Chapter 12

Bioconversion of Agricultural Wastes From the Livestock Industry for Biofuel and Feed Production

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12.1 INTRODUCTION

Microalgae are photosynthetic organisms with high productivity that assimilate eutrophying nutrients from wastewaters. They have been used for over 50 years in domestic wastewater treatment and more recently for bioremediation of manure effluents. Microalgae have gained attention as agents for nutrient bioremediation due to annual yields 7−15 times greater than soy or corn and a low biomass C/N ratio which allows the potential to convert manure nutrients into biomass in smaller land areas than crop plants.

Industrialization of livestock production began in the post war era with the development of concentrated animal feeding operations (CAFOs), whereby thousands of beef, dairy, poultry and swine, are raised in a small area until they are ready for market. The US livestock industry produces an enormous quantity of manure, as much as 500 million tons of manure per year [1]. Poor approaches to manure management, especially in CAFOs, have led to serious environmental problems including methane and CO_2 emissions [2] and eutrophication of surface and ground waters by manure

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nutrients, primarily nitrogen (N), and phosphates (P) [3]. Manure is an excellent fertilizer containing plant nutrients and amending soils with organic matter improves soil structure, aeration, moisture-holding capacity, and sequesters nutrients until plant uptake, preventing excess nutrients from entering the watershed. However, CAFOs produce large numbers of animals in small land areas allowing competitive animal production. But typically these operations have little access to affordable land to use manures as a crop fertilizer. Roughly 20% of the N and 35% of the P are recovered from manure in CAFOs using land application as crop fertilizer. The rest of the components in the manure remain unused in the fields and find their way into ground and surface waters [4]. In 2%–5% of all counties in the United States, including the agricultural regions of California, Virginia, and much of the southeast, the amount of nutrients present in the manure produced by CAFOs is greater than the entire assimilative nutrient capacity of all the cropland and pastureland available in those counties [5]. As a result, large amounts of manure are overapplied, exported to other locations, or stored on site. The primary source of agricultural nonpoint source contamination in California's groundwater is due to the improper disposal of manure wastes, by CAFOs [3]. Thus, there is a critical and urgent need to control manure-derived greenhouse gases (GHGs) and nutrient pollution while reclaiming water and nutrients.

Nutrient contamination, primarily N and P, generated by waste from CAFOs is leached from soils, lagoons, and manure storage sites. Nutrients percolate into ground waters and/or are washed into freshwater streams and reservoirs and eventually to marine waters via streams and urban storm water systems. N and P enrichment enhances algal growth, resulting in algae blooms in freshwater and red tide blooms in coastal marine waters globally [6]. The hypoxia produced by decay of such blooms coupled with, in some cases, algal toxins, leads to mortality of marine fauna [7]. Moreover, many groundwater basins in the arid southwest of the United States have been historically underutilized due to high nitrate concentrations. Significant pollution of surface and groundwater has been caused by CAFO discharges, affecting over 20 basins covering more than 10,000 square miles of land located primarily in California's Central Valley [8,9]. Drinking water with high nitrate levels is associated with acquired methemoglobinemia and gastric cancer [10]. Excess P levels in waters are also associated with cyanobacterial blooms [11].

Inadequately treated CAFO wastes impact surface water, groundwater, and air quality in every state. In fact, more than 70% of the water quality problems in surveyed rivers and lakes are believed to be a result of agricultural wastes [12]. US farms are responsible for ~7% of the GHGs emissions due to decomposition of untreated manures releasing methane and CO_2 to the atmosphere [2]. Significant pollution of surface and groundwater, caused by CAFO discharges, affect over 20 basins covering more than 10,000 square miles of land located primarily in California's Central Valley and in agricultural regions across the country [13]. Nitrate levels in California groundwater can be 7–10 times higher than the Environmental Protection Agency's (EPA's) and the World Health Organization's (WHO's) maximum contaminant level of 10 mg/L in drinking water. To address this issue, California has initiated requirements for waste discharge to help reduce contamination of surface and groundwater. These requirements are incurring significant costs to California dairy operators [14] on top of the high cost of feed, which makes up about 65% of a dairy farm's operational expenses [15]. In addition to competing with the ethanol market, corn production for animal feed also competes for water and arable land for human food production. The rising cost of feed is in part due to the global demand for the corn ethanol market. As a result of these increased costs, during the past 12 years, dairy farms in California have decreased from 2100 dairies in 2003 to just over 1500 farms today [15]. Consequently, the need to control manure-derived nutrient pollution is straining the livestock industry in the face of stricter environmental regulations.

Microalgae are more efficient for nutrient reclamation [16] than crop plants, due in part, to higher rates of growth, but also, because algae lack the large stores of structural carbon (i.e., cellulose) found in land plants. Thus, the C/N ratio of higher plants ranges from 18 to 120 (by atoms) while microalgae range from 5 to 20 [17] allowing water reclamation and nutrient recovery to be accomplished more rapidly and in a smaller area using algae rather than terrestrial plants [18].

12.2 ALGAE-BASED WASTEWATER TREATMENT

Today, the rationale of wastewater treatment has shifted from simply reducing the biological oxygen demand (BOD) of organic wastes (primary and secondary aerobic treatment) and eliminating pathogens before discharge into receiving waters. With the rising costs of energy, water, and fertilizers, coupled to growing problems of eutrophication, there is heightened interest in also reclaiming nutrients and purifying water. In traditional wastewater treatment at least 70% of the cost is due to secondary and tertiary treatment. This is due to the energy costs of oxygen transfer in secondary treatment (which mineralizes organic compounds) and to the chemicals used in tertiary treatment to remove inorganic plant nutrients. Microalgae ponding systems were developed more than 50 years ago for municipal sewage treatment [19]. The engineering design parameters were developed and the basic biological processes in bioremediation were described in high rate-ponds [20,21]. Microscopic algae convert about 2% of total solar energy to algal biomass [22]. Photosynthetically generated oxygen allows bacterial communities to decompose organic wastes to simple inorganic nutrients including the plant nutrients N, P, and carbon dioxide.

The US Department of Energy's Aquatic Species program (1979−96) that explored microalgae as a feedstock for biofuels saw a possible synergy in coupling biofuel production with bioremediation of wastewaters [23,24]. Algae-based municipal wastewater treatment is economically feasible, compared to conventional wastewater treatment, and algae systems have about 50% lower energy consumption relative to conventional mechanical treatment technologies [25−27]. However, the production of microalgae solely for biofuels is not economically feasible to date [23,28]. A major cost factor in microalgae biomass production is the provision of water and nutrients [29,30], both of which can be provided by organic wastewaters. In addition, algae assimilate nutrients from wastewater during their growth, thereby generating revenue from the treatment service [23,26,27]. Thus, a resource and economic synergy is possible. Multiple benefits of such a scenario include a high quality effluent (i.e., stripped of nutrients and pathogens), biofuels including biodiesel and methane, coproducts including high protein residues and pigments, electricity from cogeneration of methane, and the capture of carbon dioxide from the cogeneration process [31]. Thus, the economic potential of the various byproducts of this process could offset the cost of biodiesel production to produce commercially viable biofuels.

Ponds are the most common technology used for treatment of municipal, agricultural, and aquacultural wastewaters in the United States, with over 7000 public owned treatment pond systems [32]. They are a simple and relatively quick technology to install and operate. Compared to mechanical treatment technologies, ponds remove BOD and suspended solids with low cost and energy consumption. However, nutrient removal is an increasingly common regulatory requirement, and conventional ponds are not well suited for nitrogen and phosphorus removal. Newer pond technologies (e.g., paddle wheel-mixed raceway ponds and newer variants of aerated lagoons) have advanced the reliability, effectiveness, and geographical range of pond treatment systems. Although algae-based tertiary treatment is economically feasible, few municipal algae ponds attempt to control species composition or harvest the algal biomass [23]. A major hurdle to algae production for a variety of applications and, in turn, to effect nutrient removal from wastewaters is the cost of harvesting and processing the biomass. Making cost-efficient algae biofuels, a relatively low-value commodity, will require major advancements in several technologies. To date, species control using unsterilized municipal or agricultural wastewaters has not been achieved. Other major hurdles to economically feasible algae-based biomass production include the use of fast-growing strains adapted to the local environment, development of resource specific production and management systems [24] and, in the short term, coupling alga culture with mitigation of environmental problems and coproduction of high-value compounds [33].

12.3 OIL FROM ALGAE

Microalgae are a phylogenetically diverse group of photosynthetic microorganisms that vary greatly in their morphology, physiology, and environmental range. Of interest in the context of this review are that microalgae are highly productive, autotrophic, many are heterotrophic and/or mixotrophic [34,35], and they produce variable amounts of oil (lipid) primarily as triglycerides. Algal neutral storage lipids are similar in structure and molecular weight (carbon chains ranging from 12 to 22) to the oils extracted from the terrestrial plants [33]. Microalgae can have oil contents that vary from 15% to 77% of the dry weight [36] and in most strains, lipid biosynthesis is regulated by a wide range of environmental variables [18,37–40]. Thus, many species of microalgae are well suited for commercial scale biodiesel production and because they grow on marginal lands in saline or wastewaters, not suitable for agricultural irrigation [41], do not compete with food production. While most microalgae can produce biomass faster than terrestrial crops and many store excess carbon as lipids rather than structural carbohydrates, this point distracts attention from the proper metric, which is the total cost of production.

Commercial operations cultivate algae either in open ponds or in closed bioreactors. Closed bioreactors help control unwanted invaders and allow higher and more stable production. However, the >10-fold higher capital and labor costs limit their use to high-value products such as carotenoids, pharmaceutical compounds, and nutraceuticals [42]. Open ponds present a number of obstacles to monoculture production including rapid variations in temperature, light, and invasion of competing algae species, pathogens, and grazers. To date, most of the commercial operations that grow monocultures in open ponds produce algal strains adapted to harsh conditions of alkalinity and salinity including *Spirulina* [43], *Haematococcus* [44], *Dunaliella* [45], and *Chlorella* sp. [46]. Of considerable interest to the algae biomass industry are solutions to control the algae population to maintain desired strains and optimize production.

12.4 ALGAE AS LIVESTOCK FEED

Microalgae are an essential food source in nature. They are used in aquaculture operations for feeding mollusks, crustaceans, and small fish. The chemical composition of algal species used in aquaculture is well documented [47]. The proximate composition of algae varies with species [48] and is strongly influenced by light, temperature, and nutrient levels and other environmental parameters [49,50]. Thus, algal biomass production must focus on species composition, nutritional composition, and the effects of the environment on these parameters. In general, depletion of specific nutrients results in slowed algal growth, where protein levels decline while lipid and carbohydrates increase. Aquaculture operations seek algal strains rich in "highly unsaturated fatty acids (HUFAs), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (ARA, 20:4n-6), and docosahexaenoic acid (DHA, 22:6n-3)," HUFAs of importance for marine organisms and are essential fatty acids for humans [47].

Microalgae have most recently been employed as a livestock feed predominantly in the poultry industry because of the high carotenoid content of many algal species which enhances skin and egg yolk color. Algae biomass has been demonstrated to be a valuable feed supplement or substitute for conventional protein sources in multiple nutritional and toxicological tests [51]. In addition, a variety of potentially useful agricultural and pharmaceutical secondary metabolites, including antiviral [52], antitumor, antimicrobial [53], and immune-stimulatory [54] agents are produced by microalgae, improving the value of the product for consumption.

The nutritional quality of proteins is based on the amino acid composition. Ruminants can be independent of dietary amino acids since all the essential amino acids can be synthesized by rumen microorganisms. However, to achieve maximum rates of growth or milk production, dietary amino acids must be supplemented in the ration. Similarly, nonruminant animals must be supplemented with essential amino acids since they cannot synthesize them fat a rate that meets the animal's needs [55]. Most algae species have high protein content and favorable amino acid composition relative to WHO/FAO standards and have a relatively low content of potentially troublesome nonprotein nitrogen [47,51].

Carbohydrate utilization by animals depends on their digestion system. The cellulose content of algal cell walls (~ 10% of dry weight) will affect the digestibility by nonruminant animals. In land plants, cellulose may account for 20% to 50% (w/w) of the biomass [51]. Microbial fermentation, however, enables ruminants to utilize cellulose efficiently and algal feed supplements have been used successfully to increase growth rates of calves [56] and improve milk composition in dairy ewes [57]. Algal cell walls vary among taxa [58]. In the eukaryotic algae currently used for large-scale production (i.e., the *Chlorophyceae*, such as *Chlorella* and *Scenedesmus* spp.), cell walls are composed of β-1,4-glucan and cellulose [58,59]. A β-type heteropolysaccharide isolated from the marine microalgae *Isochrysis galbana* was demonstrated to have antioxidant activity [60]. Algae feeding tests performed so far indicate that their overall digestibility is high [51,61]. Algal lipids (DHA and other Omega 3s) have a positive impact as an animal feed and on healthy fat marbling in cattle [62]. Astaxanthin derived from strains, such as Haematococcus pluvialis, has been added to feed formulations to enhance the color of salmonids and poultry muscles [54].

What could limit the utility of nutritious algae–based feeds? Despite their high nutritional values and health implications, caution must be taken to avoid possible toxicity of the microalgal biomass. Of more 200,000 algal species that have been characterized, ~35,000 species appeared devoid of toxicity. Some species contain biogenic toxins, for example, purines, and nonbiogenic toxins, for example, heavy metals. Other algal species can elaborate pathological metabolites that result in neurodegenerative disorders [63]. This necessitates evaluation of species prior to their commercialization as feed supplements. Key to synergistic feed and fuel production using wastewaters (dairy effluent) is an understanding of pathogens and potential toxins (i.e., bacterial and cyanobacterial toxins) and the fate of heavy metals introduced to the system either from manure or during growth and processing of the algae biomass.

12.4.1 Manure Pathogens

Manure-borne pathogens include: *Salmonella, Listeria, Clostridium, Campylobacter* spp., pathogenic *Escherichia coli* (such as Shiga toxin-producing *E. coli*), *Mycobacterium paratuberculosis, Cryptosporidium parvum*, and many viruses [64,65]. Zoonotic pathogens associated with cattle include *Yersinia* spp., *Leptospira* spp., *Coxiella burnetii*, and the parasitic protozoa *Giardia lamblia* [66]. CAFOs produce large amounts of waste in California [67]. A dairy herd of 1000 cows can produce more >12,000,000 kg of manure/year [68]. The manure can be a reservoir for spreading pathogens, for example, via water, since it is usually stored or processed on-siter. Enteric bacterial pathogens associated with foodborne illnesses can survive for long periods in manure [69,70]. Factors, such as type of slurry, manure pH, dry matter content, temperature, numbers, and type of pathogen present and presence of competing organisms can influence survival of potentially pathogenic organisms in manure [71].

12.4.2 Cyanobacteria and Their Toxicity

Some cyanobacteria species produce hepatotoxins and neurotoxins that can kill zooplankton, humans, fish and other animals [72]. Freshwater cyanobacteria include *Anabaena circinalis, Anabaena flos-aquae, Aphanizomenon flos-aquae, Aphanizomenon ovalisporum, Cylindrospermopsis raciborskii, Gloeotrichia echinata, Lyngbya, Microcystis aeruginosa, Nodularia spumigena, Nostoc, Oscillatoria, Schizothrix*, and *Scytonema* [72]. *N. spumigena* was the first cyanobacterium to be associated with poisoning of livestock [73]. Cyanotoxins are known to bioaccumulate in some aquatic invertebrates, zooplankton, mussels, and in marine mammals [72], shellfish, prawns, and fish [74]. Since manure is rich in nutrients that can stimulate cyanobacterial growth in feed destined for livestock, presence of cyanobacteria and their toxins in algal biomass should be monitored.

12.4.3 Fate of Heavy Metals

Many algal species are known to rapidly accumulate heavy metals by biosorption and/or by active intercellular uptake [75] which leads to biomagnification in the food web of aquatic systems posing major health problems [76]. Production of algae on a large scale, especially in municipal wastewaters, can result in elevated amounts of heavy metals, for example, lead, cadmium and arsenic. Currently, there appears to be no official standards that have been established to regulate the heavy metals content of microalgal products [63]. Some algae manufacturers have voluntarily determined and introduced internal guidelines for metal levels in their products [77]. Microalgae can grow in environments rich in nutrients and accumulate metals from the wastewater. This makes them an attractive tool for sustainable, low cost, waste water treatment [78] where they can be employed to remove heavy metals from a system [79] but limit their use in feed production using effluents rich in heavy metals.

12.5 ALGAE PRODUCTION/PRODUCTIVITY ISSUES

Minimal nutritional requirements for algal growth can be estimated from the approximate molecular formula of algal biomass: $C_{(0.48)}$, $H_{(1.83)}$, $N_{(0.11)}$, $P_{(0.01)}$ [80]. The chemical compositions of municipal and dairy lagoon wastewaters typically have less N than P relative to algal biomass [22,81–83]. Although CO_2 limits algal growth in high rate oxidation ponds [23,84–88], when CO_2 is supplemented, N then typically limits algal growth on municipal wastewaters and often triggering nitrogen-fixing cyanobacterial blooms [89]. N limitation has long been known to be a trigger for lipid synthesis in some algal species [90,91]. In wastewater treatment, as N is depleted, a system with pond effluent recycling or, equivalently, a two-stage system would allow algal photosynthesis to continue to drive lipid production although total algal growth would be nutrient limited. Thus lipid productivity, the product of growth rate and lipid content, declines under nutrient stress [92–97]. Fast-growing strains, even with modest lipid levels under nutrient replete conditions, increase lipid yield and decrease harvest and processing costs [98]. In addition, robust growth of algae communities and their associated microbial consortium reduces competition by invading strains including parasitic fungi, bacteria, and viruses [99–101] which can decimate the algal fraction of the pond community.

12.5.1 Algae-Based Agricultural Wastewater Treatment

Municipal wastewater treatment using algae-bacterial systems is well established. More recently, there is a considerable volume of literature on algae-based bioremediation of raw and anaerobically digested manures from CAFOs to convert wastes into energy. As shown in Table 12.1, biomass

TABLE 12.1 Productivities of Microalgal Cultures in High Rate Algae Ponds Treating Municipal Wastes

Location	Treatment	Scale (M_2)	Duration	Productivity ($g/m^2/day$)	Dominant Species	Reference
California, USA	Secondary	70	10 Days August	25.3	Polycultures	[20]
			2 Months November–December	12.2		
California, USA	Secondary	1000	12 Months	18.4	Polycultures	[103]
Safat, Kuwait	Secondary	12	18 Months	15	Polycultures	[104]
Scotland	Secondary	13	Summer	18	*Chlorella* sp.	[105]
					Ankistrodesmus sp.	
Barcelona, Spain	Secondary	1.54	12 Months	12.7–14.8	Polycultures	[106]
Philippines	Secondary	100	12 Months	15.3	*Coelastrum* sp.	[159]
Hamilton, New Zealand	Tertiary	32	Summer	25	*Pediastrum* sp.	[107]
Haifa, Israel	Tertiary	120	Summer	33	*Micractinium* sp.	[108]
Haifa, Israel	Tertiary	150	Summer	35	*Actinastrum* sp.	[108]
Melbourne,	–	2800	30 Days March	14.3	Polycultures	[109]
Australia			30 Days April	17.4		

productivity in municipal ponds ranges from 12 to 30 g/m^2/day with considerable variation based on pond size, depth (facultative vs high rate algae ponds, HRAPs), BOD loading rate, retention time, mixing, climate, duration of experiment, and season. Table 12.2 shows comparable productivity in agricultural ponds and algae turf scrub systems. It should be noted that algae productivity values based on dry weight are overestimated when high BOD effluents are used as culture media and include detritus and heterotrophic organisms harvested. Estimates ranging from 20% to up to 25% of algae biomass harvested have been reported and variations in the ratio of heterotrophs to autotrophs is thought to be influenced by BOD levels in the influent, CO_2 supplementation, insolation, and nutrient levels [27] and productivities are often calculated on a chlorophyll basis to differentiate between heterotrophic and autotrophic biomass yields [102].

12.5.2 Anaerobic Digestion Coupled to Algae-Based Nutrient Recovery

Anaerobic digestion has been used successfully in municipal wastewater treatment for 150 years and offers several advantages to treatment of manure, food production wastes, and crop residues. Organic matter is broken down by an assortment of microbes under anaerobic conditions to soluble derivatives and into volatile fatty acids by acidogenic bacteria, which are, in turn, consumed by methanogenic archaea and converted into biogas [114]. The process converts up to 70% of organic material to biogas (60% methane and 40% CO_2) leaving a digestate rich in plant nutrients N, P, S, Fe, and K.

Digestate water quality characteristics are a function of many parameters including feedstock, loading rate, operating residence time, temperature, and of course digester type. While most digestates will contain solids, these are more prominent in primary effluents. Solids will inhibit algae productivity due to competition with bacteria for nutrients as well as shading of light due to the nature of the solids. Particles may range in size from greater than 2 mm to less than 0.05 mm [115]. Without removal of these particles, productivities may remain lower than expected. In addition, these solids tend to accumulate and the reactors get browner and less green as the additions accumulate. Algae grown on digestates for biomass may require pretreatment. Anaerobic digestates are richer in N and P nutrients relative to organic carbon and can alleviate shading and productivity issues [116].

12.5.3 Harvesting

Microalgae grown in outdoor ponds are relatively dilute compared to cultures grown in photobioreactors or heterotrophically. While a variety of harvesting systems have been in use for 50 years, most are expensive and energy intensive, limiting their use to high-value products. To date,

TABLE 12.2 Algae Productivities in Systems to Bioremediate Livestock Wastes

Location	Waste Source	Manure Treatment	Pond Types	Scale (M_2)	Duration	Loading Rate	Productivity	Species	Reference
						(g TN/m^2/day)	(g/m^2/day)		
Beltsville, MD	Dairy manure	Primary	Algae growth chambers	0.93	9 Weeks	0.64	5.35	Polyculture	[110]
		Secondary				0.75	5.34		
		Secondary				1.03	5.53		
Beltsville, MD	Swine manure	Primary	Indoor, facultative ponds	1	270 Days, April–December	0.27 ± 0.03	7.1 ± 1.0	Polyculture	
						0.48 ± 0.07	9.4 ± 2.2		[111]
						0.70 ± 0.09	8.7 ± 0.9		
						1.39 ± 0.14	9.6 ± 3.1		
Beltsville, MD	Swine manure	Primary	Indoor, raceways	1	270 Days, April–December	0.24 ± 0.01	6.8 ± 0.8	Polyculture (e.g., *Rhizoclonium* sp.)	[112]
						0.62 ± 0.01	9.2 ± 0.5		
						1.30 ± 0.01	10.7 ± 2.5		
	Dairy manure	Primary				0.33 ± 0.01	8.3 ± 2.0		
						1.60 ± 0.01	21.3 ± 2.4		
						2.30 ± 0.01	18.6 ± 1.3		
						0.33 ± 0.01	8.8 ± 1.2		
						1.60 ± 0.01	20.4 ± 2.5		
						2.30 ± 0.01	17.9 ± 2.4		

(Continued)

TABLE 12.2 (Continued)

Location	Waste Source	Manure Treatment	Pond Types	Scale (M_2)	Duration	Loading Rate	Productivity	Species	Reference
						(g TN/m^2/day)	(g/m^2/day)		
		Secondary				0.38 ± 0.01	10.5 ± 0.8		
						1.30 ± 0.01	13.9 ± 0.8		
						1.56 ± 0.01	17.3 ± 2.7		
						0.38 ± 0.01	10.3 ± 0.3		
						1.30 ± 0.01	16.6 ± 2.3		
						1.56 ± 0.01	21.0 ± 3.4		
		Secondary	HRAP	30		0.3−2.5	2.5 -24		
Veracruz, México	Swine manure	Secondary	HRAP	23.6	3 Weeks, May−June		8.9	Spirulina (*Arthrospira*)	[113]
							14.4−15.1		
					3 Weeks, September−October		6.2		
							11.8		
					3 Weeks, February−March		11.8		

HRAP, High rate algae pond.

bioflocculation of microalgae is a major improvement harvesting technologies, and in raceway ponds has been demonstrated as a chemical-free means for removing excess algal suspended solids of pond effluent [116,117], which has previously been a major drawback algae biomass production. Bioflocculation is also the preferred means to harvest algae for biofuel due to its simplicity and low-energy input [26,118,119]. Algae flocculation is discussed below in context of both physicochemical pond parameters and complex biological interactions in aquatic communities.

12.6 ECOLOGY OF ALGAE PRODUCTION

Algae in nature are associated with a consortium of bacteria in the phycosphere, a term first used by [120], based on commonalities to the rhizosphere of terrestrial plants where loose symbiotic associations between host and specific groups of microbes provides a number of mutually beneficial advantages. Algae and heterotrophic microbes are the primary producers and decomposers, respectively, forming the basic functional structure of aquatic ecosystems acting to recycle N, P, S, and C [40,121,122]. It is estimated that half of the ocean's primary productivity is secreted by phytoplankton and assimilated by heterotrophic bacteria and archaea [123]. The prymnesiophytes and dinoflagellates synthesize organic sulfur-containing molecules which are in turn are catabolized by marine bacteria playing a critical role in the global sulfur cycle [124]. Nitrogen goes through a biogeological cycle producing compounds with different oxidation states that are converted to different compounds by tightly connected networks of algal and microbial metabolism and nutrient exchange [125].

Axenic cultures of algae are difficult to isolate because in nature they are tightly associated with often specific bacterial clades [121,126–130]. Recent studies have shown that heterotrophic phycosphere symbionts not only enhance algae growth and lipid production [131,132] but also aid in autoflocculation, a major issue in cost-effective harvesting in production systems [133–135]. Bacteria associated with green algae and higher plants, termed plant growth-promoting bacteria, involve specific taxa of both symbiotic partners, presumably due to specific nutrient exchange with algae excreting specific organic carbon compounds [136] and bacteria producing essential vitamins, growth-promoting hormones and inorganic nutrients as products of decomposition [130,137,138].

While a variety of specific algae-microbe interactions have been reported, in many cases, the functional roles in the association have not yet been elucidated. The isolation of dinoflagellates and coccolithophores from a marine environment were closely associated with γ-proteobacterial members of the genus *Marinobacter*, which synthesize the siderophore vibrioferrin. Iron uptake was stimulated in cocultures suggesting that the bacteria promote algal assimilation of iron by facilitating uptake of this critical, yet in marine environments, growth-limiting nutrient [139]. This group [140,141] also teased apart a consortium between a diatom and a *Sulfitobacter* species that enhanced diatom cell division by secretion of the auxin indole-3-acetic acid, using both endogenous and diatom-secreted tryptophan. Robust growth was lost in diatoms cultured axenically suggesting a dependence on the bacteria [141]. Diatoms, a variety of unicellular green algal lineages and cyanobacteria, were reported to respond to synthetic indole-3-acetic acid (IAA) [142,143]. Members of the *Roseobacter* are among the most ubiquitous lineages in the phycosphere of marine phytoplankton. This group is metabolically diverse, with capabilities for anaerobic photosynthesis, organic sulfate catabolism, and biosynthesis of secondary metabolites with bioactive properties [144,145], including synthesis of vitamins B-1 and B-12 for which many algal groups are auxotrophic [137,146]. Fresh isolates of *Tetraselmis indica* were found to be associated with members of the *Pseudomonas*, *Acinetobacter*, and *Ruegeria* genera that promoted the growth of this strain. In addition, the bacterial consortium produced a variety of carbohydrases active on glucans, galactans, galactomannans, and pectins, suggesting a potential role in their use of cast-off algal cell walls [147]. Phylogenetic analysis of microbes closely associated with diatom and dinoflagellate isolates revealed members of the dominance of *Alphaproteobacteria* and *Gammaproteobacteria* and the *Flavobacteria–Sphingobacteria* groups which were not significantly represented in the water column. However, the functional niche of the association could not elucidated [148].

A key hurdle in sustainable large-scale algae production, in common with terrestrial crop production, is species control. Populations of zooplankton grazers, parasitic fungi [149] and infective bacteria [150], and viruses [100] are inevitable in outdoor ponds. Zooplankton herbivores can dramatically decrease algal populations in days [82,151–155]. High pH levels reaching over pH11 that occur with robust photosynthetic activity are known to inhibit herbivore populations [156]; however, CO_2 supplementation, which can double productivity in HRAPs [27], also keeps the pH neutral. High ammonium levels [157,158], low nocturnal pO_2 levels, large colonial forms of algae and associated microbial consortiums, and use of invertebrate hormonal analogs may effectively control some herbivores [159–162]. Infochemicals secreted by *Daphnia* and rotifer species stimulate an increase in colony size and autoflocculation, as defense response to predation, in *Scenedesmus* strains [15,31,33,163–165]. In contrast, organic matter excreted by *Chlorella vulgaris* acted to inhibit flocculation, to varying degrees, using five different flocculation agents [15,165]. The phycosphere association with *Roseobacter* may provide a probiotic function acting to ward off algal pathogens [144].

12.7 CONCLUSION

At present, it is unlikely that the economics of liquid fuel production from algal biomass can be uncoupled from wastewater treatment and CO_2 abatement credits. However, significant improvements in several key technologies, such as strain selection, species composition, best cultivation practices, and harvesting, are required to advance the economics of algae-based waste treatment coupled to biofuel production and coproducts derived from algae biomass. The growing efforts that look at ponding systems at the ecological level may bring much needed pond management strategies.

12.8 FUTURE OUTLOOK

The nexus of rising costs of energy, water and fertilizers, coupled to growing problems of eutrophication, has heightened interest in reclaiming nutrients and purifying water using algae-based systems that can also provide safe livestock feed, alternative proteins and nutrients, and specialty ingredients for humans, including feed and fuel, as coproducts. Algae farming is still in its infancy and management techniques in open pond systems will require insight from an ecological perspective. Integrated biological systems that can be used at the regional level with a variety of coproducts produced from algae biomass may make such systems sustainable and cost-effective in the future.

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