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Review

Recent advances in recirculating aquaculture systems and role of microalgae to close system loop

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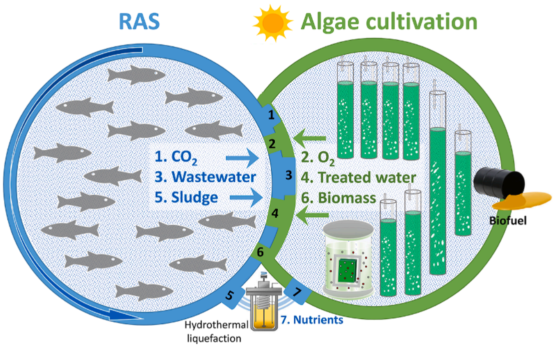
HIGHLIGHTS

- Novel integrated recirculating aquaculture systems (RAS) using microalgae is suggested.
- Microalgae decarbonate RAS by mitigating energy-demanding water treatment processes.
- Microalgae provide O_2 and sequester CO_2, boosting RAS efficiency and sustainability.
- Phycoremediation mitigates the growth-inhibiting factors of fish in RAS.
- Coupled hydrothermal liquefaction of fecal waste provides bioavailable nutrients.

GRAPHICAL ABSTRACT



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ABSTRACT

In recirculating aquaculture systems (RAS), waste management of nutrient-rich byproducts accounts for 30–50% of the whole production costs. Integrating microalgae into RAS offers complementary solutions for transforming waste streams into valuable co-products. This review aims to provide an overview of recent advances in microalgae application to enhance RAS performance and derive value from all waste streams by using RAS effluents as microalgal nutrient sources. Aquaculture solid waste can be converted by hydrothermal liquefaction (HTL), then the resultant aqueous phase of HTL can be used for microalgae cultivation. In addition, microalgae generate the required oxygen while sequestering carbon dioxide. The review suggests a novel integrated system focusing on oxygenation and carbon dioxide capture along with recent technological developments concerning efficient microalgae cultivation and nutrient recovery techniques. In such system, microalgae-based biorefineries provide environmentally-conscious and economically-viable pathways for enhanced RAS performance and conversion of effluents into high-value products.

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1. Introduction

The rapidly increasing global population, projected to reach nearly 10 billion by 2050 (Suzuki, 2019), poses significant challenges to food security and energy sustainability. In addition, global demand for freshwater is rising and an increasing proportion of fish is farmed. Traditional aquaculture and reliance on fossil fuel-based energy systems are struggling to meet the rising demands without exacerbating environmental degradation. This situation underscores the urgent need for innovative solutions to ensure a stable food supply and sustainable energy sources. Aquatic foods, from both freshwater and marine environments, play a crucial role in achieving the global food and nutrition security goals, as well as in providing more environmentally sustainable animal-source foods (Gephart et al., 2020). Globally, the per capita supply of seafood increased from 9.0 kg in 1961 to 20.2 kg in 2015 (FAO, 2024a), with rising prices indicating even stronger demand, which is expected to rise significantly in the medium term between 2030 and 2050 (Willett et al., 2019). Nevertheless, the variety and total amount of wild fish have declined over time due to the impact of human activities, which limits the efficiency of natural fishing resources (Elshobary and Ashour, 2024). Alternatively, aquaculture provides an efficient method to convert the existing feed to aquatic food, escalating the growth of fish and seafood production in the last decades (EEA, 2016). In 2022, the global aquaculture production accounted for approximately 49 % of the

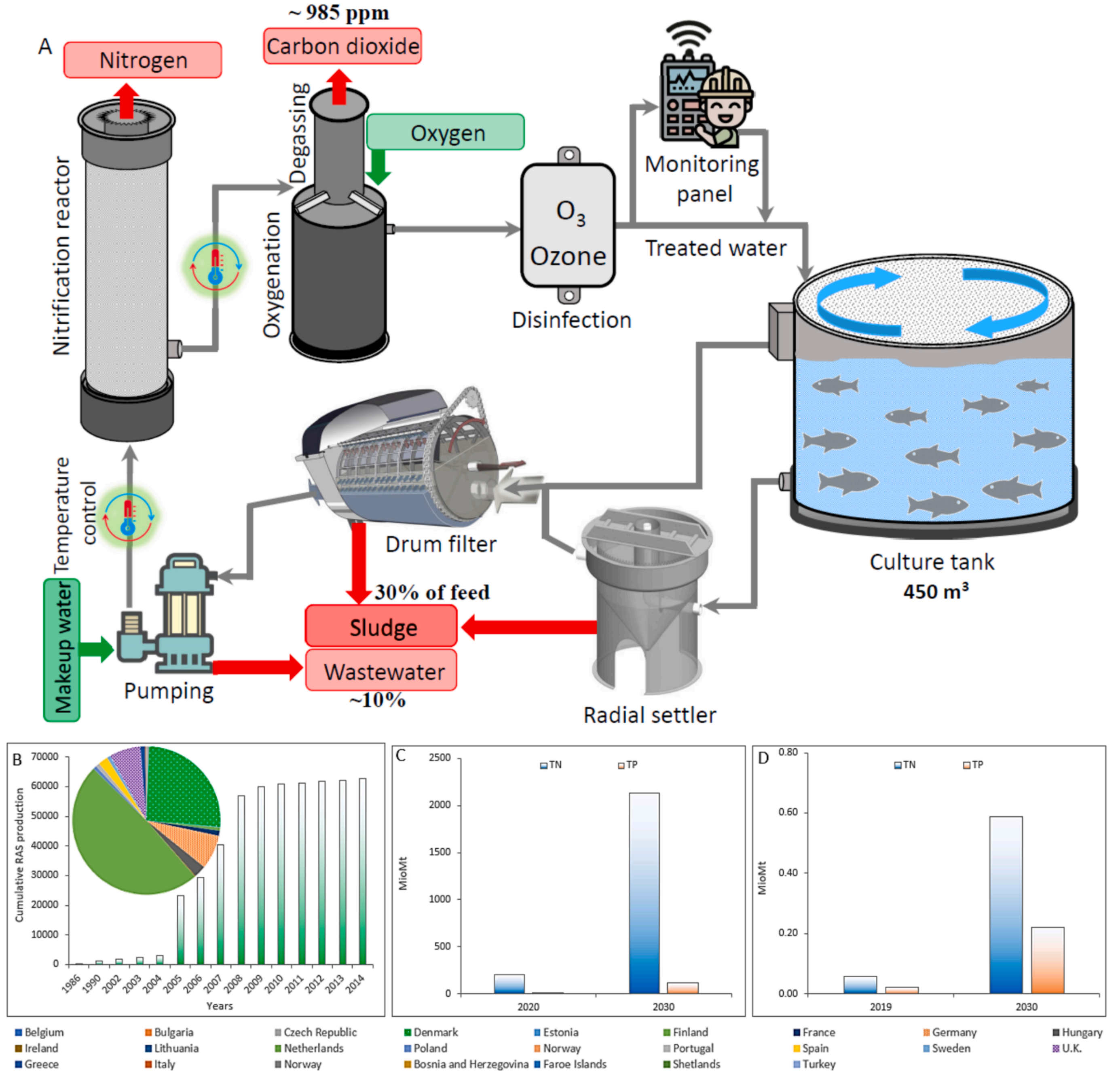

Fig. 1. Schematic diagram showing the basic components of recirculating aquaculture system (RAS, A) with the development of RAS in Europe during 1986–2014 in total and per country (B) with country legend below the figure, together with annual worldwide total nitrogen (TN) and total phosphorus (TP) generation in RAS wastewater (C) and sludge (D) in 2019 as well as the predicted values in 2030. Date are generated from (Bjørndal and Tusvik, 2017; Campanati et al., 2022; Ebeling and Timmons, 2012; FAO, 2022).

total fisheries production, where total aquaculture production in that year reached 87.5 million tonnes (FAO, 2024b). It represents a substantial increase compared to the year 2000 when aquaculture production was only 20.8 million tonnes.

The rise in fish farming globally has caused growing environmental problems because of the release of excess feed and waste from farms. Uneaten fish food and excrement from farmed fish contains high levels of phosphorus (P) and nitrogen (N). When this enters the nearby water bodies like rivers, oceans, and lakes, it leads to a buildup of phosphorus and nitrogen that can damage the aquatic ecosystems. Therefore, booming fish farm production has brought mounting environmental concerns around the world. Usually, each 1 kg of fish biomass requires between 1–3 kg of dry feed, which is not fully consumed and digested by fish and excreted as dissolved and solid waste into the water (Crab et al., 2007). The retention of ingested nutrients is influenced not only by diet composition and species but other factors such as feeding level/management, temperature, and fish size have a significant impact. For example, nutrient retention for N, P, and carbon (C) in marine fish species ranges between 13–43 %, 18–36 %, and 14–38 %, respectively (Nederlof et al., 2022). In general, it is estimated that 83 % of P and 79 % of N used for fish production in a regular aquaculture system are expelled into the aquatic ecosystem (Moura et al., 2016). Direct discharge of aquaculture effluents increases the chemical oxygen demand (COD), N and P concentrations in the water bodies leading to eutrophication, which raises many negative impacts on the environment as well as the economy (Moura et al., 2016).

Recirculating aquaculture systems (RAS) are fully-controlled environments for fish production that recycle and reuse water. Compared to conventional flow-through aquaculture systems, RAS offers several advantages including higher productivity, reduced water usage, better biosecurity, and opportunities for waste recycling. However, RAS faces economic and environmental challenges that must be addressed for widespread implementation (Ahmed and Turchini, 2021). Therefore, RAS (Fig. 1A) has been suggested in order to diminish nutrient release. There is fast development in RAS utilization in Europe, with a total cumulative of 62,779 million tonnes produced by RAS in 2014 (Fig. 1B). The most RAS-using countries are the Netherlands (49 %), Denmark (26 %), followed by Germany and the United Kingdom (7 % each) (Report AS-1201, 2024). However, this system is still not widely used globally due to the elevated water treatment costs compared to conventional aquaculture systems (Xifan et al., 2020). In addition, disposal of accumulated sludge (mainly drum filter residue) and requirements for oxygenation represent challenges to achieve a cost-effective eco-friendly system. Moreover, huge amounts of N and P are still released to the environment from the wastewater and sludge of RAS (Fig. 1C-D). Thus, innovative integrated approaches for cost-effective RAS coupled with production of value-added compounds could help to surpass the bottleneck towards global industrialization.

Aquaculture effluent of RAS (AWW) contains generally up to 100-fold less soluble nutrients than industrial or municipal wastewater that has been broadly investigated for algal biomass production. Microalgae cultivation in aquaculture waste was reported to outperform that in other waste streams such as municipal or industrial wastewater (Silkina et al., 2019). This is likely due to the higher nutrient levels and more favorable nutrient ratios present in the aquaculture effluents. Aquaculture sludges and wastewaters are rich in biological nutrients like N, P, and trace metals that are essential for microalgal growth. In contrast, many industrial and municipal wastewaters lack sufficient levels of these nutrients, are enriched with toxic chemicals, or have imbalanced N:P ratios that can limit the microalgal growth (Admirasari et al., 2022; Singh et al., 2017). Therefore, aquaculture wastes represent a more suitable and beneficial cultivation medium for microalgal biomass production. In addition, AWW is increasingly available due to increasing numbers of aquaculture fish farms using recirculating technology. Specifically, RAS represents an increasing share of farmed fish to overall fish production worldwide. As shown in Fig. 1A, RAS produces waste streams in the form of carbon dioxide (CO_2), AWW, and aquaculture-sludge (Aq-S, mainly fecal waste), which can be utilized as nutrient sources for algal growth. AWW is rich in essential chemicals required for microalgal growth such as N, P, C, iron (Fe), molybdenum (Mo), zinc (Zn), sodium (Na), nickel (Ni), magnesium (Mg), and potassium (K) (Ansari et al., 2017). Due to the increasing production of fish in RAS worldwide, recovery and use of this resource have become increasingly vital. Possible integration of microalgae cultivation with RAS could provide a potential route for enhanced economic feasibility of the system. Therefore, this review evaluates the potential of integrated microalgae-RAS, with an in-depth discussion about the trials of the current consequences. Since the characteristics of waste streams are highly essential for establishing a microalgae cultivation system, the characteristics of aquaculture effluent and Aq-S are discussed in detail. The current waste management and potential challenges associated with RAS-integrated microalgae systems are stated. The potential of microalgal cultivation to eliminate different pollutants such as heavy metals, hormones, and pharmaceuticals from RAS waste streams is also highlighted.

2. Waste valorization in recirculating aquaculture systems

Conventional aquaculture systems face sustainability challenges due to low productivity, water quality degradation, and antibiotic overuse. Alternatively, fish production using RAS is environmentally friendly and sustainable, offering higher fish quality and eco-safety (Ahmed and Turchini, 2021). In addition, RAS systems can recover nutrients from waste, transforming them into valuable biomass by integrating microalgae cultivation. This capability is essential for maintaining environmental sustainability and system efficiency. The traditional recirculation system of RAS can be subdivided into several individual units that include waste solids removal unit, culture tanks, drain system, mechanical/granular filters, settling basins and tanks, solid disposal unit, biofiltration system, oxygenation unit, CO_2 removal unit, and monitoring/control unit (Fig. 1A). All of these units work together to ensure water treatment and provide a healthy environment to the fish. Despite the aforementioned advantages, there are still chances to enhance the economic feasibility and to reduce their footprint. This can be achieved by implementing measures such as full nutrient recycling through integrated multi-trophic aquaculture (Nederlof et al., 2022), using renewable energy sources to reduce CO_2 emissions at lower operating costs (Ahmed and Turchini, 2021), and applying new treatment technologies like membrane bioreactors for efficient water reuse (Huang et al., 2024).

A conventional RAS has a minimum water exchange rate of 0.1–3.0 m^3 kg^{-1} feed (Bregnballe, 2022), with 50–70 % of the feed nitrogen being released as waste (Schneider et al., 2005). According to Ebeling et al. (2006), introducing 1 kg of feed containing 32 % crude protein into 1 m^3-capacity RAS results in the excretion of 30 g of ammonia-N into the water. Ammonia-N can be harmful to fish, so its concentration must be kept below 1 mg L^{-1} in RAS water (Labatut et al., 2007). Total ammonia nitrogen (TAN) has two forms, ammonium ion (NH_4^+) and unionized ammonia (NH_3). The toxicity of the unionized NH_3 is dependent on the dissolved CO_2 concentration and pH level of the water. The pH increases by the reduction of dissolved CO_2, which then increases the NH_3 toxicity (Labatut et al., 2007). In RAS, dissolved CO_2 is continuously generated through fish respiration and bacterial decomposition processes. To control CO_2 levels, a degassing process must be implemented (Fig. 1A), where failure to properly manage the pH and dissolved CO_2 can expose the fish to higher risks of TAN toxicity.

To sustain RAS water quality while facilitating water recycling, a sequence of water purification modules is integrated (Fig. 1A). These comprise a unit for eliminating solids, a biological filtration system for removing inorganic nitrogen, and water conditioning by oxygenation, heating, and disinfection (Bregnballe, 2022). The biological filtration system controls total ammonia concentration through autotrophic

Table 1
Bioremediation of different pollutants via various microalgae with the removal efficiency, time, mechanism, and/or growth parameters.

Pollutant	Algae Species	Removal (%)	Time	Mechanism	References
I. Heavy metals					
Arsenic	*Scenedesmus almeriensis*	40.7 %	3 h	*na*	(Saavedra et al., 2018)
	Sarcodia suiae	*na*	*na*	Adsorption	(Libatique et al., 2020)
	Chorella minutissima and *Scenedesmus* sp.	72.0 %	*na*	*na*	(Arora et al., 2017)
	Chlorella sp. + Coconut shell activated carbon	19.8 %	5 min	Adsorption	(Jiang et al., 2023)
Boron	*Scenedesmus almeriensisis*	38.6 %	10 min	*na*	(Saavedra et al., 2018)
	Chlorella regularis	12.7–80.8 %	8 days	Adsorption and accumulation	(Yan et al., 2022)
	Chlorella + triacontanol	38.0 %	20 days	Adsorption and accumulation	(Ertit Taştan et al., 2012)
Cadmium	*Phormidium ambiguum*	86.0 %	*na*	Adsorption and accumulation	(Shanab et al., 2012)
	Desmodesmus sp. and *Heterochlorella* sp.	> 58.0 %	16 days	Adsorption and accumulation	(Abinandan et al., 2019)
	Chlorococcum humicola	17.0 %	6 days	Adsorption	(Borah et al., 2020)
	Porphyra leucosticta	75.0 %	2 h	*na*	(Ye et al., 2015)
	Oedogonium westi		7 days	Biosorptiom	(Shamshad et al., 2016)
Chromium	*Pseudochlorella pringsheimii* and *Chlorella vulgaris*	80.0 %	1 day	Accumulation	(Saranya and Shanthakumar, 2019)
	Oedogonium westi	93.0 %	7 days	Adsorption	(Shamshad et al., 2016)
Cobalt	*Chlorococcum humicola*	44.0 %	*na*	Adsorption	(Borah et al., 2020)
	Haematococcus sp.	62.3 %	48 h	Adsorption & accumulation	(Kim et al., 2020)
	Vacuoliviride crystalliferum	19.5 %	48 h	Adsorption & accumulation	(Kim et al., 2020)
	Chlorella vulgaris	17.0 %	48 h	Adsorption & accumulation	(Kim et al., 2020)
	Phormidium tenue	94.0 %	30 min	Adsorption	(Abdel-Raouf et al., 2022)
Copper	*Desmodesmus* sp.	80.0 %	168 h	Adsorption & accumulation bioaccmulation	(Buayam et al., 2019)
	Chlorophyceae spp.	88.0 %	10 min	Accumulation	(Saavedra et al., 2018)
Iron	*Chlorococcum* sp.	74.5 %	6 days	Adsorption	(Borah et al., 2020)
	Scenedesmus obliquus	26.2 %	4 days	Adsorption & accumulation	(Wang et al., 2022)
	Microcystis aeruginosa	54.1 %	4 days	Adsorption & accumulation	(Wang et al., 2022)
Lead	*Oedogonium westi*	61.0–96.0 %	7 days	Adsorption	(Shamshad et al., 2016)
	Phormidium ambiguum	70.0 %	*na*	Adsorption and accumulation	(Shanab et al., 2012)
	Porphyra leucosticta	90.0–95.0 %	2 h	*na*	(Ye et al., 2015)
Manganese	*Chlorella vulgaris*	99.4 %	3 h	*na*	(Saavedra et al., 2018)
	Microcystis aeruginosa	69.2 %	6 h	Adsorption & accumulation	(Wang et al., 2022)
	Anabaena flos-aquae	72.7 %	6 h	Adsorption & accumulation	(Wang et al., 2022)
	Chlorella pyrenoidosa	72.7 %	6 h	Adsorption & accumulation	(Wang et al., 2022)
Mercury	*Scenedesmus obtusus*	95.0 %	*na*	Adsorption	(Huang et al., 2019)
	Chlorella + Coconut shell activated carbon	71.6 %	5 min	Adsorption	(Jiang et al., 2023)
	Phormidium ambiguum	97.0 %	*na*	Adsorption and accumulation	(Shanab et al., 2012)
Nickel	*Durvillaea antarctica*	32.9 %	240 min	Adsorption	(Guarín-Romero et al., 2019)
	Oedogonium westi	59.0–89.0 %	7 days	*na*	(Shamshad et al., 2016)
Zinc	Chlorophyceae spp.	91.9 %	3 h	*na*	(Saavedra et al., 2018)
II. Antibiotics					
Cefradine	*Chlorella pyrenoidosa*	76.0 %	96 h	Accumulation	(Chen et al., 2015)
	Chlorella pyrenoidosa + UV	78.0 %	24 h	Accumulation	(Du et al., 2015)
	Chlorella pyrenoidosa	76.0 %	96 h	Accumulation	(Chen et al., 2015)
Sulfamethoxazole	*Nannochloris* sp.	27.0 %	14 days	Accumulation	(Bai and Acharya, 2016)
Triclosan	*Nannochloris* sp.	100.0 %	7 days	Accumulation	(Bai and Acharya, 2016)
Tetracycline	*Chlorella vulgaris*	69.0 %	62 days	Accumulation	(de Godos et al., 2012)
Spiramycin	*Microcystis aeruginosa*	12.5–32.9 %	7 days	Accumulation	(Liu et al., 2012)
Amoxicillin	*Microcystis aeruginosa*	30.5–33.6 %	7 days	Accumulation	(Liu et al., 2012)
Ciprofloxacin	*Chlorella* sp. + Fe^{2+} + UV	>99.0 %	120 min	Accumulation	(Díaz-Quiroz et al., 2020)
Levofloxacin	*Chlorella vulgaris*	91.5 %,	11 days	Biodegradation	(Xiong et al., 2017)
III. Hormones					
17β-estradiol	*Chlorella* sp. + Fe^{2+} + UV	75.0 %	120 min	Accumulation	(Díaz-Quiroz et al., 2020)
	Selenastrum capricornutum	88.0–100.0 %	7 days	Biodegrdation	(Hom-Diaz et al., 2015)
	Nannochloris sp.	38.0 %	7 days	Accumulation	(Bai and Acharya, 2016)
17α-estradiol	*Scenedesmus dimorphus*	85.0 %	8 days	Biotransformation	(Zhang et al., 2014)
Estrone	*Scenedesmus dimorphus*	85.0 %	8 days	Biotransformation	(Zhang et al., 2014)
	Nannochloris sp.	40.0 %	7 days	Accumulation	(Bai and Acharya, 2016)
Estriol	*Scenedesmus dimorphus*	95.0 %	8 days	Biotransformation	(Zhang et al., 2014)
17α-ethinylestradiol	*Chlamydomonas reinhardtii*	71.0–100.0 %	7 days	Biodegradation	(Hom-Diaz et al., 2015)
	Desmodesmus subspicatus	68.0 %,	3 days	Biodegradation	(Maes et al., 2014)
Progesterone	*Scenedesmus obliquus*	>95.0 %,	5 days	Biodegradation	(Peng et al., 2014)
Norgestrel	*Chlorella pyrenoidosa*	60.0 %,	5 days	Biodegradation	(Peng et al., 2014)
VI. Other pollutants					
Bisphenol A	*Stephanodiscus hantzschii*	*na*	16 days	Bioaccumulation and catabolism	(Li et al., 2009)
Fluroxypyr	*Chlamydomonas reinhardtii*	57.0 %	5 days	Bioaccumulation and catabolism	(Zhang et al., 2011)

(*continued on next page*)

Table 1 (*continued*)

Pollutant	Algae Species	Removal (%)	Time	Mechanism	References
Prometryne	*Chlamydomonas reinhardtii*	40.0 %	4 days	Bioaccumulation and catabolism	(Jin et al., 2012)
Fluoranthene	*Cyclotella caspia*	85.0 %	6 days	Bioaccumulation and catabolism	(Liu et al., 2006)
Ibuprofen	*Scenedesmus obliquus* and *Chlorella vulgaris*	60.0 %	7 days	Biotransformation	(Larsen et al., 2019)

na Not available.

nitrification. Because the accumulation of nitrate as a product of nitrification is not desired, a denitrification reactor is used to reduce nitrate into nitrogen gas that is released into the atmosphere. Nevertheless, denitrification is not a valuable method because nitrogen is released as waste, and generating inorganic N fertilizers from N_2 gas demands substantial energy input. To enhance RAS sustainability, alternative methods for converting nitrate, nitrite, and ammonia need to be investigated. These may include nitrogen bio-assimilation using microorganisms such as microalgae (e.g. *Tribonema, Chlorella, Scenedesmus*) (Nederlof et al., 2022; Wang et al., 2024), bacteria (e.g. *Rhodococcus, Sphingopyxis*) (Chen et al., 2024), or their co-cultivation (Huo et al., 2020) that can be harvested and utilized as feed supplements or biofertilizers.

Removal of solids in RAS is mainly done by mechanical automated filter systems such as drum filters. Apart from other health-impairing effects of Aq-S on fish, any Aq-S will impair the nitrification process due to overgrowth of heterotrophic bacteria. Thus, Aq-S removal is extremely important to avoid oxygen depletion by heterotrophic bacterial mineralization. Direct discharge of Aq-S rich in organic carbon, nitrogen, and phosphorus into water bodies results in eutrophication and oxygen depletion in the receiving water bodies. Several strategies have been proposed for utilizing Aq-S in composting or landfill applications. However, these inefficient management practices result in significant resource loss, highlighting the need to explore more effective and sustainable alternative approaches. Despite their high nutritional value, insufficient focus has been given to Aq-S for microalgae cultivation. This is probably due to the fact that microalgae cannot utilize organically-bound nutrients directly. Hence, Aq-S requires pre-processing before such an indirect application as algae nutrients.

3. Microalgal cultivation for phycoremediation

Employing microalgae has been emerged as an economically and eco-friendly promising approach to treat conventional aquaculture effluent. This process involves generating on-site biomass, displacing the need for unsustainable fishmeal, and translating into enhanced organism survival without expanding environmental footprints (Han et al., 2019). Various algal species, such as *S. obliquus* and *C. vulgaris* (Krohn-Molt et al., 2013), *Didymogenes* sp. (Tang et al., 2023), *Acutudesmus* sp. (Mishra et al., 2022), *Chlamydomonas* sp., *Porphyridium* sp., *Spirogyra* sp., *Spirulina* sp., *Stichococcus* sp., and *Stigeoclonium* sp. (Sultana et al., 2024), showcased their ability of high removal efficiencies of nutrients, antibiotics, heavy metals, and other contaminants in aquafarming. Beyond biosorption, concurrent microalgal cultivation with fish/crustaceans in ponds confers many additional advantages. For instance, decarbonization and oxygenation, where oxygen liberating from photosynthesis prevents anoxia, lowers the cost of aeration needed (Soroosh et al., 2022), reduces the outcompeting pathogenic microbes, and thereby reduces disease and antibiotic usage. Table 1 provides a summary of different pollutants that can be effectively removed by different microalgal species.

3.1. Heavy metals

Heavy metals accumulated in RAS can exert detrimental effects on the growth of the cultured fish, inhibiting their development (Martins et al., 2009a). Since there are no available technical removal methods on the market yet, the only way to remove heavy metals is to maintain a certain level of water exchange. Thus, their accumulation is one of the main bottlenecks towards zero-water discharge in RAS. Various heavy metals like copper, molybdenum, nickel, zinc, and boron serve as essential micronutrients for microalgae that facilitate their growth by enabling cellular metabolism (Chugh et al., 2022). Microalgae possess an exceptional capacity to tolerate and remove heavy metals, making them well-suited for bioremediation (Chugh et al., 2022). Additional advantageous characteristics include high metal-binding affinity, substantially-high cellular surface area, eco-friendly nature, and regenerable value-added biomass (Dai and Wang, 2024). Cultivation of microalgae has been studied as an effective and environmentally friendly method for aquaculture heavy metals bioremediation (Table 1). Interestingly, microalgae belonging to order Cyanidiales, such as *Cyanidioschyzon*, *Cyanidium*, and *Galdieria*, showed high efficiency in heavy metal bioremediation due to their ability to tolerate extreme stresses and thrive in acidic mine drainage (Kharel et al., 2023). Accordingly, microalgae-assisted RAS would benefit from the ability of microalgae to retain heavy metals allowing further reduced water makeup in modern fish production. On the other hand, the presence of heavy metals in microalgae biomass may limit its applications, particularly in food and feed industries, due to potential health concerns. Heavy metal-rich microalgae biomass could potentially be utilized for non-food applications such as biofuel production, since this potential is further enhanced by the stress-induced lipid and carbohydrate accumulation in microalgae (Shitanaka et al., 2024).

3.2. Antibiotics and hormones

Despite the high control level in monitoring and manipulating various parameters within the closed RAS, and the presence of ultraviolet (UV) disinfection unit to minimize the impact on the environment, high stocking densities make it problematic to avoid the initiation and spread of diseases. Such diseases might lead to contagions and increase stress levels which impair animal welfare and growth as well as increase the mortalities. Antibiotics persist as the most effective therapy, despite the consequence of accumulation in fish muscles (Almeida et al., 2019). Bacterial infections negatively affect the water quality by increasing organic matter and nutrient loading in the water, which can lead to oxygen depletion, ammonia accumulation, and deterioration of water quality. Antibiotics are widely used in conventional aquaculture systems, and also in RAS, primarily to mitigate the adverse effects of water degradation on various environmental parameters and factors within the aquaculture system that can influence the health and growth of cultured organisms (see supplementary materials). This adverse effect takes place by controlling and preventing the spread of bacterial diseases among cultured organisms (Sha et al., 2022). A wide range of antibiotics including ampicillin (Amp), oxacillin (Oxa), penicillin (Pen), ceftazidime (Caz), cefazolin (CFZ), chlortetracycline (CTC), oxytetracycline (OTC), norfloxacin (NOR), ciprofloxacin (CFX), and ofloxacin (OFX) are added with fish feed or as pharmaceutical additives. However, antibiotics improve the health of the aquaculture system at the cost of human health, environmental safety, and ecological permanency. Sha et al. (2022) reported that there are no documented studies on the efficient control and/or antibiotics removal from RAS, which requires further evaluation.

Antibiotics could accumulate in the fish tissue causing oxidative stress, adversely influencing fish growth and biological activities (Zhang et al., 2020a; Zhang et al., 2021). After being retained in the body, antibiotics exhibit a challenge in terms of being metabolized, decomposed, or timely discharged, leading to bioaccumulation in the system. Antibiotics and microbes with antibiotic-resistance genes (ARGs) can be transmitted through the food chain into human, which represents a risk to human health. This could lead to chronic organ failure, sensitive reactions in the human body, and alteration of the beneficial bacterial community structure in the intestine, resulting in the weaken of human immunity. By 2050, projections suggest that antibiotic resistance may lead to approximately 10 million premature deaths and annual economic losses of about 93 trillion € globally (O'Neill, 2016). Therefore, many developed countries have restricted the applying of antibiotics in aquaculture (Love et al., 2020). Given the limited availability of alternatives as effective as antibiotics and the imperative for intensifying the aquaculture, it is plausible to anticipate continued global antibiotic use in aquaculture production, with estimated increase in antibiotic utilization of 67 % by 2030 (Van et al., 2020). For algal biomass utilization, embedded antibiotics can contribute to the accumulation of antibiotic-resistant bacteria, posing significant risks to both human and animal health. This compromises the safety and acceptability of using microalgae biomass in industries that demand high standards of product safety and purity. In addition, stringent regulatory frameworks governing antibiotic contamination levels are necessary to ensure the safety and marketability of microalgae biomass applications with high product purity standards.

Growth promoters/hormones are also commonly used in fish farms and aquatic operations to boost the yields. For example, 17α-methyltestosterone is administered to induce sex reversal in the production of monosex populations (Farias et al., 2023). Residual hormones from aquaculture sources can enter the waterbodies and have the potential to alter the endocrine systems of aquatic wildlife even at very traces concentrations (Jasrotia et al., 2021). Endocrine disrupting chemicals (EDCs) refers to external substances that disrupt the metabolism, release, production, transport, binding, or elimination of hormones within the body. Their potential to disrupt reproduction and jeopardize survival of wildlife species, as well as human health, is a major concern (Jamwal and Shekh, 2021). These EDCs lead to a range of health issues including cardiovascular disorders, neurological disorders, reproductive disorders, kidney disease, autoimmune disorders, and cancer (Thacharodi et al., 2023). Thus, there is an urgent need to reduce the utilization of these contaminants and/or sustainable remediation approaches for efficient removal from aquaculture effluent before discharge to natural water bodies.

Recent studies indicated microalgae-based as hopeful substitute for efficient antibiotics and other pollutants removal from wastewater (Table 1), which would play a significant role in RAS. There are various processes through which antibiotics can be eliminated, encompassing bioadsorption, bioaccumulation, metabolism within cells, extracellular degradation, and non-biological factors such as hydrolysis and photodegradation (Li et al., 2024). The attachment of antibiotics to extracellular polymeric substances (EPSs) and polymers secreted by microalgae facilitates bioadsorption, but this process in microalgal cells accounts for only a minor portion (1–3 %) of sulphonamides and fluoroquinolones removal (Kiki et al., 2022). Bioaccumulation, which involves the active uptake of substances into cells, is a key function of microalgae, but neither bioadsorption nor bioaccumulation is the primary route for antibiotics removal (Wang et al., 2023a). However, biodegradation, the continuous breakdown of antibiotics through dissolution, enzymatic action, and cellular ingestion without residual accumulation, is the predominant mechanism for their removal. While bacterial degradation relies on resistant bacterial strains, microalgal degradation is facilitated by intracellular metabolism and extracellular enzymatic active substances like cytochrome P450 (CYP450) (Wang et al., 2023a; Xiao et al., 2024). For instance, *C. sorokiniana* effectively removed oxytetracycline (OTC) with an efficiency up to 99 % from wastewater (Wu et al., 2022a). Additionally, it exhibited a growth-promoting effect at OTC concentrations below 50 mg L^{-1}, although growth inhibition of *C. sorokiniana* occurred at concentrations exceeding 100 mg L^{-1}. Similarly, biodegradation by *Isochrysis galbana* in RAS accounted for 86.67 % of florfenicol (FLO) (Qian et al., 2022). Moreover, the combined processes of biodegradation and biosorption by *Chlorella* sp. resulted in a significant reduction in FLO by 89.74 % in RAS without growth-inhibiting effect on the microalgae (Zhang et al., 2020b). Other investigations reported similar impacts for potential antibiotic removal by microalgae using different antibiotics such as thiamphenicol, enrofloxacin, and sulfamethazine (Chen et al., 2020; Song et al., 2020). Systems involving a symbiotic relationship between algae and bacteria significantly enhance the degradation of antibiotics. Recent studied confirmed substantial improvements in the degradation rate and efficiency of different antibiotics in such algae-bacteria symbiosis systems (Wang et al., 2023a).

Algae also have been found to remove hormones from aquaculture systems (Table 1), such as 17α-ethinylestradiol (EE2) and 17β-estradiol (E2) from mariculture wastewater (Hardegen et al., 2023). Marine microalgae including *Nannochloropsis oculata*, *Phaeocystis globosa*, *Dunaliella salina*, and *Platymonas subcordiformis* have demonstrated the ability to absorb, adsorb, and biodegrade EDCs such as nonylphenol (NP) in polluted water (Wang et al., 2019). Furthermore, the use of sonicated microalgal biomass combined with Fe^{2+} and UV light has been shown to effectively remove both antibiotics and hormones, including CFX and E2, from wastewater (Díaz-Quiroz et al., 2020). These findings indicate that microalgae can perform a substantial role in the removal of a wide variety of soluble/suspended pollutants from RAS-aquaculture effluent.

3.3. Microalgal-based decarbonization

CO_2 in the aquaculture system is primarily generated by the respiration of cultured fish and bacteria. In addition, oxidation of nitrogen in the biofiltration can generate free acids that may also raise the levels of dissolved CO_2 (Summerfelt and Sharrer, 2004). As the water exchange rates and stocking densities increase, dissolved CO_2 can become a limiting factor in the aquaculture systems. Elevated CO_2 concentrations in water lead to an increase in CO_2 levels in the fish blood, which can hinder their growth. This reduces the capacity of blood oxygen-delivery, lessens the affinity of haemoglobin to oxygen molecules, and results in the reduction of feeding ability, too much consumption of protein, and overall harmful impacts on the metabolism, development, and health of fish (Stiller et al., 2015). However, safe CO_2 concentration, which refers to the maximum level or threshold of dissolved CO_2 in the water that does not have harmful or negative impacts on the cultivated fish species, relies on the fish species, fish age, and water conditions. For striped bass (*Morone saxatilis*) and Tilapia (*Oreochromis niloticus*), CO_2 levels up to 60 mg L^{-1} showed no harmful impacts; the safe CO_2 limit for trout (*Oncorhynchus mykiss*) was 9–30 mg L^{-1}; while less than 12 mg L^{-1} CO_2 was required for Atlantic salmon (*Salmo salar*) (Mota et al., 2019). Results showed that elevated CO_2 levels above 15 mg L^{-1} result in thinner dermis and negative impacts on the development and metabolism of pikeperch (*Sander lucioperca*) (Steinberg et al., 2017). Moreover, CO_2 influences the bacteria associated with cultivated fish and biofilters by reducing the pH of the whole system (Barakat et al., 2021). Hence, it is essential to ensure efficient CO_2 removal and maintain effective system oxygenation using conventional methods such as aeration, pure oxygen injection, or oxygenation cones. These techniques are critical for achieving the appropriate dissolved oxygen concentration, which ranges from 2 to 6 mg L^{-1}, depending on the species being farmed and the stocking density (Welker et al., 2019).

Traditional aeration devices are effective at oxygenating water and eliminating CO_2 when stocking densities of farmed organisms are below 30–60 kg m^{-3}. However, to enhance the overall production, the stocking capacity of RAS farms must exceed 100 kg m^{-3}. Therefore, effective CO_2 removal is essential for the success of RAS (Ebeling and Timmons, 2010). CO_2 stripping technology is commonly applied, involving the removal of CO_2 by employing packed column where water flows down

Table 2
Chemical compositions of wastewater (AWW) and sludge from recirculating aquaculture system (RAS) in comparison with typical synthetic microalgae culture media.

Parameters	RAS AWW	 Sludge	Microalgae synthetic media BG-11	 f/2
Species	Nile tilapia	Nile /red tilapia	Freshwater species	Marine species
Conductivity (µS cm^{-1})	1142.6	3.4	*na*	*na*
Dissolved O_2 (mg L^{-1})	6.4	*na*	*na*	*na*
pH	6.3	*na*	7.4	7.5
TN (mg L^{-1})	*na*	*na*	247.0	75.0
NH_3-N (mg L^{-1})	0.3	0.8	19.0	*na*
NO_2-N (mg L^{-1})	0.3	*na*	*na*	*na*
NO_3-N (mg L^{-1})	63.0	313.0	*na*	75.0
Phosphate-P (mg L^{-1})	16.9	102.7	7.1	5.0
Chemical oxygen demand (mg L^{-1})	95.4	*na*	*na*	*na*
Alkalinity (mg L^{-1} as $CaCO_3$)	46.2	*na*	*na*	*na*
SO_4-S (mg L^{-1})	*tr*	48.4	*na*	20.0
Copper (Cu) (mg L^{-1})	*tr*	0.1	*tr*	*tr*
Manganese (Mn) (mg L^{-1})	*tr*	0.6	0.5	0.2
Zinc (Zn) (mg L^{-1})	*tr*	0.1	*na*	*tr*
Calcium (Ca) (mg L^{-1})	*tr*	151.0	9.8	10.0
Potassium (K) (mg L^{-1})	*tr*	130.9	18.0	10.0
Magnesium (Mg) (mg L^{-1})	*tr*	58.1	7.4	20.0
Sodium (Na) (mg L^{-1})	*tr*	369.9	414.0	80.0
Iron (Fe) (mg L^{-1})	*tr*	*tr*	1.4	0.4
Molybdenum (Mo) (mg L^{-1})	*tr*	0.1	0.2	*na*
Cobalt (Co) (mg L^{-1})	*tr*	*na*	*tr*	*tr*
Boron (B) (mg L^{-1})	*tr*	0.7	0.5	*na*
References	(Martins et al., 2009a)	(Rakocy et al., 2007)	(Su et al., 2022)	(Guillard, 1975)

tr the corresponding parameter is in traces below the detection limit; *na* not available/applicable.

through air-stripping column (Summerfelt et al., 2000). This method effectively lowers the ambient CO_2 levels without the need to increase water flow or decrease the stocking density, as it directly manipulates CO_2 levels and water quality. This parallel challenge is encountered in RAS farming, where significant investments are directed towards the costly removal of CO_2 from water, subsequently releasing the removed CO_2 into the atmosphere. On average, CO_2 strippers need additional electricity supplementation and relatively high maintenance expenses estimated by at least 0.31 € kg^{-1} of sold fish (Noble et al., 2012). On the other hand, microalgae production necessitates a substantial amount of CO_2 for photosynthesis and growth. Thus, microalgae could be used to capture CO_2 to mitigate the effect on the environment and promote the circular economy in RAS.

Utilization of RAS effluent systems based on microalgae offers several advantages over conventional technologies. These microalgal systems contribute to reduce direct and indirect CO_2 emissions, providing a cost-effective treatment option with reduced energy consumption. Additionally, they facilitate effective nutrient recovery from aquaculture efflent. Furthermore, the microalgal biomass obtained can be valorized and converted into value-added products like biofertilizers or utilized as feedstocks for biofuel production, specifically biodiesel. This integration of microalgal RAS into the biorefinery concept aligns with the principles of a circular economy, promoting sustainable resource utilization and waste minimization (Méndez et al., 2022). In contrast to various other forms of flue gases and wastewater, RAS CO_2 and wastewater lack harmful pollutants that could otherwise restrict the utilization of microalgae for applications beyond biofuel production (Goswami et al., 2022). A recent investigation explored the potential for enhancing nitrate capture by H*aematococcus pluvialis* and *Monoraphidium griffithii* from RAS wastewater by introducing CO_2 extracted from RAS through stripping (Pirhonen et al., 2023). The CO_2 concentration captured and supplied to microalgae was nearly twice as high as the ambient CO_2 concentration, at average ambient CO_2 level of 527 ppm. Results showed that cell densities as well as growth rates of *M. griffithii* increased with CO_2 supplementation, where growth rate showed 0.43 day^{-1} and 0.48 day^{-1} in cultivations without and with CO_2 supplement, respectively. In addition, CO_2 supplementation enhanced the growth of *H. pluvialis* from 0.44 day^{-1} to 0.52 day^{-1} (Pirhonen et al., 2023). Overall, harnessing the potential of microalgae for CO_2 utilization in RAS could be a significant contributor for commercialization of microalgae biomass production and sustainable aquaculture systems.

3.4. Microalgal-based oxygenation

Microalgae possess the unique capability to simultaneously function as biofilters, oxygen providers, and a nutritional source. As a result, dense populations of microalgae are excellent candidates for integration into aquaculture systems, both extensive and intensive. The cultivation of microalgae can be implemented directly within fishponds and their effluent streams, facilitating on-site bioremediation (Milhazes-Cunha and Otero, 2017). Alternatively, biofiltration of effluents from extensive and intensive aquaculture operations can be achieved in separate microalgae culture units. In such cases, the microalgae can either be employed as an uncontrolled agent within the effluent streams or cultivated intensively under carefully controlled conditions to enhance biomass yield and treatment efficiency. The use of microalgal biofiltration presents an opportunity to produce high-value biomass as a low-cost byproduct, thereby valorizing resources that would otherwise be lost or wasted (Milhazes-Cunha and Otero, 2017).

Microalgae possess a significant capacity to produce oxygen and could serve as a natural bio-pump for aeration in aquaculture systems, effectively enhancing oxygen levels in the water. Additionally, they play a crucial role in maintaining the microbial community within the aquatic environment (Han et al., 2019). In modern RAS systems, technical oxygen is used to supersaturate RAS water with O_2, allowing stocking densities exceeding 40 kg m^{-3}. The costs associated with technical oxygen can account for 40 cents kg^{-1} fish, representing up to 40 % of the total production costs (Badiola et al., 2012). To date, technical oxygen is the only applied method in commercial RAS to meet high oxygen demands. Photosynthetic oxygenation has been successfully applied in the remedy of industrial and domestic wastewaters as well as anaerobic digestate (Pizzera et al., 2019; Soroosh et al., 2023, 2022; Wang et al., 2023a). However, this type of oxygenation is a new approach for aquaculture systems. In a recent study balancing O_2 respiration and uptake of microalgae-bacteria associations for wastewater remediation, the highest specific microalgae respiration (sOURmM) was assessed to 14.73 mgO_2 $gVSS^{-1}$ h^{-1} by supplementation of 50 mg L^{-1} HCO_3^- (Flores-Salgado et al., 2021). However, the highest specific respiration of heterotrophic bacteria (sOURmB) was 2.37 mgO_2 $gVSS^{-1}$ h^{-1}. The mass balance of O_2 respiration and uptake needs to be put into the context of light/dark cycles applied. Longer light periods, e.g. 16:8 h showed greater biomass yield of microalgae with more oxygen production (Stunda-Zujeva et al., 2023). Hence, microalgae could produce more O_2 than what is consumed at extended light periods thereby increasing RAS O_2 levels.

4. Aquaculture waste for microalgae cultivation

4.1. Aquaculture effluent for microalgae cultivation

Due to the richness in nutrients, aquaculture effluent requires post-treatment before discharge into the natural water bodies to avoid its negative impacts on microbial diversity (Wu et al., 2022b). The composition of aquaculture effluent from RAS is shown in Table 2, in comparison with common growth media used for microalgae cultivation. It can be noted that there is a wide variation of nutrient profiles from different studies due to the impact of some critical factors such as water replacement frequency, stocking density, and feed addition. Compared to municipal waste streams and industrial wastewater, aquaculture effluent is more suitable for microalgal growth as it contains less toxic components including heavy metals and antibiotics (Hu et al., 2014). The harvested microalgal biomass can be re-used as a potential feed for aquaculture or for the production of many value-added compounds (Table 3) that could enhance the economic feasibility of the aquaculture system. Considering a 100-tonne trout farm with a volume of 450 m^3 and a daily wastewater exchange rate of 10 %, this leads to a daily production of 45 m^3 of AWW which needs to be disposed. In most modern RAS farms, this disposal adds to the overall production costs. Once aquaculture wastes have mixed with urban wastes entering the sewage waste plants, nutrient recovery becomes technically and energetically demanding.

Specifically, P-recovery extends beyond its sequestration to mitigate environmental pollution but also facilitates its reuse as a valuable resource in agricultural or industrial applications. Major elemental sorbents for phosphorus include calcium, aluminum, and iron(III) ions (Faulkner and Richardson, 2020). Most of these sorbents produce a final product which is not suitable for use as a fertilizer, due to the low P-plant availability. Another alternative for P recovery as a fertilizer is the precipitation of struvite, i.e., crystals that can be formed by precipitation of magnesium-ammonium-phosphorus (MAP) (Li et al., 2022). It is a white orthorhombic crystals composed of magnesium ion (Mg^{2+}), ammonium ion (NH_4^+), and phosphate ion (PO_4^{3-}) in equal molar amounts with formula $NH_4MgPO_4{\cdot}6H_2O$ (Korchef et al., 2011). Thus, struvite does not only allow phosphorus recovery, but also nitrogen as an essential fertilizer (Zamparas, 2021).

Struvite can also be used as a nutrient source for microalgal growth. In that context, providing struvite from biogas digestate origin at about 120 mg L^{-1} (15 mg-P L^{-1}) as an alternative P-source resulted in the equal biomass yield and biochemical composition of *Arthrospira platensis,* compared to production values obtained with control media (Markou et al., 2019). Interestingly, the replacement of K_2HPO_4, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, and $NaNO_3$ in Bold's Basal Media (BBM) by 721 mg L^{-1} struvite and 14.2 mg L^{-1} of K_2SO_4 showed higher growth rates of *C. vulgaris* compared to typical BBM (Moed et al., 2015). Similarly, *A. platensis* growth was not negatively affected when struvite replaced the mineral-based phosphorus compared to commercial Spirulina media (Beyer et al., 2023). In addition, C-phycocyanin in the struvite-based medium was significantly higher compared to that in the untreated growth media. In a recent study, it was found that protein production and the growth rates of *C. vulgaris* and *Limnospira* sp., as well as the bacterium *Rhodopseudomonas palustris*, on struvite, were higher than those observed when grown on conventional potassium phosphate (Muys et al., 2023). Thus, cultivation of microalgae directly on aquaculture effluent or through nutrients recovered from aquaculture effluent in the form of struvite presents a promising technique for microalgal biomass production coupled with RAS-aquaculture effluent treatment.

Aquaculture effluent is usually turbid in aquaculture systems owing to the high loads of suspended particles. Suspended particles smaller than 60 μm in the aquaculture effluent (which are not retained by drum filters) can be efficiently removed by protein skimmers (Kovács et al., 2023). However, suspensions containing solid particles with diameters < 60 μm (Zhang et al., 2013) can be used as a valuable nutrient source for microorganisms and successive species in the consumer chain. However, highly concentrated suspensions may interrupt the trophic processes, affecting the abundance of naturally-filtering organisms and feeding effectiveness (Levine et al., 2005; Moreira and Pires, 2016), resulting in high cloudiness of water that limits the foraging success of fish (Goździejewska et al., 2019). In addition, these particles include microorganisms such as bacteria and fungi, which could be pathogenic or grow intensively in the pond, leading to negative impacts. Thus, establishing efficient methods for micro/nano-particles removal from RAS recycled water is of great importance.

Sterilization of recycled water is an important process to eliminate microbes that could be pathogenic to fish. Utilization of UV-C radiation (280–200 nm) is an effective method for sterilizing wastewater in large-scale systems, enabling the efficient treatment of large volumes of water within a reasonable timeframe and with low energy inputs (Qin et al., 2014). In addition, the impact of ozone nanobubbles (ONBs) was examined against aquatic pathogens in the aquaculture, where exposure to 10 min lowered the load of *Aeromonas veronii* and *Streptococcus agalactiae* in fresh water by 96–97 % (Jhunkeaw et al., 2021). An additional exposure time of 20 min with ONBs treatment further reduced the bacterial load in water, achieving above 99.9 % reduction. ONBs treatment provides a high disinfection efficacy in seawater; where 1 min incubation showed over 99.99 % reduction in the tested bacteria (*Vibrio parahaemolyticus*), which reached 100 % after 5 min (Imaizumi et al., 2018). In general, high particle loads counteract with efficiency of sterilization by ozonation (Nghia et al., 2021), requiring efficient removal of nano/micro suspended particles before water reuse.

Research on the impact of sterilization methods on microalgal growth in aquaculture effluent has shown promising results. For instance, González-López et al. (2013) reported ozonation as the most effective method for sterilizing culture medium, leading to successful continuous cultures of *N. gaditana*. Similarly, Racharaks et al. (2015) reported that unsterilized medium composed of shale gas flowback water and anaerobic digestion effluent was comparable to sterilized commercial media for the growth of *N. salina* and *D. tertiolecta*. Moreover, Tejido-Nuñez et al. (2019) found that the growth and nutrient removal efficiency of *C. vulgaris* cultivated in sterilized RAS AWW exceeded that of non-sterilized water due to the grazing protozoa. These studies collectively suggest that sterilized AWW can be as effective as conventional microalgal growth media. Cavitation is one of the commercially established methods to treat/disinfect large volumes of water and is considered relatively cost-efficient. Apart from killing bacteria, cavitation treatment was demonstrated to effectively kill small planktonic crustaceans. In that regard, ONBs lowered the planktonic crustaceans in aquaculture containers by 63.3 % related to the control by destroying crustaceans of all sizes evenly (Kurita et al., 2017). Notably, cavitation has been reported to assist in the disintegration of particles and the release of nutrients (Sežun et al., 2019), which could provide additional benefits. Overall, technologies tested with respect to the liberalization of organically bound nutrients either come from an aquaponics background, aquaculture, or water sanitation research, but are still not yet commercially used for suspended particle treatment in RAS. Alternatively, the substantial adsorption capacity of microalgae and high phycoremediation potential (Section 3) could serve as a significant environmentally friendly method for removing suspended particles. Overall, investigating alternative biological methods for the removal of suspended particles presents considerable potential for advancing RAS development. In this context, the high adsorption capacity of microalgae offers invaluable support, making it an attractive option for enhancing water quality and system efficiency in RAS setups.

Microalgae-heterotrophic bacteria cultivation systems (MaB), also known as high-rate algal ponds, offer a well-established approach for wastewater treatment based on a symbiotic correlation between bacteria and microalgae (Astafyeva et al., 2022). This bidirectional exchange involves microalgae providing oxygen for bacterial growth, while

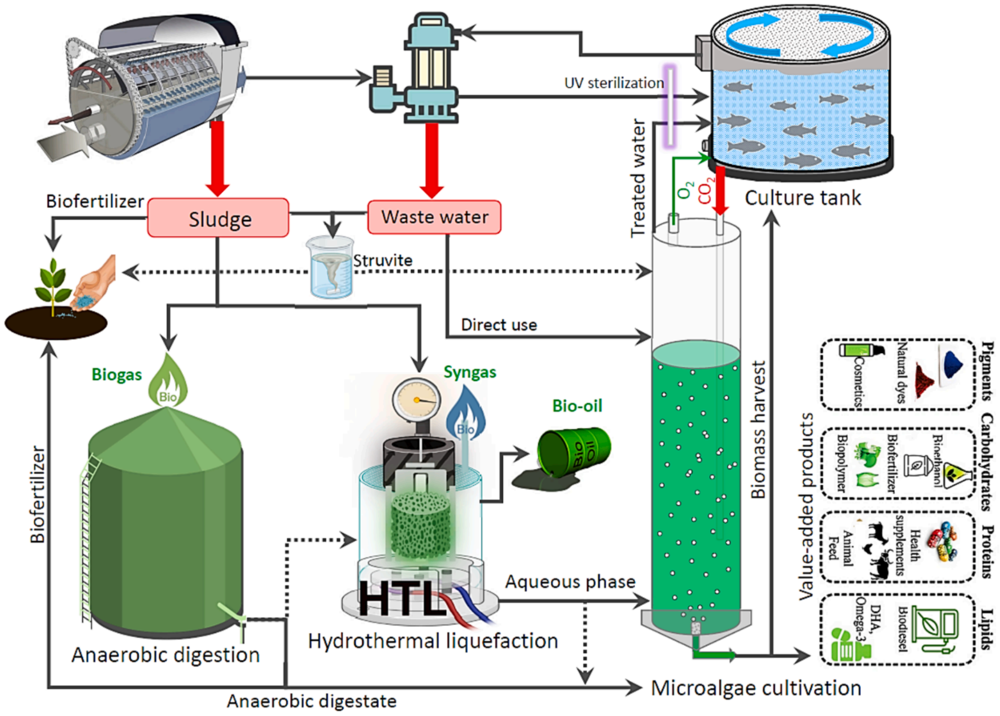

Fig. 2. Integrated routes for aquaculture waste including wastewater, sludge, and CO_2 for energy recovery coupled with microalgae cultivation.

bacteria give CO_2 as a carbon source for microalgal growth, reducing or eliminating the demand for typical aeration (Fallahi et al., 2021; Soroosh et al., 2022). Moreover, the presence of heterotrophs in the MaB system enhances the removal of chemical oxygen demand (COD) from influent wastewater, thereby improving the effluent quality (Soroosh et al., 2023). Another reason to include heterotrophs in algal reactors for wastewater treatment is their natural tendency to flourish within non-sterile microalgae cultivation systems, particularly in the presence of influent COD. Additionally, the bioflocculation of biomass from MaB facilitates efficient harvesting compared to pure microalgal cultures, as they tend to settle by gravity, thereby offering a promising avenue as a wastewater treatment and cost-effective resource recovery technology (Pell et al., 2017; Valigore et al., 2012). There is a wide variety of predominant microalgal genera used in MaB systems, which include *Actinastrum, Acutudesmus, Ankistrodesmus, Chlorella, Oocystis, Micractinium, Stigeoclonium, Microspora, Scenedesmus, Monoraphidium, Pediastrum, Phormidium* and *Coelastrum*. The bacteria detected in these systems also are widely varied, which include Firmicutes, Verrucomicrobia, Bacteroidetes, Planctomycetes, and Epsilonproteobacteria (Krohn-Molt et al., 2013; Lee et al., 2013; Tang et al., 2023).

In the context of aquaculture, the process that exploits the microalgae-bacteria symbiosis has been termed biofloc technology (BFT). It is termed as the use of biological aggregates including bacteria, microalgae, fungi, and/or protozoa held together with particulate organic matter in a matrix for the principle of improving the water quality, wastewater remediation, and disease inhibition in the intensive aquaculture systems. It represents an integrated solution for challenges of quality management, feed sustainability, and production costs (Khanjani and Sharifinia, 2020). By harnessing microbial protein produced within the biofloc, aquaculture operations can reduce the reliance on fishmeal and soybean meal in feed formulations, thus mitigating the environmental impacts associated with these resources (Panigrahi et al., 2019). The integration of biofloc from MaB with formulated diets offers a complete and sustainable food chain for aquatic organisms, resulting in improved growth performance and economic benefits for aquaculture operations. Previous studies confirmed that bioflocs can have probiotic effects that can enhance the health and physiological function of aquacultural animals. For instance, Panigrahi et al. (2019) fed the shrimp Juvenile White Tiger Prawns *Litopenaeus vannamei* (1.48 g) diets composed of five different crude protein levels (BFT, 31–47 % crude protein) in a BFT-based heterotrophic system. Results showed substantially better growth, survival, feed utilization, health status, and higher immune genes expression in the treated shrimp compared to the control group (autotrophic condition + 40 % CP diet). The aquaculture industry expansion is linked to heightened environmental impact and a heavy reliance on fishmeal in diets. This confirms the importance of utilizing BFT even more crucially in applications within RAS. Though shrimp RAS production has become increasingly relevant with increasing market share in the last decade, the main species which will probably dominate increasing RAS production in the coming years (salmon and trout) cannot be cultivated in BFT due to their species-specific water quality requirements, which limits BFT for RAS expansion. Overall, adoption of BFT represents a promising avenue for sustainable aquaculture development, offering a more environmentally friendly and cost-effective methodology compared to conventional treatment methods. However, caution should be exercised when considering the application of BFT for utilizing biomass in feed, food, or other similar purposes, considering the potential concerns related to pathogenicity.

4.2. *Aquaculture solid waste for microalgae cultivation*

In a well-managed aquaculture farm, around 30 % of the feed utilized typically becomes solid waste (Ebeling and Timmons, 2012; Miller and Semmens, 2002). As feeding rates typically rise with temperature-increased metabolic rates in fish (Volkoff and Rønnestad, 2020), solid waste production often escalates during the summer months when feeding rates are at their peak. Aside from opting for high-energy extruded feed to enhance assimilation, effective waste management strategies should prioritize the prompt removal or reutilization of solids. Principal treatment and solid waste recycling have been suggested to be done as soon as possible to reduce the fragmentation of fish feces/waste, which results in the leaching of more nutrients into the water. In addition, excessive waste accumulation spreads diseases in the fish culture (van Rijn, 2013). The most effective means of reducing downstream

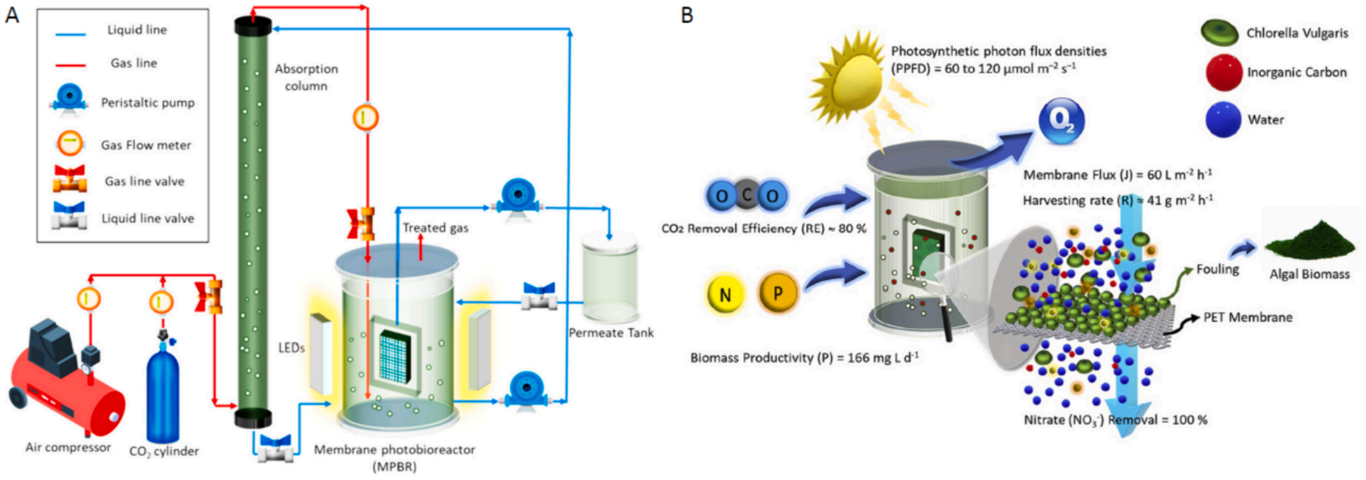

Fig. 3. A schematic diagram showing the microalgae cultivation system using membrane photobioreactor (A) and algal biomass separation through the membrane (B). Adapted from Ref. (Senatore et al., 2021) after copyright permission No. 5512030782468.

pollution is by promptly removing solids in their settleable form before discharging them into water bodies. When solid wastes settle downstream, they can cover benthic animals and diminish oxygen levels, thereby decreasing biodiversity (Miller and Semmens, 2002). Further, some integrated treatments can be applied to the solid waste fraction of aquaculture, which includes mainly anaerobic digestion and thermochemical conversion (Fig. 2). It is noteworthy to mention that these methods are widely recognized and traditionally employed for the treatment of solid waste.

Anaerobic digestion (AD) is one of the well-established technologies for biosolid waste treatment in agricultural and wastewater industries (Wang et al., 2023a). However, Aq-S produced from the backwash of RAS drum filter lacks enough alkalinity and organic load for an effective digestion process in bioreactors usually used to handle other waste, e.g. animal manure (Choudhury et al., 2022). The pre-treatment technologies applied for RAS Aq-S dewatering and thickening such as geotextile inclined belt filters, bag filters, or membrane reactors can accomplish 9–22 % solids concentration, which could allow efficient anaerobic digestion (Sharrer et al., 2010). Increasing alkalinity, to avoid digestor failure because of sudden pH decrease during acidogenesis, can be obtained by supplying bicarbonate to rise the buffering capacity (Choudhury et al., 2022). Goddek et al. (2018) compared the performance of different mineralization techniques for their organic Aq-S reduction and the ability of macro/micro-element mineralization. Results showed that aerobic and up-flow anaerobic sludge blanket (UASB) reactors are better suited for organic Aq-S reduction in relatively short time (21 days).

UASB Aq-S higher mineralization rates were reported for aerobic digestion of fish fecal Aq-S for 29 days; where concentrations of minerals significantly increased; PO_4-P by 1480 %, K by 124 %, Ca by 33 % and Mg by 181 %; in the Aq-S (Rakocy et al., 2007). In addition, NO_3-N levels increased from 2.3 to 313 mg L^{-1} due to mineralization of N after primary organic matter degradation. Moreover, biological pretreatment could enhance the solubilization of nutrients in the Aq-S. For instance, using homo-lactic *Lactobacillus plantarum* starter cultures showed high solubilization activity to 96.4 %, Ca, 93.0 % Zn, 92.2 % Fe, and 81.9 % P in the Aq-S (Jung and Lovitt, 2011). Despite AD presents a promising pathway for Aq-S recycling towards biogas production, the toxic components in the Aq-S cannot be entirely eliminated through AD, posing challenges for the disposal of anaerobic digestate. Therefore, thermochemical treatment emerges as a potential alternative or complementary approach for secure processing of Aq-S.

Thermochemical conversion processes encompass various methods such as pyrolysis, hydrothermal liquefaction (HTL), and gasification, offering a promising way to utilize the aquaculture sludge as a feedstock. Through pyrolysis, the dry feedstock is heated in the lack of oxygen, breaking down the organic matter into bio-oil, syngas, and biochar (Syed-Hassan et al., 2017). The bio-oil and syngas can be used as fuels or chemical feedstocks, while biochar has potential applications as a soil amendment. Gasification engages partial oxidation of the dry feedstock at high temperatures, converting the biomass predominately into syngas composed of hydrogen, CO_2, and methane. This syngas can then be used to generate power and heat or serve as a building block for synthetic fuels and chemicals (Syed-Hassan et al., 2017). However, gasification and pyrolysis require dry feedstock, where drying of Aq-S is energy intensive and costly process. Different from the aforementioned processes, HTL converts the wet feedstocks (moisture content around 70 % to 80 %) under high pressure (5–25 MPa) and moderate temperature (200–400 °C) into four main products namely crude bio-oil, aqueous liquid, solid char, and non-condensable gases (Xu et al., 2014), with a wide range of applications (El-Hefnawy et al., 2023). When compared to other biological or thermal conversion processes, HTL has some unique features. First, the crude bio-oil produced contains more energy than alcohol or syngas (Lachos-Perez et al., 2022). Second, the HTL can process feedstocks with high water content since it does not require the pre-drying step required in case of pyrolysis or gasification, which saves a lot of energy, cost, and drying time (Chen and Li, 2020). Additionally, HTL is considered a reliable technique for ensuring the safety of the final waste products due to its utilization of high temperatures.

Nutrient recovery from municipal sludge for microalgae culture has been explored using a two-step HTL process, which allowed efficient growth of *Euglena gracilis* and *Aurantiochytrium* sp. on the aqueous phase from HTL (Aq-HTL) rich in nutrients (Aida et al., 2016). Kumar et al., (2022) also highlight the potential of nutrients recovery from Aq-HTL municipal sludge for microalgae cultivation. Recently, the Aq-HTL from co-HTL of seaweed waste with microalgae enhanced the microalgal biomass yield by 10.3 % (El-Hefnawy et al., 2023). Thus, HTL for nutrient recovery from the Aq-S has the potential to provide a closed-loop system for crude bio-oil production and the suitability of the recycled Aq-HTL phase for microalgae cultivation as summarized in Fig. 2. Accordingly, AD and HTL serve dual purposes for Aq-S management. They facilitate the treatment and stabilization of Aq-S, and enable the recovery of nutrients from the treated sludge, which can be further utilized for microalgae cultivation, creating a closed-loop system. Therefore, both AD and HTL not only treat the Aq-S but also serve as a pretreatment to recover nutrients for subsequent microalgae cultivation, enabling a closed-loop system for waste management and bioproduct generation.

5. Microalgae-assisted recirculating aquaculture systems

The conventional technologies used in RAS developed to provide reliable performance and the algae's potential to improve the corresponding technology are shown in Table 4. In the context of an integrated RAS-microalgae system, microalgae cultivation system may be established independently of the RAS system, denoted as "uncoupled". Alternatively, a coupled system involving cultivating microalgae directly in conjunction with the fish within the RAS system can be established. The choice between uncoupled and coupled configurations depends on specific operational goals, resource utilization, and overall system efficiency considerations. For microalgal biomass production, cultivation process and dewatering stage are the two most critical steps which require further improvement to reduce the overall production cost (Kroumov et al., 2017). Different cultivation systems have been extensively discussed in previous studies (Wang et al., 2022). Closed photobioreactors (PBRs) and open raceway ponds are the two common systems used for microalgae cultivation. Despite their higher cost, closed PBRs offer several benefits over raceway ponds, such as higher growth rate, limited contamination, and better control of the cultivation conditions. In continuous cultivation mode in PBR, the biggest limitation is the natural biomass wash-out, i.e., loss of microalgal biomass due to the high dilution rate or short residence time (Discart et al., 2014). Therefore, the decoupling of microalgal biomass retention time (MRT) and the dilution rate may overcome this issue (Soroosh et al., 2022). Running the PBR in a membrane photobioreactor (MPBR) mode by installing a membrane filtration unit with the cultivation tank (Fig. 3) was suggested (Discart et al., 2014; Senatore et al., 2021).

In this type of reactors, the membrane acts as a barrier, ensuring complete retention of microalgal cells, thereby preventing their washout and enabling higher achievable biomass concentration, while the medium (water and remaining nutrients) flows through as permeate (Fig. 3). Additionally, biomass concentration can be effectively controlled by utilizing a separate filtration tank, where a portion of the retentate is returned to the MPBR (Discart et al., 2014). Due to its greater flexibility and robustness in a flow-through system, the MPBR can operate at higher dilution and growth rates, resulting in up to a nine-fold increase in biomass productivity compared to conventional photobioreactors (Bilad et al., 2014). Moreover, pre-harvesting can be achieved by applying variable concentration factors, while the remaining nutrients in the permeate can be recycled back to the reactor as a feed medium with minimal impact on growth. Using this system could achieve also a significant reduction in nutrient costs and water footprint (Discart et al., 2014). The practicality of using MPBR for wastewater treatment and microalgae cultivation has been broadly studied and multiple advantages have been put forward (Ding et al., 2022; Gao et al., 2021).

In case of aquaculture effluent, MPBR allows replacing the expensive synthetic growth medium with nutrients recovered from aquaculture, which showed a potential reduction in the overall production costs. It is estimated that using wastewater as a nutrient source could lower the production costs of microalgae by more than 60 %, from 3.6 € kg^{-1} (using conventional synthetic growth media) to 1.4 € kg^{-1} (Acién Fernández et al., 2019). In addition, CO_2 released during the process can be used by microalgae through photosynthesis, further improving the economic and ecological aspects. Due to the higher microalgal biomass yield in MPBR compared to that obtained from conventional PBR, the energetic demand of downstream processes such as harvesting and dewatering is reduced. However, the performance of MPBR highly depends on several operational conditions, such as hydraulic retention time (HRT), lighting, and solids retention time (SRT). SRT, for example, significantly influences the growth, microalgal biomass, and nutrient removal rate in MPBR systems (Xu et al., 2015), where several HRT/SRT ratios were tested and the highest algal productivity of 131.7 g m^{-3} d^{-1} was recorded at a ratio of 6 h/5 days. However, MPBR system faces challenges in maintaining long-term operation under high SRT for municipal wastewater treatment. In that context, it becomes imperative to conduct experiments assessing the long-term performance of MPBR when applied to RAS-AWW. A range of innovative technologies have been developed for uncoupled microalgae cultivation in RAS. For instance, Egloff et al. (2018) demonstrated the potential of a thin-layer PBR (TL-PBR) to continuously add water from RAS to a microalgae culture, achieving high biomass densities. In addition, Valeta and Verdegem (2015) subjected an algal turf scrubber (ATS) to high nutrient loads of catfish effluent with high ammonia nitrogen removal rate (0.656 g m^{-2} day^{-1}). These technologies were reported to offer promising solutions for efficient microalgae cultivation in a sustainable RAS (Ramli et al., 2020). Thus, advancements in technologies such as TL-PBR and algal turf scrubbers have the potential for efficient and sustainable microalgae cultivation within RAS.

Table 3
High-value products from microalgae showing the alternative sources and potential applications.

Microalgae	Product	Alternate source(s)	Applications	References
Haematococcus pluvialis, Chlorella zofingiensis	Astaxanthin	Xanthophyllomyces dendrorhous, synthetic	Pigment (aquaculture), anti-oxidant	(Schmidt et al., 2011)
Chlorella spp.	Canthaxanthin	Dietzia natronolimnaea, synthetic	Pigment (aquaculture, poultry and food)	(Koo et al., 2012)
Scenedesmus spp., *Muriellopsis* sp.	Lutein	*Tagetes* sp., Blakesleya trispora	Antioxidant	(Fernández-Sevilla et al., 2010)
Chlorella ellipsoidea; Dunalielle salina (mutant)	Zeaxanthin	Tagetes erecta, synthetic; Paprika (*Capsicum annuum*)	Pigmenter and antioxidant	(Koo et al., 2012)
Dunaliella spp.	Phytoene, phytofluene	Tomato (*Solanum lycopersicum*)	Antioxidant or cosmetic	(von Oppen-Bezalel and Shaish, 2019)
Parietochloris incisa	Arachidonic acid	*Mortiriella* spp.	Nutritional supplement	(Solovchenko et al., 2008)
Dunaliella tertiolecta Dunaliella salina	Phytosterols	Various plants	Nutraceutica	(Francavilla et al., 2010)
Aurantiochytrium sp.	Squalene	Shark liver	Cosmetics	(Kaya et al., 2011)
Porphyridium spp., *Rhodella* spp., Various cyanophytes	Polysaccharides	Guar gum, xanthan	Gelling agents; cosmaceuticals	(Arad and Levy-Ontman, 2010)
Cyanophytes, Dinophytes	Micosporine-like amino acids	Various plants	Cosmetics; Sunscreens	(Borowitzka, 2013)
Tribonema minus, Micractinium reisseri, Nannochloropsis oceanica, Oocystis pusilla, Chlorococcus infusionum	Lipids	Edible seeds	Biodiesel	(Ashour et al., 2019; Elshobary et al., 2019; Osman et al., 2023; Wang et al., 2023b)
Scenedesmus obliquus, Nanocloropsis oceanica, Spirulina platensis	Lipid-free biomass	Comemrcial feed products	Aquaculture feed	(Abomohra et al., 2014; Alprol et al., 2021; Ashour et al., 2019)
Nannochloropsis oculate, Selenastrum minutum, Phormidium pseudopristleyi, Porphyridium purpureum	Essential fatty acids	Fish oil	Nutrition, health products	(Ramesh Kumar et al., 2019)
Oscillatoria acuminata	Carbohydrates	Various plants	Biohydrogen	(Sallam et al., 2022)

Table 4

Conventional technologies used in recirculating aquaculture systems (RAS) developed to provide reliable performance and the algae potential to improve the corresponding technology (APIT).

Technology	Purpose	Advantages	Limitations	APIT
Mechanical filtration	To remove particulate matter (solids) from the water, such as fish feces, uneaten food, and debris.	Helps maintain clear water, which is essential for maintaining good health and better monitoring of the fish condition.	Mechanical filters do not remove dissolved wastes.	Algae integration helps in removal of dissolved wastes.
Biological filtration	To convert harmful ammonia produced by fish waste into nitrites and then into less harmful nitrates using beneficial bacteria.	Critical for preventing ammonia toxicity in dense populations typical of RAS systems.	Sensitive to changes in water conditions, requires time to establish a new system, and can be disrupted if not properly maintained.	Algae have a symbiotic relation with bacteria which could enhance the removal efficiency.
Chemical filtration	To remove or neutralize dissolved wastes, toxins, or antibiotics from the water using activated carbon or other chemical media.	Effective at controlling a variety of water quality issues that mechanical and biological filters can't address.	Regular replacement of the chemical media is needed; overuse can remove beneficial elements from the water.	Algae have high removal efficiency to such chemicals and toxins.
Oxygenation systems	To ensure there is ample dissolved oxygen in the water, which is crucial for fish survival and health.	Supports higher stocking densities by compensating for the oxygen consumed by fish and bacterial activity.	Energy-intensive and critical to maintain; failure can result in rapid loss of fish life.	Oxygen production and CO_2 sequestration by microalgae enhances the oxygenation process.
Temperature control	To manage water temperature, keeping it within the optimal range for the specific species cultured.	Temperature regulation is vital for metabolic rate control, growth optimization, and disease prevention.	Requires energy for heating or cooling, which can be costly; equipment failure can result in stress or loss of stock.	Not applicable
Solid waste management	To efficiently remove solid wastes from the system via techniques like settling basins, drum filters, or swirl separators.	Removes a significant source of ammonia before it enters the biological filtration stage; reduces load on filters.	Requires additional handling and disposal of waste products; efficiency depends on proper sizing and management of the removal system.	Algae integration with HTL or anaerobic digestion of solid waste ensures zero-waste route.
Water exchange	To periodically replace a portion of the system water with fresh, treated water to help manage nutrient levels and dilute pollutants.	Simple and effective way to reduce nitrate buildup and other non-filterable substances.	Water replacement incurs additional costs and can disturb the system if not done carefully. It also results in less conservation of water compared to fully recirculated systems.	Wastewater treatment using microalgae reduces the water exchange rate in RAS

In the coupled system of integrated RAS-microalgae cultivation, microalgae exert a multifaceted influence on the aquaculture environment. Their presence significantly shapes the bacterial community dynamics, fostering intricate interactions within the microbial ecosystem through metabolic activities such as photosynthesis and organic compound release. Moreover, microalgae play a crucial role in impacting fish respiration dynamics by contributing to increased oxygen production during daylight hours, ensuring a balanced and oxygen-rich environment for the aquatic species. Additionally, microalgae influence the mineral balance within the RAS by assimilating essential nutrients like nitrogen and phosphorus, contributing to overall nutrient dynamics in the system. Managing these interrelated effects is paramount for optimizing the sustainability and performance of the coupled RAS-microalgae system. It is noteworthy to mention that macroalgae also have the potential to remove inorganic compounds from aquaculture effluents efficiently. In this context, Sebök and Hanelt (2023) confirmed the successful cultivation of *Ulva lactuca* in land-based aquaculture effluent, where the growth rate increased to 4.17 d^{-1} compared to Provasoli enriched Seawater (PES) of only 2.65 d^{-1}.

Fish metabolize energy through both fat and carbohydrates, yielding an average respiratory quotient (RQ) of 0.85, i.e., 0.85 mol of CO_2 are produced per mole of O_2 consumed. Based on typical aquaculture rearing densities exceeding 50 kg fish m^{-3}, oxygen uptake rates of 4.2–13.1 mmol O_2 kg^{-1} fish h^{-1} can be calculated into 3.6–11.1 mmol CO_2 kg^{-1} fish h^{-1}. With such high stocking rates, fish respiration alone contributes up to 700 mmol CO_2 m^{-3} h^{-1}, indicating substantial production-scale carbon emissions (Skov, 2019). In eel culture, CO_2 production rate is estimated at 536 g CO_2 kg^{-1} feed (Heinsbroek and Kamstra, 1990), while Atlantic salmon releases approximately 409 g CO_2 kg^{-1} feed (Terjesen et al., 2013). Assuming a medium-sized trout farm with an annual production of 100 tones and an annual feed load of 103 tones, it corresponds to an annual production of 51 tones CO_2. The ability of microalgae to remove and metabolize CO_2 represents a great opportunity for microalgae-based CO_2 removal from RAS. However, little information is available on the concurrent capture of nutrients and CO_2 from outdoor aquaculture effluent using microalgae. A recent laboratory-scale study proposed that microalgal growth in aquaculture effluent can be enhanced by utilizing CO_2 stripped from RAS, thereby offering a potential avenue to enhance the sustainability and environmental friendliness of aquaculture production (Pirhonen et al., 2023).

Table 5

Comparison of emissions produced per kg P_2O_5 from conventional phosphorus (P) fertilizer production (CPFP) as well as P recovery techniques from sewage waste streams.

Impact category	Unit	CPFP	P-recovery techniques		
			Precipitation process with BIO-P	Sewage sludge ash process	Precipitation in centrate (Stuttgarter process)
Non-renewable cumulative energy input from fossil and nuclear resources (KEA)	MJ kg^{-1} P_2O_5	27.0	2.0	28.5	210.0
Greenhouse gas potential 100a (GWP)	kg CO_2-Eq kg^{-1} P_2O_5	1.2	0.1	1.4	15.0
Terrestrial acidification potential (TAP)	g SO_2-Eq kg^{-1} P_2O_5	19.0	*na*	*na*	*na*
Freshwater Eutrophication Potential (FEP)	g P-Eq kg^{-1} P_2O_5	24.0	*na*	*na*	*na*
Marine Eutrophication Potential (MEP)	g N-Eq kg^{-1} P_2O_5	0.4	*na*	*na*	*na*
Human toxicity potential (HTP)	kg 1,4-DCBEq kg^{-1} P_2O_5	4.5	*na*	*na*	*na*

na refers to not available.

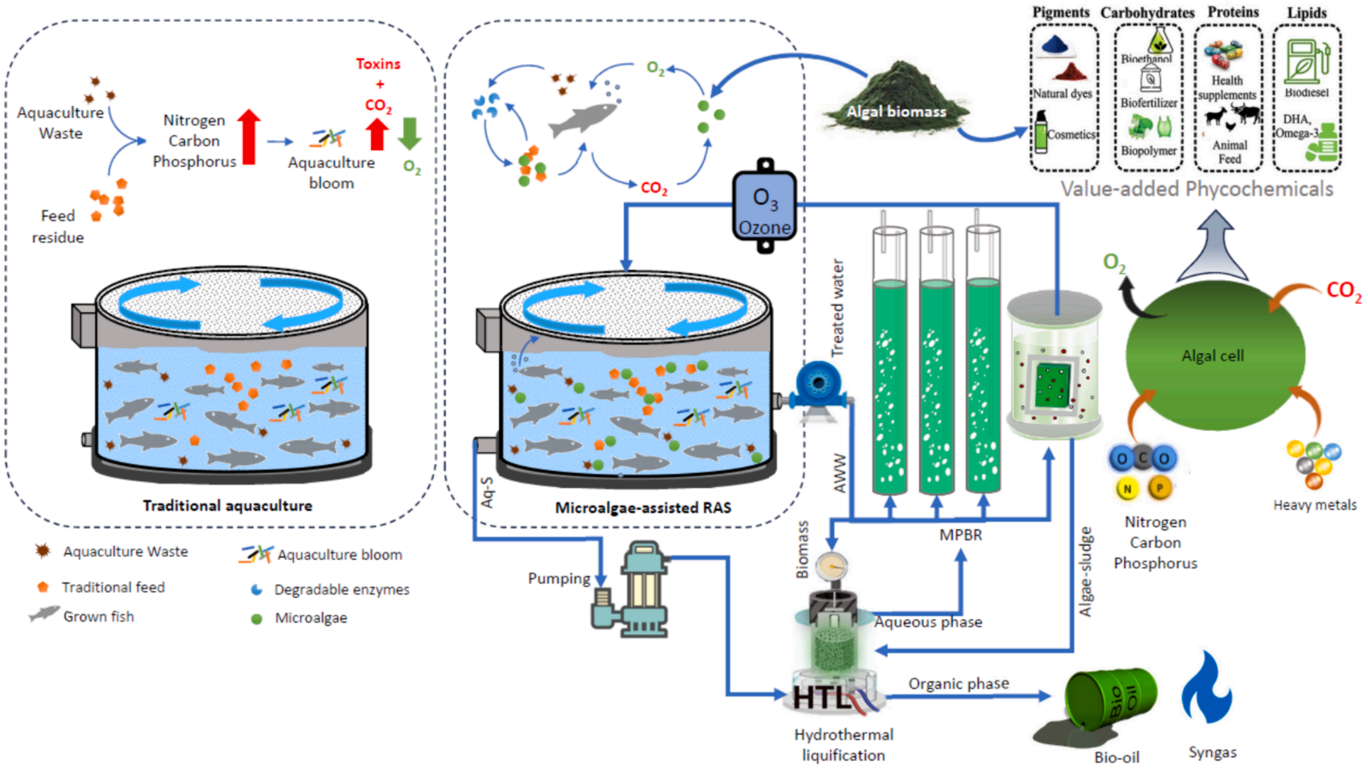

Fig. 4. The suggested closed loop microalgae-assisted RAS for energy recovery through a zero-waste route.

The study demonstrated that CO_2 supplementation accelerates the growth rates of *H. pluvialis* and *M. griffithii* by 18.2 % (0.44 to 0.52 day^{-1}) and 11.6 % (0.43 to 0.48 day^{-1}), respectively, over a 9-days trial. Based on a conversion efficiency of 1.8 kg of CO_2 per 1 kg of biomass formed (Lam et al., 2012), it is estimated that 28.3 tons of microalgal biomass are produced annually from a medium-sized trout farm. Given the diverse value-added products derived from microalgae (Abomohra and Ende, 2024), the implementation of algal biorefinery holds the potential to significantly enhance the economic feasibility of RAS. In that context, elevated CO_2 levels were found to substantially improve the productivity and nutritional quality of the macroalga *U. fasciata* (Barakat et al., 2021). By cultivating *U. fasciata* at a partial pressure of CO_2 (pCO_2) of 550 μatm, the maximum growth rate increased by 6.6 % per day compared to the ambient conditions. Furthermore, elevating pCO_2 to 550 μatm doubled the protein content to 32.43 dw% and boosted pigment levels to 2.9 mg g^{-1} versus untreated cultures (Barakat et al., 2021). The CO_2-enriched *U. fasciata* also displayed higher omega-3 and omega-6 fatty acid contents. The combination of faster growth and superior nutrient composition confirmed CO_2-stimulated *U. fasciata* as an innovative supplement to enhance the diets of farmed fish like sea bass (El-Sayed et al., 2022). Thus, integrating algae cultured under forecasted CO_2 conditions into aquafeed formulations or directly into fish ponds provides a prospective carbon mitigation technique to improve aquaculture via nutritional synergies across cultivation systems.

In addition, increasing mineral accumulation with RAS culture intensification has been related to the growth retardation of fish. For instance, negative effects of water-borne magnesium on the growth and mortality of Rainbow trout (*O. mykiss*) were observed at concentrations of 1000 mg L^{-1}, while no impacts were observed at 150 mg L^{-1} of magnesium (Shearer and Asgard, 1992). In order to remove such growth-inhibiting substances, algae can accumulate minerals/contaminants and have been used in multiple bioremediation approaches (Elshobary et al., 2019; Osman et al., 2023; Sebök and Hanelt, 2023). Hence, a microalgae-integrated aquaculture system could potentially reduce or remove many growth retardators from RAS.

Table 6
The production cost of microalgae biomass by system, plant size, and location.

System type	Production volume	Nutrient media	Production costs*	References
Open raceway	1 ha	Synthetic	12.0 € kg^{-1}	(Sui et al., 2020)
	5 ha	Synthetic	4.9 € kg^{-1}	(Acién Fernández et al., 2019)
	5 ha	Waste stream	1.3 € kg^{-1}	(Acién Fernández et al., 2019)
	405 ha	Synthetic	0.7 € kg^{-1}	(Hoffman et al., 2017)
Open raceway turf system	405 ha	Synthetic	0.5 € kg^{-1}	(Hoffman et al., 2017)
Thin layer cascade	5 ha	Synthetic	2.5 € kg^{-1}	(Acién Fernández et al., 2019)
	5 ha	Waste stream	0.7 € kg^{-1}	(Acién Fernández et al., 2019)
Photobioreactor	1500 m^2	Synthetic	46.9 € kg^{-1}	(Oostlander et al., 2020)

Calculations are based on 0.5 € kg^{-1} fishmeal.
*At exchange rate 1 $ = 0.93 € on Mai 2024.

6. Microalgal biorefinery

The composition of waste streams potentially allows to manipulate certain compounds of commercial interest. Table 3 shows a summary of many of the existing high-value products and the potential of microalgae as alternative natural resources. Industrial chemical synthesis competes with several of the algae value-added products, which are produced mainly by chemical synthesis at lower prices and, therefore, the current microalgal biorefineries are small-scale (Shitanaka et al., 2024). Despite the environmental benefits of green biosynthesis of these products, finding innovative routes to lower the production cost is of great importance. Integrated biorefinery of microalgae-assisted RAS is quite essential to improve the economic feasibility of fish farming and provide additional profit to the farmers. The targeted product can be varied based on the kind of waste used and the cultivated microalgal species. For example, lipid productivity of the green microalga *Nannochloris maculate* significantly increased using aquaculture effluent as a growth medium (Khatoon et al., 2016). In addition, *N. maculate* and *Tetraselmis chuii* showed 10 % and 8 %, respectively, higher protein contents when cultured in wastewater medium. Microalgae also have been discussed as a commercial source of high-value chemicals such as phycobilins, astaxanthin, β-carotene, and essential unsaturated fatty acids (Table 3). In addition, algal biomass after extraction of high-value compounds can be used as fish feed or as a feedstock for the production of different biofuels, ensuring a zero-waste approach. An additional value-added product that can be derived from the waste streams of RAS is a granular fertilizer sourced from the Aq-S. Nevertheless, precautions must be diligently taken to prevent the spread of diseases or the introduction of undesirable compounds, including heavy metals and antibiotics, into the agricultural system.

Struvite also can be recovered from the aquaculture effluent and used further as a fertilizer or for microalgae cultivation. In that context, struvite is already included as a commercial fertilizer in regulation EC No. 1907/2006 (REACH) and EU 2019/1009. Later in 2021, the Commission Delegated Regulation (EU)2021/2086 included precipitated phosphate salts and their derivatives as a component material category in the EU fertilizer products. A new component material category (CMC) was then included in Annex 2 of the EU Fertilizer Regulation (EU) 2019/1009: CMC 12: Precipitated Phosphate Salts and their Derivatives. The European Union aims to close substance cycle waste management ensuring ecologically sound administration of waste (European legal targets of the Directive 2008/98/EC of the European Parliament and of the Council in 19 November 2008 on waste and repealing certain Directives (OJ L 312, L 127, L 297, and L 42), which was last amended by Directive (EU) 2018/851 (OJ L 150) and shall be eligible for support. These regulations are crucial as they ensure that substances, including fertilizers like struvite, meet stringent safety and environmental standards. Thus, the utilization of aquaculture effluents for struvite production represents a strategic and environmentally conscious step toward closing the nutrient cycle in aquaculture operations.

7. Challenges and perspectives

The coupled microalgae cultivation in RAS, while offering notable advantages, comes with inherent challenges. The complexity of managing a coupled system introduces operational intricacies, requiring precise control over nutrient dynamics and environmental parameters. The competition for essential nutrients between microalgae and fish may impede optimal conditions for both components, impacting growth and fish health. Furthermore, the risk of algal blooms poses a threat to water quality and oxygen levels. The increased energy consumption to maintain suitable conditions for both fish and microalgae, coupled with potential pathogens spread through microorganisms, adds complexity to the system management. Moreover, care must be taken to monitor the quality of the harvestable microalgal biomass as well as fish due to possible transmission of contaminants though microalgae feed. For instance, *Chlorella* sp. grown in media containing sodium arsenate showed accumulation of Zn over the biosecurity values (2.4 g arsenic kg^{-1} dry mass) (Goessler et al., 1997). Other heavy metals such as chromium (Cr), lead (Pb), and cadmium (Cd) have been reported to accumulate over time and by increasing the farming intensity (Martins et al., 2009b), therefore, pose a potential risk for microalgal biomass quality. Thus, it is suggested to cultivate microalgae in a separate system to avoid direct contact with fish and mitigate the risk of contamination.

Improving RAS performance involves optimizing water quality, enhancing energy efficiency, managing stock densities, adopting advanced feed strategies, and enforcing stringent biosecurity measures to reduce emissions (Table 5). These methods improve system reliability, reduce the overall cost, and increase the production efficiency, while posing challenges like higher initial investments and management complexity. Integrating these strategies with new technologies, such as improved microalgae cultivation, is the key to sustainable growth, environmental responsibility, and economic viability in aquaculture. Fig. 4 summarizes the suggested route for a possible combination of algae-based RAS. Integrating new algal technologies with RAS enhances the economic feasibility, environmental impact, and nutrient recovery. Biofuels, gaining popularity due to concerns about CO_2 emissions and rising energy demand, offer a promising route for energy recovery through HTL, converting Aq-S into disposable by-products and microalgal growth medium (Fig. 4). In addition, using aquaculture effluent as a nutrient source could reduce the production costs and increase the system sustainability (Table 6). Although using AWW can inhibit the microalgal growth in some cases, this can be mitigated by selecting suitable microalgae or supplementing deficient nutrients.

8. Conclusions

Cultivating microalgae presents a promising means of enhancing sustainability in RAS. By assimilating dissolved nutrients like nitrogen and phosphorus, algal metabolism can improve water quality, while oxygenation via photosynthesis further benefits the reared species. Uncoupled membrane photobioreactors enable controlled algae production, where biomass can be harvested and directly fed to cultured organisms or used for biorefinery. The suggested integrated RAS-microalgae platform could mitigate the environmental impacts through chemical remediation, axial carbon capture, and recycled bioconversion. However, continued innovations for integrated bio-based treatment to balance economics and ethics are vital for actualizing sustainable RAS with value-added coproduction.

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Stephan Ende: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Joachim Henjes:** Writing – review & editing, Investigation. **Marc Spiller:** Writing – original draft, Investigation. **Mostafa Elshobary:** Writing – original draft, Formal analysis, Conceptualization. **Dieter Hanelt:** Writing – review & editing, Investigation. **Abdelfatah Abomohra:** Writing – review & editing, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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Appendix A. Supplementary data

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