity and ammonia concentration reached 55,535 NTU and 109 mg/L, respectively [37]. Microalgae grown in such an environment are not only challenged by ammonia toxicity, but also negatively impacted by the low light transmittance. In addition, some nutrients in FPW may not be digested by microalgal cells, resulting in low biomass productivity of microalgae cultivation. As reported by Yu et al. (2021), *Haematococcus pluvialis* only assimilated 52.15% nitrogen and 45.46% phosphorus in walnut shell extracts [67]. Therefore, measures should be taken to adjust the nutrient profile of FPW and create a suitable environment for microalgae cultivation.

Firstly, the most straightforward method to adjust the nutrient profile of wastewater for microalgae cultivation is adding artificial chemicals to the wastewater. This method

is intensively adopted in the experiments of microalgae-based wastewater remediation. For example, by adding $NaHCO_3$, the TN and TP removal efficiency in *Haematococcus pluvialis*-based wastewater treatment increased from 83.3% and 88.2% to 100% and 100%, respectively [66]. In a study by Huo et al. (2012), via supplementing CO_2 as the carbon source, a high removal efficiency of TN and TP was achieved [60]. However, it should be noted that the high cost of artificial chemicals may limit the practical application of this method. Normally, in the industry, low-cost substances such as agricultural waste, industrial byproducts, and food waste are employed to provide nutrients and adjust the nutrient profile of wastewater for microalgae production.

Secondly, dilution could effectively reduce the turbidity of FPW, thus enhancing the microalgal photosynthesis in wastewater. Also, ammonia toxicity in some FPW could be attenuated by dilution. In the study of growing *Chromochloris zofingiensis* in whey wastewater, it was discovered that when the dilution ratio increased from 50% to 10%, the removal efficiency of TN, phosphorus, COD, and TOC improved from 44.78%, 55.14%, 24.75%, and 21.96% to 92.69%, 77.08%, 85.47% and 92.78%, respectively [37]. Compared with other pretreatment technologies, dilution does not require advanced equipment and has a much lower investment and energy input. Particularly, in the dilution process, almost all of the nutrients could be retained in the wastewater instead of being removed, resulting in high nutrient utilization efficiency of the microalgae-based FPW treatment.

Thirdly, appropriate anaerobic digestion could convert undissolved organics into low-molecular-weight organics, which can be assimilated by microalgae more efficiently. In the step of acidogenesis during the anaerobic digestion process, nutrients in FPW could be converted into volatile fatty acids (VFAs) and alcohols. Through acidogenesis, 95% of carbohydrate, 82% of protein and 41% of lipid in dairy wastewater were degraded and 48.4% of dairy pollutants were converted into VFAs and alcohols [77]. Compared with undissolved organics, VFAs and alcohols in wastewater can be assimilated by microalgal cells in a more efficient way. Therefore, this technology has been widely applied to modify the nutrient profile of FPW and increase the utilization efficiency of nutrients [78]. In the view of the present authors, to apply this technology for microalgae cultivation, the anaerobic digestion stage and pH change should be strictly controlled. (1) The anaerobic digestion process yields CO_2, H_2, and CH_4 as terminal products. Given the low assimilation efficiency of these gases by microalgae, operational constraints should be imposed to confine the process to acidogenesis while suppressing methanogenesis. In the practical application, the progress of anaerobic digestion should be monitored by conducting real-time analysis of VFAs content or CH_4 yield. (2) In the stage of acidogenesis, the generation of VFAs is accompanied with the dramatic drop of pH value of wastewater. As a result, the acidic wastewater may not be suitable for the survival of microalgal cells. To solve this problem, in the practice, pH adjustment should be conducted by the end of acidogenesis, or pH buffer could be added.

Fourthly, ammonia stripping or ammonium slow-release could favor the astaxanthin synthesis in microalgae through reducing the N/C ratio of FPW. In meat processing wastewater, due to the present of meat residues, ammonia concentration is high, becoming a limiting factor of astaxanthin synthesis induction and even microalgae survival. Under this situation, ammonia stripping or ammonia slow-release can be employed to reduce the N/C ratio, creating a positive condition to induce astaxanthin synthesis in microalgae. (1) By introducing tiny bubbles into the wastewater, non-ionized ammonia dissolved in the water is carried out with the rising bubbles, thereby reducing the ammonia–nitrogen content in wastewater. As reported by Li et al. (2019), through introducing tiny bubbles into the piggery wastewater, ammonia concentration dropped from 450 mg/L to 225 mg/L in 8 h [79]. Critical parameters, which can determine the effectiveness and rate of ammonia

stripping, include temperature, aeration rate, bubble size, and so on [80,81]. Compared with the biological process, such as nitrification, air-bubbling-drive ammonia stripping has a much shorter time period and higher ammonia reduction rate. It should be noted that although ammonia stripping effectively optimizes the C/N ratio of wastewater by transferring ammonia into air bubbles, it concurrently depletes nutrients to some extent. In the industry, excessive ammonia stripping impairs nutrient recycling potential, thereby reducing both the algal growth rates and profit margins in wastewater treatment. (2) The novel ammonium slow-release technology employs adsorbent matrices to adsorb and then slowly release ionized ammonium. It initially modulates the nutrient profile of wastewater through ammonium adsorption and then sustains ammonia release to stabilize the C/N ratio, creating ideal conditions for astaxanthin production. According to previous studies, biochar, bentonite, and zeolite are highly effective materials for ammonia adsorption and controlled slow-release [82–84].

In theory, via effectively regulating wastewater nutrient profiles, the aforementioned technologies could boost pollutant assimilation by microalgae for enhanced wastewater treatment, concurrently establishing ideal astaxanthin biosynthesis conditions. However, up to now, not all of these technologies have been fully validated in experiments for microalgae-based astaxanthin synthesis. Therefore, in future research, the relevant parameters of wastewater pretreatment should be optimized to achieve a high yield of astaxanthin from microalgae. In the view of the present authors, aforementioned approaches related to nutrient profile adjustment have some disadvantages that should be addressed properly. For instance, the primary drawback of wastewater dilution is its high consumption of freshwater, leading to potential water resource wastage. Also, this limitation restricts its applicability in arid or semi-arid regions. In addition, although zeolite performs well in the adsorption and slow-release of ammonia in wastewater, it may significantly increase the total cost of wastewater pretreatment due to the high price of zeolite products.

4.3. Co-Growth of Microalgae with Other Microorganisms

FPW is normally enriched with undissolved organics which could not be efficiently assimilated by microalgal cells. By contrast, some bacteria or yeast exhibit a strong capability of decomposing insoluble organic matter through secreting extracellular enzymes [85]. In addition, the reproduction rates of yeast or prokaryotic bacteria are significantly higher than that of eukaryotic microalgae, resulting in a substantial improvement in wastewater treatment efficiency. Therefore, the co-cultivation of microalgae with other microorganisms has been regarded as a promising strategy for wastewater treatment.

According to previous studies, there are three approaches to establishing a synergistic relationship between microalgae and other microorganisms for wastewater treatment. Firstly, some studies inoculated microalgae in non-sterilized wastewater with bacteria and established synergistic relationships between microalgae and wastewater-borne bacteria for nutrient removal. It was observed that *Chlorella* sp. grown in non-sterilized wastewater had a much higher biomass yield (~2 g/L) than that cultivated in autoclaved wastewater (<1 g/L) [86]. In addition, compared with microalgae inoculated in autoclaved wastewater, *Chlorella* sp. grown in non-sterilized wastewater reached the maximum biomass concentration in a shorter period of time [86]. Secondly, specific bacteria can be isolated from wastewater and then co-inoculated with microalgae. Wastewater contains numerous microbial species, yet only a subset of bacteria can establish synergistic relationships with microalgae for nutrient removal and high-value biomass production, while others may produce toxins or inhibit microalgal metabolism. To mitigate the adverse effects of certain bacteria in wastewater on microalgae, specific bacterial strains can be selectively isolated for co-cultivation with microalgae to actively establish synergistic relationships.

In a study by Huo et al. (2020), two bacterial strains, *Beijerinckia fluminensi* and *Bacillus firmus*, were isolated from vinegar production wastewater and then co-inoculated with microalgae for nutrient removal in vinegar production wastewater [35]. The results showed that compared with the mono-culture of microalgae, the co-culture of microalgae with the isolated wastewater-borne bacteria improved removal efficiency of COD, TN, and TP from 62.8%, 66.7%, and 56.7% to 69.7–76.7%, 74.2–86.7%, and 61.3–74.8%, respectively [35]. Additionally, the contents of microalgae pigments (β-carotene, violoxanthin, and lutein) significantly increased as a result of the co-culture of *Beijerinckia fluminensi* and *Bacillus firmus*. Thirdly, exogenous microorganisms can be co-inoculated with microalgae into sterilized wastewater. Compared to the previous two methods, this approach remains completely unaffected by the indigenous microbial community in wastewater. For example, oleaginous yeast, *Rhodotorula glutinis*, was co-cultured with *Chlorella vulgaris* in starch processing wastewater, improving TOC removal efficiency and lipid yield to 88.6% and 1.81 g/L, respectively [34]. In the practical application, the addition of specific microorganisms can be tailored to achieve simultaneous nutrient removal and high-value biomass production based on actual requirements.

In previous studies, a couple of astaxanthin-producing microorganisms, such as *Xanthophyllomyces dendrorhous*, *Paracoccus carotinifaciens*, *Brevundimonas* sp., and *Sphingomonas* sp., have been screened and successfully applied in the industry [87]. The highest astaxanthin yield (1.19 mg/g) was achieved when *Chromochloris zofingiensis* was co-inoculated with yeast *Xanthophyllomyces dendrorhous* at a ratio of 3:1 while the astaxanthin yield of a mono-culture of microalgae was only 1.08 mg/g [88]. It should also be noted that different culture medium compositions can affect the final biomass ratio between microalgae and other microorganisms in the co-culture system. Fath et al. (2025) reported that a BBM medium demonstrated the balanced growth of both *Haematococcus pluvialis* and *Xanthophyllomyces dendrorhous*, while a BGY medium resulted in the highest cell concentration for *Haematococcus pluvialis* and limited the growth of *Xanthophyllomyces dendrorhous* [89]. Therefore, to select the suitable microbial strains for astaxanthin production in wastewater, the properties of the wastewater should be taken into consideration.

5. Practical Application of Microbial Astaxanthin in Animal Feed

The economic value of microalgae-derived astaxanthin is reflected in its extensive application within the animal feed sector. Recent studies have confirmed that astaxanthin demonstrates multiple biological functions including improving meat quality, boosting immunity, stimulating growth, and extending shelf life (Table 4).

Table 4. Functions of astaxanthin in animal feeding.

Source	Format	Dosage	Animal	Functions	Reference
Haematococcus pluvialis	Microalgae powder	Astaxanthin: 18.57 and 31.25 mg/kg	*Oncorhynchus mykiss*	(1) Compared with the control group, weight gain rate and specific growth rate of rainbow trout fed 30 mg/kg microalgae powder were much higher, reaching 251.55% and 1.05%/day, respectively; (2) Astaxanthin content in rainbow trout fed 30 mg/kg microalgae powder was around 14 mg/kg while that in the fish of control group was less than 1 mg/kg; (3) Astaxanthin supplementation (30 mg/kg microalgae powder) in diet increased the total antioxidant capacity and activity of glutathione peroxidase in liver and serum; (4) Through supplying astaxanthin in diet, malondialdehyde content in serum and liver of rainbow trout dropped from 33.2 to 16.94 nmol/mL and from 1.25 to 0.67 nmol/mL, respectively.	[90]
/	/	Astaxanthin: 49.8 mg/kg	*Oncorhynchus mykiss*	(1) Relative weight gain of fish fed astaxanthin-containing diet was 19.4%, which is slightly higher than that (18.4%) of fish fed astaxanthin-free diet.	[91]

Table 4. *Cont.*

Source	Format	Dosage	Animal	Functions	Reference
Haematococcus pluvialis	Microalgae powder	Microalgae: 50 and 100 mg/kg	*Marsupenaeus japonicus*	(1) Survival rate of *Marsupenaeus japonicus* increased from 37% to 51% by astaxanthin supplementation; (2) With dietary supplementation of astaxanthin, weight gain of kuruma prawn was improved from 281% to 348%; (3) Body astaxanthin of kuruma prawn fed *Haematococcus pluvialis* reached 128–179 mg/kg, which is much higher than that (55 mg/kg) of kuruma prawn fed astaxanthin-free diet.	[92]
Haematococcus pluvialis	Astaxanthin extract	Astaxanthin: 25, 50, 75, 100 and 150 mg/kg	*Litopenaeus vannamei*	(1) Natural astaxanthin had greater pigmentation efficiency than synthetic astaxanthin; (2) Tail muscle of shrimp fed microalgal astaxanthin contained more esterified astaxanthin (around 10–30 mg/kg) than that of shrimp fed synthetic astaxanthin; (3) Diet containing microalgal astaxanthin significantly improved redness (4.12–5.54) and yellowness (5.98–8.63) of the tail muscle of shrimp.	[93]
Haematococcus pluvialis	Astaxanthin extract	Astaxanthin: 100 and 200 mg/kg	*Oreochromis niloticus*	(1) By the end of 50-day storage, tilapia filets treated with astaxanthin had better sensory quality (e.g., brightness and texture) than those not treated with astaxanthin; (2) Tilapia supplemented with astaxanthin presented lower lipid oxidation index.	[94]
/	Natural astaxanthin	Astaxanthin: 66.7 mg/kg	Pig	(1) Natural astaxanthin shows promise for improving the length of consumer retail acceptability of pork products by delaying oxidation and surface discoloration.	[95]
/	/	Astaxanthin: 5, 10 and 20 ppm	Pig	(1) Growth performance of pigs was not improved by the dietary supplementation of astaxanthin; (2) The increase in astaxanthin supplementation reduced average fat depth and the 10th rib fat depth of pigs; (3) Pigs fed 5 or 10 ppm astaxanthin had the highest percentage of fat-free lean; (4) When the addition of astaxanthin increased from 10 ppm to 20 ppm, there was not any further improvement in carcass characteristics of pigs; (5) Based on a price of USD 9.07/lb for 10,000 ppm astaxanthin product, supplementation of 20 ppm astaxanthin in pig's diet was of no economic benefit.	[96]
Haematococcus pluvialis	Astaxanthin extract	Astaxanthin: 25 mg/kg	Weaned piglets	(1) Dietary intake of astaxanthin reduces the average percentage of collagen fibers in liver tissue to around 2%, having a protective effect on the liver of piglets; (2) Astaxanthin affected the expressions of specific genes, such as *CREB*, *NOTCH1*, *CYP7A1* and *NR1H3*, related to liver function.	[97]
Haematococcus pluvialis	Microcapsuled astaxanthin	/	*Cyprinus carpio*	(1) Weight gain rate, specific growth rate, and condition factor of fish were improved significantly by astaxanthin supplementation; (2) With astaxanthin supplementation, total superoxide dismutase activity significantly increased while malondialdehyde content decreased; (3) Compared to the control group, crude protein content in fish fed astaxanthin was higher while crude ash content was lower; (4) The muscle of fish fed astaxanthin had higher water-holding capacity.	[98]
Haematococcus pluvialis	Astaxanthin extract	Astaxanthin: 10, 20, 40, and 80 mg/kg	Chicken	(1) through affecting the hepatic mRNA levels of several redox status-controlling genes, microalgal astaxanthin modulates molecular profiles of stress.	[99]
Haematococcus pluvialis	Lyophilized microalgal cells	Astaxanthin: 50, 100 and 150 mg/kg	*Lates calcarifer*	(1) White blood cell, red blood cell, and hemoglobin content increased with the astaxanthin supplementation; (2) Increasing supplementary astaxanthin levels resulted in the drop of serum cholesterol and triglyceride levels of fish; (3) Innate immune parameters, including total immunoglobulin, lysozyme activity, respiratory burst activity, and phagocytic activity, increased with astaxanthin supplementation, reaching 35.37 g/L, 295.27 U/mL, 0.83, and 93.33%, respectively.	[100]

Table 4. *Cont.*

Source	Format	Dosage	Animal	Functions	Reference
/	Astaxanthin (purity > 98%)	Astaxanthin: 50 and 100 mg/kg	*Channa argus*	(1) Hemoglobin content, white blood cell, and red blood cell were improved by astaxanthin supplementation; (2) Through providing astaxanthin-containing diet, survival rate of *Channa argus* increased from 26% to 58% after challenge with *Aeromonas hydrophila*.	[101]

5.1. Improvement of Meat Quality

Dietary supplementation of astaxanthin can effectively enhance the quality of meat products, including their composition, sensory properties, and nutritional value.

In the experiment of feeding pigs, it was observed that pigs fed 5 or 10 ppm astaxanthin had the highest percentage of fat-free lean [96]. Also, average fat depth and the 10th rib fat depth of pigs were reduced by the increase in astaxanthin content in pig's diet [96]. In the study of Sha et al. (2025), the muscle of *Cyprinus carpio* fed microcapsuled microalgal astaxanthin had higher protein content and water-holding capacity while lower ash content [98]. Therefore, adding appropriate amounts of astaxanthin to animal feed can enhance the composition of the meat products.

In the animal feeding sector, pigmentation is another factor which is essential for achieving optimal sensory quality and color characteristics of meat products. Normally, meat products exhibiting enhanced lightness and redness are perceived as fresher and demonstrate greater consumer appeal. With the intake of an astaxanthin-supplemented diet, the accumulation of astaxanthin in animal tissues could change the color and enhance the pigmentation. As reported by Ju et al. (2011), through feeding Pacific white shrimp with an astaxanthin-rich diet, the yellowness and redness of the tail muscle were significantly improved to 5.98–8.63 and 4.12–5.45, respectively. By contrast, tail muscles of shrimp fed a basal diet and commercial diet had much lower yellowness (4.56 and 4.92) and redness (1.24 and 1.30) [93]. It should be noted that natural astaxanthin had greater pigmentation efficiency than synthetic astaxanthin [93].

A portion of astaxanthin in animal feed may accumulate in the fat and muscle of animals, thus increasing the astaxanthin content in meat products. For example, the tail muscle of shrimp fed microalgal astaxanthin contained a high content of esterified astaxanthin (around 10–30 mg/kg) [93]. Similarly, dietary intake of astaxanthin-containing feed increased astaxanthin content in the meat of *Oncorhynchus mykiss* from less than 1 mg/kg to around 14 mg/kg [90]. Typically, meat products containing astaxanthin are considered to have certain health benefits.

Last but not the least, the presence of astaxanthin in meat products can protect the meat quality during food processing. For example, when the cooking temperature was 200 °C, the presence of astaxanthin in beef meatball reduced to 47–54% of total heterocyclic aromatic amines [102]. This result suggests meat products containing astaxanthin demonstrate good thermal stability, which contributes to enhancing the nutritional value of cooked meat.

5.2. Boosting of Immunity

Dietary supplementation of astaxanthin could improve the antioxidant capacity of animals and the activity of some antioxidant enzymes [103,104]. In the experiment of supplementing microalgal astaxanthin (31.25 mg/kg) in rainbow trout's diet, the total antioxidant capacities of serum and liver reached 16.12 U/mL and 1.54 U/mg protein, which were much higher than those in the control group (8.76 U/mL and 0.68 U/mg protein) [90]. In the culture of *Cyprinus carpio*, dietary supplementation of microcapsuled microalgal astaxanthin reduced malondialdehyde content, attenuating the oxidative stress in fish species [98]. Innate immune parameters, including total immunoglobulin, lysozyme

activity, respiratory burst activity, and phagocytic activity, could also be improved by microalgal astaxanthin supplementation in *Lates calcarifer* [100]. It is noteworthy that the excessive addition of astaxanthin in feed may negatively impact the immunity of animals. For example, when the astaxanthin supplementation in the diet of *Lates calcarifer* increased from 100 to 150 mg/kg, lysozyme activity dropped slightly from 298.87 to 295.27 U/mL [100].

Astaxanthin can not only directly alleviate oxidative stress within cells but also regulate animal immunity at the molecular level. [97,99]. For example, through affecting the hepatic mRNA levels of several redox status-controlling genes, microalgal astaxanthin modulates molecular profiles of stress, improving the resistance of chickens to the harsh environment [99]. In recent years, with technological advances, a growing number of studies are focusing on the impact of microalgae-derived astaxanthin on regulating gene expression in animals.

In the experiment of providing weaned piglets with microalgal astaxanthin, the average percentage of collagen fibers in liver tissue was reduced by astaxanthin supplementation, demonstrating the protective effects of microalgal astaxanthin on the health status of pigs [97]. Particularly, the expressions of specific genes, such as *CREB*, *NOTCH1*, *CYP7A1* and *NR1H3*, related to liver function were affected by the dietary intake of astaxanthin [97]. In addition, white blood cell, red blood cell, and hemoglobin content in blood increased with the supplementation of microalgal astaxanthin, demonstrating enhanced immune function of the blood system [100]. This confirms that microalgal astaxanthin can not only modulate the overall immunity of animals but also enhance specific function in some tissues and organs.

5.3. Stimulation of Animal Growth

Some studies have reported that astaxanthin supplementation promotes animal growth [91,92]. Salarzadeh and Rajabi (2015) added *Haematococcus pluvialis* in feed for *Marsupenaeus japonicus* culture, observing a significant increase in weight gain of kuruma prawn to 348%. By contrast, the weight gain of kuruma prawn in the control group was only 281% [92]. The increase in weight gain caused by astaxanthin supplementation was also reported by Řehulka (2000) [91].

It should be noted, however, that exceptions exist. In the study of adding microalgal astaxanthin and synthetic astaxanthin in the diet of Pacific white shrimp, neither natural nor synthetic astaxanthin significantly stimulated the growth of shrimp [93]. In the culture of finishing pigs, supplementation of astaxanthin did not improve the growth performance of pigs [96]. According to previous studies, the growth-promoting effects of astaxanthin in animals exhibit some uncertainty. Therefore, its efficacy in enhancing growth should be further evaluated in practical applications.

5.4. Extension of Shelf Life

There is a positive correlation between the shelf life of meat products and the content of astaxanthin. It has been proven that meat products with high astaxanthin content have longer shelf life. Dietary supplementation of microalgal astaxanthin in tilapia's diet reduced the loss of brightness and texture of tilapia filet during storage [94]. Carr et al. (2010) discovered that dietary supplementation of natural astaxanthin in swine feed delayed oxidation and surface discoloration, thus improving the length of consumers' acceptability of pork products [95]. In addition to adding astaxanthin to animal feed, it can also be directly added as an additive to meat products to improve their shelf life. It was reported that the usage of 0.3 and 0.45 g/kg astaxanthin extracted from *Haematococcus pluvialis* had a positive effect on meat acceptance declared by consumers by the end of storage [105].

Compared with artificial preservatives, such as sodium nitrite, potassium sorbate, and sodium benzoate, astaxanthin is harmless to the human body and can be regarded as a green preservative.

The mechanisms by which astaxanthin extends the shelf life of meat products mainly include singlet oxygen quenching, free radicals/reactive oxygen species scavenging, metal cation chelating, and color stabilization. Firstly, as a highly reactive oxygen species and a primary initiator of photo-oxidation reactions, singlet oxygen plays a key role in the oxidation of meat products. With the accumulation of astaxanthin in the tissues of animals, singlet oxygen can be quenched, preventing the meat quality degradation caused by photo-oxidation reactions [106]. Normally, singlet oxygen quenching capacity (rate constant: 5.01×10^{10} $M^{-1}s^{-1}$) of *cis*-astaxanthin is much greater than that (rate constant: 2.15×10^{10} $M^{-1}s^{-1}$) of all-*trans*-astaxanthin [107]. Secondly, as a powerful antioxidant, astaxanthin can effectively neutralize free radicals and reactive oxygen species, which cause the oxidation in meat products and shorten their shelf life. Thirdly, astaxanthin could form metal ion complexes with metal cations (e.g., Zn^{+2}, Cd^{+2}, Ca^{+2}, Pb^{+2}, Cu^{+2}, and Hg^{+2}), which may cause oxidative stress in cells and accelerate the oxidation of meat products [108]. With the formation of metal ion complexes, oxidative stress in cells and degradation of meat products can be attenuated. Fourthly, the presence of astaxanthin can prevent the oxidation of myoglobin, which is responsible for the red color of meat, thus maintaining the desirable red color of meat products for a longer period [109].

6. Challenges and Prospects

While microalgae-based astaxanthin production utilizing FPW demonstrates significant ecological and economic benefits, this technical pathway presents certain challenges, such as the high risk of FPW management, low digestibility of microalgae-based feed, and instability of astaxanthin in feed production. In the coming future, to industrialize the concept of growing microalgae in FPW for astaxanthin production, the aforementioned challenged should be addressed.

6.1. High Risk of Wastewater Management

FPW is enriched with high concentrations of various organics, which can be contaminated by microorganisms, such as bacteria, fungi, and even pathogens, easily [110]. On one hand, the growth of these microorganisms consumes the nutrients in FPW, reducing the concentrations of nutrients available to microalgae. On the other hand, excessive bacteria and fungi in FPW can compete with later-inoculated microalgae, reducing both the microalgae biomass yield and the overall economic viability of the project. Sometimes, excessive reproduction of bacteria and fungi in FPW may even result in the failure of microalgae cultivation.

Cold chain transportation can effectively prevent rapid microbial proliferation in FPW, but high cost and investment significantly limit the practical application of this technology within the wastewater treatment industry [111]. Additionally, in lab research, autoclave can be adopted to rule out the effects of wastewater-borne bacteria or air-borne bacteria on microalgae growth in wastewater [112]. However, it is not practically feasible in the industry due to the high energy consumption and low treatment rate.

In the view of the present authors, on-site wastewater treatment can be conducted to integrate FPW treatment and microalgae production. Through this technological route, nutrients in FPW can be converted locally into microalgae biomass. It will eliminate the need for the long-distance transportation of FPW, reducing both costs and transportation risks. For this reason, an increasing number of food industry enterprises are focusing on establishing microalgae cultivation facilities to treat FPW and reduce their carbon emissions.

It should be mentioned that the significant variation in the physicochemical and biological properties of FPW, depending on its source, poses a challenge for developing standardized pretreatment methods. This lack of a universal approach is one of the primary technical limitations in using this wastewater for microalgae cultivation.

6.2. Low Digestibility of Microalgae-Based Feed

Most microalgal species, including *Haematococcus pluvialis* and *Chromochloris zofingiensis*, have relatively thick cell walls containing components such as cellulose and hemicellulose, which are resistant to digestion and utilization by some carnivorous animals. Accordingly, nutrients in microalgae biomass may not be assimilated by animals, resulting in low nutrient conversion efficiency. It was observed that when the inclusion level of microalgae biomass in fish diet was over 25%, growth and survival of *Micropterus salmoides* were negatively impacted [36].

To attenuate the unfavorable effects of microalgae biomass on the digestibility of feed for some animals, the cell wall of microalgae can be disrupted by bead milling, freezing-melting treatment, and chemical decomposition [113]. With the disruption of cell structures, intracellular nutrients, including astaxanthin, become more readily absorbed and utilized by animals. In addition, astaxanthin can be extracted from microalgae and then supplemented in animal feed. It is essential to consider the lipid solubility of astaxanthin in order to choose a suitable extraction solvent. Last but not the least, enzymatic treatment can be employed to pretreat microalgae biomass, facilitating the release of intracellular nutrients. Given that cellulose and hemicellulose are major constituents of the microalgal cell wall, it follows that the enzymatic pretreatment should mainly involve cellulase and hemicellulase. In application, the optimal method can be determined according to the actual situations and requirements.

6.3. Instability of Astaxanthin in Feed Production

As a super-antioxidant, astaxanthin is sensitive to hot environments and high pressure, which may accelerate its degradation [114]. With technological advancements and equipment upgrades, the manufacturing processes of animal feed are undergoing significant transformations. The advent of novel technologies and application of advanced equipment are also profoundly reshaping the utilization of microalgae-derived astaxanthin in the animal feed sector. To add microalgal astaxanthin in animal feed, the feed processing conditions should be strictly controlled. However, in recent years, extrusion technology has gained widespread adoption in the production of animal feed, particularly aquafeed [115,116]. In the feed extrusion process, pressure and temperature normally fall in a range of 34–37 atmospheres and 125–150 °C [117].

Unfortunately, up to now, few studies have comprehensively studied the effects of extrusion conditions on the biochemical properties of astaxanthin. In the view of the present authors, lipid-soluble microalgal astaxanthin can be dissolved in oil and then added in animal feed in the step of vacuum spraying. As the last step of animal feed processing, this step involves no further high-temperature or high-pressure processes, which ensures effective protection of the astaxanthin. In the future, more studies could be conducted to evaluate the practical feasibility of adding microalgal astaxanthin in the form of lipid-dissolved extract in the step of vacuum spraying.

6.4. Co-Production of Other High-Value Components

In real-world applications, the content of astaxanthin accounts for a very low percentage of the total microalgae biomass. Therefore, producing astaxanthin alone from microalgae would result in resource waste and lower profits. To increase the total profitability of microalgae cultivation, two or more high-value components could be co-produced. As

discussed above, high C/N ratio is essential to the biosynthesis of microalgal astaxanthin. Also, this inducing condition could promote the accumulation of lipids. For example, FPW with a high C/N ratio can be selected for microalgae production for the simultaneous synthesis of astaxanthin and lipid. By the end of microalgae cultivation, different components can be separated for downstream application.

Even if other high-value components do not share similar induction or synthesis conditions with astaxanthin, they can still be co-produced within microalgal cells. For instance, the synthesis condition for proteins in microalgal cells is a low C/N ratio, which is entirely opposite to the induction condition for astaxanthin. In practical applications, the co-production of astaxanthin and protein can be achieved through a two-stage cultivation model. In the first stage, the C/N ratio in FPW is reduced to facilitate the synthesis of proteins within the microalgal cells. In the second stage, specific induction conditions, such as high light intensity and salinity stress, are controlled to promote the accumulation of astaxanthin. Through this two-stage cultivation approach, it is possible to obtain microalgae biomass simultaneously rich in both proteins and astaxanthin.

7. Conclusions

This review demonstrates that microalgae-mediated FPW valorization for astaxanthin production offers a dual solution to environmental pollution and resource recovery. Astaxanthin-producing microalgal species (e.g., *Haematococcus pluvialis* and *Chromochloris zofingiensis*) could efficiently assimilate nutrients from diverse FPW streams, simultaneously mitigating eutrophication risks and carbon emissions through mixotrophic metabolism. We compared microalgae-based and plants-based carbon capture, demonstrating the promising advantages of growing microalgae to reduce the carbon emission of FPW.

An in-depth discussion of the potential technologies, including a two-stage cultivation model, nutrient profile adjustment of wastewater, and co-growth of microalgae with other microorganisms, was provided. The advancement of technology has created favorable conditions for microalgae to synthesize astaxanthin, achieving not only a high productivity of astaxanthin but also the efficient removal of nutrients. Astaxanthin application in animal feed significantly improves the meat pigmentation, antioxidant capacity, immune response, and shelf life of meat products. This approach delivers significant economic and ecological benefits.

Future research should prioritize on-site wastewater treatment, microalgae biomass digestibility, and astaxanthin stabilization techniques to advance industrial adoption. This progress toward a circular bio-economy not only reduces the environmental footprint of the food processing industry but also creates sustainable value chains in the animal farming sector.

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